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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to stable probiotic compositions for special dietary uses, for example, an infant formula.

BACKGROUND OF THE INVENTION

[0002] There are currently a variety of probiotic microorganisms (also called probiotics) for supplementing gastrointestinal tracts of animals, including humans. These microorganisms may modulate a natural microflora within an animal's gut for a desirable biological effect.

[0003] One of the challenges to providing an effective amount of probiotic bacteria to a host is the preservation of their viability under the harsh conditions of typical industrial manufacturing processes and long-term storage at high temperature and humidity. Although there have been developments concerning encapsulation and formulation techniques for delivery of biological materials into digestive systems of animals, there has been little development in encapsulation or stabilization techniques that protect the viability of probiotics during manufacturing processes, distribution and storage. There is a need for a stabilization technique that enables probiotic bacteria to survive upon exposure to various harsh environments, especially those associated with elevated temperature and humidity.

[0004] In addition, the inherent moisture of a probiotic product itself poses another challenge in that probiotics generally are sensitive to water activity, especially in combination with high temperature. To date, no technology or technique has been identified to provide significant protection of probiotics under intermediate moisture conditions (i.e., water activity of about 0.2 and higher, or up to about 0.4 or higher) and high temperatures during distribution and storage (e.g., temperatures of at least about 30°C, or up to about 40°C or higher) when incorporated into products such as nutritional products. As such, there is a need for stable probiotic compositions suitable for distribution in various geographic locations, including those in tropical climates, where the viability of probiotics could be compromised.

[0005] Additional challenges include regulatory limitations on the use of conventional food ingredients in special dietary formulations suitable for consumption by people like infants, young children and elderly people. Conventional synthetic encapsulation and stabilizing compounds and even some natural compounds such as gum acacia, alginate, milk proteins and certain sugars such as trehalose are not recommended for use in these special dietary formulations. A recommended list of nutritional compounds allowed for special dietary uses is regulated by the joint FAO/WHO Codex Alimentarius Commission.

[0006] US 2013/296165 A1 discloses dry probiotic compositions comprising hydrolyzed pea protein, sucrose at 42.6 % of the matrix, cyclodextrin, alginate and sodium alginate and showing good viability.

[0007] CA 2 420 095 A1 relates to methods of protection microorganisms against lethal and sub-lethal damage caused by exposure to low temperatures. Protection involves oligo/polysaccharides.

[0008] What is desired therefore are stable probiotic compositions suitable for special dietary uses comprising probiotic microorganisms such as probiotic bacteria and other ingredients and stabilization techniques for making such compositions.

SUMMARY OF THE INVENTION

[0009] The present invention provides stable dry probiotic compositions for special dietary uses and their preparation methods as set forth in the claims.

[0010] According to one aspect of the invention, a dry composition is provided comprising one or more viable probiotic microorganisms, one or more hydrolyzed proteins, one or more disaccharides, one or more oligosaccharides, and one or more polysaccharides, wherein the composition does not comprise trehalose,
wherein the composition comprises at least 40% of the one or more hydrolyzed proteins, based on the total dry weight of the composition, and the one or more hydrolyzed proteins are selected from the group consisting of hydrolyzed casein, hydrolyzed whey protein, hydrolyzed pea protein, hydrolyzed soy protein, and combinations thereof,
wherein the composition comprises less than 30% of the one or more disaccharides, based on the total dry weight of the composition, and the one or more disaccharides are selected from the group consisting of sucrose, lactose, and combinations thereof,
wherein the composition comprises 1-30% of the one or more oligosaccharides, based on the total dry weight of the composition, and the one or more oligosaccharides are selected from the group consisting of inulin, maltodextrins, dextrans, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS), and combinations thereof,
wherein the composition comprises 0.1-40% of the one or more polysaccharides, based on the total dry weight of the composition, and the one or more polysaccharides are selected from the group consisting of carrageenan, guar gum, gum acacia, locust bean gum, starches, modified starches, and combinations thereof,
wherein the composition has viability of at least $1x10^{10}$ CFU/g, and wherein the composition has a viability loss of less than 1 log unit/g after 3 months at a temperature of 40 °C and a relative humidity of 33%.

[0011] The composition may provide a probiotic benefit to a host in a special dietary product. The special dietary product may be selected from the group consisting of an infant formula, a

follow-on formula, processed cereal based food, canned baby food, an animal supplement or treatment, and/or a special food for a medical purpose. In particular embodiments, the special dietary product is an infant formula.

