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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2005 (08.12.2005)

PCT

(10) International Publication Number
WO 2005/115171 A1

(51) International Patent Classification[7]: A23L 1/30, 1/307, 1/308, 1/054, 1/09, 1/236, A23K 1/165, A61K 31/733, 31/7016, 31/702, 31/715, 31/716

(21) International Application Number: PCT/EP2005/005317

(22) International Filing Date: 14 May 2005 (14.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
04012289.7 25 May 2004 (25.05.2004) EP

(71) Applicant *(for all designated States except US)*: **COGNIS IP MANAGEMENT GMBH** [DE/DE]; Henkelstr. 67, 40589 Düsseldorf (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RULL PROUS, Santiago** [ES/ES]; Francesc Carbonell n° 32-4a-2°, E-08034 Barcelona (ES). **FABRY, Bernd** [DE/DE]; Bruchstrasse 13, 41352 Korschenbroich (DE). **BELL, Doris** [DE/DE]; Am Ellerforst 43, 40625 Düsseldorf (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** ORAL AND/OR TOPICAL COMPOSITIONS COMPRISING PREBIOTICS AND STEROLS

(57) **Abstract:** Suggested are new advantageous oral and/or topical compositions, comprising (a) prebiotics and (b) sterols or their esters.

WO 2005/115171 A1

Oral and/or topical compositions

Field of the invention

The present invention is related to the area of alimentation and concerns oral and/or topical compositions comprising sterols or their esters and prebiotics, dietary supplements and food compositions comprising sterols or their esters and prebiotics, and the use of mixtures comprising sterols or their esters and prebiotics for improving the stimulation of the growth of healthy bacteria.

Background of the invention

Probiotics contain live bacteria and represent an important part of the complex world of foods that are good for health. Its the bacteria and the metabolites which they produce that give these products their health promoting properties. The best known example of a probiotic is yoghurt. The experimental data for yoghurt is still not as conclusive as one would like, however, human studies related to the consumption of dietary milk products show increased milk digestibility, quicker recovery from certain types of diarrhoea, enhanced immune function, relation in certain cancers, and possible lowering of blood cholesterol levels.

Bacteria found in products like yoghurt, kefir or fermented vegetables usually aren't found in the human intestine. In fact, the intestinal environment is often a hostile one for these foreign bacteria. Because of this, bacteria eaten in probiotic products don't colonise the intestine but are flushed through and eliminated from the body.

The bacteria living in the intestine make up a very large and very diverse population. The numbers of each kind of bacteria change depending on age, diet, health status, and use of drugs and supplements. The effects are linked to the ability of the bacteria to adhere to the intestinal wall and use the semi-digested food that it passing through the intestines. It is not surprising to found that the bacterial population in the intestines of vegetarians is much different compared to that of meat eaters. Because some bacteria have specific nutrient require-

ments it has been proposed that adding these particular foods or nutrients to the diet could be a way of increasing the numbers of specific bacteria. Such additives are called "prebiotics". Thus, to be effective, prebiotics must escape digestion in the upper gastrointestinal tract and be used by a limited number of the micro-organisms comprising the colonic microflora. In the large intestine prebiotics are converted into short-chain fatty acids like capronic or caprylic acid. Said acids are used by the human body as an energy source. Beside this, the short-chain acids are known to inhibit inflammatories of the intestine, which represents a kind of cancer prophylaxis. In addition, prebiotics increase the resorption time in the intestine which leads to an improve uptake of minerals. Typical examples for well-known prebiotics are oligosaccharides, e.g. in 1995 Gibson et al found that oligofructose and inulin, when fed to humans, selectively stimulated the growth of bifidobacteria without influencing the numbers of lactobacillus. Since prebiotics mainly stimulate the growth of bifidobacteria, for which reason the also are referred to as bifidogenetic factors.

Although various types of prebiotics are known from the literature and can be found in the market there is still an increasing need for more active alternatives or additives which support the various activities of existing products in synergistic manner. Therefore, the object of the present invention has been to provide a new system of prebiotic compounds which shows a synergistic stimulation of the growth of healthy bacteria, preferably bifido and lactic bacteria both and improves the health status of the human body.

Detailed description of the invention

The present invention refers to oral and/or topical compositions, comprising

(a) prebiotics and
(b) sterols or their esters.

Surprisingly it has been observed that mixtures of said sterols or sterol esters and prebiotics show a synergistic behaviour with respect to stimulation of growth of bacteria selected from the group consisting of *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum* and *Bifidobacterium adolescentis* on one hand, and *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Streptococcus faecium, and Streptococcus thermophilus* on the other.

