(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 September 2021 (23.09.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/186258 A1

(51) International Patent Classification:
F26B 3/20 (2006.01) *F26B 3/24* (2006.01)
A23C 9/20 (2006.01) *A23L 29/30* (2016.01)
C07H 1/06 (2006.01) *A23L 33/125* (2016.01)

(21) International Application Number:
PCT/IB2021/050703

(22) International Filing Date:
29 January 2021 (29.01.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2020 70175 20 March 2020 (20.03.2020) DK

(71) Applicant: **GLYCOM A/S** [DK/DK]; Kogle Allé 4, 2970 Hørsholm (DK).

(72) Inventor: **CHASSAGNE, Pierre**; 9 Rue du Commerce, 63110 Beaumont (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

WO 2021/186258 A1

(54) **Title:** METHODS FOR PROVIDING A SOLID HMO PRODUCT, AND SOLID HMO PRODUCTS OBTAINED THEREBY

(57) **Abstract:** Various methods are provided for providing a solid HMO product, said methods including drum-drying, belt drying, preferably vacuum bed drying, or granulation. Solid HMO products are obtained via said methods, as well as solid HMO products per se. The methods can provide solid HMO products which are different to those achieved via other methods.

METHODS FOR PROVIDING A SOLID HMO PRODUCT, AND SOLID HMO PRODUCTS OBTAINED THEREBY

TECHNICAL FIELD

Various methods for providing a solid HMO product are provided, as well as solid HMO products obtained via such methods, and novel solid HMO products themselves.

BACKGROUND

Human milk oligosaccharides (HMOs) are a heterogeneous mixture of soluble glycans found in human milk. They are the third most abundant solid component after lactose and lipids in human milk and are present in concentrations of 5-25 g/l (Bode: *Human milk oligosaccharides and their beneficial effects*, in: Handbook of dietary and nutritional aspects of human breast milk (Zibadi et al., eds.), pp. 515-31, Wageningen Academic Publishers (2013)).

Human milk oligosaccharides (HMOs) have become of great interest in the past few years due to their important functions in human development. To date, the structures of at least 115 HMOs have been determined (see Urashima et al.: Milk Oligosaccharides, Nova Biomedical Books, *New York*, 2011, ISBN: 978-1-61122-831-1; Chen *Adv. Carbohydr. Chem. Biochem.* **72**, 113 (2015)), and considerably more are probably present in human milk. The thirteen core structures identified to date, for the at least 115 HMOs, are listed in Table 1:

No	Core name	Core structure
1	lactose (Lac)	Galβ1-4Glc
2	lacto-*N*-tetraose (LNT)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc
3	lacto-*N*-neotetraose (LNnT)	Galβ1-4GlcNAcβ1-3Galβ1-4Glc
4	lacto-*N*-hexaose (LNH)	Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
5	lacto-*N*-neohexaose (LNnH)	Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
6	para-lacto-*N*-hexaose (para-LNH)	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
7	para-lacto-*N*-neohexaose (para-LNnH)	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
8	lacto-*N*-octaose (LNO)	Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
9	lacto-*N*-neooctaose (LNnO)	Galβ1-4GlcNAcβ1-3(Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4Glc

10	iso-lacto-*N*-octaose (iso-LNO)	Galβ1-3GlcNAcβ1-3(Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
11	para-lacto-*N*-octaose (para-LNO)	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
12	lacto-*N*-neodecaose (LNnD)	Galβ1-3GlcNAcβ1-3[Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAcβ1-6]Galβ1-4Glc
13	lacto-*N*-decaose (LND)	Galβ1-3GlcNAcβ1-3[Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAcβ1-6]Galβ1-4Glc

Table 1. Core HMO structures

Low cost ways have been sought for making industrial quantities of as many as possible of the HMOs, so that their uses in nutritional and therapeutic formulations for infants, as well as possibly children and adults, could be discovered, developed and exploited by researchers worldwide. A few HMOs have recently been chemically synthesized in high yields, while other methods have used fermentation techniques using cultured microorganisms. WO 2013/185780 and WO 2019/110800 disclose spray-drying of HMOs and mixtures of HMOs.

Certain crystalline HMOs have shown to be relatively unstable when stored for extended periods without refrigeration. They have tended to melt and thereby become sticky and form agglomerations.

Accordingly, it is also an object of the present invention to provide a method for providing solid HMO products with enhanced stability, so that they can be stored for extended periods without refrigeration, for example at temperatures of up to 30 ºC or even higher, preferably up to 40 ºC or even higher.

SUMMARY

In a first aspect the present invention relates to a method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said process comprising a step of drum-drying an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis.

Preferably, the aqueous composition comprises one or more HMOs and optionally HMO precursors and/or by-products of HMO synthesis, and method thereby provides the solid HMO product comprising one or more HMOs that optionally comprises HMO precursors and/or by-products of the HMO synthesis.

In a second aspect, the present invention relates to a method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said process comprising a step of belt drying, preferably

vacuum belt drying, an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis.

Preferably, the aqueous composition comprises one or more HMOs and optionally HMO precursors and/or by-products of HMO synthesis, and method thereby provides the solid HMO product comprising one or more HMOs that optionally comprises HMO precursors and/or by-products of the HMO synthesis.

In a third aspect, the present invention relates to a method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said process comprising a step of granulating an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis to form a granulated composition, followed by a step of drying said granulated composition.

Preferably, the aqueous composition comprises one or more HMOs and optionally HMO precursors and/or by-products of HMO synthesis, and method thereby provides the solid HMO product comprising one or more HMOs that optionally comprises HMO precursors and/or by-products of the HMO synthesis after drying the granulated composition.

Furthermore, a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs) is provided, obtained by the methods described herein.

A drum-dried, a belt dried, preferably vacuum belt dried, or a granulated solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs) is also provided, obtained by the methods described herein.

Also provided is a solid HMO product comprising one or more human milk oligosaccharides (HMOs), said solid HMO product having a glass transition temperature (T_g) more than 100 ºC, such as from around 105 ºC to around 160 ºC, e.g. around 100-150 ºC or around 120-140 ºC. The term “around” in this context means a deviation from the indicated value of +/- 2.5 ºC.

A food product, in particular a nutritional formulation, e.g. an infant nutritional formulation, is also provided, which comprises a solid HMO product as described herein.

Further features of the invention are evident from the following description, numbered aspects, examples and claims.

FIGURES

Figure 1 shows the XRPD spectrum of the material obtained in example 3.

DETAILED DISCLOSURE

Unless otherwise defined, the term “around” is to be defined as a value that deviates from 0.1 % to 5 % from the indicated value, such as 0.1-2.5 % or 0.1-1.0 %.

As above, the present technology provides three methods for providing a solid HMO product. The solid HMO product comprises one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis. Preferably, the solid HMO product comprises one or more synthetic human milk oligosaccharides (HMOs), and is obtained via drying (by various means) an aqueous composition comprising said one or more human milk oligosaccharides (HMOs).

The first method comprises a step of drum-drying an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis. Solid HMO product is thus obtained.

The second method comprises a step of belt drying, preferably vacuum belt drying, an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis. Solid HMO product is thus obtained.

The third method comprises a step of granulating an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis to form a granulated composition, followed by a step of drying said granulated composition. Solid HMO product is thus obtained.

All methods described herein use an aqueous composition comprising the one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis. The aqueous composition may be an aqueous solution or an aqueous suspension of the human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis, and is preferably an aqueous solution of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis.

The aqueous composition suitably comprises two or more HMOs, such as e.g. three or more HMOs, or four or more HMOs. The aqueous composition may comprise even 5 or more HMOs. The HMOs may be selected from the group consisting of LNT, LNnT, 2‘-FL, 3-FL, DFL, LNFP I, 3‘-SL, 6‘-SL, FSL, LST a, LST b, and DS-LNT.

The aqueous composition may include additional components such as e.g. salts, pH regulating agents or solubilising agents. The pH of the aqueous composition is suitably between 3-7, preferably between 4-6.

In each case, the drying step suitably takes place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %.

In one particular aspect, the one or more synthetic HMOs are produced by means of fermentation, prior to said drying step.

Suitably, according to this aspect, one or more purification steps are carried out on the HMO fermentation broth, enzymatic reaction milieu or synthetic reaction mixture, prior to said drying step. The purification steps carried out on the fermentation broth, enzymatic reaction milieu or synthetic reaction mixture may be selected from one or more of:

- removing solid material e.g. from the HMO synthesis milieu, such as fermentation material (such as proteins and DNA) or enzymatic reaction material (proteins);
- removing salts and charged molecules (such as small DNA fragments, organic acids, peptides);
- removing uncharged or non-charged material (e.g. lipids, polysaccharides e.g. endotoxin, colorants from fermentation);
- removing HMO precursors and/or by-products;
- separating HMOs, if an HMO mixture is produced;
- removing an excess of water.

In aspects, said one or more synthetic HMOs are produced by means of fermentation and the fermentation broth is further processed to prepare an aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs).

In one embodiment, especially if the drying step is belt drying, preferably vacuum belt drying, as disclosed in the present application, the fermentation broth is treated by the following steps prior to drying:

a) clarifying the broth to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris from a fermentation process; then

b) removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent purification step, from the aqueous solution obtained in step a).