[0012] The viable probiotic microorganism may be selected from the group consisting of live probiotic bacteria, fungi, and yeast.

[0013] The composition may comprise at least 50% of the one or more hydrolyzed proteins, based on the total dry weight of the composition. The one or more hydrolyzed proteins may be selected from the group consisting of milk proteins, plant proteins, and combinations thereof. The one or more hydrolyzed proteins may be selected from the group consisting of hydrolyzed casein, hydrolyzed whey protein, hydrolyzed pea protein, hydrolyzed soy protein, and combinations thereof.

[0014] The composition may comprise less than 20% of the one or more disaccharides, based on the total dry weight of the composition. The one or more disaccharides may be selected from the group consisting of sucrose, lactose, and combinations thereof.

[0015] The composition may comprise 5-30% of the one or more oligosaccharides, based on the total dry weight of the composition. The one or more oligosaccharides may be selected from the group consisting of inulin, maltodextrins, dextrans, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS), and combinations thereof.

[0016] The composition may comprise 1-10% of the one or more polysaccharides, based on the total dry weight of the composition. The one or more polysaccharides may be selected from the group consisting of carrageenan, guar gum, gum acacia, locust bean gum, starches, modified starches, and combinations thereof.

[0017] The composition may further comprise one or more additional agents. The composition may comprise 0.5-10% of the one or more additional agents, based on the total weight of the composition. The one or more additional agents may be selected from the group consisting of carboxylic acid salts, tocopherols, and combinations thereof. The carboxylic acid salts may be selected from the group consisting of ascorbic acid salts and citric acid salts. The one or more additional agents may comprise one or more tocopherols and one or more carboxylic acid salts at a weight ratio from 1:4 to 4:1. Preferably, the one or more additional agents comprise vitamin E and sodium ascorbate at a weight ratio of 4:1.

[0018] According to another aspect of the invention, a method for preparing the composition of the present invention is provided. The method comprises one or more drying processes selected from the group consisting of air drying, vacuum-drying, fluid bed drying and spray-drying.

[0019] According to yet another aspect of the invention, a method for preparing the composition of the present invention is provided. The method comprises: (a) combining the

one or more viable probiotic microorganisms, the one or more hydrolyzed proteins, the one or more disaccharides, the one or more oligosaccharides, and the one or more polysaccharides in an alkali aqueous solvent to form a slurry; (b) snap-freezing the slurry in liquid nitrogen to form solid frozen particles in the form of beads, droplets or strings; (c) primary drying step of the solid frozen particles by evaporation, under vacuum, while maintaining the temperature of the particles above their freezing temperature, whereby a primarily dried formulation is formed; and (d) secondary drying of the primarily dried formulation at full strength vacuum and a heat source temperature of 20°C or higher for a time sufficient to reduce the water activity of the primarily dried formulation to 0.3 Aw or lower. As a result, the composition of the invention is prepared. The method may further comprise sterilizing the one or more hydrolyzed proteins, the one or more disaccharides, the one or more oligosaccharides, and the one or more polysaccharides before step (a). The method may further comprise cutting, crushing, milling or pulverizing the composition into a free flowing powder. The particle size of the powder may be less than about 1000 µm.

[0020] In the method of the present invention, the composition may comprise an effective amount of the one or more viable probiotic microorganisms for providing a probiotic benefit to a host in a special dietary product. The method may further comprise making the special dietary product with the composition. The special dietary product may be selected from the group consisting of an infant formula, a follow-on formula, processed cereal based food, canned baby food, an animal supplement or treatment, and/or a special food for a medical purpose. In particular embodiments, the special dietary product is preferably an infant formula.

BREIF DESCRIPTION OF THE DRAWINGS

[0021] Figure 1 shows storage stability of samples of Example 2 under accelerated storage conditions of 40°C and 33%RH.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention provides novel stable dry probiotic compositions, preferably for special dietary uses, and methods for making such compositions. These compositions provide desirable stability and protection to probiotic microorganisms. The probiotic microorganisms may be protected during manufacturing processes for making consumable products, through distribution channels, and under extreme storage conditions. Most probiotic formulators utilize in their products an extremely high count of bacterial cells, which may sometimes be as high as 10 and even 100 times more than an effective dose, with the understanding that a significant number of the cells ultimately lose viability and die during the manufacturing processes, transportation, and storage.

