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Zorea(10) **Pub. No.: US 2011/0008493 A1**(43) **Pub. Date: Jan. 13, 2011**(54) **HEAT RESISTANT PROBIOTIC
COMPOSITIONS AND HEALTHY FOOD
COMPRISING THEM**(75) Inventor: **Yohai Zorea, Eilat (IL)**

Correspondence Address:

ALSTON & BIRD LLP**BANK OF AMERICA PLAZA, 101 SOUTH
TRYON STREET, SUITE 4000
CHARLOTTE, NC 28280-4000 (US)**(73) Assignees: **Israel Rubin; Doron Sasson;
Henri Zimand**(21) Appl. No.: **12/637,487**(22) Filed: **Dec. 14, 2009**(30) **Foreign Application Priority Data**

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Provided are granules for incorporating probiotic bacteria into health food products, particularly bakery probiotic products. The granules contain a core of bacteria to be released in a viable state in the small intestine, coated with layers protecting the bacteria against high temperature during baking, and against adverse conditions along the gastrointestinal tract.

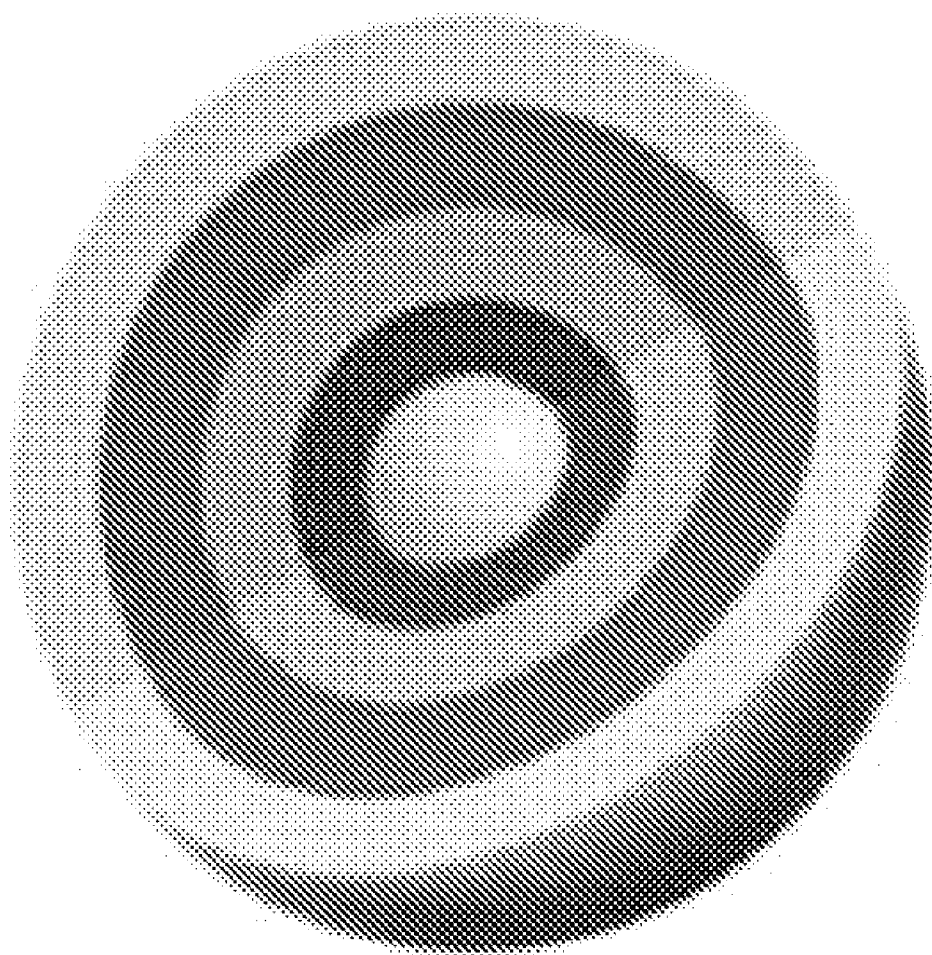


Fig. 1

HEAT RESISTANT PROBIOTIC COMPOSITIONS AND HEALTHY FOOD COMPRISING THEM

FIELD OF THE INVENTION

[0001] The present invention relates to the health food products, particularly, to bakery probiotic products. Provided is a method of preparing a product which undergoes heat treatment in at least one stage of its preparation, while keeping a sufficient amount of probiotic microorganisms.