In step a), the broth is clarified in a conventional manner, e.g. by centrifugation or filtration. Preferably the aqueous medium is first flocculated and then centrifuged or filtered to remove

any remaining insoluble particulates and contaminants, as well as cells and cell components and insoluble metabolites and debris.

In step b), proteins and related impurities are removed from the aqueous medium obtained previously in a conventional manner, e.g. by ultrafiltration, nanofiltration, tangential flow high-performance filtration, tangential flow ultrafiltration, affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, gel filtration, size exclusion chromatography and/or active charcoal treatment. The active charcoal treatment helps to remove or at least reduce the amount of colorizing agents and/or water soluble contaminants, such as salts, if required. Ion exchange chromatography efficiently removes charged components such as salts, colour bodies, proteins, amino acids, lipids and DNAs.

Accordingly, the method my further comprise the following separation/purification steps of the fermentation broth in any order prior to drying, preferably belt drying, more preferably vacuum belt drying:

i) ultrafiltration (UF),

ii) nanofiltration (NF), and

iii) treatment with an ion exchange resin.

Advantageously, step i) is conducted before step ii). More advantageously, the step i) is conducted before any of the steps ii) and iii). Preferably, the method is performed in the order where step ii) follows step i) and step iii) follows step ii). The method may further comprise an active charcoal treatment after UF, NF or ion exchange resin treatment.

In one aspect of interest, the one or more synthetic HMOs are produced by means of fermentation, and the fermentation broth is fed directly to the drum dryer as the aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs), without any intermediate purification steps.

The HMO content of the aqueous composition, and whether a solution or slurry is formed, depends the solubility of the HMO in question.

Typically for 2'-FL, the minimum concentration is estimated at Brix 50. Preferably, the process will be run on a saturated solution (ex: Brix 58-65), it but can also be imagined to be run on a supersaturated metastable solution or an aqueous slurry (up to 85 % dry matter).

Synthetic human milk oligosaccharides (HMOs)

The human milk oligosaccharides referred to in the present technology are synthetic, i.e. produced by chemical or biochemical processes in vitro or in vivo. The synthetic HMOs used in the present methods and products may be selected from one or more of LNT, LNnT, 2'-FL, 3-

FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b, and DS-LNT, preferably LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL and 6'-SL.

The synthetic HMOs may be neutral or acidic (sialylated).

The term "neutral human milk oligosaccharide" means a non-sialylated (therefore neutral) complex carbohydrate found in human breast milk (Urashima et al.: *Milk oligosaccharides*, Nova Biomedical Books, 2011; Chen *Adv. Carbohydr. Chem. Biochem.* **72**, 113 (2015)) comprising a core structure being a lactose unit at the reducing end that is a) substituted with one or two α-L-fucopyranosyl moieties, b) substituted with a galactosyl residue, or c) elongated, via its 3'-OH group, by an N-acetylglucosamine, a lacto-N-biose (Galβ1-3GlcNAc) or an N-acetyllactosamine (Galβ1-4GlcNAc) moiety. The N-acetyllactosamine containing derivatives can be further substituted with N-acetyllactosamine and/or lacto-N-biose (lacto-N-biose is always a non-reducing terminal). The N-acetyllactosamine and the lacto-N-biose containing derivatives can optionally be substituted by one or more α-L-fucopyranosyl moieties.

Examples of neutral trisaccharide HMOs include 2'-O-fucosyllactose (2'-FL, Fucα1-2Galβ1-4Glc), 3-O-fucosyllactose (3-FL, Galβ1-4(Fucα1-3)Glc) or lacto-N-triose II (GlcNAcβ1-3Galβ1-4Glc); examples of neutral tetrasaccharide HMOs include 2',3-di-O-fucosyllactose (DFL, Fucα1-2Galβ1-4(Fucα1-3)Glc), lacto-N-tetraose (LNT, Galβ1-3GlcNAcβ1-3Galβ1-4Glc) or lacto-N-neotetraose (LNnT, Galβ1-4GlcNAcβ1-3Galβ1-4Glc); examples of neutral pentasaccharide HMOs include lacto-N-fucopentaose I (LNFP I, Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc), lacto-N-fucopentaose II (LNFP II, Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc), lacto-N-fucopentaose III (LNFP III, Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc), lacto-N-fucopentaose V (LNFP V, Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc), lacto-N-fucopentaose VI (LNFP VI, Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc); examples of neutral hexasaccharide HMOs include lacto-N-difucohexaose I (LNDFH I, Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc), lacto-N-difucohexaose II (LNDFH II, Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc), lacto-N-difucohexaose III (LNDFH III, Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc), lacto-N-hexaose (LNH, Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc), para-lacto-N-hexaose (pLNH, Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc), lacto-N-neohexaose (LNnH, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc) or para-lacto-N-neohexaose (pLNnH, Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc).

The term "sialylated human milk oligosaccharide" means a sialylated complex carbohydrate found in human breast milk (Urashima et al.: *Milk oligosaccharides*, Nova Biomedical Books, 2011; Chen *Adv. Carbohydr. Chem. Biochem.* **72**, 113 (2015)) comprising a core structure being a lactose unit at the reducing end that can be elongated by one or more β-N-acetyl-lactosaminyl and/or one or more β-lacto-N-biosyl units, and which core structure is substituted by an α-N-acetyl-neuraminyl (sialyl) moiety and optionally can be substituted by an α L-

fucopyranosyl moiety. In this regard, the acidic HMOs have at least one sialyl residue in their structure.

Examples of acidic HMOs include 3'-sialyllactose (3'-SL), 6'-sialyllactose (6'-SL), 3-fucosyl-3'-sialyllactose (FSL), LST a, fucosyl-LST a (FLST a), LST b, fucosyl-LST b (FLST b), LST c, fucosyl-LST c (FLST c), sialyl-LNH (SLNH), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II) and disialyl-lacto-N-tetraose (DS-LNT).

Different mixtures of HMOs produced enzymatically are described in earlier patent applications WO 2016/199071, WO 2017/221208, WO 2017/103850, WO 2016/157108, WO 2016/063262, which are incorporated by reference.

Any artificial blends of HMOs synthesised by any available methods are also included in the scope of the present invention.

HMO precursors

The term "HMO precursor" in the present context refers to a compound being involved in the biosynthetic pathway of one or more HMOs according to the invention, which are produced and naturally present in the host cell or imported into the cell from the extracellular medium. Some non-limiting examples of HMO precursors are listed below:

Precursor:	Product:
UDP-GlcNAc	LNT, LNnT, LNFP-I, LNFP-II, LNFP-III, LNFP-V, LNFP-VI, LNDFH-I, LNDFH-II, pLNH, F-pLNH I, 3'-SL, 6'-SL, pLNnH, (F)LST a, (F)LST b, (F)LST c, (F)LST d
UDP-Gal	LNT, LNnT, LNFP-I, LNFP-II, LNFP-III, LNFP-V, LNFP-VI, LNDFH-I, LNDFH-II, pLNH, F-pLNH I, pLNnH, LST a, LST b, LST c, LST d
GDP-fucose	LNFP-I, LNFP-II, LNFP-III, LNFP-V, LNFP-VI, LNDFH-I, LNDFH-II, F-pLNH I, 2'-FL, 3-FL, DFL, FLST a, FLST b, FLST c, FLST d

Precursors also include lactose, fucose, sialic acid and derivatives thereof. In some embodiments, a precursor may be a by-product of an HMO fermentation.

By-products of the HMO synthesis

By the term "by-products" is meant carbohydrate or carbohydrate-like structures that are formed during fermentation beside the HMO of interest. For example, in fermentation of 2'-FL, fucosylated carbohydrates other than 2'-FL, e.g. DFL, can be found as by-products in high-titre aqueous fermentation broths in a DFL/2'-FL ratio of 2:8 to 1:9 by weight. The fucosylated carbohydrates other than 2'-FL, in the aqueous solution, can be any other monofucosylated

lactoses that can be formed during fermentation as a result of a deficient, defective or impaired fucosylation other than an α-1,2-fucosylation on the galactose moiety of lactose (e.g. one leading to 3-O-fucosyllactose, 3-FL), or of fucose migration of 2'-FL under the cultivation condition or post-fermentative operations, or of fucose hydrolysis from multifucosylated, preferably difucosylated, lactose. Such an other fucosylated lactose can also be a multifucosylated, preferably difucosylated, lactose that can be formed as a result of overfucosylation of lactose under the cultivation condition. The difucosylated lactose is preferably 2,2'-di-O-fucosyllactose or 2',3-di-O-fucosyllactose (DFL), particularly DFL as a characteristic by-product formed in a fermentative production of 2'-FL. This aqueous solution can also contain carbohydrate-like contaminants like 2'-O-fucosyl lactulose, lactose, lactulose, fucose, glucose, galactose and the like. These contaminants can be formed during fermentation or in post-fermentative purification/isolation steps, e.g. by rearrangement (e.g. 2'-O-fucosyl lactulose, lactulose) or by hydrolysis (e.g. fucose, glucose, galactose, lactose), or can be unconsumed educts or ingredients added during the fermentation (e.g. glucose as a carbon source). These contaminants are typically in a concentration of not more than 1-2 w/w% (individually), or 5-7 w/w% (altogether) in the aqueous solution of a desired HMO intended for drying. The aqueous solution typically can further contain up to 15 w/w%, preferably up to 10 w/w%, more preferably no more than 5 w/w%, particularly no more than 1 w/w%, of the HMO precursor, e.g. lactose as unused acceptor added to the fermentation broth.