[0023] The term "special dietary use" as used herein refers to making or applying a special

dietary product to a host. Preferably, the special dietary product is recommended by the joint FAO/WHO Codex Alimentarius Commission in a document entitled "Standard For Infant Formula and Formulas For Special Medical Purposes Intended for Infants, CODEX STAN 72-1981" ("US Standard Codex 72"). Examples of a special dietary product include an infant formula, a follow-on formula, processed cereal based food, canned baby food, and special food for a medical purpose. Preferably, the special dietary product is an infant formula.

[0024] The host may be any animal, including a fish, an avian, e.g., a chicken, or a mammal such as a ruminant, a pig, or a companion animal such as an equine, canine, or feline. In particular embodiments the mammal is a human. The human host may be an infant, a child or an elderly person. Preferably, the human host is an infant.

[0025] The term "infant" as used herein refers to a human from birth to about 12 months old.

[0026] The term "child" as used herein refers to a human from about 12 months old to about 12 years old.

[0027] The term "elderly person" as used herein refers to a human at least about 55, 60, 65 or 70 years old, preferably at least about 65 years old.

[0028] The terms "probiotic microorganism" and "probiotic" are used herein interchangeably, and refer to a live microorganism that provides or confers a probiotic benefit to a host when administered to the host in an effective amount. The term "effective amount" as used herein refers to an amount of a probiotic microorganism that is sufficient to achieve a desirable probiotic benefit in a host when administered to the host via, for example, a dietary product such as a special dietary product. The probiotic microorganism may be selected from the group consisting of live probiotic bacteria, fungi, and yeast. The desirable probiotic benefit may be any beneficial health or nutritional effect, for example, maintaining a healthy gastrointestinal flora, enhancing immunity, preventing allergies and cold and protecting against diarrhea, atopic dermatitis and urinary infections.

[0029] The term "viability" as used herein refers to the ability of a probiotic microorganism in a composition to form colonies on a nutrient media appropriate for the growth of the probiotic microorganism, and may be expressed as colony forming units (CFU) over the weight of the composition, e.g., CFU/g.

[0030] The term "relative humidity (RH)" as used herein refers to the amount of water vapor in the air, often at a given temperature. Relative humidity is usually less than that is required to saturate the air, and is often expressed in percentage of saturation humidity.

[0031] The term "dry" as used herein refers to a physical state of a substance, for example, the composition of the present invention, that is dehydrated or anhydrous, e.g., substantially lacking liquid. The substance, for example, the composition of the present invention, may be dried by one or more drying processes, for example, air drying, vacuum drying, fluidized bed

drying, spray drying, and lyophilization.

[0032] The term "water activity (Aw)" as used herein refers to the availability of water in a substance, for example, the composition of the present invention, which represents the energy status of water in the substance. It may be defined as the vapor pressure of water above a substance divided by that of pure water at the same temperature. Pure distilled water has a water activity of exactly one, i.e., Aw=1.0. A dry substance may have an Aw of about 0.5 or lower, preferably about 0.3 or lower, more preferably about 0.2 or lower, most preferably about 0.1 or lower.

[0033] A dry composition is provided. The composition comprises one or more viable probiotic microorganisms, one or more hydrolyzed proteins, one or more disaccharides, one or more oligosaccharides, and one or more polysaccharides. The composition has an initial viability of at least $1x10^9$, $1x10^{10}$, $1x10^{11}$ or $1x10^{12}$ CFU/g, preferably $1x10^{10}$ CFU/g. The composition has a viability loss of less than 1 log unit/g after a predetermined period of time under predetermined conditions. Preferably, the composition does not comprise trehalose.

[0034] The composition may comprise an effective amount of the one or more viable probiotic microorganisms for providing a probiotic benefit to a host in a special dietary product. The special dietary product may be an infant formula, a follow-on formula, processed cereal based food, canned baby food, or special food for a medical purpose, preferably an infant formula.

[0035] The predetermined period of time may be about 1, 2, 3 or 4 weeks, or 1, 2, 3, 4, 5, 6, 12, 18, 24 or 36 months, preferably about 1, 2 or 3 months, more preferably 1 or 3 months. A specified time period may include a shorter or longer time period that is within 10% of the specified time period. The term "3 months" as used herein refers to a time period of about 84-90 days. The term "2 months" as used herein refers to a time period of about 56-60 days. The term "1 month" as used herein refers to a time period of about 28-30 days.

[0036] The predetermined conditions may include a predetermined temperature and a predetermined relative humidity (RH). The predetermined temperature may be at least about 25, 37, 40, 45, 50 or 55 °C. The predetermined relative humidity (RH) may be at least about 10%, 20%, 30%, 33%, 35%, 40%, 50%, 60%, 70% or 80%.