Prebiotics

Prebiotics are defined as non-digestible food ingredients that may beneficially affect the host be selectively stimulating the growth and/or the activity of a limited number of bacteria in the colon. The following describes the various oligosaccharides which can be taken into account as suitable prebiotics (component a) :

- Fructooligosaccharides

Fructooligosaccharides or FOS typically refer to short-chain oligosaccharides comprised of D-fructose and D-glucose, containing from three to five monosaccharide units. FOS, also called neosugar and short-chain FOS, are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme. FOS are resistant to digestion in the upper gastrointestinal tract. They act to stimulate the growth of *Bifidobacterium* species in the large intestine. FOS are marketed in the United States in combination with probiotic bacteria and in some functional food products.

- Inulins

Inulins refer to a group of naturally-occurring fructose-containing oligosaccharides. Inulins belong to a class of carbohydrates known as fructans. They are derived from the roots of chicory *(Cichorium intybus)* and Jerusalem artichokes. Inulins are mainly comprised of fructose units and typically have a terminal glucose. The bond between fructose units in inulins is a beta-(2-1) glycosidic linkage. The average degree of polymerisation of inulins marketed as nutritional supplements is 10 to 12. Inulins stimulate the growth of *Bifidobacterium* species in the large intestine.

- Isomaltooligosaccharides

Isomaltooligosaccharides comprise a mixture of alpha-D-linked glucose oligomers, including isomaltose, panose, isomaltotetraose, isomaltopentaose, nigerose, kojibiose, isopanose and higher branched oligosaccharides. Isomaltooligosaccharides are produced by various enzymatic processes. They act to stimulate the growth of *Bifidobacterium* species and *Lactobacillus* species in the large intestine. Isomalto oligosaccharides are marketed in Japan as dietary supplements and in functional foods. They are being developed in the United States for similar uses.

- Lactilol

Lactilol is a disaccharide analogue of lactulose. Its pharmaceutical use is in the treatment of constipation and hepatic encephalopathy. Lactilol is also used in Japan as a prebiotic. It is resistant to digestion in the upper gastrointestinal tract and it is fermented by a limited number of colonic bacteria, resulting in an increase in the biomass of bifidobacteria and lactobacilli in the colon. Lactilol is known chemically as 4-0-(beta-D-galactopyranosyl)-D-glucitol. Lactilol is not approved for the treatment of hepatic encephalopathy or constipation in the U.S., and its use as a prebiotic is considered experimental. Lactilol is used in Europe as a food sweetener.

- Lactosucrose

Lactosucrose is a trisaccharide comprised of D-galactose, D-glucose and D-fructose. Lactosucrose is produced enzymatically by the enzymatic transfer of the galactosyl residue in lactose to sucrose. Lactosucrose is resistant to digestion in the stomach and small intestine. It is selectively utilized by intestinal *Bifidobacterium* species resulting in significant induction of growth of these bacteria in the colon. Therefore, under physiological conditions, lactosucrose acts on the intestinal microflora as a growth factor for *Bifidobacterium* species. Lactosucrose is also known as 4G-beta-D-galactosylsucrose. It is widely used in Japan as a dietary supplement and in functional foods, including yoghurt. Lactosucrose is being developed in the United States for similar uses.

- Lactulose

Lactulose is a semi-synthetic disaccharide comprised of the sugars D-lactose and D-fructose. The sugars are joined by a beta-glycosidic linkage, making it resistant to hydrolysis by human digestive enzymes. Lactulose is, however, fermented by a limited number of colonic bacteria. This can lead to changes in the colonic ecosystem in favour of bacteria, such as lactobacilli and bifidobacteria, which may confer some health benefits. Lactulose is a prescription drug in the United States for the treatment of constipation and hepatic encephalopathy. It is marketed in Japan for use as a dietary supplement and in functional foods. Its use in the United States as a prebiotic substance is still experimental.

- Pyrodextrins

Pyrodextrins comprise a mixture of glucose-containing oligosaccharides that is derived from the hydrolysis of starch. Pyrodextrins have been found to promote the proliferation of *Bifidobacterium* species in the large intestine. They are resistant to digestion in the upper gastrointestinal tract. Pyrodextrins are being developed for the nutritional supplement market place.

