

(19) World Intellectual Property
Organization
International Bureau



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

PCT

(10) International Publication Number
WO 2005/115170 A1

(51) International Patent Classification⁷: **A23L 1/30**,
1/307, 1/308, 1/09, A61K 31/733, 31/716, 31/702, 35/78,
31/35, 7/48

(21) International Application Number:
PCT/EP2005/005316

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

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
04012288.9 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]; Fransesc 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, 40627 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 "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ORAL AND /OR TOPICAL COMPOSITIONS

(57) Abstract: Suggested are new advantageous oral and/or topical compositions, comprising (a) prebiotics and (b) polyphenols or
plant extracts rich in polyphenols.



WO 2005/115170 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 defined active principles and prebiotics, dietary supplements and food compositions comprising said actives and prebiotics, and the use of mixtures comprising said actives 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. It's 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 is passing through the intestines. It is not surprising to find that the bacterial population in the intestines of vegetarians is much different compared to that of meat eaters. Because some bacteria have specific nutrient requirements 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) polyphenols or plant extracts rich in polyphenols.

Surprisingly it has been observed that mixtures of various types of polyphenols preferably of plant origin 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 by 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-O-(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

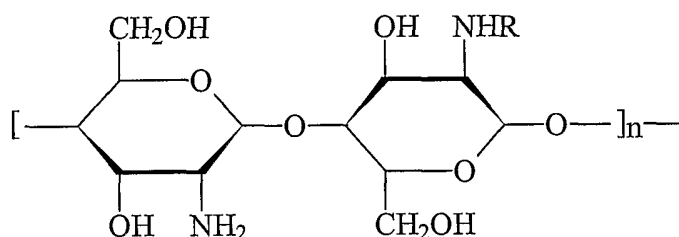
Transgalactooligosaccharides (TOS) are a mixture of oligosaccharides consisting of D-glucose and D-galactose. TOS are produced from D-lactose via the action of the enzyme beta-galactosidase obtained from *Aspergillus oryzae*. TOS are resistant to digestion in the upper gastrointestinal tract and stimulate 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.



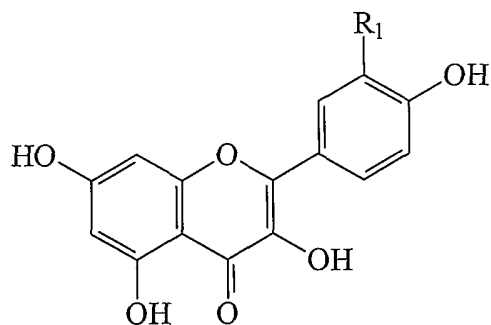
Chitosan is obtained by deacetylation of chitin and shows molecular weights in the range of 50.000 up to 2.000.000.

Polyphenols and plant extracts rich in polyphenols

In the following examples for polyphenols and plant extracts which are rich in said polyphenols are given in order to illustrate the invention, but not to limit the invention to the selected species:

- Ginkgo biloba

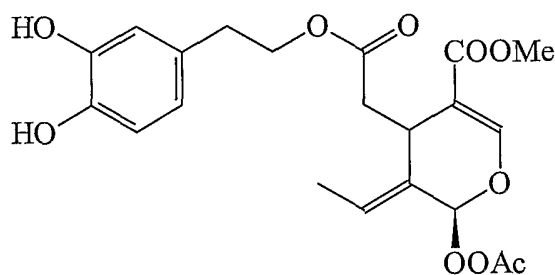
The active ingredients of the extract are flavonoid glycosides, which among others contain (iso)quercetin glycosides, kaempferol, kaempferol-3-rhamnosides, isorhamnetin, luteoline glycosides, sitosterol glycosides and predominantly hexacyclic terpene lactones, consisting of ginkgolides A, B, C, J, M and bilobalides.