[0037] The predetermined conditions may be accelerated storage conditions. For example, the predetermined conditions may include at least about 40 °C and at least about 33%RH, or at least about 45 °C and at least about 33%RH.

[0038] The composition may have a viability loss of less than 1 log unit/g after about 3 months at about 40 °C and 33%RH, or after 1 month at about 45 °C and 33%RH.

[0039] The composition may comprise about 1-30%, 10-25%, 10-20% or 15-20% of the one or more viable probiotic microorganisms, based on the total dry weight of the composition. Suitable probiotic microorganisms include, but are not limited to, yeasts such as

Saccharomyces, Debaromyces, Candida, Pichia and *Torulopsis;* moulds such as *Aspergillus, Rhizopus, Mucor, Penicillium* and *Torulopsis;* and bacteria such as the genera *Bifidobacterium, Clostridium, Fusobacterium, Melissococcus, Propionibacterium, Streptococcus, Enterococcus, Lactococcus, Staphylococcus, Peptostrepococcus, Bacillus, Pediococcus, Micrococcus, Leuconostoc, Weissella, Aerococcus, Oenococcus* and *Lactobacillus.* Specific examples of suitable probiotic microorganisms may be represented by the following species and include all culture biotypes within those species: *Aspergillus niger, A. oryzae, Bacillus coagulans, B. lentus, B. licheniformis, B. mesentericus, B. pumilus, B. subtilis, B. natto, Bacteroides amylophilus, Bac. capillosus, Bac. ruminocola, Bac. suis, Bifidobacterium adolescentis, B. animalis, B. breve, B. bifidum, B. infantis, B. lactis, B. longum, B. pseudolongum, B. thermophilum, Candida pintolepesii, Clostridium butyricum, Enterococcus cremoris, E. diacetylactis, E. faecium, E. intermedius, E. lactis, E. muntdii, E. thermophilus, Escherichia coli, Kluyveromyces fragilis, Lactobacillus acidophilus, L. alimentarius, L. amylovorus, L. crispatus, L. brevis, L. case L. curvatus, L. cellobiosus, L. delbrueckii ss. bulgaricus, L farciminis, L. fermentum, L. gasseri, L. helveticus, L. lactis, L. plantarum, L. johnsonii, L. reuteri, L. rhamnosus, L. sakei, L. salivarius, Leuconostoc mesenteroides, P. cereviseae (damnosus), Pediococcus acidilactici, P. pentosaceus, Propionibacterium freudenreichii, Prop. shermanii, Saccharomyces cereviseae, Staphylococcus carnosus, Staph, xylosus, Streptococcus infantarius, Strep. salivarius. thermophilus, Strep. Thermophilusand and Strep. Lactis.* Preferably, the probiotics are lactic acid bacteria and bifido bacteria.

[0040] The composition may comprise at least about 40% or 50%, preferably at least about 50% of the one or more hydrolyzed proteins, based on the total dry weight of the composition. For example, the composition may comprise about 40-80%, 40-70%, 50-70% or 50-60%, preferably 40-80%, of the hydrolyzed protein.

[0041] The terms "hydrolyzed protein" and "protein hydrolysate" are used herein interchangeably, and refer to proteins broken down by hydrolysis or digestion into shorter peptide fragments and/or amino acids. The hydrolysis or digestion may be carried out by a strong acid, a strong base, an enzyme or a combination thereof. The hydrolyzed protein may be from an animal or a plant, preferably from a mammal, more preferably from a dairy source. The hydrolyzed proteins may be milk proteins, plant proteins, or a mixture thereof.

[0042] The hydrolyzed protein may be partially or extensively hydrolyzed, preferably extensively hydrolyzed. The hydrolyzed protein may be a mixture of polypeptides and amino acids. In some embodiments, at least about 60%, 70%, 80%, 90%, 95% or 99%, preferably at least about 70%, of the hydrolyzed protein has a molecular weight lower than about 100,000, 75,000, 50,000, 25,000, 10,000, 5,000, 1,000 or 500 Dalton, preferably about 50,000 Dalton, more preferably about 10,000 Dalton, more preferably about 2,000 Dalton. For example, at least about 50%, 60%, 70%, 80% or 90%, preferably at least about 70%, of the hydrolyzed protein has a molecular weight lower than about 2,000 Daltons.