- Soy oligosaccharides

Soy oligosaccharides refer to oligosaccharides found in soybeans and also in other beans and peas. The two principal soy oligosaccharides are the trisaccharide raffinose and the tetrasaccharide stachyose. Raffinose comprises one molecule each of D-galactose, D-glucose and D-fructose. Stachyose consists of two molecules of D-galactose, one molecule of D-glucose and one molecule of D-fructose. Soy oligosaccharides act to stimulate the growth of *Bifidobacterium* species in the large intestine. They are marketed in Japan as dietary supplements and in functional foods. They are being developed in the United States for similar uses.

- Transgalactooligosaccharides

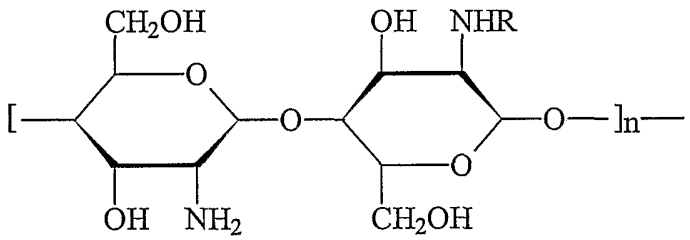
Transgalactooligosaccharides (TOS) are a mixture of oligosaccharides consisting of D-glucose and D-galactose. TOS are produced from D-lactose via the action of the enzyme beta-galactosidase obtained from *Aspergillus oryzae*. TOS are resistant to digestion in the upper gastrointestinal tract and stimulate the growth of bifidobacteria in the large intestine. TOS are marketed in Japan and Europe as dietary supplements and are used in functional foods. They are being developed for similar use in the United States.

- Xylooligosaccharides

Xylooligosaccharides are comprised of oligosaccharides containing beta (1→ 4) linked xylose residues. The degree of polymerisation of xylooligosaccharides is from two to four. Xylo oligosaccharides are obtained by enzymatic hydrolysis of the polysaccharide xylan. They are marketed in Japan as prebiotics and are being developed for similar use in the United States.

- Biopolymers

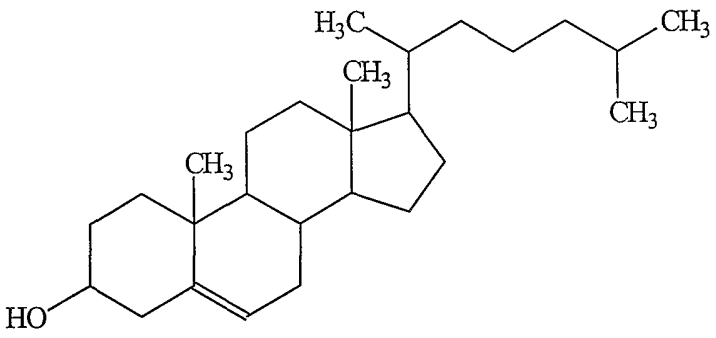
Suitable biopolymers like e.g. beta-glucans include those originating from plants including cereals such as oats and barley, fungi, yeast, and bacteria. In addition, microbial cell wall preparations and whole cells rich in beta glucans are also suitable sources for beta glucan preparations useful for the present invention. Monomer residues in glucans can have 1-3 and 1-4, or 1-3 and 1-6 linkages (that is the monomer units are joined through 1,3, 1,4 or 1,6 bonds) and the percent of each type can vary. Preferably, beta glucans derived from yeast, particularly from *Saccharomyces*, preferably *Saccharomyces cerevisiae*, are used for the present invention. It will be appreciated, however, that other beta glucans would also be suitable. Further examples for suitable biopolymers are chitin and its derivatives, preferably oligoglucosamin and chitosan which represents a typical hydrocolloid.



Chitosan is obtained by deacetylisation of chitin and shows molecular weights in the range of 50.000 up to 2.000.000.

Sterols and sterol esters

Sterols – also called sterins - represent steroids showing a single hydroxyl group linked to the C-3. In addition sterols, which consist of 27 to 30 carbon atoms, may show a double bond, preferably in 5/6 position. The hydrogenation of the double bond ("hardening") leads to sterols which are usually called stanols. The figure below shows the structure of the best known member of the sterol family, cholesterol, which belongs to the group of zoosterols.



Due to their superior physiological activity, the plant sterols, so-called phytosterols, like ergosterol, stigmasterol, and especially sitosterol and its hydrogenation product sitastanol, are the preferred species. In addition instead of the sterols or stanols their esters with saturated or unsaturated fatty acids having 6 to 26 carbon atoms and up to 6 double bonds can be used. Typical examples are the esters of β-sitosterol or β-sitostanol with capric acid, caprylic acid, 2-ethylhexanoic acid, caprinic acid, lauric acid, isotridecylic acid, myristic acid, palmitic acid, palmoleic acid, stearic acid, isostearic acid, oleic acid, elaidinic acid, petroselinic acid, linolic acid, linoleic acid, elaeostearic acid, arachidonic acid, gadoleinic acid, behenic acid and erucic acid.