The spectrum of fermentation by-products of other HMO structures, like sialylated or neutral non-fucosylated HMOs, could be different from the spectrum of by-products of the fucosylated HMOs fermentation, as the spectrum of precursors and by-products, in general, depends on the desired HMO structure and substrate specificity of the enzyme(s) used for the production of the HMO. Non-limiting examples of HMOs and HMO mixtures, where one HMO is a desired HMO and other HMOs are "by-products" that can be generated in fermentation of the desired HMO using some selected enzymes, are presented in Table 2 below.

Enzyme/Gene	HMO example
ß-1,3-N-acetylglucosaminyltransferase/ *lgtA*	LNT, LNnT, LNFP-I, LNFP-II, LNFP-III, LNFP-V, LNFP-VI, LNDFH-I, LNDFH-II, pLNH, F-pLNH I, pLNnH
ß-1,4-galactosyltransferase/ *galT*	LNnT, LNFP-III, LNFP-VI, pLNH, F-pLNH I, pLNnH
ß-1,3-galactosyltransferase/ *cpslBJ*	LNT, LNFP-I, LNFP-II, LNFP-V, LNDFH-I, LNDFH-II, pLNH, F-pLNH I
α-1,3-fucosyltransferase/ *MAMA_R764*	2'-FL, 3-FL, DFL, LNFP-I, LNFP-III, LNFP-V, LNFP-VI, LNDFH-II, F-pLNH I
α-1,3-fucosyltransferase/ *Mg791*	2'-FL, 3-FL, DFL, LNFP-I, LNFP-III, LNFP-V, LNFP-VI, LNDFH-II, F-pLNH I
α-1,3-fucosyltransferase/ *Moumou_00703*	2'-FL, 3-FL, DFL, LNFP-I, LNFP-III, LNFP-V, LNFP-VI, LNDFH-II, F-pLNH I

α-1,3-fucosyltransferase/ *futA*	2'-FL, 3-FL, DFL, LNFP-I, LNFP-III, LNFP-V, LNFP-VI, LNDFH-II, F-pLNH I
α-1,2-fucosyltransferase/ *futC*	2'-FL, DFL, LNFP-I, LNDFH-I
α-1,3-fucosyltransferase *fucT*	2'-FL, 3-FL, DFL, LNFP-I, LNFP-III, LNFP-V, LNFP-VI, LNDFH-II, F-pLNH I
α-1,4-fucosyltransferase/ *fucTIII*	LNDFH-I, LNDFH-II
α-1,3/4-fucosyltransferase/	LNFP-II, LNDFH-I, LNDFH-II
CMP-Neu5Ac synthetase/ *neuA*	3'-SL, 6'-SL
sialic acid synthase/ *neuB*	3'-SL, 6'-SL
GlcNAc-6-phosphate-2-epimerase/ *neuC*	3'-SL, 6'-SL

Table 2.

In some embodiments, the terms "precursor" and "by-product" may relate to the same molecule.

Drum drying

Drum drying is a technique which involves depositing the aqueous composition comprising the one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis onto a heated surface of a large drum. The drum rotates while water evaporates. A doctor blade is used to scrape dried product from the drum surface.

In one configuration, the drum dryer comprises one or more applicator rollers. The aqueous composition is deposited in the nip between applicator rollers and/or in the nip between the applicator rollers and the drying drum. There are other configurations of drum dryer without applicator rollers. For instance, drum dryers may incorporate a dip feed or a sprayer or splasher to apply the composition to the heated drum.

In one aspect, therefore, the drum-dryer used in the step of drum-drying does not comprise any applicator rollers. In another aspect, the drum-dryer used in the step of drum-drying is a double drum dryer.

A drum dryer may be single-drum (with one heated drum) or double-drum (with two heated drums, where the composition to be dried is fed into the nip between heated drums).

The present technology encompasses vacuum drum dryers, which – as they operate at reduced pressures – can advantageously avoid issues relating to high temperatures. Vacuum drum dryers typically operate at 20-60 mbars pressure, allowing the product to be exposed to low temperatures (40-120 ºC, preferably 60-90 ºC). Besides operating at lower temperatures, vacuum drum dryers are also more sanitary (as they operate within a closed environment) and

possibly more suitable for infant food production. Suitably, therefore the drum-dryer used in the step of drum-drying is a vacuum drum dryer.

Drying using a drum dryer takes place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %, such as 2-3 %.

The person skilled in the art is able to adjust the operating parameters of a drum dryer to obtain the desired properties (e.g. moisture content, fineness) of the solid HMO product. For instance, the drum-drying step may take place at a temperature of 100 to 160-170 ºC, more preferably at a temperature of 110-170 ºC, even more preferably at a temperature of 110-140 ºC or 120-160 ºC, at atmospheric pressure. The heated drum usually rotates at a speed of from 0.3 rpm to several (i.e. 2-5 or even 9-10) rpm, providing a residence time of the aqueous composition on the surface of the heated drum of several seconds to 20-180 sec. Surprisingly, no significant degradation or coloration of the HMO was noticed running drum dryer at atmospheric pressure, with temperature above 100 ºC.

Belt drying, vacuum belt drying

In belt dryers, products which are mostly wet are diffused equally on a wire mesh belt into the infeed chamber of the belt dryer. Most of the belt dryers have a wire mesh belt in a horizontal position which conveys the product through the drying sections. Gas or air flows circulate through the drying sections over the wet products. Each section within the dryer can be equipped with a heat exchanger or either a ventilating fan which enables the process operators to control each section separately. A belt dryer contains at least one entry point (infeed head), the conveyor belt to transport the product onto a porous wire mesh belt for air permeability and the discharge end for output. A belt dryer works with an air flow through or over the product to dry the product.

A vacuum belt dryer is a belt dryer that operates under vacuum. It comprises a belt upon which an aqueous composition is deposited, and where water is driven off using a combination of heat and low pressure. The belt is heated, typically by means of heating plates arranged above or below the belt which provide heat transfer via radiation.

The vacuum belt dryer used in the present technology may comprise various temperature zones, e.g. at least two, or at least three temperature zones. The operating temperature in each zone typically decreases from the first zone to the second and further zones.

Again, the person skilled in the art may adjust the operating parameters of a vacuum belt dryer, to obtain the desired properties. Vacuum belt drying may take place at a temperature of 90-160 ºC, preferably at a temperature of 110-140 ºC (temperature of the plate), and at a pressure of 10-30 mbar. When vacuum belt drying, the aqueous composition typically has a residence time

on the belt of 20-25 minutes. The feed flow of the aqueous composition to the vacuum belt dryer is typically between 150-180 g/min.

Belt drying, preferably vacuum belt drying, is a surprisingly effective industrial drying method that – in spite of the high temperature and the long residence time applied - does not provoke the thermal degradation of the HMOs and/or any other non-desirable change in the composition of HMOs. This is demonstrated in comparison tests with freeze drying which is known to be a gentle drying method (see examples).

Granulation followed by drying

An overview of granulation technologies is found in Salman et al. *Handbook of Powder Technology* vol. 11, Granulation, Elsevier 2006. Granulation is the process of forming grains or granules from a powdery or solid substance, producing a granular material. Typically, granulation involves agglomeration of fine particles into larger granules, typically of size range between 0.2 and 4.0 mm depending on their subsequent use.

High shear granulators in general fall into two classes, namely horizontal axis and vertical axis, and can be either continuously operated or batch operated. High shear granulators use an impeller to vigorously agitate the powder and produce high-density granules. They are commonly found in the pharmaceutical, agrochemical and industries due to their ability to handle difficult feed formulations, including high viscosity binder fluids and fine cohesive powders. Impellers rotate at high speed (between 100 and 1500 rpm) on either a vertical or horizontal axis to create the agitation required for granulation. Typically, a secondary smaller impeller, called a chopper, is used. This rotates at much higher speeds (around 1500 rpm). Binder addition to high shear granulators can be in the form of a liquid spray or pouring.

Fluidized beds may also used be used for granulation. Fluidized-bed granulation in particular is very common, where atomizable liquids (e.g. , suspensions, solutions, emulsions or melts) can be converted into free-flowing granular solids by integration of a number of processes like wetting, drying, size enlargement, shaping and homogenization or separation into a single step of the process chain by using high heat and mass transfer.

Another granulation process is extrusion-spheronisation (E-S). E-S is used to manufacture spherical or cylindrical pellets by extruding a semi-solid wet powder mass through a single die, a series of dies or a screen featuring many holes, then breaking up and rounding the extrudate on a rotating friction plate.

A rolling drum granulator is one of the most widely used granulating equipment devices, in which size enlargement is achieved by collisions in a bed of moist particles undergoing rolling motion. The rolling drum is the simplest continuous granulation device, and is widely used in the granulation of fertiliser and in the balling of iron ore.

Granulation can be carried out on an aqueous solution; in such cases, a solid dried carrier is required (e.g. solid HMO product from a previous batch). Suitably, the granulation step takes place at a temperature of 70-150 ºC at atmospheric pressure; or a temperature of 30-100 ºC under vacuum (50-100 mbars).