[0043] Proteins suitable for making hydrolyzed proteins for the composition of the present invention include egg proteins, gelatin, milk proteins, casein, whey protein, albumen, soy

protein, pea protein, rice protein, wheat protein, and other plant proteins. Preferably, the proteins are those recommended for special dietary uses.

[0044] Examples of the hydrolyzed proteins include hydrolyzed casein, hydrolyzed whey protein, hydrolyzed pea protein, hydrolyzed soy protein, and combinations thereof. In one preferred embodiment, the hydrolyzed protein comprises hydrolyzed casein or pea proteins, at least about 80% of which has a molecular weight of less than about 2,000 Daltons.

[0045] The composition comprises a carbohydrate mixture of disaccharides, oligosaccharides and polysaccharides, in which the probiotic microorganism is embedded. Without being bound by theory, it is believed that a matrix formed by combining a carbohydrate mixture and extensively hydrolyzed proteins as described herein allows faster drying and contributes to a desirable amorphous and rigid structure of the resulting dry composition.

[0046] The composition comprises less than 30%, and may comprise less than about 20% or 10%, preferably less than 20%, of the one or more disaccharides, based on the total dry weight of the composition. For example, the composition may comprise about 1-30%, 1-20%, 1-10%, 5-30%, 5-20%, 5-10%, 10-20%, 10-15% or 10-20%, preferably about 5-30%, of the disaccharide.

[0047] The disaccharides are preferably those recommended for special dietary uses. The disaccharide may be lactose, sucrose, maltose, fructose, or a combination thereof, preferably lactose or sucrose, more preferably lactose. The disaccharide is preferably not trehalose. In some preferred embodiments, the composition of the invention does not comprise trehalose.

[0048] The composition comprises 1-30%, and may comprise about 1-20%, 1-10%, 5-30%, 5-20%, 5-10%, 10-20%, 10-15% or 10-20% of the one or more oligosaccharides, based on the total dry weight of the composition. Preferably, the composition comprises 5-30% of the oligosaccharides.

[0049] Oligosaccharides are soluble fibers often considered as prebiotics in nutritional applications. Advantageously, soluble fibers pass through the stomach undigested and become available for digestion by the gut microflora. The incorporation of soluble fibers may also help to protect the probiotic from digestive enzymes and high acidity of the stomach.

[0050] The oligosaccharides are preferably those recommended for special dietary uses. The oligosaccharide may be inulin, maltodextrin, dextran, fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), mannan-oligosaccharide (MOS), or a combination thereof, preferably maltodextrin or inulin, more preferably inulin.

[0051] The composition comprises 0.1-40%, and may comprise about 0.5-30%, 1-30%, 1-20%, 1-10%, 1-5% or 5-10% of the one or more polysaccharides, based on the total dry weight of the composition. Preferably, the composition comprises 1-10% of the polysaccharide. The polysaccharides are preferably those recommended for special dietary uses. The

polysaccharide may be carrageenan, guar gum, gum acacia, locust bean gum, starch, modified starch, or a combination thereof, preferably locust bean gum or guar gum, more preferably locust bean gum. Preferably, the polysaccharide is not alginate or chitosan. In some preferred embodiments, the composition does not comprise alginate or chitosan. In some other preferred embodiments, the composition does not comprise trehalose or alginate.

[0052] In some embodiments, the composition comprises 0.1-20% of polysaccharides, 5-30% of oligosaccharides, and 1-20% of disaccharides, on the total dry weight of the composition. In particular, the composition may comprise 0.1-20% of locust bean gum, 5-30% of Inulin and 1-20% lactose, based on the total dry weight of the composition.

[0053] The composition of the present invention may further comprise one or more additional agents. The additional agent may provide an additional benefit to the probiotic microorganism, the host or both. For example, the additional agent may provide a therapeutic or immunogenic effect to the host. The addition agent may be selected from the group consisting of vitamins, antioxidants, trace elements, sterols, magnesium stearate, fumed silica, surfactants, peptides and steroids and combinations thereof.

[0054] The composition may comprise 0.1-20%, 0.5-20%, 1-20%, 0.1-10%, 0.5-10%, 1-10% or 1-5% of the additional agent, based on the total weight of the composition. The additional agent is preferably an agent recommended for special dietary uses.

[0055] The additional agent may be selected from the group consisting of carboxylic acid salts, tocopherols, and combinations thereof. The carboxylic acid salts may be selected from the group consisting of ascorbic acid salts and citric acid salts. In some embodiments, the additional agent comprises one or more tocopherols and one or more carboxylic acid salts at a weight ratio from 4:1 to 1:4. For example, the additional agent comprises vitamin E and sodium ascorbate at a weight ratio of 4:1.

