



(51) International Patent Classification:

A23C 9/12 (2006.01) C12N 9/38 (2006.01)
A23C 9/13 (2006.01) C12N 15/52 (2006.01)
A23L 33/21 (2016.01)

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/EP2024/072052

(22) International Filing Date:

02 August 2024 (02.08.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/IB2023/000472

04 August 2023 (04.08.2023) IB

(71) Applicant: **COMPAGNIE GERVAIS DANONE**
[FR/FR]; 17 boulevard Haussmann, 75009 PARIS (FR).

(72) Inventors: **KNOL, Jan**; Elstar 88, 6708 LZ Wageningen (NL). **LEVY, Patricia**; Carrer de Buenos Aires, 21, L'Eixample, 08029 Barcelona (ES). **ROSON, Beatriz**; Alameda Porcelana street, n°185, Apartment 22F, São Caetano do Sul São Paulo (BR). **MOTA MACHADO, Natasha**; 374, Grevillea street, Campinas, São Paulo (BR).

(74) Agent: **GEVERS & ORES**; Immeuble Palatin 2, 3, Cours du Triangle - CS80165, 92939 Paris La Défense Cédex (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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

Published:

- with international search report (Art. 21(3))
- with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: SYMBIOTIC MILK PRODUCTS CONTAINING A PROBIOTIC AND GALACTO-OLIGOSACCHARIDES

(57) Abstract: The present invention relates to fermented milk products containing galacto-oligosaccharides (GOS) and a lactic acid bacterium such as a probiotic L casei or L. paracasei, compositions for producing the same and methods for producing the same.

SYMBIOTIC MILK PRODUCTS CONTAINING A PROBIOTIC AND GALACTO-OLIGOSACCHARIDES

Field of the invention

The present invention relates to fermented milk products containing galacto-oligosaccharides (GOS) and a lactic acid bacterium such as a probiotic *Lactobacillus casei* or *Lactobacillus paracasei*, compositions for producing the same and methods for producing the same.

Technical background

Lactobacillus casei and *Lactobacillus paracasei* (also known as *Lacticaseibacillus (para)casei*) are lactic acid bacteria which are commonly used as probiotics. Probiotics are defined as "living microorganisms, which when consumed in adequate amounts, have a beneficial effect on the health of the host" (Report of the joint expert consultation CAM/OMS on the evaluation of the health and nutritional properties of probiotics in foods, 2001). This definition implies that, in order to exert its beneficial effect, the microorganism concerned must be present in a sufficient quantity in the ready-to-eat product and should stay alive after consumption. The maintenance of the viability and of the capacity to growth of probiotic bacteria after consumption thus constitutes an essential element of the quality of these products.

Prebiotics, such as galacto-oligosaccharides (GOS), are ingredients providing health benefits when fermented by the native microflora in the gastrointestinal tract of a subject.

Combination of probiotics and prebiotics can give rise to a synergistic effect. In particular, the effect of combined GOS and probiotics can be beneficial for modulation of gut microbiota composition and for modulation of immune response. It has been shown that this synergistic effect depends on the ability of probiotic bacteria to grow on and consume GOS in the gastrointestinal tract of the subject. The effect of such symbiotic products have been evidenced in particular in infants, so that several infant formula compositions comprising GOS and probiotic bacteria, for example, have been used commercially.

There is a constant need to provide further symbiotic compositions. In particular, there is a need for products that can improve the efficiency of a probiotic by facilitating its survival or its growth after its ingestion by an individual.

Summary of the invention

The present invention allows the production in a simple process of a symbiotic milk product, i.e. a milk product comprising a probiotic and GOS fibers which will be used as a growth substrate in the gastrointestinal tract by the probiotic.

5 The present invention follows from the unexpected finding made by the Inventors that the fermentation of a milk substrate by a probiotic, which is *Lactobacillus casei* or *Lactobacillus paracasei*, in presence of a beta-galactosidase having transgalactosylating activity allows the production of stable GOS, which are not digested by the probiotic during the production or shelf life of the product but can be used as a growth substrate by the probiotic in the human gastrointestinal tract. The GOS produced
10 by the enzymatic reaction simultaneously with the fermentation will not be digested by the probiotic in the acidic conditions of the fermentation, but they will be consumed when the pH is less acid in the gastrointestinal tract of the consumer. In addition, the invention allows a reduction of lactose content, which is particularly advantageous for individuals with lactose intolerance.

An aspect of the invention thus relates to a composition for producing a fermented milk product
15 containing galacto-oligosaccharides (GOS) fibers comprising:

- a milk substrate containing lactose,
- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *Lactobacillus paracasei*, and
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert
20 lactose into GOS fibers that can be metabolized by the probiotic.

Another aspect of the invention relates to a method for producing a fermented milk product containing GOS fibers comprising:

- a step of fermentation of the composition as defined above in conditions allowing the β -galactosidase having transgalactosylating activity to convert lactose into GOS fibers.
- 25 Another aspect of the invention relates to a fermented milk product containing GOS fibers obtained by the method as defined above.

Another aspect of the invention relates to a fermented milk product containing GOS fibers comprising:

- a fermented milk substrate,
- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *Lactobacillus paracasei*,
30 and *Lactobacillus paracasei*,
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic, and
- GOS fibers resulting from the conversion of lactose originally present in the milk.

Detailed description of the invention

As used herein, the words "comprise", "comprising" and the like are to be construed in an inclusive sense, that is to say, in the sense of "including, but not limited to", opposed to an exclusive or exhaustive sense.

- 5 As used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the term "x% (w/w)" or "x% w/w" is equivalent to "x g per 100 g". Unless indicated otherwise, all % value shall be taken to indicate x% w/w.

- 10 Unless defined otherwise, all technical and scientific terms have and should be given the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

- As used herein, the expression "fermented milk product" shall be taken to mean a product or a composition derived from dairy milk by the acidifying action of at least one lactic acid bacterium, such as a yogurt (e.g., a set, stirred or drink yogurt), or a fresh cheese such as a white cheese or a "petit-Suisse". It can also be a strained fermented milk such as a strained yoghurt (e.g., a concentrated or
15 Greek-style yoghurt). It can also be a fermented milk product wherein the total protein content is between 0 and 10 g/100g of product and the fat content between 0 and 5 g/100g of product.