Oral and/or topical compositions

The oral and/or topical compositions according to the present invention may comprise the prebiotics and the sterols or their esters in a weight ratio of 99 to 1 to 50 : 50 and more particularly 95 : 10 to 75 : 25. The highest synergistic effects, however, are observed at ratios of 92 : 8 to 80 : 20. In general, the compositions can be used in a concentration of up to about 10, particularly 0.5 to 8 and more particularly 1 to 2 % b.w. – calculated on the probiotic microorganisms being present in the final food composition. One percent, however, has been found to be particularly suitable.

In a special embodiment of the present invention said compositions are macro- or microencapsulated. "Microcapsules" are understood to be spherical aggregates with a diameter of about 0.1 to about 5 mm which contain at least one solid or liquid core surrounded by at least one continuous membrane. More precisely, they are finely dispersed liquid or solid phases coated with film-forming polymers, in the production of which the polymers are deposited onto the material to be encapsulated after emulsification and coacervation or interfacial polymerization. In another process, liquid active principles are absorbed in a matrix ("micro-

sponge") and, as microparticles, may be additionally coated with film-forming polymers. The microscopically small capsules, also known as nanocapsules, can be dried in the same way as powders. Besides single-core microcapsules, there are also multiple-core aggregates, also known as microspheres, which contain two or more cores distributed in the continuous membrane material. In addition, single-core or multiple-core microcapsules may be surrounded by an additional second, third etc. membrane. The membrane may consist of natural, semisynthetic or synthetic materials. Natural membrane materials are, for example, gum arabic, agar agar, agarose, maltodextrins, alginic acid and salts thereof, for example sodium or calcium alginate, fats and fatty acids, cetyl alcohol, collagen, chitosan, lecithins, gelatin, albumin, shellac, polysaccharides, such as starch or dextran, polypeptides, protein hydrolyzates, sucrose and waxes. Semisynthetic membrane materials are inter alia chemically modified celluloses, more particularly cellulose esters and ethers, for example cellulose acetate, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose and carboxymethyl cellulose, and starch derivatives, more particularly starch ethers and esters. Synthetic membrane materials are, for example, polymers, such as polyacrylates, polyamides, polyvinyl alcohol or polyvinyl pyrrolidone. Examples of known microcapsules are the following commercial products (the membrane material is shown in brackets) *Hallcrest Microcapsules* (gelatin, gum arabic), *Coletica Thalaspheres* (maritime collagen), *Lipotec Millicapseln* (alginic acid, agar agar), *Induchem Unispheres* (lactose, microcrystalline cellulose, hydroxypropylmethyl cellulose), *Unicerin C30* (lactose, microcrystalline cellulose, hydroxypropylmethyl cellulose), *Kobo Glycospheres* (modified starch, fatty acid esters, phospholipids), *Softspheres* (modified agar agar), *Kuhs Probiol Nanospheres* (phospholipids) and *Primaspheres* or *Primasponges* (chitosan, anionic polymers). The encapsulation of the compositions according to the present invention is preferred in case the active should be liberated at the same part of the intestine. Therefore, one skilled in the art can easily select the adequate encapsulation system by comparing the stability of the capsules under the pH-conditions of the respective part of the intestine.

Food compositions

A further object of the present invention relates to food compositions, comprising

(a) prebiotics and
(b) sterols or their esters.

The compositions may further comprise certain plant extracts, like extracts of *Camellia sinensis* (Green tea) or *Olea europensis* (Olive tree) which are rich in actives like polyphenols, oleuropein and hydroxtyrosol.

Industrial application

A final object of the present invention is related to the use of mixtures, comprising

(a) prebiotics and
(b) sterols or their esters

for stimulating the growth of healthy bacteria, for example in the stomach (if applied oral) or on skin (if administered topical) and for improving the status of the human body, for example with respect to

- reduction of *Heliobacter pylon* infection,
- reduction of allergic symptoms,
- relief from constipation,
- relief from inflammatory bowel syndrom and inflammatories of the intestine,
- beneficial effects from mineral metabolism, particularly bone density and stability (osteoporosis prevention),
- cancer prevention, and
- reduction of cholesterol and triacylglycerol plasma concentrations.