[0056] In some embodiments, the composition comprises 40-80% hydrolyzed proteins, 5-30% disaccharides, 5-30% oligosaccharides and 1-10% polysaccharides, based on the total weight of the composition. In a preferred embodiment, the composition comprises 54% of hydrolyzed pea protein, 8% lactose, 14% inulin and 3% locust bean gum, based on the total weight of the composition. The composition may further comprise 4% of an additional agent comprising vitamin E and sodium ascorbate at a weight ratio of 4:1, based on the total weight of the composition.

[0057] The composition of the present invention may be prepared by techniques known in the art. The preparation method may include processes such as mixing, freezing, freeze-drying, ambient air drying, vacuum drying, spray drying, or a combination thereof. The resulting probiotic composition, whether alone or integrated into a special dietary product, possesses enhanced viability when exposed to a wide range of temperatures and humidity conditions.

[0058] The probiotic microorganism used to prepare the composition is preferably a

fermentation harvest that is concentrated to a wet paste-like consistency having a solid content of about 5-30% w/v. The probiotic concentrate can be in a form of wet, frozen or thawed paste before being combined with other ingredients. Starting with a probiotic microorganism in a dry form is an alternative.

[0059] The preparation of a stable probiotic composition may include concentrating a selected probiotic, mixing ingredients with the concentrated probiotic to form a slurry, snap-freezing the slurry in liquid nitrogen to form particles in the form of droplets, strings or beads, drying the particles by evaporating the moisture in the particles under a regimen of reduced pressure while supplying heat to the particles, and then packaging or combining the resulting stable probiotic composition into a special dietary product, which may be a nutritional product such as an infant formula.

[0060] One suitable mixing process may be adding a dry mixture of all ingredients except the probiotic microorganism in the composition directly into a concentrate culture or media solution comprising the probiotic microorganism to form a slurry. The dry mixture may be pre-dissolved in a water solution adjusted to pH of 8-9 with a concentrated alkali solution (e.g., 1M or 5M sodium hydroxide (NaOH) solution) at 20-80 °C. In the slurry, the dry weight mass of the probiotic microorganism may constitute about 5-30% w/v while the dry mixture may constitute about 70-95% or 80-90% w/v. The total solid content in the slurry may be about 20-60% or 30-50%. The amount of polysaccharides in the dry mixture may be adjusted to achieve a desired viscosity of the slurry allowing efficient drying while avoiding rubbery formation or excessive foaming that may occur during drying. A desirable density of the slurry may be achieved by any means known in the art, for example, by degassing under vacuum or injecting gas such as air, nitrogen, carbon dioxide, or argon.

[0061] The slurry may be snap-frozen to from about -30°C to about -180°C, or snap-frozen in liquid nitrogen by atomizing, dripping or injecting into a liquid nitrogen bath. The resulting particles in the form of beads, strings or droplets may be collected and dried in a freeze drier or vacuum drier, or alternatively stored in a deep freezer (e.g., between -30°C and -80°C) for later use in a frozen form or for later drying, e.g., by freeze drying or vacuum drying.

[0062] In general, the drying process techniques that are useful include freeze drying, or evaporative drying of a thawed slurry in a vacuum oven or centrifugal evaporator while the temperature of the slurry or the drying product is maintained above its freezing temperature (e.g., -20 to -5 °C), followed by milling to desirable particle size. Preferably, the probiotic microorganism is coated by non-crystallized amorphous materials in the particles. The advantage of coating the probiotic microorganism with materials in an amorphous state is to increase physical stability of the particles and reduce deleterious crystalline formation within the particles. It should be noted that achieving a non-crystallized amorphous structure is not a prerequisite for long term stability as some microorganisms may fare better in a more crystalline state. In a suitable exemplary embodiment, the snap-frozen slurry may be loaded onto trays at a loading capacity from about 0.1 kg/sqft to about 1.5 kg/sqft and then immediately transferred to a vacuum drying chamber where the drying process may proceed in

three major steps including: (a) an optional short temperature acclimation and structure stabilizing step of the frozen particles under a vacuum pressure of less than <1000 mTORR, (b) primary drying, or primary evaporative drying, under vacuum and at a temperature of the particles above their freezing point, and (c) secondary drying under full strength vacuum pressure and an elevated heat source temperature for a time sufficient to reduce the water activity of the resulting dry composition to, for example, 0.3 Aw or less. The resulting dry composition may be glassy amorphous.