The granulation step and the drying step may be performed in the same apparatus, e.g. a fluidised-bed apparatus. The granulation process may therefore be fluidized-bed granulation or extrusion-spheronisation (E-S) granulation. Suitably, the granulation step is wet granulation.

Solid HMO product

Suitably, the solid HMO product of the invention comprises around 40 % or more, such as around 50 % or more, preferably around 60 % or more by weight of synthetic HMOs, as a percentage of the total weight of the solid HMO product.

Some crystallisation of the synthetic HMOs could occur during the drying, due to the concentration step. The solid HMO product may therefore be amorphous solid HMO product, or crystalline solid HMO product, or a mixture of amorphous and crystalline solid HMO product.

Additional method steps

The general methods provided herein may be supplemented by additional method steps, performed on the solid HMO product; i.e. post-drying.

In one aspect, the methods described herein further comprise the step of milling the solid HMO product to a solid HMO powder. Milling may provide a solid HMO powder with a various particle size distribution (D90) depending on the type of mill used e.g. impact mill, ball mill, forced sieve, or jet mill. As a further aspect, the methods may further comprise the step of sieving the solid HMO powder and separating the HMO powder into at least a first HMO powder fraction and a second HMO powder fraction. Sieving may provide HMO powder fractions with a particle size distribution (D90) depending the type of sieve used. The sieving or classification can be adapted to any customer requirement. From a food safety prospective a max. 1 mm sieving would be required, preferably 0.5–0.7 mm.

Food product

The solid HMO product may also be formulated in a food product, in particular a nutritional formulation, e.g. an infant nutritional formulation.

Nutritional formulations comprising solid HMO product may be foods, drinks or feeds. The nutritional formulation may contain edible micronutrients, vitamins and minerals as well. The amounts of such ingredients may vary depending on whether the formulation is intended for use with normal, healthy infants, children, adults or subjects having specialized needs (e.g. suffering from metabolic disorders). Micronutrients include for example edible oils, fats or fatty acids

(such as coconut oil, soy-bean oil, monoglycerides, diglycerides, palm olein, sunflower oil, fish oil, linoleic acid, linolenic acid etc.), carbohydrates (such as glucose, fructose, sucrose, maltodextrin, starch, hydrolyzed cornstarch, etc.) and proteins from casein, soy-bean, whey or skim milk, or hydrolysates of these proteins, but protein from other source (either intact or hydrolysed) may be used as well. Vitamins may be chosen from the group consisting of vitamin A, B1, B2, B5, B6, B12, C, D, E, H, K, folic acid, inositol and nicotinic acid. The nutritional formulation may contain the following minerals and trace elements: Ca, P, K, Na, Cl, Mg, Mn, Fe, Cu, Zn, Se, Cr or I.

In a preferred embodiment the nutritional formulation is an infant nutritional formulation. Infant nutritional formulation means a foodstuff intended for particular nutritional use by infants during the first 4-6 months of life and satisfying by itself the nutritional requirements of infants. It may contain one or more probiotic *Bifidobacterium* species, prebiotics such as fructooligosaccharides and galactooligosaccharides, proteins from casein, soy-bean, whey or skim milk, carbohydrates such as lactose, saccharose, maltodextrin, starch or mixtures thereof, lipids (e.g. palm olein, sunflower oil, safflower oil) and vitamins and minerals essential in a daily diet. The infant formula contains solid HMO product in a total amount of 0.1-3.0 g/100 g formula.

In another preferred embodiment the nutritional formulation may be a food supplement comprising solid HMO product. The food supplement may comprise one or more probiotics in an amount sufficient to achieve the desired effect in an individual, preferably in children and adults. The food supplement may also contain vitamins, minerals, trace elements and other micronutrients as well. The food supplement may be for example in the form of tablets, capsules, pastilles or a liquid. The supplement may contain conventional additives selected from but not limited to binders, coatings, emulsifiers, solubilising agents, encapsulating agents, film forming agents, adsorbents, carriers, fillers, dispersing agents, wetting agents, jellifying agents, gel forming agents, etc. The daily dose of solid HMO product ranges from 0.1 to 3.0 g.

The following numbered aspects of the invention are provided:

Aspect 1. A method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said method comprising a step of drum-drying an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis.

Aspect 2. The method according to aspect 1, further comprising the step of milling the solid HMO product to a solid HMO powder.

Aspect 3. The method according to aspect 2, further comprising the step of sieving the solid HMO powder and separating the HMO powder into at least a first HMO powder fraction and a second HMO powder fraction.

Aspect 4. The method according to any one of the preceding aspects, wherein the solid HMO product comprises around 40 % or more, such as around 50 % or more, preferably around 60 % or more by weight of synthetic HMOs, as a percentage of the total weight of the solid HMO product.

Aspect 5. The method according to any one of the preceding aspects, wherein the synthetic HMOs are selected from one or more of LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b, and DS-LNT, preferably LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL and 6'-SL.

Aspect 6. The method according to any one of the preceding aspects, wherein the aqueous composition is an aqueous solution or an aqueous suspension of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, preferably an aqueous solution of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis.

Aspect 7. The method according to any one of the preceding aspects, wherein the aqueous composition comprises two or more HMOs, such as e.g. three or more HMOs, or four or more HMOs.

Aspect 8. The method according to any one of the preceding aspects, wherein the aqueous composition comprises preferably 5 or more HMOs, from the group consisting of LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b, and DS-LNT.

Aspect 9. The method according to any one of the preceding aspects, wherein the drum-drying step takes place at a temperature of 100-160 ºC, preferably at a temperature of 110-140 ºC, at atmospheric pressure.

Aspect 10. The method according to any one of the preceding aspects, wherein – in said drum-drying step – the aqueous composition has a residence time on the drum of 20-180 seconds.

Aspect 11. The method according to any one of the preceding aspects, further comprising the step of producing said one or more synthetic HMOs by means of fermentation, enzymatic reaction or synthetic reaction, prior to said step of drum-drying.

Aspect 12. The method according to aspect 11, wherein one or more purification steps are carried out on the HMO fermentation broth, enzymatic reaction milieu or synthetic reaction mixture, prior to said step of drum-drying.

Aspect 13. The method according to aspect 12, wherein said one or more purification steps carried out on the fermentation broth, enzymatic reaction milieu or synthetic reaction mixture are selected from one or more of:

- removing solid material;
- removing salts and charged molecules;
- removing uncharged or non-charged material;
- removing HMO precursors and/or by-products;
- separating HMOs, if an HMO mixture is produced;
- removing an excess of water.

Aspect 14. The method according to any one of aspects 11-13, wherein said one or more synthetic HMOs are produced by means of fermentation and the fermentation broth is further processed to prepare an aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs).

Aspect 15. The method according to aspect 13 or aspect 14, wherein the method comprises:

a) clarifying the broth to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris from a fermentation process; then

b) removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent purification step, from the aqueous solution obtained in step a).

Aspect 16. The method according to aspect 15, wherein the method comprises:

i) ultrafiltration (UF),

ii) nanofiltration (NF), and

iii) treatment with an ion exchange resin.

Aspect 17. The method according to aspect 11, wherein the one or more synthetic HMOs are produced by means of fermentation, and wherein the fermentation broth is fed directly to the drum dryer as the aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs), without any intermediate purification steps.

Aspect 18. The method according to any one of the preceding aspects, wherein the drying step takes place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %.

Aspect 19. The method according to any one of the preceding aspects, wherein the drum-dryer used in the step of drum-drying does not comprise any applicator rollers.

Aspect 20. The method according to any one of the preceding aspects, wherein the drum-dryer used in the step of drum-drying is a double drum dryer.

Aspect 21. The method according to any one of aspects 1-19, wherein the drum-dryer used in the step of drum-drying is a vacuum drum dryer.

Aspect 22. A drum-dried solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the method of any one of aspects 1-21.

Aspect 23. A solid HMO product comprising one or more human milk oligosaccharides (HMOs), said solid HMO product having a glass transition temperature (T_g) higher than 120 ºC.

Aspect 24. The solid HMO product according to any one of aspects 22-23, wherein the solid HMO product comprises one or more amorphous HMOs, or a mixture of amorphous and crystalline HMOs, or a crystalline HMO.

Aspect 25. A food product, in particular a nutritional formulation, e.g. an infant nutritional formulation, comprising a solid HMO product according to any one of aspects 21-23.

Aspect 26. A method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said method comprising a step of belt drying, preferably vacuum belt drying, an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis.

Aspect 27. The method according to aspect 26, further comprising the step of milling the solid HMO product to a solid HMO powder.

Aspect 28. The method according to aspect 27, further comprising the step of sieving the solid HMO powder and separating the HMO powder into at least a first HMO powder fraction and a second HMO powder fraction.

Aspect 29. The method according to any one of aspects 26-28, wherein the solid HMO product comprises around 40 % or more, such as around 50 % or more, preferably around 60 % or more by weight of synthetic HMOs, as a percentage of the total weight of the solid HMO product.

Aspect 30. The method according to any one of aspects 26-29, wherein the synthetic HMOs are selected from one or more of LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b, and DS-LNT, preferably LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL and 6'-SL.