Isorhamnetin ($R^1 = H$), Kaempferol ($R^1 = OH$), Ginkgolid A ($R^1 = OMe$)

- Oleacea europensis

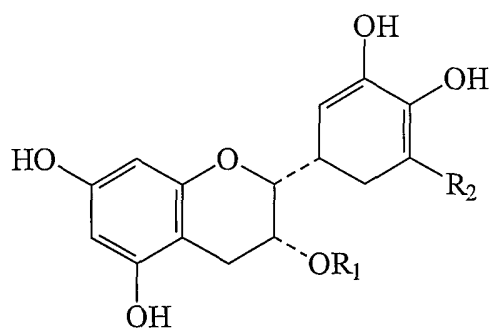
The main constituent of the leaves of the olive tree (*Oleacea europensis*) is the antioxidant oleuropein, which is also the main source for hydroxytyrosol.



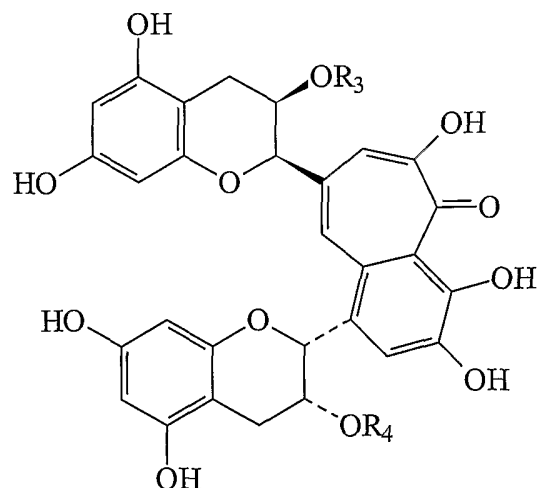
Oleuropein

- Camellia sinensis

Polyphenoles of the catechin and flavonoid type, so-called „tea-tannins“ represent the main active principles of extracts of Green Tea (*Camellia sinensis*) :



Catechins

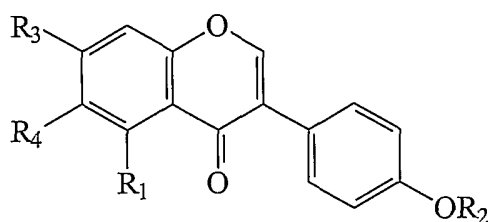


Flavonoids

	R1	R2	R3	R4
(-)-Epicatechin	H	H		
(-) Epigallocatechin	H	OH		
(-) Epicatechin gallate	Galloyl	H		
(-) Epigallocatechin gallate	Galloyl	OH		
Theflavin			H	H
Theaflavin monogallate A			Galloyl	H
Theaflavin monogallat B			H	Galloyl
Theaflavin digallate			Galloyl	Galloyl

- Trifolium pratense

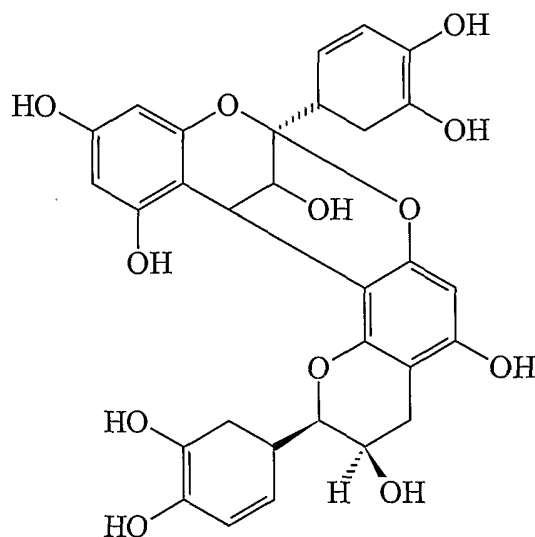
The main active principles of red clover (*Trifolium pratense*) are isoflavones, like e.g. daidzein, genestein, formononetin and biochanin as well as their glucosides like ononin or sissotrin:



Isoflavonglucosides	R ₁	R ₂	R ₃	R ₄
Daizidin	H	H	Glucose	H
Genistin	H	H	Glucose	OH
Ononin	H	CH ₃	Glucose	H
Sissostrin	H	CH ₃	Glucose	OH

- Litchi sinensis

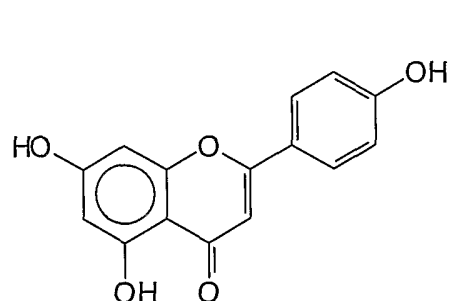
Extracts of pericarps from Litchi (*Litchi sinensis*) are well known for its high content of flavon derivatives like e.g. 2-phenyl-4H-1-benzopyrans, flavanen, flavan-3-ols (catechins, catechin oligomeren), flavan-3,4-diols (leucoanthocyaniden), flavons, flavonols and flavonons. The main component, however, represent condensed tannins, so-called procyanodols (OPC). These compounds comprise 2 to 8 monomers of the catechin or epicatechin-type, like e.g. procyanidins, proanthocynidins, procyanidoel, oligoprocyanidins, leucoanthocyanidins, leucodelphinins, leucocyanins and anthocyanogens. OPC, mainly the preferred proanthocyanidin A2 (OPC A2) behave like vitamin P, especially with respect to MMP inhibition.



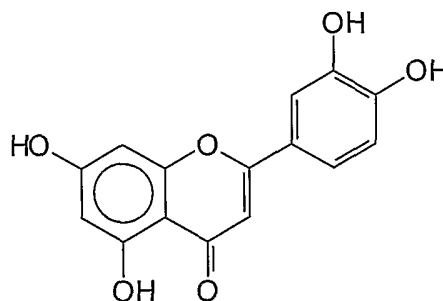
Oligomeric proanthocyanidin

- Passiflora incarnata

Extracts of passion flower (*Passiflora incarnata*) are rich in flavons of the apigenin and luteolin-type and their C-glycosides:



Apigenin

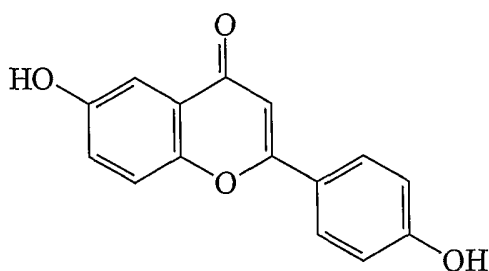


Luteolin

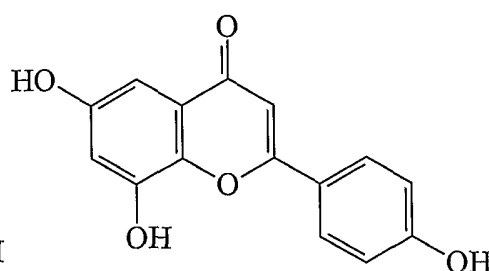
In addition they comprise 2"-B-D-glucosides, schaftosides and iso-schaftosides, isovitexin, isoorientin, vicianin-2, incenin-2, daponanin and trace elements like calcium, phosphor und iron.

- Medicago sativa

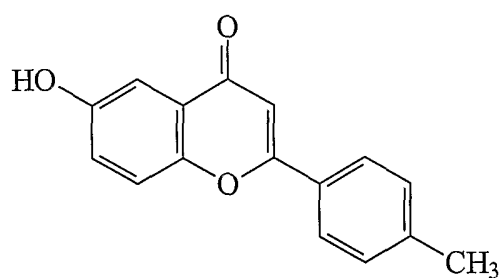
Extracts of Alfalfa (*Medicago sativa*) are rich in isoflavons like e.g. daidzein, genestein, formononetin, biochanin A und tricin :