As used herein, the terms "galacto-oligosaccharides" or "GOS" refer to oligosaccharides composed of different galactosyl residues (usually from 2 to 9 units) and a terminal glucose linked by (3-glycosidic bonds, such as (β-(1-2), (β-(1-3), (β-(1-4), and (β-(1-6).

- 20 As used herein, the term "beta-galactosidase having transgalactosylating activity" refers to one or more enzymes with enzymatic activity of enzyme class EC 3.2.1.23, also called beta-D-galactoside galactohydrolase, exo-(1→4)-beta-D-galactanase or lactase, which catalyses the hydrolysis of terminal non-reducing beta-D-galactose residues in beta-D-galactosides, as well as transgalactosylation by transferring a galactose moiety of a beta-D-galactoside to another sugar molecule. In presence of a
25 milk substrate, the enzyme works with two main mechanisms: firstly, it hydrolyses the lactose into 2 monosaccharaides, one molecule of glucose and one molecule of galactose; secondly, it polymerizes several galactoses on lactose to obtain GOS and glucose remains as not polymerized.

- As used herein, EC (Enzyme Committee) numbers refer to the definition of enzymatic activity and nomenclature given by the Nomenclature Committee of the International Union of Biochemistry and
30 Molecular Biology as in force on 3rd July 2019.

As used herein, the word "fiber" refers to carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine. Examples of fibers are inulin,

oligosaccharides such as galactooligosaccharides (GOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), manno-oligo-saccharides, gluco-oligosaccharides, polydextrose, natural gums such as guar gum and acacia gum, mucilages, pectins, arabinogalactan, beta-glucans, tagatose, and resistant starch.

5 Composition for producing a fermented milk product

In an aspect, the invention relates to a composition for producing a fermented milk product containing galacto-oligosaccharides (GOS) fibers comprising:

- a milk substrate containing lactose,
- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei*
10 and *Lactobacillus paracasei* and
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic.

The lactic acid bacterium according to the invention preferably belongs to an *Aerococcaceae*, *Carnobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae*, *Streptococcaceae* or
15 *Bifidobacteriaceae* family and more preferably to an *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, *Weissella* or *Bifidobacterium* genus. More preferably, the lactic acid bacterium according to the invention belongs to a *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus delbruckei*, in particular *L. delbruckei* supsb. *bulgaricus* or *lactis*,
20 *Lactobacillus diolivorans*, *Lactobacillus fermentum*, *Lactobacillus fructivorans*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus jensenii*, *Lactobacillus kunkeei*, *Lactobacillus mali*, *Lactobacillus nagelii*, *Lactobacillus paracasei*, en particulier *L. paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus vini*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus*, *Streptococcus lactis*, *Streptococcus raffinolactis*, *Streptococcus cremoris*, *Bifidobacterium adolescentis*, *Bifidobacterium*
25 *animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, or *Bifidobacterium longum* species.

In a preferred embodiment, the lactic acid bacterium is a probiotic, more preferably a probiotic selected from the group comprising *Lactobacillus casei* and *Lactobacillus paracasei*.

As used herein, the term "probiotic" designates any lactic acid bacterium that has a beneficial effect
30 on the health of the host.

In an embodiment, the milk substrate may be a milk, i.e. non-human milk, preferably cow milk. For example, the milk substrate may be skimmed milk, semi-skimmed milk and/or whole milk.

In an embodiment, the milk substrate may be obtained from milk which has been modified in composition by adjustment of fat content, protein content, lactose content and/or water content.

In an embodiment, the milk substrate may correspond to an aqueous composition comprising one or more dairy ingredients. The term "dairy ingredients" relates to ingredients derived from a non-human mammal milk, such as cow milk, goat milk, ewe milk, camel milk, donkey milk. For avoidance of doubt, the term "dairy ingredients" includes milk, i.e. non-human mammal milk, preferably cow milk. Preferably, the dairy ingredients are ingredients derived from cow milk. Examples of dairy ingredients include milk, milk fat, milk powder, skim milk, milk proteins, dairy curd, cream, buttermilk, condensed milk and combinations thereof. The milk may be a whole milk, semi-skimmed milk or a skimmed milk.

In an embodiment, the milk substrate corresponds to an aqueous composition obtained by reconstituting milk powder, lactose, whey permeate and/or sweet whey into an aqueous liquid, such as water and/or cow milk. By "milk powder", it is understood non-human mammal milk powder, preferably cow milk powder. For example, the milk substrate may be skimmed milk powder, semi-skimmed milk powder and/or whole milk powder reconstituted into water. The cow milk may be skimmed milk, whole milk, semi-skimmed milk or combination thereof.

In an embodiment, the milk substrate comprises at least 4 % (w/w) lactose by dry weight of the milk substrate. Preferably, the milk substrate comprises at least 8 % (w/w), at least 10 % (w/w) or at least 15 % (w/w) lactose by dry weight of the milk substrate. More preferably, the milk substrate comprises at least 20 % (w/w), preferably at least 25 % (w/w) or at least 30 % (w/w) lactose by dry weight of the milk substrate.

The concentration of lactose in the composition impacts positively the GOS production and prevents for longer time their degradation, as enough lactose still remain in the medium.

In an embodiment, the β -galactosidase having a transgalactosylating activity is a microbe derived β -galactosidase, in particular derived from bacteria or fungi.

In an embodiment, the β -galactosidase having a transgalactosylating activity is a *Bifidobacterium* derived β -galactosidase.

As an illustrative example, a β -galactosidase having a transgalactosylating activity appropriate to be used in the present invention is disclosed in WO 01/90317.

In an embodiment, the β -galactosidase is a protein having at least 90% sequence identity with the amino acid sequence SEQ ID NO: 1.

