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(54) Title: METHOD FOR PRODUCING PRODUCTS CONTAINING GALACTOOLIGOSACCHARIDES AND USE THEREOF

(57) Abstract: The invention relates to a method for production of galactooligosaccharides-containing milk-based products that as a part of a regular diet has beneficial physiological effects, and hence is capable of enhancing colon health and improving gastrointestinal conditions. The invention further relates to a use of the product as such, having laxative or bifidogenic effect, or as prebiotic ingredient for use in the preparation of functional foodproducts.

METHOD FOR PRODUCING PRODUCTS CONTAINING GALACTOOLIGOSACCHARIDES AND USE THEREOF

FIELD OF THE INVENTION

The present invention relates to a method for producing galactooligosaccharides-containing milk-based products that as a part of a regular diet has beneficial physiological functional effects such as laxative or bifidogenic effect, and hence is capable of enhancing colon health and improving gastrointestinal conditions. More particularly, the present invention relates to a method for producing a milk-based product containing fructo-galactooligosaccharides.

The invention further relates to a use of a milk-based product obtained by the method of the invention, having laxative or bifidogenic effect, as such or as prebiotic ingredient for use in the preparation of functional food products.

BACKGROUND OF THE INVENTION

Prebiotic oligosaccharides are usually defined as glycosides that contain three to ten sugar moieties (sugar residues). However, many disaccharides possess similar functional properties to those of higher sugars, and are often major components of food-grade oligosaccharide products. In general, prebiotic oligosaccharides are hydrolysed or non-hydrolysed carbohydrates comprising sugar residues interconnected via beta 1-1, 1-2, 1-3, 1-4, 1-6 and/or alpha 1-6 linkages. Most preferably, such oligosaccharides contain 2-10 sugar residues in the saccharide backbone.

Conventionally galactooligosaccharides are defined as di- to octasaccharides composed of 1-7 galactose moieties linked to a glucose molecule at the reducing end of glucose. Galactooligosaccharides can be typically expressed by the formula Gal-$(Gal)_n$-Glc wherein Gal denotes a galactose residue, Glc, a glucose residue, and n, an integer of 0 to 7. Even transgalactosylated disaccharides containing galactose and glucose formed from lactose, with different β-glycoside bonds, are considered dietary fibre because they have physiological characteristics similar to those of higher galactooligosaccharides.

Nomenclature of Carbohydrates by International Union of Pure and Applied Chemistry (IUPAC) recommends that oligosaccharides are called disaccharides, trisaccharides etc. according to the number of the monosaccharide units joined to each other by glycosidic linkages. Further, the borderline

between polysaccharides and oligosaccharides cannot be drawn strictly. The term 'oligosaccharide' is commonly used to refer to a defined structure of a sugar molecule, whereas 'polysaccharide' denotes a polymer of unspecified length or a homologous mixture.

The prebiotic bifidogenic effects of galactooligosaccharides or mixtures of galactooligosaccharides and fructo-oligosaccharides on the colonic microbiota are well known, and galactooligosaccharides are therefore considered advantageous to human health. Furthermore, EP 1597978 proposes several advantageous physiological effects for compositions comprising synergistically active amounts of polyfructose and galactooligosaccharides mixtures.

Uses generally reported for galactooligosaccharides are calcium absorption in gut, infant formulae, drinks, bread, and beverages for curing constipation. The main physiologically functional effects of oligosaccharides are considered to be low calorie, prevention of tooth decay, intestinal control by enhancement of bifidobacteria and dietary fibre-like effects. It is generally known that carbohydrates with low sugar residues are fermented easily and at relatively uniform rate. Part of the mixture will not be fermented and passes to subsequent parts of the intestine, where it will contribute to the stool bulk and will not increase substantially the viscosity of the faeces.

Conventionally, food-grade oligosaccharides are manufactured using enzymatic processes (by enzymatic synthesis) from simple sugars by transglucosylation reactions. Galactooligosaccharides, such as Oligomate and TOS-100 (Yakult Honsha, Japan), Cup-Oligo (Nissin Sugar Manufacturing Company, Japan), P7L (Snow Brand Milk Products, Japan), TOS-Syrup (Borculo Whey Products, The Netherlands, Borculo Domo Ingredients/Friesland) and others are produced commercially from lactose using the galactosyltransferase activity of β-galactosidase (lactase) (EC 3.2.1.23). β-galactosidase catalyzes both hydrolysis of lactose and transgalactosylation reactions.