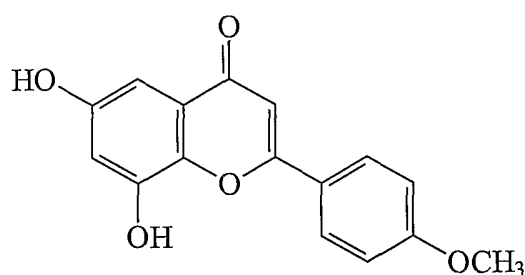
Daidzein



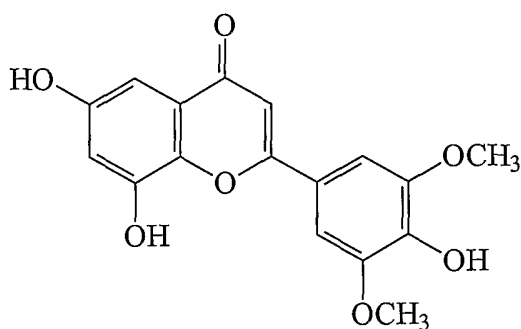
Genestein



Formononetin



Biochanin A



Tricin

Oral and/or topical compositions

The oral and/or topical compositions according to the present invention may comprise the prebiotics and the polyphenols 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 micro-organisms 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 micro-encapsulated. "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, 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 Thalaspheeres* (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) polyphenols or plant extracts rich in polyphenols.

Industrial application

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

- (a) prebiotics and
- (b) polyphenols or plant extracts rich in polyphenols

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 *Helicobacter pylori* 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:

Extract A : Extract of *Trifolium pratense* (Red clover)

Extract B : Extract of *Camellia sinensis* (Green tea)

Extract C : Extract of *Oleacea europensis* (Olive tree)

Extract D : Extract of *Ginkgo biloba* (Ginkgo tree)

All extracts show an active content of about 20 % b.w. and are commercially available from Cognis Deutschland GmbH & Co. KG.

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 (amount of extract calculated on active content):

Table 1
Effect of 1 % prebiotic, plant extracts and prebiotic/plant extract 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
Extract A	-	-	-	-	-	1.0	-	-	-	0.2	-	-	-	-
Extract B	-	-	-	-	-	-	1.0	-	-	-	0.1	0.1	-	-
Extract C	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1	-
Extract D	-	-	-	-	-	-	-	-	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	2.3 x 10 ⁶	2.7 x 10 ⁶	3.4 x 10 ⁶	4.1 x 10 ⁷	4.3 x 10 ⁷	4.0 x 10 ⁷	4.0 x 10 ⁷	4.4 x 10 ⁷

Starting from a control of $1.0 \cdot 10^6$ 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 plant extracts does only have a weak effect on the stimulation of cell growth (Comparative Examples C5-C8). Adding however mixture of prebiotics and plant extracts 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 : polyphenols of about 90 : 10.

Table 2
Effect of 1 % prebiotic, plant extract and prebiotic/plant extract 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
Extract A	-	-	-	-	-	1.0	-	-	-	0.2	-	-	-	-
Extract B	-	-	-	-	-	-	1.0	-	-	-	0.1	0.1	-	-
Extract C	-	-	-	-	-	-	-	1.0	-	-	-	-	0.1	-
Extract D	-	-	-	-	-	-	-	-	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	4.2 x 10^5	4.3 x 10^5	4.4 x 10^5	4.5 x 10^5	6.5 x 10^5	6.3 x 10^6	6.7 x 10^6	6.6 x 10^6	6.9 x 10^6