SEQ ID NO: 1
MAVRRLGGRI VAAATVALS IPLGLLTNSA WAVEDATRSD STTQMSSTPE VVYSSAVDSK QNRTSDFDAN WKFMLSDSVQ AQDPAFDDSA WQQVDLPHDY SITQKYSQSN EAESAYLPGG TGWYRKSFTI DRDLAGKRIA INFDGVYMNA TVWFNGVKLG THPYGYSPFS FDLTGNAKFG GENTIVVKVE NRLPSSRWYS GSGIYRDVTL TVTDGVHVG NGVAIKTPSL ATQNGGDVTM NLTTKVANDT EAAANITLKQ TVFPKGGKTD AAIGTVTTAS KSIAAGASAD VTSTITAASP KLWSIKNP NL YTVRTEVLNG GKVLDTYDTE YGFRWTGFDA TSGFSLNGEK VKLKGVSMMH DQGS LGAVAN RRAIERQVEI LQKMGVNSIR TTHNPAAKAL IDVCNEKGVL VVEEVFDMWN RSKNGNTEDY GKWFGQAIAG DNAVLGGDKD ETWAKFDLTS TINRDRNAPS VIMWSLGNEM MEGISGSVSG FPATSAKLVA WTKAADSTRP MTYGDNKIK NWNESNTMGD NLTANGGVVG TNYSDGANYD KIRTTHPSWA IYGETASAI NSRGIYNRTT GGAQSSDKQL TSYDNSAVGW GAVASSAWYD VVQRDFVAGT YVWTGFDYLG EPTPWNGTGS GAVGSLAVAE ELVLRHRRHR RLPEDTYIFY QSQWNDVHT LHILPAWNEN VVAKGSGNNV PVVVYDAAK VKLYFTPKGS TEKRLIGES FTKKTTAAGY TYQVYEGSDK DSTAHKNMYL TWNV PWAEGT ISAEAYDENN RLIPEGSTEG NASVTTTGKA AKLKADADRK TITADGKDL YIEVDVTDAN GHIVPDAANR VTFDVKGAGK LVGVDNGSSP DHDSYQADNR KAFSGKVLAI VQSTKEAGEI TVTAKADGLQ SSTVKIATTA VPGTSTEKT VRSFYYSRNY VKTGNKPILP SDVEVRYSDG TSDRQNV TWD AVSDDQIAKA GSFSVAGTVA GQKISVRVTM IDEIGALLNY SASTPVGTPA VLPGRPAVL PDGTVTSANF AVHWTKPADT VYNTAGTVKV PGTATVFGKE FKVTATIRVQ RSQVTIGSSV SGNALRLTQN IPADKQSDTL DAIKDGSTTV DANTGGGANP SAWTNWAYS AGHNTAEITF EYATEQQLGQ IVMYFFRDSN AVRFPDAGKT KIQISADGKN WTDLAATETI AAQESSDRVK PYTYDFAPVG ATFKVKT VTN ADTTTPSGVV CAGLTEIELK TATSKFVTNT SAALSSLTVN GTKVSDSVLA AGSYNTPAI ADVKAEGEGN ASVTVLPAHD NVIRVITESE DHVTRKTFTI NLGTEQEFP DSDERDYPAA DMTVTVGSEQ TSGTATEGPK KFAVDGNTST YWHSNWTPTT VNDLWIAFEL QKPTKLDALR YLPRPAGSKN GSVTEYKVQV SDDGTNWTDA GSGTWTTDYG WKLAEFNQPV TTKHVRLKAV HTYADSGNDK FMSASEIRLR KAVDTTDISG ATVTVPKLT VDRVDADHPA TFATKDVTVT LGDATLRYGV DYLLDYAGNT AVGKATVTVR GIDKYS GTVA KTFTIELKNA PAPEPTLTSV SVKTKPSKLT YVVGDAFDPA GLVLQHDRQA DRPPQPLVGE QADERGLTCG TRCDRVEQLR KHENREAHRT GLDHLEFVGA ADGAVGEQAT FKVHVHADQG DGRHDDADER DIDPHVPVDH AVGELARAAC HHVIGLRVDT HRLKASGFQI PADDMAEIDR ITGFHRFERH VG

Table 1. Example of appropriate β -galactosidase with a transgalactosylating activity

In an embodiment, the β -galactosidase is a protein having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the amino acid sequence SEQ ID NO: 1.

- In an embodiment, the β -galactosidase is a protein consisting of the amino acid sequence SEQ ID NO: 1.

In an embodiment, the β -galactosidase is the Nurica™ enzyme (commercialized by Dupont).

In an embodiment, the β -galactosidase is the Zymstar™ enzyme (commercialized by Dupont).

In an embodiment, the β -galactosidase is the NS43104 enzyme (commercialized by Novozyme).

The GOS produced by the action of the β -galactosidase and the digestion of said GOS by the probiotic can be measured by methods that are well-known by a skilled person in the art.

- 5 In the present invention, “*Lactobacillus casei*” refers to strains belonging to the *Lactobacillus casei* species, which has been reclassified as the *Lacticaseibacillus casei* species (Zheng *et al.*, Int J Syst Evol Microbiol 70:2782-2858, 2020).

- 10 In the present invention, the “*Lactobacillus paracasei*” species refers to strains previously classified as belonging to the *Lactobacillus casei* species, which has been reclassified as *Lacticaseibacillus paracasei* species (Zheng *et al.*, 2020).

In a preferred embodiment, the probiotic is a *L. paracasei* strain.

Typically, said given *L. paracasei* is a probiotic strain with immunomodulatory properties.

In a preferred embodiment, the *L. paracasei* strain is selected from the group comprising *L. paracasei* CNCM I-1518 and *L. paracasei* CNCM I-3689.

- 15 As used herein, the term “CNCM I-” followed by a 4 digit number shall be taken to refer to a strain deposited at the Collection Nationale de Cultures de Microorganismes (CNCM) 25 rue du Docteur Roux, Paris, France under the Budapest Treaty with an accession number corresponding to said 4 digit number, e.g. CNCM I-1518.

- 20 *L. paracasei* CNCM I-1518 is disclosed in EP0794707 and was deposited, according to the Budapest Treaty, at the Collection Nationale de Cultures de Microorganismes (CNCM) 25-28 rue du Docteur Roux, 75724 Paris, France, on September 28, 1994 with the reference CNCM I-1518.

L. paracasei CNCM I-3689 is disclosed in WO2009122042 and was deposited, according to the Budapest Treaty, at deposited at the Collection Nationale de Cultures de Microorganismes 25-28 rue du Docteur Roux, 75724 Paris, France, on November 9, 2006 with the reference CNCM I-3689.

- 25 CNCM I-1518 and CNCM I-3689 strains both have immuno-modulatory properties (see for example WO2009/130423).

L. paracasei strains obtained by mutagenesis or by genetic transformation of CNCM I-1518 or CNCM I-1518 strains, which keep its probiotic properties, are also encompassed in the present invention.

- 30 In an embodiment, the composition as defined above further comprises a compound to improve flavor, texture and/or color.

In an embodiment, the composition as defined above further comprises dextrose.

Dextrose is used to bring caramel notes and color.