It is commonly known to obtain a sugar mixture containing oligosaccharides of high purity with a small amount of unreacted lactose. At high lactose concentrations, the transgalactosylation reaction predominates, and lactose is converted to galactooligosaccharides by the action of β-galactosidase. Generally, the main products are trisaccharides, namely 4'-galactosyllactose or 6'-galactosyllactose with a substantial amount of transgalactosylated disaccharides.

Commercially available galactooligosaccharides are generally mixtures of lactose, glucose, galactose, constituting usually more than 55% on the dry matter of the oligosaccharides. The components in the final products and the efficiency of transgalactosylation highly depend on the enzymes and specific conditions used in the reaction, such as pH, temperature and quantity of enzyme.

Conventional enzymic production of oligosaccharides has been largely studied and described in the background art, one particular point of interest being a production of oligosaccharides from lactose as described in EP 266177 B1. In this document, oligosaccharides are produced by reacting lactose with β-galactosidase from *Aspergillus oryzae* at a lactose concentration in the reaction mixture of 50 to 90 weight%, and at a reaction temperature within a range from 55°C to a temperature lower than the inactivation temperature of β-galactosidase. According to the publication, a product containing high-purity oligosaccharides is obtained with small amounts of unreacted lactose and by produced monosaccharides.

EP 0263700 B2 discloses a method for producing oligosaccharides to obtain a sweet saccharide mixture providing sweetness and add oligosaccharides to food and drinks with a lower increase in calories than that of conventional additives. In a two-step process disclosed in the publication, a lactose solution or lactose-containing substance having a lactose concentration of 10 to 50 weight% is treated with at least two kinds of β-galactosidases produced by different microorganisms.

EP 0323201 B1 describes a method for producing a processed milk containing galactooligosaccharides. β-galactosidase from *Streptococcus thermophilus* or *Lactobacillus bulgaricus* catalysed conversion of at least 15% of the lactose originally contained in the animal milk into galactooligosaccharides.

EP 0458358 B1 discloses a process for producing skim milk powder containing 10–15% by weight of galactooligosaccharide. The process comprises the steps of concentrating skim milk to obtain concentrated milk with a solid content of 20–50% by weight, adding ß-galactosidase (0.1–200 units/ml, 20–50°C), heating the resulting reaction mixture 30 seconds to 15 minutes to a temperature of 70–85°C and spray-drying. No significant viscosity increase of the reaction mixture is reported.

US Patent 4873229 describes a production of a powder having a galactooligosaccharide content of 20 to 50% by weight. The powder is to be used in a stock feed containing from 0.1 to 2% of galactooligosaccharides.

Moreover, there are publications relating to optimisation of a lactulose production, with a special aim to increase the proportion of lactulose in respect to other oligosaccharides and sugars produced in the lactulose production. Lactulose is widely used in the food and pharmaceutical fields, having a bifidogenic effect and prebiotic character. Lactulose is a disaccharide which can be obtained by enzymatic transgalactosylation from lactose to fructose by chemical synthesis. Furthermore, oligosaccharides such as lactulose are produced in small amounts during heat treatment (UHT) of milk. An optimal ratio of lactose to fructose can increase relative lactulose yield. Further, also other oligosaccharides than lactulose are formed in conventional lactulose production processes. However, due to the low yield of lactulose and extensive side reactions in the production of lactulose, the separation and purification of lactulose is complex. A production of lactulose has been described, e.g., by Vaheri and Kauppinen in Acta Pharm. Fenn. 1978, 87: 75-83, where lactulose has been enzymically produced from lactose using fructose as an acceptor. A maximum lactulose concentration of 25 mmol/L (8% of initial lactose concentration) is reported.

Further, Lee et al. in Appl. Microbiol. Biotechnol. 2004, 64: 787-793 investigated a lactulose production from lactose and fructose with several commercial β-galactosidases, with the highest productivity obtained with an enzyme from permeabilized *Kluyveromyces lactis* cells. With 40% (w/v) of lactose and 20% (w/v) of fructose, approximately 20 g/L of lactulose was produced in 3 hours at 60°C and pH 7.0. It was shown that high lactose concentrations and high temperatures are preferable for lactulose synthesis. The highest lactulose concentration of 42 mmol/L (4% of an initial lactose concentration) was obtained.