Starting from a control of $2.8 \cdot 10^5$ 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 plant extracts does only have a weak effect on the stimulation of cell growth (Comparative Examples C13-C16). Adding however, mixture of prebiotics and plant extracts 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 : polyphenols 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 an extract of Green Tea. 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) polyphenols or plant extracts rich in polyphenols.
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 polyphenols are selected from the group consisting of (iso)flavons, (iso)flavonols, (iso)flavonones, (iso)flavonoids, catechins, ginkgolides A, B, C, bilobalides, oligoprocyanidins and their glycosides.
4. Compositions according to any of the claims 1 to 3, **characterised in** that said polyphenols are selected from the group consisting of (iso)quercetin, kaempferol, isorhamnetin, luteolin, oleuropein, hydroxytyrosol, (epi)catechin(gallate), (epi)gallocatechin(gallate), theaflavin(gallate), daidzein, genestein, formononetin, biochanin A, tricin, proanthocyanidin A2, apigenin, luteolin and their glycosides.
5. Compositions according to any of the claims 1 to 4, **characterised in** that component (b) represents extracts of *Ginkgo biloba*, *Camellia sinensis*, *Trifolium pratense*, *Oleacea europensis*, *Litchi sinensis*, *Passiflora incarnata*, *Medicago sativa* and their mixtures.
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) polyphenols or plant extracts rich in polyphenols

10. Use of mixtures, comprising

- (a) prebiotics and
- (b) polyphenols or plant extracts rich in polyphenols

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

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/005316

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L1/30 A23L1/307 A23L1/308 A23L1/09 A61K31/733
A61K31/716 A61K31/702 A61K35/78 A61K31/35 A61K7/48

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 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, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 200346 Derwent Publications Ltd., London, GB; Class B03, AN 2003-485823 XP002306600 & JP 2002 335926 A (IKKO KAGAKU KK) 26 November 2002 (2002-11-26) abstract	1-6, 9
A		7
X	DATABASE WPI Section Ch, Week 200215 Derwent Publications Ltd., London, GB; Class D13, AN 2002-111697 XP002306601 & KR 2001 079 226 A (SAM BO NATURAL CO LTD) 22 August 2001 (2001-08-22) abstract	1-6, 9

☒ Further documents are listed in the continuation of box C.

☒ 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

13 June 2005

Date of mailing of the international search report

29/06/2005

Name and mailing address of the ISA

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

Authorized officer

Saunders, T

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2005/005316

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/077671 A (CENTRAL SOYA CO) 25 September 2003 (2003-09-25) page 2, paragraph 3; example 2 -----	1-4,6,9
X	WO 02/47493 A (AVENTIS PHARMA GMBH) 20 June 2002 (2002-06-20) page 29, line 9 - line 10; claim 1 -----	1-4,9
X	DATABASE WPI Section Ch, Week 200347 Derwent Publications Ltd., London, GB; Class B04, AN 2003-498138 XP002306602 & JP 2003 055234 A (HANA HEALTH KK) 26 February 2003 (2003-02-26) abstract -----	1-5,9
X	DATABASE WPI Section Ch, Week 200411 Derwent Publications Ltd., London, GB; Class B04, AN 2004-102946 XP002306603 & JP 2004 010605 A (TANAKA Y) 15 January 2004 (2004-01-15) abstract -----	1-5,9
X	DATABASE WPI Section Ch, Week 200239 Derwent Publications Ltd., London, GB; Class D13, AN 2002-353022 XP002306604 & CN 1 336 142 A (TANG Z) 20 February 2002 (2002-02-20) abstract -----	1-4,9
X	US 6 713 116 B1 (RITTER GUIDO ET AL) 30 March 2004 (2004-03-30) examples 1,2 -----	1-3,6,9
X	WO 00/64282 A (CORRADINI CLAUDIO ; TORTORA DIOMEDE ANTONIO (IT); DUGO GIOVANNI (IT)) 2 November 2000 (2000-11-02) claims 1,6 -----	1,2,6,9
X	EP 0 524 484 A (PLANTEXTRAKT PFLANZEN EXTRAKTI) 27 January 1993 (1993-01-27) claim 1 -----	1-3,9
X	PATENT ABSTRACTS OF JAPAN vol. 2003, no. 12, 5 December 2003 (2003-12-05) & JP 2004 075692 A (ITSUKOU KAGAKU KK), 11 March 2004 (2004-03-11) abstract -----	1,2,9
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2005/005316