Examples

Examples 1 to 10, Comparative Examples C1 to C18

The stimulation of growth of micro-organisms has been studied by enumerating *bifidobacterium* and *lactobacilli* in vitro in the presence of various test substances. More specifically, aliquots (1 mL) of human faecal homogenates (10 g per 100 mL diluent) were added to diluted WC broth (diluted 50:50 with 0.05M phosphate buffer) to which were added the test mixtures and a lactobacillus or bifidobacterium strain. For each of the combinations, parallel tubes were prepared with one set being inoculated with *Bifidobacterium spp* or *Lactobacillus spp.* All mixtures were then incubated for up to 24 hours and bacterial numbers enumerated. The results are presented in Tables 1 and 2:

Table 1
Effect of 1 % prebiotic, sterols and prebiotic/sterol mixture on *Bifidobacterium*

	0	C1	C2	C3	C4	C5	C6	C7	C8	1	2	3	4	5
Inulin	-	1.0	-	-	-	-	-	-	-	0.8	0.9	-	-	-
Lactosucrose	-	-	1.0	-	-	-	-	-	-	-	-	0.9	-	-
Lactolin	-	-	-	1.0	-	-	-	-	-	-	-	-	0.9	-
Betaglucan	-	-	-	-	1.0	-	-	-	-	-	-	-	-	0.9
β-Sitosterol	-	-	-	-	-	1.0	-	-	-	0.2	-	-	-	-
β-Sitostanol	-	-	-	-	-	-	1.0	-	-	-	0.1	0.1	-	-
β-Sitosterol-E.	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1	-
β-Sitostanol-E.	-	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1
Bacterial numbers (CFU/ml)	1.0 x 10^6	1.5 x 10^7	1.1 x 10^7	1.6 x 10^7	1.2 x 10^7	3.3 x 10^6	2.4 x 10^6	2.7 x 10^6	3.2 x 10^6	4.1 x 10^7	4.1 x 10^7	4.1 x 10^7	4.1 x 10^7	4.4 x 10^7

β-Sitosterol-E. = β-Sitosterolpalmitate; β-Sitostanol-E. = β-Sitostanolstearate

Starting from a control of 1.0 10^6 CFU/ml (0) the addition of 1 % b.w. of various prebiotics (Comparative Examples C1-C4) increases the CFU by a factor of 10, while the addition of the sterols does only have a weak effect on the stimulation of cell growth (Comparative Examples C5-C8). Adding however mixture of prebiotics and sterols to the samples, the CFU numbers were multiplied by a factor of about 40 (Inventive Examples 1 to 5). The highest synergistic effect can be seen at a relation prebiotic : sterol of about 90 : 10.

Table 2
Effect of 1 % prebiotic, sterol and prebiotic/sterol mixture on *Lactobacterium*

	0	C9	C10	C11	C12	C13	C14	C15	C16	6	7	8	9	10
Inulin	-	1.0	-	-	-	-	-	-	-	0.8	0.9	-	-	-
Lactosucrose	-	-	1.0	-	-	-	-	-	-	-	-	0.9	-	-
Lactolin	-	-	-	1.0	-	-	-	-	-	-	-	-	0.9	-
Betaglucan	-	-	-	-	1.0	-	-	-	-	-	-	-	-	0.9
β-Sitosterol	-	-	-	-	-	1.0	-	-	-	0.2	-	-	-	-
β-Sitostanol	-	-	-	-	-	-	1.0	-	-	-	0.1	0.1	-	-
β-Sitosterol-E.	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1	-
β-Sitostanol-E.	-	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1
Bacterial numbers (CFU/ml)	2.8 x 10^5	1.4 x 10^6	1.1 x 10^6	1.5 x 10^6	1.1 x 10^6	4.4 x 10^5	4.4 x 10^5	4.4 x 10^5	4.5 x 10^5	6.1 x 10^6	6.2 x 10^6	6.2 x 10^6	6.2 x 10^6	6.7 x 10^6

β-Sitosterol-E. = β-Sitosterolpalmitate; β-Sitostanol-E. = β-Sitostanolstearate

Starting from a control of 2.8 10^5 CFU/ml (0) the addition of 1 % b.w. of various prebiotics (Comparative Examples C9-C12) increases the CFU by a factor of 4, while the addition of the sterols does only have a weak effect on the stimulation of cell growth (Comparative Examples C13-C16). Adding however, mixture of prebiotics and sterols to the samples, the CFU numbers were multiplied by a factor of about 15 (Inventive Examples 6 to 10). The highest synergistic effect can be seen again at a relation prebiotic : sterol of about 90 : 10.