Mayer et al. reported in J. Agric. Food Chem. 2004, 52:6983-6990 that a lactulose yield of 46 mmol/L (44% relative to lactose) was obtained with the hyperthermostable β-glucosidase from *Pyrococcus furiosus.* Lactulose was shown to be the major transglycosylation disaccharide. Less than 5% of other oligosaccharides were detected.

Major problems associated with the use of the conventional galactooligosaccharides are abdominal adverse effects such as flatulence, abdomi-

nal pain and bloating. Earlier findings with galactooligosaccharides indicate that functional high value-added products and applications with fructo-galactooligosaccharides would be very welcome as a part of a regular/common diet. Such functional, prebiotic-containing products are nowadays providing an attractive alternative to the consumers. Furthermore, efficient producing methods to assure products without added sugar are valuable.

Thus, there is a special need for a novel, economic, inexpensive and efficient total process for direct natural production of food products and ingredients containing prebiotic galactooligosaccharides comprising fructo-galactooligosaccharides with therapeutic value.

BRIEF DESCRIPTION OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method for directly producing a mixture of galactooligosaccharides containing fructo-galactooligosaccharides into a milk-based product that as a part of a regular diet has beneficial physiological functional effects such as laxative or bifidogenic effect, and hence is capable of enhancing colon health and improving gastrointestinal conditions.

It is a further object of the present invention to provide a method for an economic, inexpensive and efficient production of galactooligosaccharides containing fructo-galactooligosaccharides, in a processed milk product such as yoghurt, curdled milk, curd, sour milk, "viili", buttermilk and other sour milk products. According to the invention other edible products such as milk, flavoured milk, beverages, ice-cream etc. are available. In accordance with the present invention, products are also applicable as capsules, pills or tablets that allow the use as convenient part or supplement, for example, of the every-day diet.

It is a further object of the present invention to provide a milk-based product as such, having laxative or bifidogenic effect, or as prebiotic ingredient for use in the preparation of functional food products.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method for producing a milk-based product containing a mixture of galactooligosaccharides which are represented by the general formula of $(Gal)_n$-x, where x is a fructose, glucose or galactose residue, and n is 1-8, comprising the following steps:

a) adding fructose and optionally lactose to a milk-based raw material to obtain a mixture,

b) treating the mixture with a β-galactosidase to obtain a reaction mixture,

c) terminating an enzymatic reaction of the reaction mixture.

In the present invention, "milk-based product" means any liquid or semi-solid milk-based product having a varing fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk, and whey without any processing, or a processed milk, yoghurt, curdled milk, curd, sour milk, "viili", buttermilk and other sour milk products.

In the context of the present invention, the term "galactooligosaccharides" will be used hereinafter to mean a mixture of galactooligosaccharides (abbreviation GOS) represented by the general formula of $(Gal)_n$-x, where x is a fructose, glucose or galactose residue, and n is 1-8, unless otherwise specified. It is to be understood that fructo-galactooligosaccharides are represented by said formula, when x is fructose.

The concept "food products" is intended to cover all consumable products that can be solid, jellied or liquid form, and to cover both ready-made products and products to which the galactooligosaccharide-containing milk-based product (concentrate or powder) of the invention is added during consumption as an additive or a prebiotic ingredient or part of the product. Food products can for instance be products of dairy industry and beverage industry. Typical products include milk products, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk, other sour milk products, filling of snack bars, etc. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; ice-cream; milk-containing food such as sweets, and other types of products such as animal feed.

The method of the present invention is based on a finding that β-galactosidases are capable of converting lactose to oligosaccharides. Without binding to any theory, reactions caused in the β-galactosidase treatment in the method of the invention are hydrolytic reactions of lactose into glucose and galactose, and β-galactosyl transfer reaction to various galactooligosaccharides in the presence of fructose and lactose. The components in the final products highly vary depending on the factors such as quality and quantity of β-

galactosidase enzymes, and specific conditions used in the process, such as pH, temperature. It is desirable that the reaction is performed under a condition which provides a highest yield of fructo-galactooligosaccharides in the galactooligosaccharide mixture. However, the optimum conditions for an economic, inexpensive and efficient production process of fructo-galactooligosaccharides vary depending on the factors such as the quality of β-galactosidase and composition of the raw milk. In particular, lactose content of the raw milk and the ratio of lactose to fructose are important factors for an effective total process for producing a mixture of galactooligosaccharides containing an essential amount of fructo-galactooligosaccharides.