In a specific embodiment, the invention relates to a composition as defined above comprising:

- a skimmed milk powder,

5 - a *L. paracasei* strain, preferably the *L. paracasei* strain deposited with the CNCM under the number I-1518,

- a β -galactosidase having a transgalactosylating activity, preferably a protein consisting of the amino acid sequence SEQ ID NO 1, and

- water.

10 In an embodiment, the invention relates to a composition as defined above, said composition further comprising dextrose.

In a specific embodiment, the composition as defined above can be prepared by mixing water, skimmed milk powder and dextrose, pre-heating the mixture, homogenizing the mixture, sterilizing/caramelizing the mixture, adding the probiotic and the β -galactosidase to the mixture.

15 In an embodiment, the composition as defined above comprises only one lactic acid bacterium which is a probiotic, preferably selected from the group comprising *L. casei* and *L. paracasei*.

In an embodiment, the composition as defined above comprises only one lactic acid bacterium which is a probiotic selected from the group comprising *L. paracasei* CNCM I-1518 and *L. paracasei* CNCM I-3689.

20 Method for producing a fermented milk product containing GOS fibers

In another aspect, the invention relates to a method for producing a fermented milk product containing GOS fibers comprising:

- a step of fermentation of the composition as defined above in conditions allowing the β -galactosidase having transgalactosylating activity to convert lactose into GOS fibers.

25 In a preferred embodiment, the composition is fermented at 37°C +/- 1°C, preferably at 37°C +/- 0.5°C, for at least about 3 days, preferably for about 5 days (120h).

This temperature allows the fermentation conducted by the probiotic simultaneously with the enzymatic reaction made by the β -galactosidase having transgalactosylating activity.

The fermentation step is monitored so that it can be stopped when the desired population of probiotic
30 and/or the desired quantity of GOS fibers and/or the desired pH are reached.

In an embodiment, the invention relates to a method as defined above, said method further comprising:

- a step of inactivating the β -galactosidase having a transgalactosylating activity when a desired quantity of GOS fibers or reduction of lactose content is reached.

- 5 In an embodiment, the β -galactosidase having a transgalactosylating activity is inactivated when at least a quantity of 1.4 gram of GOS fibers per 100 grams of composition is reached.

In an embodiment, the β -galactosidase having a transgalactosylating activity is inactivated when the lactose content is less than 1% (w/w), preferably less than 0.1% (w/w).

- 10 In an embodiment, the invention relates to a method as defined above, wherein the β -galactosidase having a transgalactosylating activity is inactivated by the acidification of the composition, preferably when pH reaches 3.8 or less.

In an embodiment, the invention relates to a method as defined above, wherein the β -galactosidase having a transgalactosylating activity can be inactivated by heat treatment.

- 15 In an embodiment, the invention relates to a method as defined above, wherein the β -galactosidase having a transgalactosylating activity is not inactivated by heat treatment.

In an embodiment, the fermentation step is stopped when the composition reaches pH 3.6 +/- 0.05.

In an embodiment, the invention relates to a method as defined above, wherein a lactase enzyme is added.

Such a lactase enzyme can be used to digest residual lactose.

- 20 In a preferred embodiment, the invention relates to a method as defined above, wherein after the fermentation step, the composition comprises protein 5.7% (w/w), GOS 3.2 % (w/w), lactose 1.2 % (w/w).

In an embodiment, the invention relates to a method as defined above, said method further comprising:

- 25 - a step of adding at least an extra fiber, preferably an extra fiber that is not metabolized by the probiotic but is metabolized by the gut microbiome of a consumer.

- In an embodiment, the extra fiber is selected from the group comprising inulin, fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), manno-oligo-saccharides, gluco-oligosaccharides, polydextrose, natural gums such as guar gum and acacia gum, mucilages, pectins, arabinogalactan, beta-glucans, tagatose, and resistant starch, preferably polydextrose.
- 30

In a preferred embodiment, the extra fiber is added so that the final product comprises at least 1.5 grams, preferably 3.0 grams, more preferably 3.3 grams of total fibers per 100 grams of product.

In an embodiment, the invention relates to a method as defined above, said method further comprising:

- 5 - a step of adding sugar.

In an embodiment, the invention relates to a method as defined above, said method further comprising:

- a step of adding a syrup.

In an embodiment, the syrup comprises water, liquid sugar, polydextrose and citric acid.

10 Fermented milk products containing GOS fibers

In another aspect, the invention relates to a fermented milk product containing GOS fibers obtained by the method as defined above.

In another aspect, the invention relates to a fermented milk product containing GOS fibers comprising:

- a fermented milk substrate,
15 - a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *L. paracasei*,
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic, and
- GOS fibers resulting from the conversion of lactose originally present in the milk substrate.

- 20 In an embodiment, the probiotic is selected from the group comprising *L. paracasei* CNCM I-1518 and *L. paracasei* CNCM I-3689.

In an embodiment, the invention relates to a fermented milk product containing GOS fibers comprising:

- a fermented milk substrate,
25 - a probiotic selected from the group comprising *L. paracasei* CNCM I-1518 and *L. paracasei* CNCM I-3689
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic, and
- GOS fibers resulting from the conversion of lactose originally present in the milk substrate.

- 30 In an embodiment, the invention relates to a fermented milk product as defined above, said product further comprising at least an extra fiber, preferably an extra fiber that is not metabolized by the probiotic but is metabolized by the gut microbiome of a consumer.

In an embodiment, the invention relates to a fermented milk product as defined above, wherein said extra fiber is selected from the group comprising inulin, fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), mannooligo-saccharides, gluco-oligosaccharides, polydextrose, natural gums such as guar gum and acacia gum, mucilages, pectins, arabinogalactan, beta-glucans, tagatose, and resistant starch, preferably polydextrose.

In an embodiment, the invention relates to a fermented milk product as defined above, said product comprising at least 1.5 grams, preferably 3.0 grams, more preferably 3.3 grams, of total fibers per 100 grams of product.

In an embodiment, the invention relates to a fermented milk product as defined above, said product comprising at least 1×10^8 CFU of the probiotic per 100 grams of product.

As used herein, the term “cfu” or “CFU” shall be taken to be an abbreviation of the term “colony forming unit”.

In an embodiment, the invention relates to a fermented milk product as defined above, said product further comprising added sugar.

In an embodiment, the invention relates to a fermented milk product as defined above, said product further comprising a syrup, preferably a syrup comprising water, liquid sugar, polydextrose and citric acid.