Example 11
Yoghurt composition

Soy milk is added to 15-75 parts by volume of cow milk to make 100 parts of the mixture. The mixture is then pasteurised at about 90 °C. for 15 seconds and then cooled. The cooled, pasteurised mixtures are then inoculated with 3 to 5 percent by volume of a yoghurt culture having 1:1 ratio of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis*. The incubation is carried out at about 42 °C. In about 2 hours thickening will occur. The fermentation is carried out for about 5.5 hours. The yoghurt compositions thus obtained is treated with 1 % - calculated on the amount of micro-organisms being present – of a 9:1 mixture of inulin and β-sitosterol. The products firm consistency and have a flavour like or substantially indistinguishable from that of a corresponding yoghurt composition using 100 percent of fresh cow milk. A small amount of citric acid can be added to the fermentation mixture to enhance the flavour of the final yoghurt composition. A suitable amount of citric acid is 0.5 percent based on the weight of the composition.

Claims

1. Oral and/or topical compositions, comprising

   (a) prebiotics and
   (b) sterols or their esters.

2. Compositions according to claim 1, **characterised in** that said prebiotics (component a) are selected from the group consisting of fructooligosaccharides, inulins, isomaltooligosaccharides, lactilol, lactosucrose, lactulose, pyrodextrins, soy oligosaccharides, transgalactooligosaccharides, xylooligosaccharides and biopolymers.

3. Compositions according to claims 1 and/or 2, **characterised in** that said sterols or sterol esters (component b) represent phytosterols or phytosterol esters.

4. Compositions according to any of the claims 1 to 3, **characterised in** that component (b) represents sitosterol or sitostanol.

5. Compositions according to any of the claims 1 to 4, **characterised in** that component (b) represents esters of sterols or stanols with saturated or unsaturated fatty acids having 6 to 26 carbon atoms and up to 6 double bonds.

6. Compositions according to any of the claims 1 to 5, **characterised in** that they comprise component (a) and (b) in weight ratios of from 99 : 1 to 50 : 50.

7. Compositions according to any of the claims 1 to 5, **characterised in** that said mixtures are used in amounts of up to 10 % b.w. calculated on the micro-organisms being present in the final food composition.

8. Compositions according to any of the claims 1 to 6, **characterised in** that said mixtures are macro- or micro-encapsulated.

9. Food compositions, comprising

   (a) prebiotics and
   (b) sterols or their esters.

10. Use of mixtures, comprising

(a) prebiotics and
(b) sterols or their esters

for stimulating the growth of healthy bacteria and improving the status of the human body.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/005317

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 A23L1/307 A23L1/308 A23L1/054 A23L1/09
A23L1/236 A23K1/165 A61K31/733 A61K31/7016 A61K31/702
A61K31/715 A61K31/716

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 7 A23L A23K A61K

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 practical, search terms used)
EPO-Internal, PAJ, WPI Data, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/30359 A (SJOEBERG KJELL ; TRIPLE CROWN AB (SE)) 3 May 2001 (2001-05-03) the whole document	1-9
X	US 2003/068357 A1 (GUGGER ERIC T ET AL) 10 April 2003 (2003-04-10)	1-7,9
Y	the whole document	8
X	US 5 780 451 A (FULLER MARTHA KAY ET AL) 14 July 1998 (1998-07-14)	1-7,9,10
Y	column 10, line 50 - column 11, line 6; claims 1-18; tables 8,14	8
X	US 5 455 235 A (OKAMOTO TOSHIHIKO ET AL) 3 October 1995 (1995-10-03)	1-7,9,10
Y	claim 1; table 6	8

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document 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
29 August 2005	12/09/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Jong, E

4

Form PCT/ISA/210 (second sheet) (January 2004)