It is well known that β-galactosidases can be produced by a variety of molds, bacteria or yeasts. In the method of the present invention, any β-galactosidase known in the art can be used. Examples of suitable sources of microorganisms producing β-galactosidase are disclosed, for example, in EP 0263700 A2. Especially, commercial β-galactosidase preparations originating from bacteria (*Escherichia coli, Bacillus* sp), yeast (*Candida pseudotropicalis, Kluyveromyces lactis, Kluyveromyces marxianus*) or mold (*Aspergillus niger, A. oryzae*) can be used. Preferably, β-galactosidase produced by yeast *Kluyveromyces lactis* is used for the production of galactooligosaccharides in fermented milk products, such as yoghurt.

An amount of the β-galactosidase is not specifically limited. Amounts in a range of 12000–30000 ONPG-units/L of β-galactosidase produced by *Kluyveromyces lactis* (GODO-YNL2, Godo Shusei Company Limited, Japan), for example, are suitable in the present invention.

The method of the present invention can be executed on milk having an ordinary content of lactose, i.e. approximately 4.8% (w/v). However, it is also possible to add lactose to a raw milk to be treated with β-galactosidase from an outer source. Added lactose to be used in the method can be edible-grade lactose such as lactose-containing whey powder, permeate, etc.

Needless to say, it is desirable that the reaction with a β-galactosidase is executed under such a condition as to provide a high yield of galactooligo-saccharides in a milk-based product. Preferably, it is desired that a mixture of galactooligosaccharides is obtained comprising a substantial amount of fructo-galactooligosaccharides. In the present invention, initial weight ratio of lactose to fructose is about 0.1–5. Preferably, for the production of yoghurt containing galactooligosaccharides, the initial weight ratio of lactose to fructose

is about 1.2–1.5, and more preferably 1.30–1.38 (example 1). The respective ratio for the production of prebiotic ingredient, such as sweetened condensed milk (example 4) or sweet skimmed milk powder (example 5) containing galactooligosaccharides, is about 0.95–1.5, and more preferably 1.25–1.4.

As is usual in the preparation of conventional milk-based products, conventional heat treatment methods are also used for the milk-based products produced in the present invention, such as pasteurization (for example 72°C, 15 s), ESL (for example 130°C, 1–2 s), UHT (for example 138°C, 2–4 s) or high temperature pasteurization (95°C, 5 min). An example of heat treatment to be used in the method according to the present invention is high temperature pasteurization at 94°C for 4 min with production of yoghurt containing galactooligosaccharides (example 1).

The method of the invention is preferably performed in fermented milk products, like yoghurts. The process/fermentation conditions (starters, temperature, pH, time etc.) for the production of fermented milk products or prebiotic ingredients are selected to meet the requirements of the final product and a starter used in the method so as to form a sufficient amount of galactooligosaccharides to produce the desired effect. The selection of suitable conditions belongs to the knowledge of a person skilled in the art. Conventionally, fermentation for yoghurt production is carried out at about 20°C to 45°C. The fermentation is allowed to continue until the pH is 4.2 to 4.6. In case of fermented milk products, fermentation normally takes from 2 to 7 hours with yoghurts, up to 24 hours with sour cream.

As stated above, it is desirable that the reaction with a β-galactosidase is executed under such a condition as to provide a high yield of galactooligosaccharides in a milk-based product. For this purpose, it is necessary that an enzymatic activity of β-galactosidase used is completely lost and thus, an enzymatic reaction completely terminated after an appropriate reaction time in order to not to cause an adverse reaction of galactooligosaccharides into monosaccharides. The enzymatic reaction of the reaction mixture can be terminated by heating the mixture, for example, to a temperature of 85°C for 60 seconds. On the other hand, the enzymatic reaction can also be terminated by an adjustement of pH to a range of inactivation pH of β-galactosidase. Inactivation of an enzyme can be accomplished by way of an active action, or by self-inactivation. Specifically, in the preparation of fermented milk products, β-galactosidase reacts during the fermentation as long as the pH of mixture is

within the favourable activity and stability range of the enzyme used. For example, β-galactosidase produced by yeast *Kluyveromyces lactis* (GODO-YNL2, Godo Shusei Company Ltd, Japan) reacts during the yoghurt fermentation as long as the pH of the mixture is above inactivation pH 5.5 (example 1).