In an embodiment, the invention relates to a fermented milk product as defined above, wherein the quantity of total carbohydrates does not exceed 15 grams, preferably 11 grams, per 100 grams of product.

In an embodiment, the invention relates to a fermented milk product as defined above, which has a low lactose content.

In an embodiment, the invention relates to a fermented milk product as defined above, wherein the quantity of lactose does not exceed 1.0 gram, preferably 0.1 gram, per 100 grams of product.

In an embodiment, the invention relates to a fermented milk product as defined above, further comprising a lactase enzyme.

In a particular embodiment, the invention relates to a fermented milk product as defined above, wherein said product does not comprise added sugar.

In a particular embodiment, the fermented milk product comprises 43 % (w/w) of a composition as defined above and 57 % (w/w) of a syrup.

In an embodiment, the fermented milk product has a protein content from 1% to 15% (w/w).

In an embodiment, the fermented milk product has a fat content from 0% to 5% (w/w).

The various features of the present invention referred to in individual aspects above apply, as appropriate, to other aspects *mutatis mutandis*. Consequently, features specified in a given aspect of the invention may be combined with features specified in other aspects as appropriate.

- 5 The invention will be further illustrated by the following non-limiting Figures and Examples.

Description of the figures

Figure 1. Fermentation of composition A.

Figure 2. Evolution of lactose content during fermentation of composition A.

Figure 3. Fermentation of composition B.

5 **Figure 4.** Evolution of lactose content during fermentation of composition B.

Examples

Example I - Composition A

5 Production process of the composition A

Cooled fluid milk is automatic transferred to the mixing tank. Then, dextrose and skimmed milk powder are manually charged to the mixing tank. Ingredients are dispersed and blended for 15 minutes, and physicochemical analysis performed to check the right mixture composition. The mix is hydrated for 1 hour at 6 °C, under smoothly agitation to guarantee a homogeneous mixture. The standardized mix
10 passes through filters (0.5 mm) to remove any possible physical contamination, then sterilized for food safety security. The Maillard reaction, which gives the specific caramel taste and color to the product, happens right after the sterilization of the standardized mix and takes place in the previously sterilized mass tank. It consists in cooking the mix at 96°C to reach the color target. At this temperature, the glucose and amino acids from milk proteins react to form « caramel » products and change the mix
15 color (browning). Once the designed color is achieved, the mix is cooled down to the fermentation temperature (37°C) before inoculation and enzyme addition. The fermentation step takes more than 3 days (>72 hours) for GOS production and 5 days (120 hours) to reach the breaking pH. Fermented mass is cooled in-tank, by circulating chilled water in the double wall jacket, while providing a continuous agitation. This way of cooling the mass, gives the opportunity to use the fermentation tank as a storage
20 tank before mixing with the Sugar syrup.

Composition A was prepared with the following ingredients (Table 2).

Ingredients	Quantity	
Milk 0.05% fat	5 419.93 L	86.887 %
Skimmed milk powder	550.00 kg	8.335 %
Dextrose	96.75 kg	1.500 %
CNCM I-1518	6 packets	0.040 %
Enzyme Nurica™	2.00 kg	0.0300 %
Water	206.92 L	3.208 %
Total	6 450.00 kg	100%

Table 2. Ingredients of composition A.

25

Composition A was fermented at 37°C for 120h (Figure 1).

As shown in figure 1, the quantity of GOS remains stable, i.e. they are not digested by the *L. paracasei* strain at this pH conditions. This is particularly advantageous in that GOS are a substrate for growth for *L. paracasei* in less acidic conditions (see for example, Watson *et al.*, J Appl Microbiol, 114:1132-1146, 2012). Consequently, GOS are stable in the fermented product but they will be metabolized by the *L. paracasei* strain in the gastrointestinal tract when ingested by the consumer.

Lactose in the end of fermentation was 1,19% (Figure 2).

The β -galactosidase was inactivated when pH reaching 3.8.

After fermentation, a syrup has been added to the composition A. The syrup comprises the following ingredients (Table 3).

Ingredients	Quantity	
Decorated potable water	5 645.00 L	66.026 %
Liquid sugar (66.5 % total sucrose)	1 698.88 L	26.316 %
Polydextrose Litesse Powder	369.36 kg	4.320 %
Citric acid granulated	0.855 kg	0.010 %
Citric Vanilla flavor	9.542 kg	0.112 %
Canne sugar flavor	0.752 kg	0.009 %
Water	274.28 L	3.208 %
Total	8 550.00 kg	100%

Table 3. Ingredients of the syrup.

The addition of polydextrose is advantageous in that this will be beneficial for the intestinal flora but it will not be consumed by *L. paracasei*, i.e. the probiotic will not compete with the intestinal microflora as regards with this substrate.

A fermented milk product containing galacto-oligosaccharides (GOS) fibers has been obtained by mixing the fermented composition A (43%) with the syrup (57%).

Example 2 - Composition B

Production process of the composition B

Cooled water is automatic transferred to the mixing tank. Then, dextrose and skimmed milk powder are manually charged to the mixing tank. Ingredients are dispersed and blended for 15 minutes, and physicochemical analysis performed to check the right mixture composition. The mix is hydrated for 1 hour at 6 °C, under smoothly agitation to guarantee a homogeneous mixture. The standardized mix passes through filters (0.5 mm) to remove any possible physical contamination, then sterilized for food safety security. The Maillard reaction, which gives the specific caramel taste and color to the product, happens right after the sterilization of the standardized mix and takes place in the previously sterilized mass tank. It consists in cooking the mix at 96°C to reach the color target. At this temperature, the glucose and amino acids from milk proteins react to form « caramel » products and change the mix color (browning). Once the designed color is achieved, the mix is cooled down to the fermentation temperature (37°C) before inoculation and enzyme addition. The fermentation step takes more than 3 days (>72 hours) for GOS production and 5 days (120 hours) to reach the breaking pH. Fermented mass is cooled in-tank, by circulating chilled water in the double wall jacket, while providing a continuous agitation. This way of cooling the mass, gives the opportunity to use the fermentation tank as a storage tank before mixing with the Sugar syrup.

Composition B was prepared with the following ingredients (Table 4).