BACKGROUND OF THE INVENTION

[0002] Probiotics are live microbial food supplements which beneficially affect the host by supporting naturally occurring gut flora, by competing harmful microorganisms in the gastrointestinal tract, by assisting useful metabolic processes, and by strengthening the resistance of the host organism against toxic substances. A number of organisms is used in probiotic foods, an example being bacterial genera *Lactobacillus* or *Bifidobacterium*. Probiotic organisms should survive for the lifetime of the product, in order to be effective, and further they should survive the whole way through the gastrointestinal tract to the colon. Probiotic organisms are usually incorporated into milk products, such as yogurts. The need is felt to deliver the beneficial microorganisms in other foodstuff types, for example in baked products. However, the main problem in preparing baked health food is the processing temperature, which is usually so high (exceeding 180° C.) that it nearly sterilizes the products. WO 94/00019 describes a method of preparing a baked product containing living microorganisms, comprising cooling a baked product and injecting into it a living suspension. WO 2009/069122 of the same inventors as the present invention describes a process for preparing baked food, comprising encapsulating probiotic granules, thereby enhancing their stability. It is an object of the present invention to provide a process for preparing a nutritionally acceptable composition comprising probiotic microorganisms, the composition being resistant to high temperatures. Another object of the invention is to provide a bakery product comprising viable bacteria in a sufficient amount.

[0003] It is a still another object of the present invention to provide a process for preparing a prebiotic bakery product, without need of injecting viable microorganisms into the bakery product after the baking process.

[0004] It is a further object of the invention to provide bakery products containing live probiotic microorganisms during the whole process of baking.

[0005] It is a still further object of the invention to provide bakery products comprising heat-stabilized probiotic composition.

[0006] It is also a further object of the invention to provide prebiotic bakery products exhibiting a long shelf life.

[0007] Other objects and advantages of present invention will appear as description proceeds.

SUMMARY OF THE INVENTION

[0008] The present invention provides a probiotic granule comprising i) a core comprising probiotic bacteria and a substrate in which said bacteria are absorbed; ii) an inner layer of vegetable oil coating said core; and iii) two outer layers, coating said core and said inner layer, comprising at least two different polymers. Said substrate and said two

different polymers are preferably nutritionally acceptable polymers. Said core may further comprise one or more supplemental agents for said bacteria. Preferably, the agents support the growth of said bacteria, and may comprise prebiotic materials, such as oligosaccharides. Said two outer layers in the granules of the invention comprise two different polymers. The polymers may be of fibrous or of gelatinous character. Advantageously, at least one of the outer layers comprises a fibrous polysaccharide, and at least one of the outer layers comprises a gelatinous polysaccharide. The prebiotic granule of the present invention comprises a prebiotic microorganism; the organism is preferably bacterial. Said bacteria advantageously comprise a strain selected from the group consisting of *Bacillus coagulans* GBI-30, 6086; *Bifidobacterium animalis* subsp. *lactis* BB-12; *Bifidobacterium breve* Yakult; *Bifidobacterium infantis* 35624; *Bifidobacterium animalis* subsp. *lactis* HN019 (DR10); *Bifidobacterium longum* BB536; *Escherichia coli* M-17; *Escherichia coli* Nissle 1917; *Lactobacillus acidophilus* DDS-1; *Lactobacillus acidophilus* LA-5; *Lactobacillus acidophilus* NCFM; *Lactobacillus casei* DN114-001 (*Lactobacillus casei* Immunitas(s)/Defensis); *Lactobacillus casei* CRL431; *Lactobacillus casei* F19; *Lactobacillus casei* Shirota; *Lactobacillus paracasei* St11 (or NCC2461); *Lactobacillus johnsonii* La1 (= *Lactobacillus* LC1); *Lactococcus lactis* L1A; *Lactobacillus plantarum* 299V; *Lactobacillus reuteri* ATTC 55730 (*Lactobacillus reuteri* SD2112); *Lactobacillus rhamnosus* ATCC 53013 (discovered by Gorbach & Goldin (=LGG)); *Lactobacillus rhamnosus* LB21; *Saccharomyces cerevisiae* (boulardii) Iyo; *Lactobacillus rhamnosus* GR-1 & *Lactobacillus reuteri* RC-14; *Lactobacillus acidophilus* NCFM & *Bifidobacterium bifidum* BB-12; and a mixture thereof.

[0009] The invention provides a method for manufacturing a probiotic granule comprising a core, containing probiotic bacteria and a substrate in which said bacteria are absorbed, surrounded by an inner oily layer and two outer polymer layers, the method comprising i) mixing a suspension of prebiotic bacteria with a cellulose-based substrate and with supplemental agents for the bacteria, thereby obtaining a core mixture; ii) coating particles of said core mixture with a vegetable oil, thereby obtaining oil-coated particles; iii) coating said oil-coated particles with a first polymer layer, which first polymer layer confers stability to said bacteria under the conditions of upper gastrointestinal tract, thereby obtaining particles coated with two layers; and iv) coating said two-layer particles with a second polymer layer, which second polymer layer increases the stability of the bacteria in said core under the conditions of baking. Said bacteria may comprise one or more bacterial strains, and are admixed to and absorbed on a microbiologically compatible polymer, which as also nutritionally acceptable and approvable, an example being a polysaccharide, such as cellulose based material. Materials supporting the stability or growth of said bacteria may be added into the mixture. Preferably included are probiotic supporters known as prebiotics, for example maltodextrin, trehalose, etc. The coating steps may utilize techniques known in the field, including fluidized bed coating, spraying, etc. When creating the coated layers, solutions or suspensions may be employed, as well as powders, etc. Said coating steps ii) to iv) result in a mass increase of from 10 to 100% relatively to the mass of the core, for example between 15 and 50%. In a preferred embodiment, a method for manufacturing a prebiotic granule comprises i) mixing an aqueous suspension of prebiotic bacteria comprising at least one strain of