The yoghurt product prepared as described in example 1 of this publication and containing galactooligosaccharides has been found to be extremely suitable for a purpose of the invention. As demonstrated in example 3, in a clinical trial (N = 41) with elderly subjects suffering from moderate constipation, defecation was significantly easier during a period of an intake of galactooligosaccharides-containing yoghurt (10 g GOS/day) compared to the placebo yoghurt containing no galactooligosaccharides ($p = 0.025$) (p indicates a statistical value). The bowel frequency also slightly increased ($p = 0.084$), while the transit time seemed to become shorter with the galactooligosaccharides-containing yoghurt (3.1 days vs. 3.4 days; $p = 0.39$). Results are in accordance with earlier clinical studies with conventional galactooligosaccharides; see for example Teuri et al., J. Nutr Sci Vitaminol 1998: 44, 465-471; Teuri and Korpela, Ann Nutr Metab 1998, 42: 319-327; Sairanen et al. Eur. J. Nutr, 5 October 2005; Deguchi et al., Jpn J. Nutr. 1997, 55:13-22; Shitara Med Biol. 1988, 177: 371-373. However, major problems associated with use of the conventional galactooligosaccharides were now surprisingly avoided, with hardly any abdominal adverse effects such as flatulence, abdominal pain and bloating.

Thus, the present invention is also directed to a use of the milk-based products produced by the method of the present invention, having laxative or bifidogenic effects, as such or as a prebiotic ingredient in the preparation of functional food products.

As stated above, the milk-based products described above can be used as such to achieve the desired effect. Said products can also be concentrated and use as prebiotic ingredients. Further, the products can also be dried and use in the form of powder or lyophilisate. The products can also be preferably used in the preparation of functional food products, health and wellness edible products, or other corresponding products. Possible forms are capsules, pills or tablets, for example, manufactured in conventional processes used in the preparation of pharmaceutical products.

According to the invention, the milk-based product containing galactooligosaccharides is used in a sufficient amount to achieve the desired effect.

When using the product obtained by method described in example 1, the amount to use varies within a wide range. It may be from 2 to 20 g in daily dose, being preferably approximately 5 to 12 g per day.

Prebiotic ingredients, concentrate, or powder of the milk-based products of the invention containing galactooligosaccharides can be added to a food product during its preparation or to a finished food product. The food products in question thus contain the desired galactooligosaccharides. Milk-based products of the invention fully correspond in taste and behaviour (other than laxative or bifidogenic effect) with the corresponding conventional products.

Milk-based products of the invention are primarily suitable for use for human adults and infants. The positive effects of the products are also beneficial to animals, especially pets and production animals. Examples of these include dogs, cats, rabbits, horses, cows, pigs, goats, sheep and poultry.

The invention will be described in more detail by means of the following examples.

Example 1. Preparation of yoghurt

8,8 l of standardized milk having a solid content of 9.0%, a lactose content of 4.8%, and a fat content of 0.5% was heated to 50°C. 540 g of lactose, 706 g of fructose and 45 g of thickener was dissolved in the milk. The mixture was pasteurized at 94°C for 4 minutes and cooled to 41°C. 159 000 ONPG-units of β-galactosidase produced by *Kluyveromyces lactis* (GODO-YNL2, Godo Shusei Company Limited, Japan) and starter culture were added to the mixture. The mixture was fermented under conventional fermentation conditions to a pH of 4.2–4.6 and thereafter cooled to refrigeration temperatures (1°C–10°C). The enzyme reacts during the fermentation as long as the pH of the mixture is above 5.5. After fermentation, typical sugar composition of the yogurt base is: Fructose 5.3%, galactooligosaccharides 3.3%, glucose 3.3%, galactose 2.0% and lactose 1.0%. This sweet yogurt base can be flavoured with aromas or jams as conventional yoghurts. There is no need for added sugar. The yogurt can be used as such to relief constipation and as prebiotic fermented milk product.

Reference Example 2. Preparation of yoghurt

8,8 l of standardized milk having a solid content of 9.0%, a lactose content of 4.8%, and a fat content of 0.5% was pasteurized at 94°C for 4 min-

utes and cooled to 41°C. 159 000 ONPG-units of β-galactosidase produced by *Kluyveromyces lactis* (GODO-YNL2, Godo Shusei Company Limited, Japan) and starter culture were added to the mixture. The mixture was fermented under standard fermentation conditions to a pH of 4.2–4.6 and thereafter cooled to refrigeration temperatures (1°C–10°C). After fermentation, typical sugar composition of the non-flavoured yogurt base is: glucose 2.2%, galactose 1.7% (total sugars 5.0%), galactooligosaccharides less than 0.3% and lactose less than 1%. No fructo-galactooligosaccharides are formed.