Ingredients	Quantity	
Decorated potable water	5 067.97 L	78.573 %
Skimmed milk powder	1 075.00 kg	16.649 %
Dextrose	96.75 kg	1.500 %
CNCM I-1518	6 packets	0.040 %
Enzyme Nurica™	2.00 kg	0.0300 %
Water	206.92 L	3.208 %
Total	6 450.00 kg	100%

Table 4. Ingredients of composition B.

Composition B is fermented at 37°C for 111h (Figure 3).

As shown in figure 2, the quantity of GOS remains stable, i.e. they are not digested by the *L. paracasei* strain at this pH conditions. This is particularly advantageous in that GOS are a substrate for growth for *L. paracasei* in less acidic conditions (see for example, Watson *et al.*, J Appl Microbiol, 114:1132-

1146, 2012). Consequently, GOS are stable in the fermented product but they will be metabolized by the *L. paracasei* strain in the gastrointestinal tract when ingested by the consumer.

Lactose in the end of fermentation was 1,52% (Figure 4).

5

After fermentation, a syrup has been added to the composition B (same syrup as used in Example 1, see Table 3).

10

A fermented milk product containing galacto-oligosaccharides (GOS) fibers has been obtained by mixing the fermented composition B (43%) with the syrup (57%).

GOS concentration was monitored in the final product (Table 5).

Shelf life time	12 days	51 days
Analyses date	03/04/2023	12/05/2023
GOS (%)	2	2

Table 5. Stability of GOS in the final product.

15

GOS concentration was stable in the final product when kept at 10 °C for 51 days. There was no relevant GOS amount variation in the product shelf life. Interestingly, the GOS produced remain stable in the product, with no need of adding a stabilizer.

20

TRAITE DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
AUX FINS DE LA PROCEDURE EN MATIERE DE BREVETS

FORMULE INTERNATIONALE

DESTINATAIRE :

CIRDC
15, avenue Galilée
92350 LE PLESSIS-ROBINSONRECEPISSE EN CAS DE DEPOT INITIAL,
délivré en vertu de la règle 7.1 par
l'AUTORITE DE DEPOT INTERNATIONALE
identifiée au bas de cette pageNOM ET ADRESSE
DU DEPOSANT

I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT : DN-114 001	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE : I - 1518
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous*chiffre I était accompagné :	
☐ d'une description scientifique	
☐ d'une désignation taxonomique proposée	
(Cocher ce qui convient)	
III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le (date du dépôt initial) ¹	
IV. RECEPTION D'UNE REQUETE EN CONVERSION	
La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous chiffre I le 28 septembre 1994 (date du dépôt initial) et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de Budapest le 30 décembre 1994 (date de réception de la requête en conversion)	
V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : CNCM Collection Nationale de Cultures de Microorganismes	Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) : Mme Y. CERISIER Directeur Administratif de la CNCM
Adresse : INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15	Date : Paris, le 06 février 1995

¹ En cas d'application de la règle 6.4.d), cette date est la date à laquelle le statut d'autorité de dépôt internationale a été acquis.

COPIE POUR INFORMATION

WO 2025/032004 BUDAPEST TREATY ON THE INTERNATIONAL **PCT/EP2024/072052**
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF
PATENT PROCEDURE

INTERNATIONAL FORM

ADDRESSEE

COMPAGNIE GERVAIS DANONE
BP 63
126, rue Jules Guesde
92302 LEVALLOIS-PERRET

RECEIPT IN CASE OF INITIAL DEPOSIT
delivered in pursuance of rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF APPLICANT

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the APPLICANT DN-114 121	Accession number allocated by the INTERNATIONAL DEPOSITARY AUTHORITY CNCM I-3689
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. was accompanied by: ☒ a scientific description ☒ a proposed taxonomic designation (place an X in a appropriate square)	
III. RECEPTION AND ACCEPTANCE	
The present International Depositary Authority accepts the microorganism identified under I., which it received on November 9, 2006 (date of initial deposition) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I. above was received by this International Depositary Authority on _____ (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion)	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Collection Nationale de Cultures de Microorganismes (CNCM) Address : INSTITUT PASTEUR 25 rue du Docteur Roux F-75724 PARIS Cédex 15 (France)	Signature(s) of the person(s) competent to represent the International Depositary Authority, or of the authorized employee(s): Georges Wagener signature Date : Paris, December 8, 2006

¹ Where rule 6.4.d) applies, this date is the date on which the status of the International Depositary Authority has been acquired.

CLAIMS

1. A composition for producing a fermented milk product containing galacto-oligosaccharides (GOS)

5 fibers comprising:

- a milk substrate containing lactose,

- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *Lactobacillus paracasei*, and

- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert
10 lactose into GOS fibers that can be metabolized by the probiotic.

2. The composition according to claim 1, comprising:

- a milk substrate containing lactose,

- a probiotic selected from the group comprising *Lactobacillus casei* and *Lactobacillus paracasei*, and

- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert
15 lactose into GOS fibers that can be metabolized by the probiotic.

3. The composition according to claim 1 or 2, wherein the β -galactosidase is a microbe derived β -galactosidase, preferably a bacterium derived β -galactosidase, more preferably a *Bifidobacterium*
20 derived β -galactosidase.

4. The composition according to any one of claims 1 to 3, wherein the β -galactosidase is a protein having at least 90% sequence identity with the amino acid sequence SEQ ID NO 1.

5. The composition according to any one of claims 1 to 4, wherein the probiotic is selected from the group comprising *L. paracasei* CNCM I-1518 and *L. paracasei* CNCM I-3689, preferably *L. paracasei* CNCM I-1518.
25

6. The composition according to any one of claims 1 to 5, said composition further comprising dextrose.
30

7. The composition according to any one of claims 1 to 6, said composition comprising:

- a skimmed milk powder,

- a *L. paracasei* strain, preferably the *L. paracasei* strain deposited with the CNCM under the number I-1518,

- a β -galactosidase having a transgalactosylating activity, preferably a protein consisting of the amino acid sequence SEQ ID NO: 1, and
- water.

5 **8.** A method for producing a fermented milk product containing GOS fibers comprising:

- a step of fermentation of the composition as defined in any one of claims 1 to 7, in conditions allowing the β -galactosidase having transgalactosylating activity to convert lactose into GOS fibers.

9. A method according to claim 8, said method further comprising:

- 10 - a step of inactivating the β -galactosidase having a transgalactosylating activity when a desired quantity of GOS fibers is reached.