Aspect 31. The method according to any one of aspects 26-30, wherein the aqueous composition is an aqueous solution or an aqueous suspension of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, preferably an aqueous solution of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis.

Aspect 32. The method according to any one of aspects 26-31, wherein the aqueous composition comprises two or more HMOs, such as e.g. three or more HMOs, or four or more HMOs.

Aspect 33. The method according to any one of aspects 26-32, wherein the aqueous composition comprises preferably 5 or more HMOs, from the group consisting of LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b, and DS-LNT.

Aspect 34. The method according to any one of aspects 26-33, wherein the vacuum belt dryer operates at a temperature of 90-160 ºC, preferably at a temperature of 110-140 ºC, and at a pressure of 10-30 mbar.

Aspect 35. The method according to any one of aspects 26-34, further comprising the step of producing said one or more synthetic HMOs by means of fermentation, enzymatic reaction or synthetic reaction, prior to said step of belt drying, preferably vacuum belt drying.

Aspect 36. The method according to aspect 35, wherein one or more purification steps are carried out on the HMO fermentation broth, enzymatic reaction milieu or synthetic reaction mixture, prior to said step of belt drying, preferably vacuum belt drying.

Aspect 37. The method according to aspect 36, wherein said one or more purification steps carried out on the fermentation broth, enzymatic reaction milieu or synthetic reaction mixture are selected from one or more of:

- removing solid material;
- removing salts and charged molecules;
- removing uncharged or non-charged material;
- removing HMO precursors and/or by-products;
- separating HMOs, if an HMO mixture is produced;
- removing an excess of water.

Aspect 38. The method according to any one of aspects 35-37, wherein said one or more synthetic HMOs are produced by means of fermentation and the fermentation broth is further processed to prepare an aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs).

Aspect 39. The method according to aspect 37 or aspect 38, wherein the method comprises:

a) clarifying the broth to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris from a fermentation process; then

b) removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent purification step, from the aqueous solution obtained in step a).

Aspect 40. The method according to aspect 39, wherein the method comprises:

i) ultrafiltration (UF),

ii) nanofiltration (NF), and

iii) treatment with an ion exchange resin.

Aspect 41. The method according to aspect 35, wherein the one or more synthetic HMOs are produced by means of fermentation, and wherein the fermentation broth, as the aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs), is fed directly to the belt dryer, preferably vacuum belt dryer, without any intermediate purification steps.

Aspect 42. The method according to any one of aspects 26-41, wherein the drying step takes place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %.

Aspect 43. The method according to any one of aspects 26-42, wherein the vacuum belt dryer comprises at least three temperature zones.

Aspect 44. A belt dried, preferably vacuum belt dried solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the method of any one of aspects 26-43.

Aspect 45. A solid HMO product comprising one or more human milk oligosaccharides (HMOs), said solid HMO product having a glass transition temperature (T_g) more than 100 ºC, such as from around 105 ºC to around 160 ºC , e.g. around 100-150 ºC, around 120-140 ºC.

Aspect 46. The solid HMO product according to any one of aspects 44-45, wherein the solid HMO product comprises one or more amorphous HMOs, or a mixture of amorphous and crystalline HMOs, or a crystalline HMO.

Aspect 47. A food product, in particular a nutritional formulation, e.g. an infant nutritional formulation, comprising a solid HMO product according to any one of aspects 44-46.

Aspect 48. A method for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said method comprising a step of granulating an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis to form a granulated composition, followed by a step of drying said granulated composition.

Aspect 49. The method according to aspect 48, wherein the granulation step is wet granulation.

Aspect 50. The method according to any one of aspects 48-49, wherein the granulation step and the drying step are performed in the same apparatus.

Aspect 51. The method according to any one of aspects 48-50, further comprising the step of milling the solid HMO product to a solid HMO powder.

Aspect 52. The method according to aspect 51, further comprising the step of sieving the solid HMO powder and separating the HMO powder into at least a first HMO powder fraction and a second HMO powder fraction.

Aspect 53. The method according to any one of aspects 48-52, wherein the solid HMO product comprises around 40% or more, such as around 50% or more, preferably around 60% or more by weight of synthetic HMOs, as a percentage of the total weight of the solid HMO product.

Aspect 54. The method according to any one of aspects 48-53, wherein the synthetic HMOs are selected from one or more of LNT, LNnT, 2‘-FL, 3-FL, DFL, LNFP I, 3‘-SL, 6‘-SL, FSL, LST a, LST b, and DS-LNT, preferably LNT, LNnT, 2‘-FL, 3-FL, DFL, LNFP I, 3‘-SL and 6‘-SL.

Aspect 55. The method according to any one of aspects 48-54, wherein the aqueous composition is an aqueous solution or an aqueous suspension of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, preferably an aqueous solution of said one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis.

Aspect 56. The method according to any one of aspects 48-55, wherein the aqueous composition comprises two or more HMOs, such as e.g. three or more HMOs, or four or more HMOs.

Aspect 57. The method according to any one of aspects 48-56, wherein the aqueous composition comprises preferably 5 or more HMOs, from the group consisting of LNT, LNnT, 2‘-FL, 3-FL, DFL, LNFP I, 3‘-SL, 6‘-SL, FSL, LST a, LST b, and DS-LNT.

Aspect 58. The method according to any one of aspects 48-57, wherein the granulation step takes place at a temperature of 70-150 ºC at atmospheric pressure.

Aspect 59. The method according to any one of aspects 48-58, wherein said granulation is fluidized-bed granulation or extrusion-spheronisation (E-S) granulation.

Aspect 60. The method according to any one of aspects 48-59, further comprising the step of producing said one or more synthetic HMOs by means of fermentation, enzymatic reaction or synthetic reaction, prior to said step of granulation.

Aspect 61. The method according to aspect 60, wherein one or more purification steps are carried out on the HMO fermentation broth, enzymatic reaction milieu or synthetic reaction mixture, prior to said step of granulation.

Aspect 62. The method according to aspect 61, wherein said one or more purification steps carried out on the fermentation broth, enzymatic reaction milieu or synthetic reaction mixture are selected from one or more of:

- removing solid material;
- removing salts and charged molecules;
- removing uncharged or non-charged material;
- removing HMO precursors and/or by-products;
- separating HMOs, if an HMO mixture is produced;
- removing an excess of water.

Aspect 63. The method according to any one of aspects 48-62, wherein said one or more synthetic HMOs are produced by means of fermentation and the fermentation broth is further processed to prepare an aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs).

Aspect 64. The method according to aspect 62 or aspect 63, wherein the method comprises:

a) clarifying the broth to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris from a fermentation process; then

b) removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent purification step, from the aqueous solution obtained in step a).

Aspect 65. The method according to aspect 64, wherein the method comprises:

i) ultrafiltration (UF),

ii) nanofiltration (NF), and

iii) treatment with an ion exchange resin.

Aspect 66. The method according to aspect 63, wherein the one or more synthetic HMOs are produced by means of fermentation, and wherein the fermentation broth is fed directly to the granulator as the aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs), without any intermediate purification steps.

Aspect 67. The method according to any one of aspects 48-66, wherein the granulation and drying steps take place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %.

Aspect 68. A granulated, dried solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the method of any one of aspects 48-67.

Aspect 69. A solid HMO product comprising one or more human milk oligosaccharides (HMOs), said solid HMO product having a glass transition temperature (T_g) of around 120 ºC, or higher.

Aspect 70. The solid HMO product according to any one of aspects 68-69, wherein the solid HMO product comprises one or more amorphous HMOs, or a mixture of amorphous and crystalline HMOs, or a crystalline HMO.

Aspect 71. A food product, in particular a nutritional formulation, e.g. an infant nutritional formulation, comprising a solid HMO product according to any one of aspects 68-70.

Aspect 72. A method for producing crystalline HMO in a predetermined polymorphic form from an aqueous composition of said HMO, said method comprising the steps of:

- optionally, seeding said aqueous composition of said HMO with a seed crystal of said HMO in said predetermined polymorphic form, and
- drying said aqueous composition to a water content of less than 10 w/w%

thereby producing crystalline HMO in said predetermined polymorphic form.

Aspect 73. The method according to aspect 72, wherein the aqueous composition of said HMO has a Brix of between 45 and 90, preferably 60 and 80, preferably about 75.

Aspect 74. The method according to aspect 72 or aspect 73, wherein the drying is belt drying, preferably vacuum belt drying.

Aspect 75. The method according to any one of aspects 72-74, wherein the HMO is 2'-FL.

Aspect 76. The method according to any one of aspects 72-75, wherein the polymorphic form is polymorph II.

Aspect 77. The method according to any one of aspects 72-76, wherein the aqueous composition is a fermentation broth from a fermentation process for producing said HMO.

The technology has been described with reference to a number of aspects and embodiments. These aspects and embodiments can be combined as desired by the skilled person, while remaining within the scope of the present invention, as defined by the appended claims. In particular, features relating to the methods described herein are also applicable to the solid HMO products, inasmuch as the method features give rise to identifiable differences in the solid HMO products themselves.