[0063] The terms "lyophilization" and "freeze drying" are used herein interchangeably and refer to the preparation of a composition in dry form by rapid freezing and dehydration in the frozen state (sometimes referred to as sublimation). Lyophilization takes place at a temperature that results in the crystallization of ingredients in the composition.

[0064] The term "primary drying" as used herein refers to drying a product at a temperature of the product substantially lower than the temperature of a heat source, i.e., heat source temperature or shelf temperature, to make a primarily dried product. Typically, the bulk of primary drying may be carried out by extensive evaporation, while the product temperature remains significantly lower than the temperature of the heat source.

[0065] The term "secondary drying" as used herein refers to drying a primarily dried product at a temperature of the product near the temperature of a heat source, i.e., heat source temperature or shelf temperature, to make a dry product. This process may take place under vacuum sufficient to reduce the water activity of the resulting dry product. In a typical drying process, a secondary drying step reduces the water activity of the formulation to, for example, an Aw of 0.3 or less.

[0066] In one embodiment, the composition of the present invention is prepared by a method comprising (a) combining one or more viable probiotic microorganisms, one or more hydrolyzed proteins, one or more disaccharides, one or more oligosaccharides, and one or more polysaccharides in an alkali aqueous solvent to form a slurry; (b) snap-freezing the slurry in liquid nitrogen to form solid frozen particles in the form of beads, droplets or strings; (c) primary drying step of the solid frozen particles by evaporation, under vacuum, while the temperature of the particles is maintained above their freezing temperature, whereby a primarily dried formulation is formed; and (d) secondary drying of the primarily dried formulation at full strength vacuum and a heat source temperature of 20°C or higher for a time sufficient to reduce the water activity of the primarily dried formulation to 0.3 Aw or lower, whereby the composition is prepared.

[0067] The method may further comprise sterilizing the one or more hydrolyzed proteins, the one or more disaccharides, the one or more oligosaccharides and the one or more polysaccharides before step (a). The sterilization may be achieved by any method known in the art. For example, heating under pressure a mixture of the hydrolyzed protein, the disaccharide, the oligosaccharide and the polysaccharide, and followed by cooling before step (a).

[0068] The method may further comprise solubilizing the one or more hydrolyzed proteins, the one or more disaccharides, the one or more oligosaccharides and the one or more polysaccharides before step (a).

[0069] The method may further comprise cutting, crushing, milling or pulverizing the composition into a free flowing powder. The particle size of the powder may be less than about 10,000, 1,000, 500, 250 or 100 µm, preferably less than about 1,000 µm, more preferably less than about 250 µm.

[0070] The dry composition of the present invention may be used directly as a flake, or grounded into a powder and sieved to an average particle size from about 1-10,000 µm, preferably 10-1,000 µm.

[0071] The composition of the present invention may be administrated as a concentrated powder or a reconstituted liquid (e.g., a beverage). It may also be incorporated either in flake or powder form into an existing food product.

[0072] The method may further comprise making a special dietary product with the composition of the present invention, which comprises an effective amount of the one or more viable probiotic microorganisms for providing a probiotic benefit to a host in the special dietary product. Examples of the special dietary product may include an infant formula, a follow-on formula, processed cereal based food, canned baby food, and special food for a medical purpose. Preferably, the special dietary product is an infant formula.

[0073] The resulting dry stable powder comprising probiotics may be agglomerated with molten fats. The dry powder may be placed in a planetary mixer at 40°C and molten fats such as cocoa butter, natural waxes or palm oil, stearic acid, stearine or a mixture thereof may be added slowly to the warm powder. The mixture may be cooled down to below the melting temperature of the fats while mixing continues until a visually uniform size of agglomerated powder is achieved. The weight mass of the molten fats in the resulting composition may be about 20-70%, preferably 30-50%.

EXAMPLE 1. Preparation of dry and stable composition

[0074] Hydrolyzed pea protein (65 g, Marcor, Carlstadt, NJ) was dissolved in 100 ml warm distilled water (75°C). The pH of the pea solution was adjusted to 8.5 using a 20% concentrated NaOH solution. Locust Bean gum (3 g, Tic gum, Belcamp, MD), lactose (10 g, Foremost Farms, Rothschild, WI), Inulin (17 g, Cargill Minneapolis, MN), a mixture of vitamin E and sodium ascorbate at 4:1 w/w (5 g) were dry blended and added to the pea solution under continuous mixing at 500 rpm with an impeller mixer. The solution was cooled down and maintained at a temperature between 35°C and 40°C under continuous mixing.