Lactobacillus or *Bifidobacterium* genus with microcrystalline cellulose, maltodextrin, and trehalose, thereby obtaining a core mixture; ii) coating particles of said core mixture with a hydrogenated vegetable oil, thereby obtaining oil-coated particles; iii) coating said oil-coated particles with a first polymer layer and with a second polymer layer; iv) wherein said two polymers layers are different and comprise at least two materials selected from cellulose, modified cellulose, polysaccharide and or synthetic polymers and a mixture thereof.

[0010] Importantly, the invention relates to a prebiotic composition comprising granules having a core, containing probiotic bacteria and a substrate in which said bacteria are absorbed, surrounded by an inner oily layer and two outer polymer layers. The prebiotic composition according to the invention exhibits a high resistance to the increased temperature. When relating here to a high resistance to the increased temperature, or when relating to a high heat-stability, intended is survival of the prebiotic bacteria within the granules compared to free bacteria, and particularly survival of the prebiotic bacteria within the granules admixed in a food product compared to free bacteria. In one aspect of the invention, the prebiotic bacteria in the core of the three-layer granule survives exposures of the granules to temperatures higher than ambient temperature. The heat stability of the prebiotic composition according to the invention is sufficiently high to ensure that a part of the initial bacterial load admixed in a prebiotic food product of the invention remains viable even after all necessary manufacturing steps. Such steps may include baking.

[0011] The invention provides a healthy food product or a food additive comprising a prebiotic composition, as above described, comprising the stable probiotic granules. Said product may preferably comprise a bakery product, for example pastry or bread. Said product may also comprise tuna, chocolate, fruit juices, and dairy products.

[0012] A healthy food product according to the invention may comprise pastry, bread, flour, flour products, baked goods, frozen baking products, yogurt, dairy products, chocolate, nectars, fruit juices, and tuna. The food product according to the invention, comprising probiotic granules, may be exposed to higher than ambient temperature during the production process.

BRIEF DESCRIPTION OF THE DRAWING

[0013] The above and other characteristics and advantages of the invention will be more readily apparent through the following examples, and with reference to the appended drawing, wherein:

[0014] FIG. 1. shows a schema of a multiple-layered capsule according to one embodiment of the invention, to be comprised in healthy food; the encapsulation is designed to provide probiotic bacteria with maximum heat resistance during the heating step of the manufacturing process, when providing said food, and also with highly biological efficacy in the lower GI tract after leaving the stomach intact; the white core comprises probiotic bacteria and absorbing substrate; the first dark layer adjacent to the core is a vegetable oil layer, supplying food to bacteria; a light layer adjacent to the oil layer is an optional isolating layer; a dark grey layer adjacent

to the isolating layer is an acid-resistant layer; and the outer light layer is a heat-resistant layer.

DETAILED DESCRIPTION OF THE INVENTION

[0015] It has now been surprisingly found that probiotic bacteria may be formulated in cores of three-layer granules, thereby obtaining probiotic compositions providing viable probiotic organisms even after baking, the composition being further stable on storage and capable of administering viable bacteria to colon after the oral administration.

[0016] The invention provides granular probiotics to be used as healthy food additives. The present invention is particularly directed to a process for the preparation of baked food, such as probiotic pastry. A suspension of prebiotic microorganisms is mixed with a suitable carrier material to form a core part of particles to be coated. The granulation process may employ a suitable granulator, or alternatively a fluidized bed. The drying process may comprise lyophilization. The prebiotic granules according to the invention may have a wide range of dimensions. A non-limiting example of a prebiotic granule according to the invention is an essentially spherical particle having a mean diameter of about from 0.1 to about 1000 microns. Without wishing to be limited by any theory, it is believed that admixing the probiotic microorganisms with a microbiologically acceptable polymer, such as a cellulose derivative, in a particle core to be further coated with a triple layer of microbiologically acceptable materials results in an increased heat resistance of the microorganism, wherein the increased resistance may result both from lowered heat conductivity and from cell stabilization. The probiotic microorganisms processed according to the invention may even resist baking heat for a predetermined baking temperature and baking time. It is believed that the inner oily layer and the first outer layer further protect the probiotic microorganisms during their passage through the upper gastrointestinal tract (enteric coating layer), enabling the release of the probiotics in either small intestine, or colon or both. The structure of the granular probiotic composition of the invention ensures a relatively high stability (bacterial viability) on storage before its use in preparing food products, as well as inside food products on their storage. Furthermore, said structure ensures desirable release of the viable bacteria in the lower gastrointestinal tract of a person eating the healthy food, for example healthy bakery product. Furthermore, the whole beneficial effect may be further enhanced when including in the probiotic composition also oligosaccharides (called prebiotic) supporting the growth of the beneficial microorganism. Optionally the upper gastrointestinal resistant coating layer (the enteric coating layer) may be separated from the outer heat resistant coating layer by an intermediate inert coating layer in order to prevent any possible base-acid reaction between them. The encapsulated probiotic compositions of the present invention may also be coated with one or more pH-sensitive coating compositions commonly referred to in the art as "enteric coatings," according to conventional procedures in order to delay the release of pro-biotic bacteria. Suitable pH-sensitive polymers include those which are relatively insoluble and impermeable at the pH of the stomach, but which are more soluble or disintegrable or permeable at the pH of the small intestine and colon. Such pH-sensitive polymers include polyacrylamides, phthalate derivatives such as acid phthalate of carbohydrates, amylose acetate phthalate, cellulose acetate phthalate (CAP), other cellulose ester phthalates, cellulose ether phthalates,