EXAMPLES

Vacuum belt drying

Example 1

2'-FL-containing broth was generated by fermentation using a genetically modified E. coli strain of $LacZ^-$, $LacY^+$ phenotype, wherein said strain comprises a recombinant gene encoding an alpha-1,2-fucosyltransferase enzyme which is able to transfer fucose of GDP-fucose to the internalized lactose and genes encoding a biosynthetic pathway to GDP-fucose.

The fermentation was performed by culturing the strain in the presence of exogenously added lactose and a suitable carbon source, thereby producing 2'-FL which was accompanied with DFL and unreacted lactose as major carbohydrate impurities in the fermentation broth.

The broth was pH adjusted to pH 3.6 and subjected to ultrafiltration (15 kD, Kerasep Ceramic membranes). The UF permeate was further concentrated and diafiltrated by NF (Trisep UA 60 membranes) before being treated by an ion exchanger for demineralization and discoloration. The ion exchanger is a strongly acidic cation exchanger resin (Dowex 88) connected to a weakly basic anion exchanger resin (Dowex 66). The treated product was further decolorized by active charcoal (CPG-L F 20x40) before being concentrated by nanofiltration and vacuum evaporation. Product can be dried by spray drying or crystallized with the aid of acetic acid as described in WO 2016/095924. In the case of spray drying the final composition will include the carbohydrate impurities contained in the fermentation broth (lactose, DFL).

A 2'-FL solution (Brix 58) was prepared by dissolving spray dried/crystalline powder (ratio 2:1) at 60 ºC. The resulting solution was adjusted to pH 4.2 with NaOH. A part of the so-obtained solution was freeze dried whereas another part was dried on a vacuum belt dryer pilot unit (ZWAG, Switzerland, 20 mbars, heating zone 1 130 ºC, heating zone 2 120 ºC, heating zone 3 90 ºC, cooling zone 30 ºC, residence time 20 min, feed flow 150 g solution /min) to get a dried solid. The solid was further manually milled and sieved (1 mm) to give a white powder (residual humidity by Karl-Fisher: 6.0 %).

Particle size distribution:

D10	35 µm
D50	213 µm
D90	475 µm

The XRPD spectrum (recorded on Philips model PW1710/PW1820 instrument, using Cu Kα radiation (λ=0.15418 nm) shows the sample is overwhelmingly (>95 %) amorphous, containing a small amount of crystalline 2'-FL form II.

The freeze dried and the vacuum belt dried samples were analyzed by HPAE chromatography. The data showed that the concentrations of the respective ingredients practically did not differ in both samples (after normalization on dry matter). Specifically, concentrations of impurities that may be products of a possible thermal degradation and/or rearrangement process of 2'-FL (e.g. fucose, lactose, lactulose, fucosyl lactulose, galactose, glucose, fucosyl galactose, fructose) as well as concentrations of unspecified impurities, respectively, were practically the same in the samples. These data indicate that the harsher conditions of belt drying surprisingly does not change the quality of the obtainable solid 2'-FL product.

Example 2

2'-FL-containing broth was generated by fermentation using a genetically modified *E. coli* strain of $LacZ^-$, $LacY^+$ phenotype, wherein said strain comprises a recombinant gene encoding an alpha-1,2-fucosyltransferase enzyme which is able to transfer fucose of GDP-fucose to the internalized lactose and genes encoding a biosynthetic pathway to GDP-fucose. The fermentation was performed by culturing the strain in the presence of exogenously added lactose and a suitable carbon source, thereby producing 2'-FL which was accompanied with DFL and unreacted lactose as major carbohydrate impurities in the fermentation broth.

The broth was pH adjusted to pH 3.6 and subjected to ultrafiltration (15 kD, Kerasep Ceramic membranes). The UF permeate was further concentrated and diafiltrated by NF (Trisep UA 60 membranes) before being treated by an ion exchanger for demineralization and discoloration. The ion exchanger is a strongly acidic cation exchanger resin (Dowex 88) connected to a weakly basic anion exchanger resin (Dowex 66). The treated product was further decolorized by active charcoal (CPG-L F 20x40) before being concentrated by nanofiltration and vacuum evaporation. Product can be dried by spray drying or crystallized with the aid of acetic acid as described in WO 2016/095924. In the case of spray drying the final composition will include the carbohydrate impurities contained in the fermentation broth (lactose, DFL).

A supersaturated 2'-FL solution (Brix 74) was prepared by dissolving spray dried/crystalline powder (ratio 3:1) at 90 ºC. The resulting solution was cooled to 70 ºC and adjusted to pH 4.2 with NaOH. A part of the so-obtained solution was freeze dried whereas another part was dried on a vacuum belt dryer pilot unit (ZWAG, Switzerland, 20 mbars, heating zone 1 130 ºC, heating

zone 2 120 ºC, heating zone 3 90 ºC, cooling zone 30 ºC, residence time 20 min, feed flow 150 g solution/min) to get a dried solid. The solid was further manually milled and sieved (1 mm) to give a white powder (residual humidity residual humidity by Karl-Fisher: 4.3 %).

DSC analysis: no glass transition temperature between 0 and 120 ºC.

Particle size distribution:

D10	33 µm
D50	228 µm
D90	514 µm

The freeze dried and the vacuum belt dried samples were analyzed by HPAE chromatography. The data showed that the concentrations of the respective ingredients practically did not differ in both samples (after normalization on dry matter). Specifically, concentrations of impurities that may be products of a possible thermal degradation and/or rearrangement process of 2'-FL (e.g. fucose, lactose, lactulose, fucosyl lactulose, galactose, glucose, fucosyl galactose, fructose) as well as concentrations of unspecified impurities, respectively, were practically the same in the samples. These data indicate that the harsher conditions of belt drying surprisingly does not change the quality of the obtainable solid 2'-FL product.

Example 3

2'-FL-containing broth was generated by fermentation using a genetically modified *E. coli* strain of LacZ-, LacY+ phenotype, wherein said strain comprises a recombinant gene encoding an alpha-1,2-fucosyltransferase enzyme which is able to transfer fucose of GDP-fucose to the internalized lactose and genes encoding a biosynthetic pathway to GDP-fucose. The fermentation was performed by culturing the strain in the presence of exogenously added lactose and a suitable carbon source, thereby producing 2'-FL which was accompanied with DFL and unreacted lactose as major carbohydrate impurities in the fermentation broth.

The broth was pH adjusted to pH 3.6 and subjected to ultrafiltration (15 kD, Kerasep Ceramic membranes). The UF permeate was further concentrated and diafiltrated by NF (Trisep UA 60 membranes) before being treated by an ion exchanger for demineralization and discoloration. The ion exchanger is a strongly acidic cation exchanger (Dowex 88) connected to a weakly basic anion exchanger (Dowex 66). The treated product was further decolorized by active charcoal (CPG-L F 20x40) before being concentrated by nanofiltration and vacuum evaporation to brix 76. The solution was seeded (2'-FL seed polymorph II) and cooled down to room temperature for 2 days.

The obtained suspension was rewarmed to 50 ºC and was dried on a vacuum belt dryer pilot unit (ZWAG, Switzerland, 20 mbars, heating zone 1 130 ºC, heating zone 2 120 ºC, heating zone 3 90 ºC, cooling zone 30 ºC, residence time 20 min, feed flow 150 g solution /min) to get a

dried solid. The solid was further manually milled and sieved (1 mm) to give a white powder (residual humidity residual humidity by Karl-Fisher: 2.5 %)

The XRPD spectrum (recorded on Philips model PW1710/PW1820 instrument, using Cu Kα radiation (λ=0.15418 nm) shows the sample is well crystalline 2'-FL form II (see Figure 1; the peaks with asterisk * belong to reference 2'-FL polymorph II that is described in WO 2011/150939).

Particle size distribution:

D10	63 µm
D50	364 µm
D90	601 µm

CLAIMS

1. A process for providing a solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), HMO precursors and/or by-products of HMO synthesis, said process comprising a step of

- belt drying, preferably vacuum belt drying,
- drum-drying, or
- granulating

an aqueous composition comprising said one or more human milk oligosaccharides (HMOs), HMO precursors and/or by-products of the HMO synthesis.

2. The process according to claim 1, wherein the aqueous composition comprises one or more HMOs and optionally HMO precursors and/or by-products of HMO synthesis, and the process thereby provides the solid HMO product comprising one or more HMOs that optionally comprises HMO precursors and/or by-products of the HMO synthesis.

3. The process according to claim 1 or claim 2 comprising a step of belt drying, preferably vacuum belt drying.

4. The process according to claim 1 or claim 2 comprising a step of granulating the aqueous composition to form a granulated composition, followed by a step of drying said granulated composition.

5. The process according to any one of the preceding claims, further comprising the step of milling the solid HMO product to a solid HMO powder.

6. The process according to claim 5, further comprising the step of sieving the solid HMO powder and separating the HMO powder into at least a first HMO powder fraction and a second HMO powder fraction.

7. The process according to any one of the preceding claims, wherein the solid HMO product comprises around 40 % or more, such as around 50 % or more, preferably around 60 % or more by weight of synthetic HMOs, as a percentage of the total weight of the solid HMO product.

8. The process according to any one of the preceding claims, wherein the synthetic HMOs are selected from one or more of LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL, 6'-SL, FSL, LST a, LST b and DS-LNT, preferably LNT, LNnT, 2'-FL, 3-FL, DFL, LNFP I, 3'-SL and 6'-SL.