10. A method according to claims 8 or 9, said method further comprising:

- 15 - a step of adding at least an extra fiber, preferably an extra fiber that is not metabolized by the probiotic but is metabolized by the gut microbiome of a consumer.

11. A method according to claim 10, wherein said extra fiber is selected from the group comprising galactooligosaccharides (GOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), manno-oligo-saccharides, gluco-oligosaccharides, polydextrose, natural gums such as guar gum and acacia gum, mucilages, pectins, arabinogalactan, beta-glucans, tagatose, and resistant starch, preferably polydextrose.

12. A method according to any one of claims 8 to 11, said method further comprising:

- a step of adding sugar.

13. A method according to any one of claims 8 to 12, said method further comprising:

- a step of adding a syrup, preferably a syrup comprising water, liquid sugar, polydextrose and citric acid.

14. A fermented milk product containing GOS fibers obtained by the method as defined in any one of claims 8 to 14.

15. A fermented milk product containing GOS fibers comprising:

- a milk substrate,

- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *L. paracasei*,
- a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic, and
- 5 - GOS fibers resulting from the conversion of lactose originally present in the milk substrate.

16. The fermented milk product according to claim 15, comprising:

- a milk substrate,
- a lactic acid bacterium, such as a probiotic selected from the group comprising *Lactobacillus casei* and *L. paracasei*,
- 10 - a β -galactosidase having a transgalactosylating activity, said β -galactosidase being able to convert lactose into GOS fibers that can be metabolized by the probiotic, and
- GOS fibers resulting from the conversion of lactose originally present in the milk substrate.

15 **17.** The fermented milk product according to any one of claims 14 to 16, said product further comprising at least an extra fiber, preferably an extra fiber that is not metabolized by the probiotic but is metabolized by the gut microbiome of a consumer.

20 **18.** The fermented milk product according to claim 17, wherein said extra fiber is selected from the group comprising galactooligosaccharides (GOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), manno-oligo-saccharides, gluco-oligosaccharides, polydextrose, natural gums such as guar gum and acacia gum, mucilages, pectins, arabinogalactan, beta-glucans, tagatose, and resistant starch, preferably polydextrose.

25 **19.** The fermented milk product according to any one of claims 14 to 18, said product comprising at least 1.5 grams, preferably 3.0 grams, more preferably 3.3 grams, of total fibers per 100 grams of product.

30 **20.** The fermented milk product according to any one of claims 14 to 19, said product comprising at least 1×10^8 cfu of the probiotic per 100 grams of product.

21. The fermented milk product according to any one of claims 14 to 20, said product further comprising added sugar.

22. The fermented milk product according to any one of claims 14 to 21, said product further comprising a syrup, preferably a syrup comprising water, liquid sugar, polydextrose and citric acid.

23. The fermented milk product according to any one of claims 14 to 20, wherein said product does
5 not comprise added sugar.

24. The fermented milk product according to any one of claims 14 to 23, wherein the quantity of total carbohydrates does not exceed 15 grams, preferably 11 grams, per 100 grams of product.

10 **25.** The fermented milk product according to any one of claims 14 to 24, wherein the quantity of lactose does not exceed 1.0 gram, preferably 0.1 gram, per 100 grams of product.