hydroxypropylcellulose phthalate (HPCP), hydroxypropyl-ethylcellulose phthalate (HPECP), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCAS, methylcellulose phthalate (MCP), polyvinyl acetate phthalate (PVAcP), polyvinyl acetate hydrogen phthalate, sodium CAP, starch acid phthalate, cellulose acetate trimellitate (CAT), styrene-maleic acid dibutyl phthalate copolymer, styrene-maleic acid/polyvinylacetate phthalate copolymer, styrene and maleic acid copolymers, polyacrylic acid derivatives such as acrylic acid and acrylic ester copolymers, polymethacrylic acid and esters thereof, polyacrylic and methacrylic acid copolymers, shellac, and vinyl acetate and crotonic acid copolymers. Preferred pH-sensitive polymers include shellac, phthalate derivatives, CAT, HPMCAS, polyacrylic acid derivatives, particularly copolymers comprising acrylic acid and at least one acrylic acid ester, polymethyl methacrylate blended with acrylic acid and acrylic ester copolymers, and vinyl acetate, crotonic acid copolymers alginic acid and alginates such as ammonia alginate, sodium, potassium, magnesium or calcium alginate. A particularly preferred group of pH-sensitive polymers includes CAP, PVAcP, HPMCP, HPMCAS, anionic acrylic copolymers of methacrylic acid and methylmethacrylate, and osmopolymers comprising acrylic acid and at least one acrylic acid ester. Cellulose acetate phthalate may be applied as an enteric coating to the encapsulated pro-biotic compositions of the invention to provide delayed release of pro-biotic bacteria until the dosage form has exited the stomach. The CAP coating solution may also contain one or more plasticizers, such as diethyl phthalate, polyethyleneglycol-400, triacetin, triacetin citrate, propylene glycol, and others as known in the art. Preferred plasticizers are diethyl phthalate and triacetin. The CAP coating formulation may also contain one or more emulsifiers, such as polysorbate-80.

[0017] Anionic acrylic copolymers of methacrylic acid and methylmethacrylate are also particularly useful enteric coating materials for delaying the release of pro-biotic bacteria until they have moved to a position in the GI tract which is distal to the stomach. Copolymers of this type are available from Rohm America, Inc., under the trade names EUDRAGIT-L and EUDRAGIT-S. EUDRAGIT-L and EUDRAGIT-S are anionic copolymers of methacrylic acid and methylmethacrylate. The ratio of free carboxyl groups to the esters is approximately 1:1 in EUDRAGIT-L and approximately 1:2 in EUDRAGIT-S. Mixtures of EUDRAGIT-L and EUDRAGIT-S may also be used. For coating these acrylic coating polymers can be dissolved in an organic solvent or mixture of organic solvents or suspended in aqueous media. Useful solvents for this purpose are acetone, isopropyl alcohol, and methylene chloride. It is generally advisable to include 5-20 wt % plasticizer in coating formulations of acrylic copolymers. Useful plasticizers include polyethylene glycols, propylene glycols, diethyl phthalate, dibutyl phthalate, castor oil, and triacetin. EUDRAGIT-L is preferred because it dissolves relatively quickly at intestinal pH. In addition to the pH-sensitive polymers listed above, delayed release coatings may consist of a mixture or blend of two or more pH-sensitive polymers or may consist of a mixture of one or more pH-sensitive polymers and one or more non-pH-sensitive polymers. Addition of a non-pH-sensitive polymer to the pH-sensitive polymer is useful in modulating the duration of the delay or rate of release of pro-biotic bacteria from the granule, bead or pellets. For example, the delay can be lengthened by blending an aqueous-insoluble polymer with the pH-sensitive polymers, while the delay can be shortened

by blending a water-soluble polymer with the pH-sensitive polymers. Preferred non-pH-sensitive aqueous insoluble polymers include cellulose esters, cellulose ethers, polyacrylates, polyamides, polyesters, and vinyl polymers. Preferred non-pH-sensitive aqueous-soluble polymers include hydroxyalkyl-substituted celluloses such as HPC, HEC and HPMC, PVA, PEG, PEO, PEG/PPG copolymers, and aqueous-soluble polyamides, polysaccharides, and polyacrylates.