9. The process according to any one of the preceding claims, wherein the aqueous composition is an aqueous solution or an aqueous suspension of said synthetic HMOs, HMO

precursors and/or by-products of HMO synthesis, preferably an aqueous solution of said one or more synthetic HMOs, HMO precursors and/or by-products of HMO synthesis.

10. The process according to any one of the preceding claims, wherein the drying step takes place to a moisture content in the solid HMO product of below 15 %, preferably below 10 %, more preferably below 7 %, most preferably below 6 %.

11. The process according to any one of the preceding claims, further comprising the step of producing said one or more synthetic HMOs by means of fermentation, enzymatic reaction or synthetic reaction, prior to said step of drum drying, belt drying, preferably vacuum belt drying, or granulation.

12. The process according to claim 11, wherein one or more purification steps are carried out on the HMO fermentation broth, enzymatic reaction milieu or synthetic reaction mixture, prior to said step of drum drying, belt drying, preferably vacuum belt drying, or granulation.

13. The process according to claim 12, wherein said one or more purification steps carried out on the fermentation broth, enzymatic reaction milieu or synthetic reaction mixture are selected from one or more of:

- removing solid material;
- removing salts and charged molecules;
- removing uncharged or non-charged material;
- removing HMO precursors and/or by-products;
- separating HMOs, if an HMO mixture is produced;
- removing an excess of water.

14. The process according to any one of claims 1-13, wherein said one or more synthetic HMOs are produced by means of fermentation and the fermentation broth is further processed to prepare an aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs).

15. The process according to claim 13 or claim 14, wherein the method comprises:

a) clarifying the broth to remove suspended particulates and contaminants, particularly cells, cell components, insoluble metabolites and debris from a fermentation process; then

b) removing substantially all the proteins, as well as peptides, amino acids, RNA and DNA and any endotoxins and glycolipids that could interfere with the subsequent purification step, from the aqueous solution obtained in step a).

16. The process according to claim 15, wherein the method comprises:

i) ultrafiltration (UF),

ii) nanofiltration (NF), and

iii) treatment with an ion exchange resin.

17. The process according to claim 11, wherein the one or more synthetic HMOs are produced by means of fermentation, and wherein the fermentation broth, as the aqueous composition comprising said one or more synthetic human milk oligosaccharides (HMOs), is fed directly to the drum dryer, belt dryer, preferably vacuum belt dryer, or granulator, without any intermediate purification steps.

18. The process according to any one of the preceding claims, wherein the solid HMO product comprises one or more amorphous HMOs, or a mixture of amorphous and crystalline HMOs, or a crystalline HMO.

19. A drum-dried solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the process of any one of claims 1, 2 or 5-18.

20. A belt dried, preferably vacuum belt dried, solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the process of any one of claims 1-3 or 5-18.

21. A granulated, dried solid HMO product comprising one or more synthetic human milk oligosaccharides (HMOs), obtained by the process of any one of claims 1 or 4-18.

22. A solid HMO product comprising one or more human milk oligosaccharides (HMOs), said solid HMO product having a glass transition temperature (T_g) around 120 ºC, or higher.

23. A food product, in particular a nutritional formulation, e.g. an infant nutritional formulation, comprising a solid HMO product according to any one of claims 19-21.

24. A method for producing crystalline HMO in a predetermined polymorphic form from an aqueous composition of said HMO, said method comprising the steps of:

- optionally, seeding said aqueous composition of said HMO with a seed crystal of said HMO in said predetermined polymorphic form, and
- drying said aqueous composition to a water content of less than 10 w/w%,

thereby producing crystalline HMO in said predetermined polymorphic form.

25. The method according to claim 24, wherein the aqueous composition of said HMO has a Brix of between 45 and 90, such as between 60 and 80, preferably about 75.

26. The method according to claim 24 or claim 25, wherein the drying is belt drying, preferably vacuum belt drying.

27. The method according to any one of claims 24-26, wherein the HMO is 2'-FL.

28. The method according to any one of claims 24-27, wherein the polymorphic form is polymorph II.

29. The method according to any one of claims 24-28, wherein the aqueous composition is a fermentation broth from a fermentation process for producing said HMO.

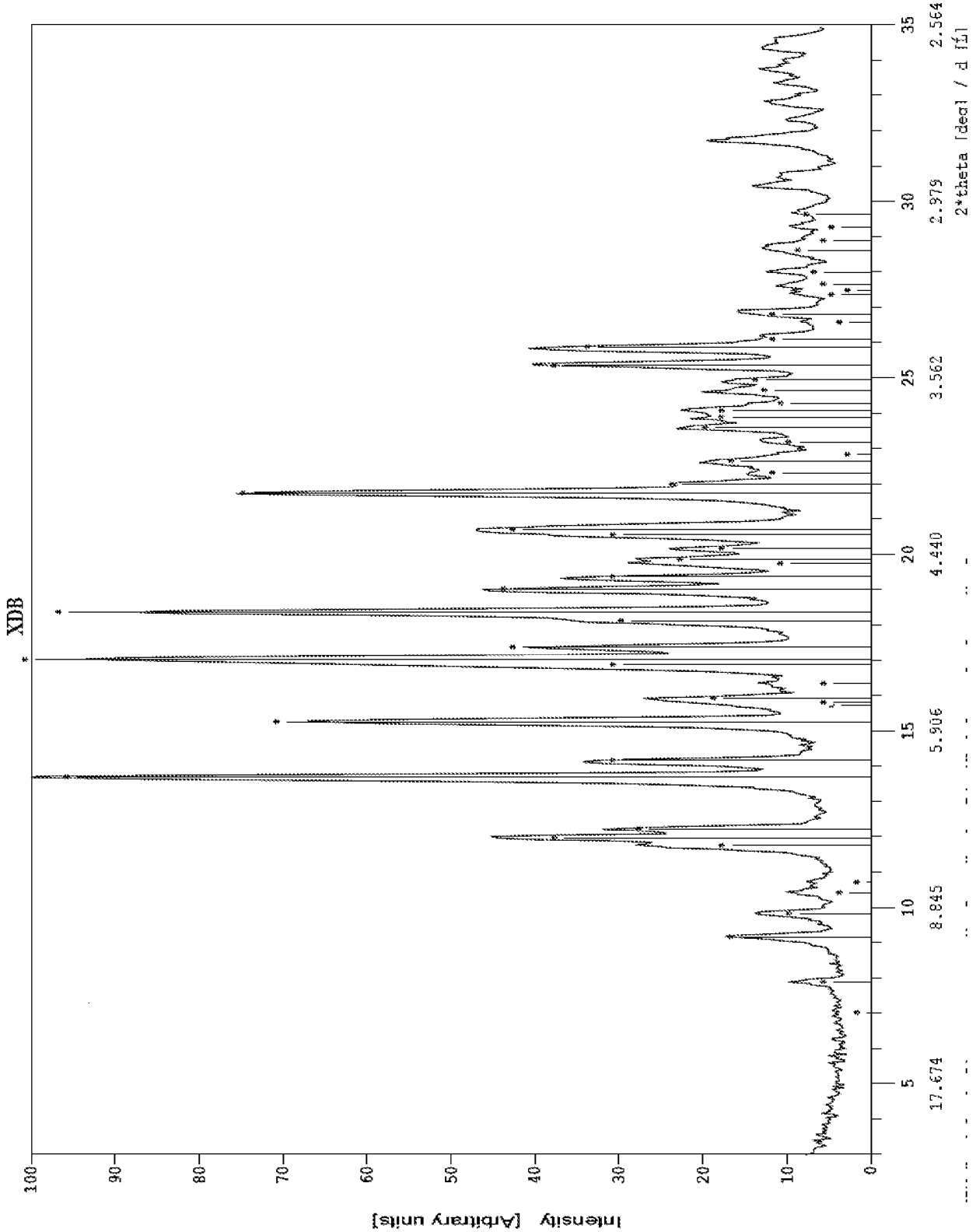

XDB
Intensity [Arbitrary units]
2*theta [deg] / d [Å]
17.674
8.845
5.906
4.440
3.562
2.979
2.564

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/050703

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

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: A23C, A23L, C07H, F26B

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

SE, DK, FI, NO classes as above

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

EPO-Internal, PAJ, WPI data, CHEM ABS Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016065324 A1 (EVOLVE BIOSYSTEMS INC), 28 April 2016 (2016-04-28); paragraphs [0025]-[0028], [0038], [0053]	1-2, 4-19, 21, 23
Y	--	3, 20
X	EP 2272374 A1 (NESTEC SA), 12 January 2011 (2011-01-12); paragraphs [0040]-[0043]	1, 9-10
	--	
Y	CN 104783028 A (SICHUAN YISHENGYUAN TECHNOLOGY CO LTD), 22 July 2015 (2015-07-22); (abstract) Retrieved from: WPI database; Original document: p.4, last paragraph; p. 5-6 experiments	3, 20, 26
	--	

☒ 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
24-02-2021	25-02-2021

Name and mailing address of the ISA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Tony Zhao Telephone No. + 46 8 782 28 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/050703

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

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

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

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

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

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

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

See Continuation of Box III

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.:

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 No. III

According to Rule 13.1 PCT, an international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.