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 2002/187134 A1 (RANGANATHAN NATARAJAN ET AL) 12 December 2002 (2002-12-12) page 3, paragraphs 21,22; claims 1-16 page 4, paragraph 32 page 5, paragraph 43-45	8
X	DATABASE WPI Section Ch, Week 200146 Derwent Publications Ltd., London, GB; Class B05, AN 2001-430654 XP002303887 & KR 2001 001 290 A (PACIFIC SYSTEMS INC) 5 January 2001 (2001-01-05)	1-7
Y	abstract	8
X	US 5 776 524 A (REINHART GREGORY A) 7 July 1998 (1998-07-07)	1,2,9,10
Y	table 1	8
X	GARLEB K A ET AL: "EFFECT OF FRUCTOOLIGOSACCHARIDE CONTAINING ENTERAL FORMULAS ON SUBJECTIVE TOLERANCE FACTORS, SERUM CHEMISTRY PROFILES, AND FAECAL BIFIDOBACTERIA IN HEALTHY ADULT MALE SUBJECTS" MICROBIAL ECOLOGY IN HEALTH & DISEASE, CHICHESTER, GB, vol. 9, no. 6, 1996, pages 279-285, XP008023764	1-3,5,9, 10
Y	page 284, column 2, paragraph 2; table 1	8
X	MAKI K C ET AL: "Food products containing free tall oil-based phytosterols and oat beta -glucan lower serum total and LDL cholesterol in hypercholesterolemic adults." JOURNAL OF NUTRITION 133 (3) 808-813 2003 CHICAGO CENT. FOR CLINICAL RES., CHICAGO, IL, USA. E-MAIL KMAKI(A)PROTOCARE.COM, 2003, XP002303885	1-3,5-7, 9
Y	table 1	8
X	US 6 383 514 B1 (WEITKEMPER NORBERT ET AL) 7 May 2002 (2002-05-07) column 2, line 65 - column 3, line 40; claims 1-20	1-10
X	DATABASE WPI Section Ch, Week 200360 Derwent Publications Ltd., London, GB; Class B05, AN 2003-630167 XP002341213 & JP 2003 113089 A (GOTO CORP YG) 18 April 2003 (2003-04-18) abstract	1-10

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANONYMOUS: "Pseudo ceramides used in cosmetics, etc., - to prevent drying of skin under normal atmospheric conditions" WPI WORLD PATENT INFORMATION DERWENT, DERWENT, GB, vol. 15, no. 97, 1997, XP002089064 abstract	1-6,10
A	ANONYMOUS: "Prebiotics" INTERNET ARTICLE, 'Online! 4 June 2003 (2003-06-04), XP002303886 Retrieved from the Internet: URL:http://www.pdrhealth.com/drug_info/nmdrugprofiles/nutsupdrugs/pre_0326.shtml> 'retrieved on 2004-11-02! the whole document	1-10
L	& INTERNET ARTICLE, 'Online! 4 June 2003 (2003-06-04), Retrieved from the Internet: URL:http://web.archive.org/web/20030604182836/http://pdrhealth.com> 'retrieved on 2004-11-02! To prove publication date of XP002303886	
A	WO 97/29763 A (PROCTER & GAMBLE) 21 August 1997 (1997-08-21) claims 1-10	1-10
A	US 5 380 717 A (OHKUMA KAZUHIRO ET AL) 10 January 1995 (1995-01-10) column 4, lines 22-30; claims 1-11	1-10
A	EP 0 265 970 A (SUNTORY LTD) 4 May 1988 (1988-05-04) claims 1-5	1-10
A	MIZOTA T: "LACTULOSE AS A GROWTH PROMOTING FACTOR FOR BIFIDOBACTERIUM AND ITS PHYSIOLOGICAL ASPECTS" BULLETIN - INTERNATIONAL DAIRY FEDERATION, BRUSSELS, BE, vol. 313, 1996, pages 43-48, XP000677675 ISSN: 0259-8434 the whole document	1-10
A	OHKUSA T ET AL: "Long-term ingestion of lactosucrose increases Bifidobacterium sp. in human fecal flora" DIGESTION, BASEL, CH, vol. 56, no. 5, 1995, pages 415-420, XP009028691 ISSN: 0012-2823 page 416, column 1, paragraph 2 page 419, column 2, paragraph 4	1-10

-/--

4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROBERFROID M B: "FUNCTIONAL FOODS: CONCEPTS AND APPLICATION TO INULIN AND OLIGOFRUCTOSE" BRITISH JOURNAL OF NUTRITION, CAMBRIDGE, GB, vol. 87, no. SUPPL 2, 2002, pages S139-S143, XP009008910 pages 141-142	1-10
A	US 5 294 458 A (FUJIMORI ISAO) 15 March 1994 (1994-03-15) claims 1-3	1-10

INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP2005/005317

Box 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. [X] Claims Nos.: 10 (partly)
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 10 is directed to a method of treatment of the human/animal body (Rule 39.1(iv) PCT), the search has been carried out and based on the alleged effects of the composition.

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 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:

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 an additional fee, this Authority did not invite payment of any additional fee.