[0018] Various additives may be included in such coatings, including emulsifiers, plasticizers, surfactants, fillers and buffers. Finally, the polymeric coating may be described as being "quasi-enteric" in the sense that it remains substantially intact for a significant period of time (e.g., greater than an hour) after the dosage form exits the stomach, thereafter becoming sufficiently pro-biotic bacteria-permeable to permit gradual release of pro-biotic bacteria by diffusion through the coating.

Intermediate Coating

[0019] Optionally a formulation according to the present invention features an intermediate layer between the enteric layer and the outer heat resistant layer. The intermediate coating layer of the composition according to the present invention substantially entirely covers the enteric coating of each individual unit. The intermediate layer is provided in order to prevent direct contact between the enteric layer and the outer heat resistant layer thus preventing any interaction between them. The intermediate coating layer according to any of the embodiments of the present invention optionally and preferably comprises one of aqueous soluble polymers which includes but is not limited to polyvinyls such as povidone (PVP: polyvinyl pyrrolidone), polyvinyl alcohol, copolymer of PVP and polyvinyl acetate, cross-linked polyvinyls, HPC (hydroxypropyl cellulose) (more preferably a low molecular weight), HPMC (hydroxypropyl methylcellulose) (more preferably a low molecular weight), CMC (carboxy methyl cellulose) (more preferably a low molecular weight), ethylcellulose, MEC (methyl ethyl cellulose), CMEC (carboxy methyl ethyl cellulose), HEC (hydroxyethyl cellulose) HEMC (hydroxy methyl ethyl cellulose), polyethylene oxide, acacia, dextrin, magnesium aluminum silicate, starch, polyacrylic acid, polyhydroxyethylmethacrylate (PHEMA), polymethacrylates and their copolymers, gum, water soluble gum, polysaccharides, cross-linked polysaccharides, peptides or cross-linked peptides, protein or cross-linked proteins, gelatin or cross-linked gelatin, hydrolyzed gelatin or cross-linked hydrolyzed gelatin, collagen or cross-linked collagen, modified cellulose, polyacrylic acid or cross-linked polyacrylic acid and/or mixtures thereof.

Outer Heat Resistant Coating

[0020] Such polymers may be linear, branched, or crosslinked. They may be homopolymers or copolymers or graft copolymers or block copolymers, single or a blend. Although they may be synthetic polymers, preferably, such polymer may be naturally occurring polymers such as polysaccharides, cross-linked polysaccharides, gums, modified polysaccharides modified starch and modified cellulose. polysaccharide can be selected from the group consisting of chitin, chitosan, dextran, pullulan, gum agar, gum arabic, gum karaya, locust bean gum, gum tragacanth, carrageenans, gum ghatti, guar gum, xanthan gum and scleroglucan, starches, dextrin and maltodextrin, hydrophilic colloids such