This yogurt base can be flavoured with aromas or jams.

Example 3. The effect of yoghurt containing galactooligosaccharides on the colon health and gastrointestinal conditions

The aim was to investigate whether a yoghurt prepared as described in Example 1 and containing galactooligosaccharides relieves constipation. Forty-one elderly subjects (mean age 68 years; range 60–79 years) with self-reported constipation completed this randomised, double-blind cross-over intervention consisting of two three-week intervention periods. The statistical analyses have been carried out only for the first period due to a carry-over effect. During the intervention, the participants daily ingested either two galactooligosaccharides-containing yoghurts (GOS) (10 g GOS/day) as described in Example 1, or two placebo yoghurts without galactooligosaccharides but otherwise similar to the galactooligosaccharides-containing yoghurts.

Bowel habits were followed by a symptom diary, and the intestinal transit time was measured using Sitzmark® capsules.

Defecation was significantly easier during the galactooligosaccharides-containing yoghurt period compared to the placebo yoghurt period ($p = 0.025$). There was also a tendency towards increased bowel frequency ($p = 0.084$), while the transit time seemed to become shorter with the galactooligosaccharides-containing yoghurt (3.1 days vs. 3.4 days; $p = 0.39$). There was no difference between the galactooligosaccharide yoghurt group and the placebo yoghurt group regarding abdominal adverse effects (flatulence, abdominal pain, bloating).

Table 1. Defecation frequency (bowel movements per five days), difficulty in defecation (from 0 = easy to 3 = difficult; possible range 0-15) and intestinal symptoms (from 0 = easy to 3 = difficult; possible range for each symptom 0-15).). Number of study subjects was 23 in the GOS yoghurt group and 18 in the placebo yoghurt group.

	Baseline		End of intervention		p-value
	Placebo	GOS	Placebo	GOS	
	Mean ± SD	Mean ± SD	Mean (95% CI)	Mean (95% CI)	
Number of bowel movements	4.2 ± 1.7	4.6 ± 3.3	-0.2 (-1.2 to 0.4)	0.5 (-0.4 to 1.4)	0.084
Difficulty of defecation	8.8 ± 3.0	11.0 ± 3.0	-0.1 (-2.1 to 1.7)	-3.5 (-4.7 to -2.0)	0.025
Flatulence	6.2 ± 3.5	5.9 ± 3.7	-0.2 (-2.2 to 1.6)	0.9 (-0.7 to 2.1)	0.41
Abdominal pain	2.6 ± 3.8	2.3 ± 3.4	-0.6 (-2.8 to 0.2)	0.1 (-0.9 to 1.5)	0.46
Bloating	4.0 ± 4.3	4.2 ± 4.4	-1.2 (-3.3 to -0.2)	-0.4 (-1.4 to 0.8)	0.34

SD = standard deviation, 95% CI = 95% confidential interval

The results set forth in Table 1 show that the yoghurt prepared by a method of the present invention has a beneficial influence on gastrointestinal conditions.

The results are discussed in more detail on pages 8 and 9 above.

Example 4. Preparation of sweetened condensed milk

28.3 kg of fructose and 18.9 kg of lactose was added to 412.8 l of skimmed milk containing 0.1% of fat, 4.8% of lactose, and 8.8% of milk solids. The mixture was pasteurized (72°C, 15 s) and evaporated to a dry matter content of 60%, and thereafter cooled to 40°C. 15 350 kONPG-units of β-galactosidase produced by *Kluyveromyces lactis* (GODO-YNL2, Godo Shusei Company Limited) was added to the mixture. The enzyme was allowed to react for 6 hours, and the reaction mixture was then heated to 80°C for 1 minute so that the enzyme was inactivated. The sugar components of the mixture was analysed whereby the content of galactooligosaccharides was 11.0%, lactose 6.6%, glucose 4.5%, galactose 4.0% and fructose 16.9%. The mixture thus obtained can be used as such as sweetened condensed milk containing galactooligosaccharides, as a base for yoghurt manufactured with conventional production methods, or as a prebiotic ingredient for food products such as fermented milk drink, whey beverage or milk beverage.