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/03150 A (UNIV MONTANA) 8 February 1996 (1996-02-08) page 7, line 32 - page 8, line 4 page 9, line 25 - line 27; claim 6; example 2	1-4, 6, 8-10
X	PATENT ABSTRACTS OF JAPAN vol. 2000, no. 09, 13 October 2000 (2000-10-13) & JP 2000 157183 A (NAKAJIMA FUMIO), 13 June 2000 (2000-06-13) abstract	1-6, 9
X	WO 03/013438 A (MILLENNIUM BIOTECHNOLOGIES INC) 20 February 2003 (2003-02-20) examples	1-4, 6
X	US 2001/018077 A1 (SHANER EDWARD O) 30 August 2001 (2001-08-30) paragraph '0011!	1-4
X	DATABASE WPI Section Ch, Week 200146 Derwent Publications Ltd., London, GB; Class B05, AN 2001-430654 XP002306605 & KR 2001 001 290 A (PACIFIC IND CO) 5 January 2001 (2001-01-05) abstract	1-4
A	US 5 071 653 A (KAKUDA TAKAMI ET AL) 10 December 1991 (1991-12-10) claim 1	1, 3-5, 9, 10
A	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 abstract	1, 2, 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/005316

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

Although claims 1-10 are directed to a method of treatment of the human or animal body, 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

Information on patent family members

International Application No

PCT/EP2005/005316

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 2002335926	A	26-11-2002	JP 3523220 B2	26-04-2004
KR 2001079226	A	22-08-2001	NONE	
WO 03077671	A	25-09-2003	AU 2003222286 A1	29-09-2003
			BR 0308313 A	05-04-2005
			CA 2479244 A1	25-09-2003
			EP 1482810 A2	08-12-2004
			WO 03077671 A2	25-09-2003
			US 2003190401 A1	09-10-2003
WO 0247493	A	20-06-2002	DE 10109798 A1	12-09-2002
			EP 1214893 A1	19-06-2002
			AU 2193402 A	24-06-2002
			WO 0247493 A2	20-06-2002
			EP 1355539 A2	29-10-2003
			JP 2004515508 T	27-05-2004
			US 2002146463 A1	10-10-2002
JP 2003055234	A	26-02-2003	NONE	
JP 2004010605	A	15-01-2004	NONE	
CN 1336142	A	20-02-2002	NONE	
US 6713116	B1	30-03-2004	NONE	
WO 0064282	A	02-11-2000	IT RM990260 A1	27-10-2000
			IT RM990646 A1	23-04-2001
			AU 4777100 A	10-11-2000
			EP 1094722 A1	02-05-2001
			WO 0064282 A1	02-11-2000
EP 0524484	A	27-01-1993	DE 4123124 A1	14-01-1993
			EP 0524484 A1	27-01-1993
JP 2004075692	A	11-03-2004	NONE	
WO 9603150	A	08-02-1996	AT 277638 T	15-10-2004
			DE 69533593 D1	04-11-2004
			EP 0797451 A2	01-10-1997
			WO 9603150 A1	08-02-1996
			US 6087092 A	11-07-2000
			US 5614501 A	25-03-1997
JP 2000157183	A	13-06-2000	NONE	
WO 03013438	A	20-02-2003	US 6503506 B1	07-01-2003
			CA 2457624 A1	20-02-2003
			CN 1568196 A	19-01-2005
			EP 1432436 A2	30-06-2004
			JP 2005501061 T	13-01-2005
			WO 03013438 A2	20-02-2003
US 2001018077	A1	30-08-2001	NONE	
KR 2001001290	A	05-01-2001	NONE	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2005/005316

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 5071653	A	10-12-1991	JP	2211863 A	23-08-1990
			JP	2815628 B2	27-10-1998