as pectin, high methoxy pectin, low methoxy pectin, phosphatides such as lecithin. The cross-linked polysaccharide can be selected from the group consisting of insoluble metal salts or cross-linked derivatives of alginate, pectin, xanthan gum, guar gum, tragacanth gum, and locust bean gum, carrageenan, metal salts thereof, and covalently cross-linked derivatives thereof. The modified cellulose may be selected from the group consisting of cross-linked derivatives of hydroxypropylcellulose, hydroxypropyl methylcellulose, hydroxyethylcellulose, methylcellulose, carboxymethyl cellulose, and metal salts of carboxymethylcellulose. More preferably such polymers may be cationic polymers. Samples of cationic polymers include but are not limited to cationic polyamines, cationic polyacrylamide, cationic polyethyleneimine, cationic polyvinyl alcohol which is a methyl chloride quaternary salt of poly(dimethylamino ethyl acrylate)/polyvinyl alcohol graft copolymer or a methyl sulfate quaternary salt of poly(dimethylamino ethyl acrylate)/polyvinyl alcohol graft copolymer, a series of dry blends of PVA with N-(3-chloro-2-hydroxypropyl)-N,N,N-trimethylammonium chloride, available from Dow Chemical Company under the name QUAT®-188, containing varying amounts of water and of NaOH, cationic polyvinylpyrrolidone, gelatin, polyvinylpyrrolidone, copolymer of polyvinylacetate and polyvinylpyrrolidone, copolymer of polyvinylalcohol and polyvinylpyrrolidone, polyethyleneimine, polyallylamine and its salts, polyvinylamine and its salts, dicyandiamide-polyalkylenepolyamine condensate, polyalkylenepolyamine-dicyandiamideammonium condensate, dicyandiamide-formalin condensate, an addition polymer of epichlorohydrin-dialkylamine, a polymer of diallyldimethylammonium chloride ("DADMAC"), a copolymer of dimethylaminoethyl methacrylate and neutral methacrylic esters available from Rohm Pharma (Degusa) under the name Eudragit E, a copolymer of diallyldimethylammonium chloride-SO₂, polyvinylimidazole, polyvinylpyrrolidone, a copolymer of vinylimidazole, polyamidine, chitosan, cationized starch, cationic polysaccharides such as cationic guar and cationic hydroxypropyl guar, polymers of vinylbenzyltrimethylammoniumchloride, (2-methacryloyloxy ethyl)trimethylammoniumchloride, polymers of dimethylaminoethyl methacrylate, a polyvinylalcohol with a pendant quaternary ammonium salt, cationic polyvinylformamide cationic polyvinylacetamide, cationic polyvinylmethylformamide, cationic polyvinylmethylacetamide, poly(dimethylaminopropylmethacrylamide) (DMPAM), poly(dimethylaminoethylacrylate), poly(acryloylethyltrimethylammonium chloride), poly(acrylamidopropyltrimethylammonium chloride) (polyAPTAC), poly(methacrylamidopropyltrimethylammonium chloride) (polyMAPTAC), and its salts, poly(vinylpyridine) and its salts, poly(dimethylamine-co-epichlorohydrin), poly(dimethylamine-co-epichlorohydrin-co-ethylendiamine), poly(amidoamine-epichlorohydrin), cationic starch, copolymers which contain N-vinylformamide, allylamine, diallyldimethylammonium chloride, N-vinylacetamide, N-vinylpyrrolidone, N-methyl-N-vinylformamide, N-methyl-N-vinylacetamide, dimethylaminopropyl methacrylamide, dimethylaminoethyl acrylate, diethylaminoethyl acrylate, acryloylethyltrimethylammonium chloride or methacryl amidopropyltrimethylammonium chloride in the form of polymerized units and, if required, in cleaved form, and salts thereof and combinations thereof.

[0021] The invention enables to manufacture various healthy food products without separating the admixing heating steps. Enables is, for example, the preparation of bread dough containing the probiotic granules, avoiding any awkward injecting steps of prior art methods. The mass ratio between the probiotic composition and the rest of the dough may be, for example, 1:100.

[0022] The encapsulated pro-biotic bacteria according to the present invention may be incorporated into flour, flour products, bake goods, yogurt, tuna, frozen baking products, chocolate, hot drinks, nectars and fruit juices, and other products that during the handling and/or production process may be exposed to higher temperature than an ambient (room temperature).

[0023] The invention will be further described and illustrated in the following examples.

EXAMPLES

Example 1

Materials

[0024]

Materials:	Function:
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Hydrogenated vegetable oil	First coat layer agent
Ethylcellulose E100	Second coating layer polymer
Sodium alginate	Second coating layer polymer and heat-resisting polymer
Calcium chloride	Heat-resisting component (cross-linking agent)

Method

1. Absorption of Bacteria on Microcrystalline Core Substrate

[0025] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C.

2. The First Coating Layer Using a Hydrogenated Vegetable Oil

[0026] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose hydrogenated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0027] The coating was carried out using a solution of ethylcellulose E100 and sodium alginate with a ratio of 85:15 respectively in ethanol with a concentration of total solid of 6% (w/w). The end point of the coating process was targeted

to obtain a 20% weight gain by the coating. The coating process was performed using a fluidized bed coater at 40° C.

4. The Third Coating Layer—Heat Resistant Coating

[0028] Calcium alginate was used as heat-resisting polymer for the third coating layer. First an aqueous solution of sodium alginate (3% w/w) and calcium chloride (5% w/w) were separately prepared. Then both sodium alginate and calcium chloride solutions were alternatively sprayed on the resulting coated bacteria until a weight gain of 20% (w/w) was obtained.

Example 2

Materials

[0029]

Ingredients	Function
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Hydrogenated vegetable oil	First coating layer agent
High viscosity sodium alginate	Second coating layer polymer
Chitosan	Heat-resisting polymer
Hydrochloride acid (HCl)	pH-adjusting agent

Method:

1. Absorption of Bacteria on Microcrystalline Core Substrate

[0030] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C. in order to avoid the exposure of bacteria to high temperatures and thus high-temperature damage.

2. The First Coating Layer Using a Hydrogenated Vegetable Oil

[0031] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose hydrogenated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0032] Sodium alginate was used as an enteric polymer. An aqueous solution of sodium alginate (2% w/w) was prepared. The sodium alginate solution was sprayed on resulting coated bacteria until a weight gain of 15% was obtained.

4. The Third Coating Layer—Heat Resistant Coating

[0033] Chitosan was used as the heat-resisting polymer for the third coating layer. First an aqueous solution of chitosan (4% w/w) in pH 5 using HCl was prepared. The resulting

solution was sprayed on the resulting coated bacteria until a weight gain of 20% (w/w) was obtained.