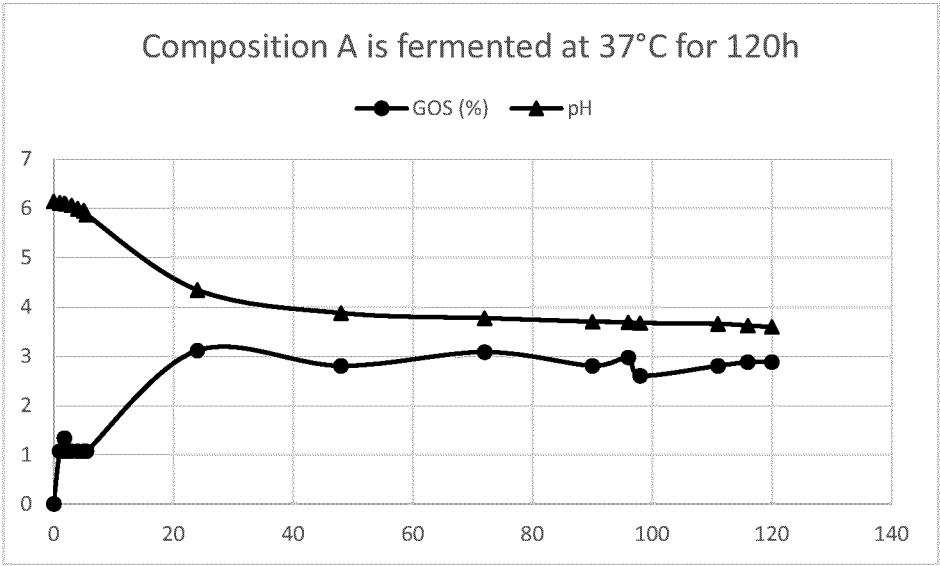


FIGURE 1

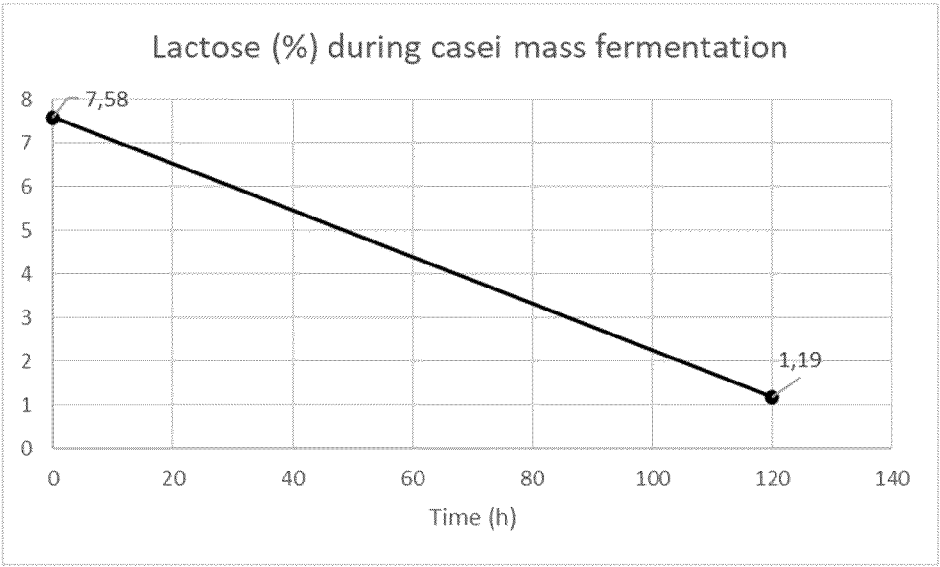


FIGURE 2

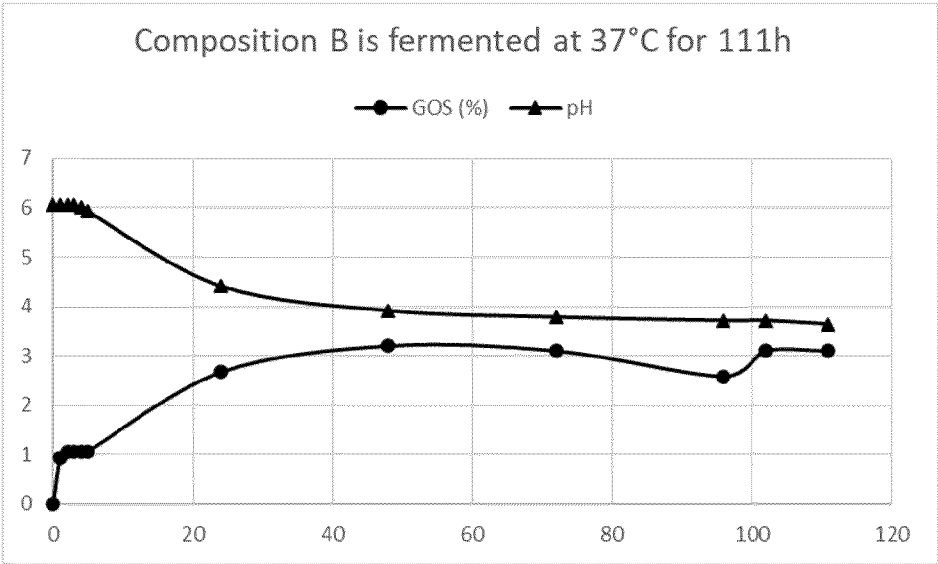


FIGURE 3

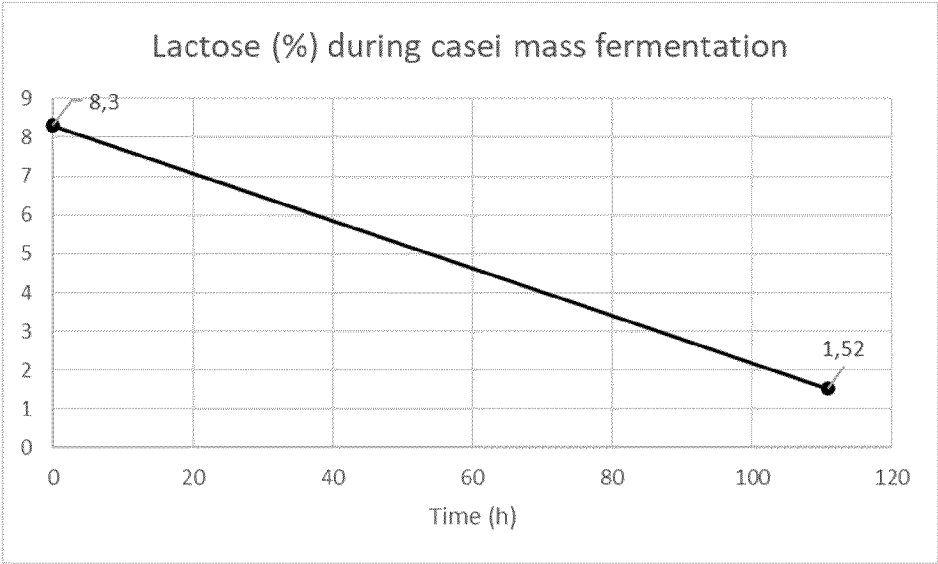


FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/072052

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/072052

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A23C9/12	A23C9/13
	A23L33/21	C12N9/38
		C12N15/52
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
A23C C12N A23L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal, FSTA, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/189568 A2 (CHR HANSEN AS [DK])	1, 3, 4, 6,
	15 September 2022 (2022-09-15)	8-25
Y	claims 1-15; example 3	2, 5, 7

Y	CN 114 258 950 A (BRIGHTNESS MILK INDUSTRY STOCK LTD COMPANY)	2, 5, 7
	1 April 2022 (2022-04-01)	
	claims 1-10; example 1	

	- / - -	
☒ 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		
"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
23 October 2024		06/11/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Schlegel, Birgit

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/072052

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALVAREZ ANNE-SOPHIE ET AL: "Safety and functional enrichment of gut microbiome in healthy subjects consuming a multi-strain fermented milk product: a randomised controlled trial", SCIENTIFIC REPORTS, vol. 10, no. 1, 29 September 2020 (2020-09-29), XP093123597, US ISSN: 2045-2322, DOI: 10.1038/s41598-020-72161-w abstract -----	5
A	WO 2023/102101 A1 (COCA COLA CO [US]; LI ALLEN [US] ET AL.) 8 June 2023 (2023-06-08) the whole document -----	1-24
A	US 2014/308391 A1 (KNOL JAN [NL] ET AL) 16 October 2014 (2014-10-16) paragraphs [0005] - [0010], [0017], [0022], [0026], [0040] - [0042]; claims 1-24 -----	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/072052

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022189568 A2	15-09-2022	AU 2022234865 A1 CN 116897209 A EP 4305185 A2 WO 2022189568 A2	21-09-2023 17-10-2023 17-01-2024 15-09-2022
CN 114258950 A	01-04-2022	NONE	
WO 2023102101 A1	08-06-2023	CN 116250562 A EP 4440320 A1 WO 2023102101 A1	13-06-2023 09-10-2024 08-06-2023
US 2014308391 A1	16-10-2014	BR PI0819299 A2 CA 2706105 A1 CN 101917873 A EP 2217095 A2 JP 2011503225 A RU 2010125197 A US 2010330040 A1 US 2014308391 A1 US 2016338397 A1 WO 2009065905 A2 WO 2009067000 A1	07-10-2014 28-05-2009 15-12-2010 18-08-2010 27-01-2011 27-12-2011 30-12-2010 16-10-2014 24-11-2016 28-05-2009 28-05-2009