According to Rule 13.2 PCT when a group of inventions is claimed, the requirement of Rule 13.1 PCT shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. In this context, “special technical features” means those technical features with which each invention contributes over the prior art.

If the independent claim lacks novelty and/or an inventive step it is investigated whether there are any common special technical features that unite the dependent claims. This is investigated irrespective of whether one or more dependent claims lack novelty and/or an inventive step beyond the prior art as defined in the document D1. If these dependent claims lack a common special technical feature, the application is considered to include several independent inventions.

The following separate inventions were identified:

1: Claims 1-2, 5-18, 22-23 (partially) and 3, 19-20 directed to a process for providing a solid HMO product comprising a step of belt drying or drum-drying an aqueous composition comprising said HMO product.

2: Claims 1-2, 5-18, 22-23 (partially) and 4, 21 directed to directed to a process of providing a solid HMO product comprising a step of granulating an aqueous composition comprising said HMO product.

3: Claims 24-29 directed to a method of producing a crystalline HMO in a predetermined polymorphic form from an aqueous composition of said HMO.

The present application has been considered to contain 3 inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

Invention 1 relates to the problem of preparing a solid HMO product. This problem is solved by belt-drying or drum-drying a HMO product.

Invention 2 relates to the problem of preparing a solid HMO product. This problem is solved by granulating the HMO product.

Invention 3 relates to the problem of preparing a solid, crystalline HMO product of a certain polymorphic form. This problem is solved by drying the HMO product to a certain water content.

The single general concept of the present application is the teaching of a method of preparing a solid HMO product.

.../...

Continuation of: Box No. III

Document D1 discloses a composition comprising colostrum oligosaccharides from various mammals, amongst them human colostrum oligosaccharides, which comprises human milk oligosaccharides and lactose. D1 further discloses that colostrum oligosaccharides can be dried by drum drying (see [0025]). D1 also discloses that the colostrum oligosaccharides, isolated from mammalian sources can be processed using ultrafiltration and that enzymes can be added to degrade lactose. Both the ultrafiltration and the enzyme degradation step would result in an aqueous composition comprising colostrum oligosaccharides. A method for preparing a solid HMO product by drum drying an aqueous composition comprising HMOs and/or lactose is therefore considered implicitly disclosed by D1.

Thus, the single general concept is known and cannot be considered as a single general inventive concept in the sense of Rule 13.1 PCT.

No other features can be distinguished which can be considered as the same or corresponding special technical features in the sense of Rule 13.2 PCT.

Thus, the application lacks unity of invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/050703

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 20160255849 A1 (DIEKHAUS MARTIN ET AL), 8 September 2016 (2016-09-08); paragraphs [0177]-[0180]	3, 20, 26
	--	
A	US 20130035481 A1 (PEREZ FIGUEROA IGNACIO ET AL), 7 February 2013 (2013-02-07); paragraphs [0322]-[0323]	1-29
	--	
A	WO 2019110806 A1 (JENNEWEIN BIOTECHNOLOGIE GMBH), 13 June 2019 (2019-06-13); page 18, line 7 - page 18, line 8; pages 20-25	1-29
	--	
X	US 20190292211 A1 (JENNEWEIN STEFAN ET AL), 26 September 2019 (2019-09-26); paragraphs [0135]-[0136]	22
	--	
X	EP 2922860 A2 (INALCO S A S DI GIOVANNI CIPOLETTI & C ET AL), 30 September 2015 (2015-09-30); pages 25-35	24-25, 27-29
Y		26
	--	
P, X	Jennewein Biotechnologie GmbH; Mischa Baier; Tobias Seitz ; Markus Helfrich; Stefan Jannewein - A Method for drying human milk oligosaccharides - Prior art Register, XP007023342; RID 31305334; whole document	1-29
	--	

International application No.
PCT/IB2021/050703

Continuation of: second sheet

International Patent Classification (IPC)

F26B 3/20 (2006.01)
A23C 9/20 (2006.01)
C07H 1/06 (2006.01)
F26B 3/24 (2006.01)
A23L 29/30 (2016.01)
A23L 33/125 (2016.01)

WO	2016065324	A1	28/04/2016	AU	2015335601	A1	01/06/2017
				BR	112017008284	A2	19/12/2017
				CA	2965663	A1	28/04/2016
				CN	107249611	A	13/10/2017
				EP	3209308	A1	30/08/2017
				MX	2017005260	A	30/01/2018
				SG	11201703298	A	30/05/2017
				SG	10202002593	A	28/05/2020
				US	20200397837	A1	24/12/2020
				US	20170304375	A1	26/10/2017
				US	10716816	B2	21/07/2020
EP	2272374	A1	12/01/2011	AR	077213	A1	10/08/2011
				AU	2010264848	A1	19/01/2012
				BR	PI1015227	A2	25/08/2015
				CL	2011003280	A1	01/06/2012
				CN	105746703	A	13/07/2016
				CN	102458134	A	16/05/2012
				EP	2470027	B1	17/04/2019
				ES	2533617	T3	13/04/2015
				ES	2727021	T3	11/10/2019
				MX	2011013997	A	08/06/2012
				MY	161448	A	14/04/2017
				SG	176775	A1	30/01/2012
				US	20160000105	A1	07/01/2016
				US	20120189733	A1	26/07/2012
				WO	2010149557	A1	29/12/2010
CN	104783028	A	22/07/2015	NONE			
US	20160255849	A1	08/09/2016	AU	2016201347	B2	08/03/2018
				BR	102016004641	A2	06/09/2016
				CA	2922229	A1	02/09/2016
				CN	105935082	A	14/09/2016
				DE	102016002279	B4	03/09/2020
				DK	3078276	T3	01/04/2019
				JP	2016165273	A	15/09/2016
				JP	6799930	B2	16/12/2020
				NZ	717614	A	23/03/2018

US	20130035481	A1	07/02/2013	AU	2011217614	A1	02/08/2012
				BR	112012020791	A2	03/05/2016
				CA	2789821	A1	25/08/2011
				CN	102822186	A	12/12/2012
				EP	2536736	A4	30/10/2013
				JP	2013519694	A	30/05/2013
				KR	20120138772	A	26/12/2012
				MX	2012009306	A	12/09/2012
				RU	2012140011	A	27/03/2014
				US	9237764	B2	19/01/2016
				WO	2011100979	A1	25/08/2011

WO	2019110806	A1	13/06/2019	AU	2018380959	A1	18/06/2020
				AU	2018380962	A1	11/06/2020
				AU	2018380957	A1	11/06/2020
				AU	2018380956	A1	11/06/2020
				AU	2018380960	A1	04/06/2020
				BR	112020011300	A2	17/11/2020
				BR	112020011296	A2	24/11/2020
				BR	112020011242	A2	17/11/2020
				BR	112020011236	A2	17/11/2020
				BR	112020011202	A2	17/11/2020
				CN	111432663	A	17/07/2020
				CN	111447843	A	24/07/2020
				CN	111465331	A	28/07/2020
				CN	111447845	A	24/07/2020
				CN	111447844	A	24/07/2020
				DE	202018006272	U1	20/12/2019
				DK	201900113	U8	05/11/2020
				DK	201900079	U1	15/10/2019
				EP	3720301	A1	14/10/2020
				EP	3720300	A1	14/10/2020
				EP	3720299	A1	14/10/2020
				EP	3720298	A1	14/10/2020
				EP	3720297	A1	14/10/2020
				KR	20200096770	A	13/08/2020
				KR	20200096793	A	13/08/2020
				KR	20200096779	A	13/08/2020
				KR	20200096772	A	13/08/2020
				KR	20200096771	A	13/08/2020
				SG	11202004914	A	29/06/2020
				SG	11202004659	A	29/06/2020
				SG	11202004655	A	29/06/2020
				SG	11202004650	A	29/06/2020
				SG	11202004414	A	29/06/2020
				US	20200384041	A1	10/12/2020
				US	20200383367	A1	10/12/2020
				WO	2019110801	A1	13/06/2019
				WO	2019110804	A1	13/06/2019
				WO	2019110803	A1	13/06/2019
				WO	2019110800	A1	13/06/2019

US	20190292211	A1	26/09/2019	BR	112016007342	B1	17/11/2020
				CN	109705175	A	03/05/2019
				CN	105814070	A	27/07/2016
				EP	2857410	A1	08/04/2015
				EP	3063159	A1	07/09/2016
				JP	2016535724	A	17/11/2016
				JP	6666243	B2	13/03/2020
				KR	20160090791	A	01/08/2016
				MX	2016004288	A	18/01/2017
				PH	12016500594	A1	13/06/2016
				RU	2685537	C2	22/04/2019
				RU	2016109548	A	09/11/2017
				US	20160237104	A1	18/08/2016
				US	10435427	B2	08/10/2019
				WO	2015049331	A1	09/04/2015
EP	2922860	A2	30/09/2015	IT	FI20120143	A1	13/01/2014
				US	10851128	B2	01/12/2020
				US	20200048295	A1	13/02/2020
				US	20200048294	A1	13/02/2020
				US	20190284219	A1	19/09/2019
				US	10308675	B2	04/06/2019
				US	20160060283	A1	03/03/2016
				WO	2014009921	A3	13/03/2014