Example 3

Materials

[0034]

Ingredients	Function
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Hydrogenated vegetable oil	First coating layer agent
Low viscosity sodium alginate	Second coating layer polymer
Chitosan	Heat-resisting polymer
Hydrochloride acid (HCl)	pH-adjusting agent

Method:

1. Absorption of Bacteria on Microcrystalline Core Substrate

[0035] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C. in order to avoid the exposure of bacteria to high temperatures and thus high-temperature damage.

2. The First Coating Layer Using a Hydrogenated Vegetable Oil

[0036] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose hydrogenated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0037] Sodium alginate was used as an enteric polymer. An aqueous solution of sodium alginate (2% w/w) was prepared. The sodium alginate solution was sprayed on resulting coated bacteria until a weight gain of 15% was obtained.

4. The Third Coating Layer—Heat Resistant Coating

[0038] Chitosan was used as the heat-resisting polymer for the third coating layer. First an aqueous solution of chitosan (4% w/w) in pH 5 using HCl was prepared. The resulting solution was sprayed on the resulting coated bacteria until a weight gain of 20% (w/w) was obtained.

Example 4

Materials

[0039]

Ingredients	Function
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Saturated vegetable oil	First coating layer agent
High viscosity sodium alginate	Second coating layer polymer
Chitosan	Heat-resisting polymer
Silicon dioxide	Glidant
Hydrochloride acid (HCl)	pH-adjusting agent

Method:

1. Absorption of Bacteria on Microcrystalline Core Substrate

[0040] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C. in order to avoid the exposure of bacteria to high temperatures and thus high-temperature damage.

2. The First Coating Layer Using a Saturated Vegetable Oil

[0041] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose saturated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0042] Sodium alginate was used as an enteric polymer. An aqueous solution of sodium alginate (2% w/w) was prepared. The sodium alginate solution was sprayed on resulting coated bacteria until a weight gain of 15% was obtained.

4. The Third Coating Layer—Heat Resistant Coating

[0043] Chitosan was used as the heat-resisting polymer for the third coating layer. First an aqueous solution of chitosan (4% w/w) in pH 5 using HCl was prepared. Then after complete dissolution of chitosan silicon dioxide (1% w/w) was added. The resulting solution was sprayed on the resulting coated bacteria until a weight gain of 25% (w/w) was obtained.

Example 5

Materials

[0044]

Ingredients	Function
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Hydrogenated vegetable oil	First coating layer agent
High viscosity sodium alginate	Second coating layer polymer
Chitosan	Heat-resisting polymer
Hydrochloride acid (HCl)	pH-adjusting agent

Method:

1. Absorption of Bacteria on Microcrystalline Core Substrate

[0045] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C. in order to avoid the exposure of bacteria to high temperatures and thus high-temperature damage.

2. The First Coating Layer Using a Hydrogenated Vegetable Oil

[0046] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose hydrogenated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0047] Sodium alginate was used as an enteric polymer. An aqueous solution of sodium alginate (2% w/w) was prepared. The sodium alginate solution was sprayed on resulting coated bacteria until a weight gain of 15% was obtained.

4. The Third Coating Layer—Heat Resistant Coating

[0048] Chitosan was used as the heat-resisting polymer for the third coating layer. First an aqueous solution of chitosan (4% w/w) in pH 5 using HCl was prepared. The resulting solution was sprayed on the resulting coated bacteria until a weight gain of 30% (w/w) was obtained.

Example 6

Materials

[0049]

Ingredients	Function
<i>Lactobacillus acidophilus</i>	A Probiotic bacteria
<i>Bifidobacterium</i>	A Probiotic bacteria

-continued

Ingredients	Function
Microcrystalline cellulose (MCC)	Core substrate
Maltodextrin	Supplement agent for the bacteria
Trehalose	Supplement agent for the bacteria
Hydrogenated vegetable oil	First coating layer agent
High viscosity sodium alginate	Second coating layer polymer
Chitosan	Heat-resisting polymer
Hydrochloride acid (HCl)	pH-adjusting agent

Method:**1. Absorption of Bacteria on Microcrystalline Core Substrate**

[0050] *Lactobacillus acidophilus* and *Bifidobacterium* were absorbed on MCC substrate based on a ratio of 38:62 respectively. For this purpose an aqueous-based suspension of 30% of the bacteria and maltodextrin and trehalose was prepared. The concentration of bacteria was about 15% (w/w) in that suspension. The absorption process was carried out at an outlet temperature <35° C. in order to avoid the exposure of bacteria to high temperatures and thus high-temperature damage.

2. The First Coating Layer Using a Hydrogenated Vegetable Oil

[0051] The coating was carried out using a fluidized bed coater based on a Hot-Melt method. For this purpose hydrogenated vegetable oil was sprayed on the Bacteria-absorbed MCC substrate at 40° C. to obtain a 40% weight gain. The inlet air flow was adjusted to be low.