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.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

formation on patent family members

International Application No
PCT/EP2005/005317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0130359 A	03-05-2001	SE 517769 C2	16-07-2002
		AU 1321101 A	08-05-2001
		EP 1227816 A1	07-08-2002
		WO 0130359 A1	03-05-2001
		SE 9903915 A	30-04-2001
US 2003068357 A1	10-04-2003	NONE	
US 5780451 A	14-07-1998	AT 212194 T	15-02-2002
		AU 689555 B2	02-04-1998
		AU 1884495 A	23-10-1995
		CA 2187628 A1	12-10-1995
		DE 69525150 D1	14-03-2002
		DE 69525150 T2	05-09-2002
		DK 754001 T3	13-05-2002
		EP 0754001 A1	22-01-1997
		ES 2171535 T3	16-09-2002
		HK 1011914 A1	14-02-2003
		NZ 281878 A	24-02-1997
		PT 754001 T	31-07-2002
		US 5952314 A	14-09-1999
		WO 9526646 A1	12-10-1995
		US 6468987 B1	22-10-2002
US 5455235 A	03-10-1995	AU 657405 B2	09-03-1995
		AU 3768793 A	18-11-1993
		CA 2110993 A1	28-10-1993
		DE 69312851 D1	11-09-1997
		DE 69312851 T2	08-01-1998
		EP 0593774 A1	27-04-1994
		ES 2106329 T3	01-11-1997
		JP 2789069 B2	20-08-1998
		JP 6038707 A	15-02-1994
		WO 9320718 A1	28-10-1993
		KR 256150 B1	01-07-2000
US 2002187134 A1	12-12-2002	CA 2447376 A1	21-11-2002
		CN 1509144 A	30-06-2004
		EP 1397044 A1	17-03-2004
		JP 2004533442 T	04-11-2004
		WO 02091833 A1	21-11-2002
		US 2004105848 A1	03-06-2004
		US 2004106185 A1	03-06-2004
		US 2004161422 A1	19-08-2004
		US 2004197352 A1	07-10-2004
KR 2001001290 A	05-01-2001	NONE	
US 5776524 A	07-07-1998	NONE	
US 6383514 B1	07-05-2002	DE 19700796 A1	04-06-1998
		AU 713665 B2	09-12-1999
		AU 5322998 A	22-06-1998
		AU 714993 B2	13-01-2000
		AU 5553198 A	22-06-1998
		CA 2272754 A1	04-06-1998
		CA 2273341 A1	04-06-1998
		DE 59707175 D1	06-06-2002

ormation on patent family members

International Application No
PCT/EP2005/005317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6383514 B1		DE 59708286 D1	24-10-2002
		DK 941097 T3	19-08-2002
		DK 952837 T3	27-01-2003
		WO 9823275 A1	04-06-1998
		WO 9823277 A1	04-06-1998
		EP 0941097 A1	15-09-1999
		EP 0952837 A1	03-11-1999
		ES 2176805 T3	01-12-2002
		ES 2182137 T3	01-03-2003
		JP 2001504505 T	03-04-2001
		JP 2001508046 T	19-06-2001
		NO 992562 A	27-05-1999
		NO 992564 A	27-05-1999
		NZ 335990 A	27-10-2000
		NZ 335974 A	23-06-2000
		US 6444659 B1	03-09-2002
JP 2003113089 A	18-04-2003	NONE	
WO 9729763 A	21-08-1997	AU 1854297 A	02-09-1997
		CA 2246371 A1	21-08-1997
		CN 1211189 A	17-03-1999
		EP 0881905 A1	09-12-1998
		JP 11504049 T	06-04-1999
		WO 9729763 A1	21-08-1997
US 5380717 A	10-01-1995	JP 2007645 C	11-01-1996
		JP 3244365 A	31-10-1991
		JP 7028694 B	05-04-1995
		AT 114934 T	15-12-1994
		DE 69105546 D1	19-01-1995
		DE 69105546 T2	20-04-1995
		DE 443788 T1	30-04-1992
		EP 0443788 A1	28-08-1991
		ES 2044797 T1	16-01-1994
		KR 181312 B1	01-02-1999
EP 0265970 A	04-05-1988	JP 2549638 B2	30-10-1996
		JP 63112979 A	18-05-1988
		AT 79401 T	15-08-1992
		CA 1300023 C	05-05-1992
		DE 3781087 D1	17-09-1992
		DE 3781087 T2	04-02-1993
		EP 0265970 A2	04-05-1988
		ES 2043632 T3	01-01-1994
		US 5939309 A	17-08-1999
US 5294458 A	15-03-1994	JP 5115250 A	14-05-1993