Example 5. Preparation of a sweet skimmed milk powder

Sweetened condensed milk containing galactooligosaccharides was prepared as described in Example 4, and dried with conventional methods to a powder. The powder can be used as such or as a base for yoghurt manufactured with conventional production methods or as a prebiotic ingredient for food products such as fermented milk drink, whey beverage or milk beverage. The product containing the daily requirement of active ingredient is to be used as such.

CLAIMS

1. A method for producing a milk-based product containing a mixture of galactooligosaccharides which are represented by the general formula of $(Gal)_n$ -x, where x is a fructose, glucose or galactose residue, and n is 1-8, comprising the following steps:

a) adding fructose and optionally lactose to a milk-based raw material to obtain a mixture,

b) treating the mixture with a β-galactosidase to obtain a reaction mixture,

c) terminating an enzymatic reaction of the reaction mixture.

2. A method according to claim 1 wherein a weight ratio of lactose to fructose in the mixture ranges from about 0.1 to 5, preferably from about 1.2 to 1.5, more preferably 1.30 to 1.38.

3. A method according to claim 1 wherein a weight ratio of lactose to fructose in the mixture ranges from about 0.95 to 1.5, more preferably 1.25 to 1.4.

4. A method according to any of claims 1 to 3 wherein β-galactosidase is from strain *Kluyveromyces lactis.*

5. A method according to any of claims 1 to 4 wherein a milk-based product consisting essentially of fructo-galactooligosaccharides is produced.

6. A method according to any of claims 1 to 5 wherein the milk-based raw material is skimmed milk.

7. A method according to any of claims 1 to 6 wherein the milk-based product is a fermented milk product.

8. A method according to claim 7 wherein the enzymatic reaction is terminated as an inactivation pH of the β-galactosidase is reached in the reaction mixture.

9. A method according to any of claims 1 to 6 wherein the milk-based product is a condensed milk.

10. A method according to claim 9 wherein the β-galactosidase is allowed to react for 6 hours.

11. A method according to claim 10 wherein the enzymatic reaction is terminated by heating the reaction mixture to a temperature of 85°C for 1 minute.

12. A method according to any of claims 1 to 11 wherein the milk-based product is concentrated to a concentrate or lyophilisate or dried to a powder.

13. A method according to claim 12 wherein the milk-based product is skimmed milk powder.

14. Use of a milk-based product produced by a method according to any of claims 1 to 13 as such or as a prebiotic ingredient in the preparation of functional food products.

15. Use of claim 14 wherein the functional food product is fermented milk product, fermented milk drink, whey beverage or milk beverage.

16. Use of claim 14 wherein the product is for animal use.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2007/050449

A. CLASSIFICATION OF SUBJECT MATTER

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)

IPC8: C12P, A23C

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

FI, SE, NO, DK

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

EPO-INTERNAL, WPI, BIOSIS, EMBASE, MEDLINE, AGRICOLA, CABA, CAPLUS, FROSTI, FSTA, NUTRACEUT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0458358 A1 (SNOW BRAND MILK PROD CO LTD) 27 November 1991 (27.11.1991) whole document cited in the application	1-6, 8-16
Y	EP 0323201 A2 (YAKULT HONSHA KK) 05 July 1989 (05.07.1989) whole document cited in the application	1-10, 14-16
Y	KIM, Y.-S. et al., "Lactulose production from lactose and fructose by a thermostable beta-galactosidase from Sulfolobus solfataricus", Enzyme and Microbial Technology, 2 August 2006, Vol. 39, No. 4, pages 903-908 whole document	1-16

☒ 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
20 November 2007 (20.11.2007)	22 November 2007 (22.11.2007)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2007/050449

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE, Y.-J. et al., "Lactulose production by beta-galactosidase in permeabilized cells of Kluyveromyces lactis", Applied Microbiology and Biotechnology, 2004, Vol. 64, pages 787-793 whole document cited in the application	1-16
A	HARTIKAINEN, M. et al., "Laktoosiin perustuvat oligosakkaridit", Kemia-Kemi, 1988, Vol. 15, No. 3, pages 218-222, abstract	1-16

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Information on patent family members

International application No.
PCT/FI2007/050449

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A23C 17/02 (2006.01)
A23C 19/032 (2006.01)
A23C 21/02 (2006.01)
A23G 9/34 (2006.01)
A23G 9/36 (2006.01)