3. The Second Coating Layer—an Enteric Coating

[0052] Sodium alginate was used as an enteric polymer. An aqueous solution of sodium alginate (2% w/w) was prepared. The sodium alginate solution was sprayed on resulting coated bacteria until a weight gain of 25% was obtained.

4. The Third Coating Layer—Heat Resistant Coating

[0053] Chitosan was used as the heat-resisting polymer for the third coating layer. First an aqueous solution of chitosan (4% w/w) in pH 5 using HCl was prepared. The resulting solution was sprayed on the resulting coated bacteria until a weight gain of 20% (w/w) was obtained.

Example 7

[0054] Encapsulated probiotic bacteria granules were tested for heat resistance. Accordingly, the resulting encapsulated bacteria granules from Example 6 were exposed to 85° C. for 45 minutes. Then CFU/g was determined using a counting procedure described as follows.

[0055] *Lactobacillus acidophilus* and *Lactobacillus bifidus* counting procedure:

[0056] 10 g of sample was suspended in 90 ml phosphate buffer and placed in a Stomacher for 10 min. Then the resulting suspension was shaken for 90 min. The mixture was then serially (decimally) diluted and finally poured into an appropriate plate culture media. MRS growth media containing either cystein or maltose were respectively used for *acidophilus* and *bifidus*. The resulting plates were then incubated for 3

days under anaerobic conditions. Finally the bacteria were counted and CFU/g was calculated accordingly.

[0057] Results:

	<i>Lactobacillus acidophilus</i>	<i>Bifidobacterium bifidum</i>
Uncoated—before coating process*(initial CFU/g)	3.6×10^{10}	7.2×10^9
After coating **(CFU/g)	1.6×10^7	1.2×10^7
After Heating*** (CFU/g)	1.4×10^7	5.4×10^6

*The weight ratio between two bacteria types in the final product is 1:1.

**The bacteria blend constitutes 10% (w/w) of the final product.

***The heating process was carried out at 80° C. for 45 minutes.

[0058] While this invention has been described in terms of some specific examples, many modifications and variations are possible. It is therefore understood that within the scope of the appended claims, the invention may be realized otherwise than as specifically described.

1. A probiotic granule comprising

- a core comprising probiotic bacteria and a substrate in which said bacteria are absorbed;
- an inner layer of vegetable oil coating said core; and
- two outer layers, coating said core and said inner layer, comprising at least two different polymers.

2. A probiotic granule according to claim 1, wherein said substrate and said two different polymers are nutritionally acceptable polysaccharides.

3. A probiotic granule according to claim 1, wherein said core further comprises one or more supplemental agents for said bacteria.

4. A probiotic granule according to claim 3, wherein said agents are prebiotic oligosaccharides.

5. A probiotic granule according to claim 1, wherein one of said outer layers comprises a fibrous polysaccharide.

6. A probiotic granule according to claim 1, wherein one of said outer layers comprises a gelatinous polysaccharide.

7. A probiotic granule according to claim 1, wherein said bacteria comprise a genus selected from *Lactobacillus* and *Bifidobacterium*.

8. A method for manufacturing the granule of claim 1, comprising

- mixing a suspension of prebiotic bacteria with a cellulose-based substrate and with supplemental agents for the bacteria, thereby obtaining a core mixture;
- coating particles of said core mixture with a vegetable oil, thereby obtaining oil-coated particles;
- coating said oil-coated particles with a first polymer layer, which first polymer layer confers stability to said bacteria under the conditions of upper gastrointestinal tract, thereby obtaining particles coated with two layers; and
- coating said two-layer particles with a second polymer layer, which second polymer layer increases the stability of the bacteria in said core under the conditions of baking.

9. A method according to claim 8, wherein each of said coating steps ii) to iv) result in a mass increase of from 10 to 100% relatively to the mass of the core.

10. A method according to claim 8, comprising

- mixing an aqueous suspension of prebiotic bacteria comprising at least one strain of *Lactobacillus* or *Bifidobac-*

terium genus with microcrystalline cellulose, maltodextrin, and trehalose, thereby obtaining a core mixture;

ii) coating particles of said core mixture with a hydrogenated vegetable oil, thereby obtaining oil-coated particles;

iii) coating said oil-coated particles with a first polysaccharide layer and with a second polysaccharide layer;

wherein said two polysaccharides layers are different and comprise at least two of cellulose, alginate, chitosan, or a mixture thereof.

11. A prebiotic composition comprising granules according to claim 1.

12. A prebiotic composition according to claim 11, exhibiting high heat resistance and long storage stability.

13. A food product or a food additive comprising a prebiotic granule according to claim 1.

14. A food product according to claim 13, selected from the group consisting of pastry, bread, flour, flour products, baked goods, frozen baking products, yogurt, dairy products, chocolate, nectars, fruit juices, and tuna.

15. A food product according to claim 14, exposed to higher than ambient temperature during the production process.

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