[0075] This resulted stabilizing composition was translucent with a consistency of syrup and amber in color. The syrupy solution was transferred to a dual planetary mixer (DPM, 1qt, Ross Engineering, Inc. Savannah, GA) equipped with controlled temperature jacket. The mixer jacket temperature was 37°C. Frozen bacteria (100g, Bifidobacterium sp.) were added under mixing at 45 rpm for 2-3 minutes, or until all the bacteria were well thawed and homogenously distributed. The probiotic mixture was cooled down to 4°C and kept at this temperature for 30-60 minutes. The mixture was dripped and snap-frozen in a liquid nitrogen bath to form frozen beads, where were harvested from the liquid nitrogen and stored at -80°C for later drying.

[0076] For drying, the frozen beads were spread on pre-cooled trays (-20°C) at a loading capacity of 800 g/sqft and then immediately placed on shelves in a freeze drier (Model 25 SRC, Virtis, Gardiner, NY). Vacuum was then adjusted to between 1800-2200 mTORR and the shelf temperature was raised to +20°C. These temperature and vacuum pressure settings were maintained for 12 hours. Before primary drying, the temperature of the frozen beads was optionally acclimatized to about -20°C by applying a vacuum pressure at about 1000 mTORR to allow the temperature of the frozen beads to acclimate for about 10 minutes. The primary drying step was then followed by adjusting the vacuum pressure to 2000-2700 mTORR and the shelf temperature to +20°C. These temperature and vacuum pressure settings were maintained for 12 hours. A secondary drying step was then followed at full strength vacuum (150-200 mTORR) and the shelf temperature was maintained at 40°C for additional 12 hours. The formulation was completely dried and its water activity as measured by a Hygropalm Aw1 instrument (Rotonic Instrument Corp., Huntington, NY) was Aw 0.23. The dry material was then milled and sieved to particle size ≤ 250 µm and stored at 4°C.

EXAMPLE 2. Storage stability of the dry probiotic composition

[0077] Samples comprising the dry stable composition of probiotic bacteria from Example 1 or commonly freeze dried bacteria suspension in 10% trehalose were placed in a desiccator under accelerated storage conditions of 40°C and 33%RH. Samples were taken periodically for microbial CFU assessment using standard microbiological dilutions and LMRS agar plating procedures. Figure 1 shows the storage stability under accelerated storage conditions of 40°C and 33%RH. The unprotected probiotic bacteria completely lost its viability within the first few weeks under the accelerated storage conditions, while the dry composition of the probiotic bacteria of the present invention lost only 0.70 log unit/g after 84 days at 40°C and 33%RH.

EXAMPLE 3. Effects of carbohydrates and proteins on storage stability

[0078] The compositions of the present invention eliminates the need for those carbohydrate stabilizers that are not included in the recommended list of nutritional compounds allowed for special dietary uses, according to the joint FAO/WHO Codex Alimentarius Commission. The following examples 3(a)-3(d) do not fall within the scope of the claims and are provided for

information. Example 3(e) is according to the invention.

1. **a. A composition comprising Maltodextrin:** A composition containing 50 g *pea protein hydrolysate* (Marcor, Carlstadt, NJ) 33g maltodextrin (Tate & Lyle, Decatur, Il) 10g inulin (Cargill, Minneapolis, MN), 2g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. The Pea protein hydrolysate dissolved in 100 grams of distilled water and pH adjusted to 7.5. The carbohydrate compounds were dry blended and Added to the pea protein solution. The mixture was heated to 70°C to dissolve all the compounds and the solution cooled down to 37°C. Frozen beads of *L. rhamnosus* sp. (100 g) were added to the solution and the slurry dried as described in Example 1. The initial count of live bacteria in the dry composition was 9.92 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample had 1.19 log unit/g loss after 1 month thus, failed the challenge.
2. **b. A composition comprising Wheat protein isolate:** Another composition comprising 25 g *pea protein hydrolysate* (Marcor, Carlstadt, NJ), 33g *Prolite* 200 (wheat protein isolate, Archer Daniels Midland Company Decatur, IL) 25g maltodextrin (Tate & Lyle, Decatur, IL) 10g inulin (Cargill, Minneapolis, MN), 2g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing *L. rhamnosus* sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 10.17 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample resulted 1.71 log unit/g loss after two weeks thus, failed the challenge test.
3. **c. A composition comprising Whey protein isolate:** Another composition containing 25g *pea protein hydrolysate* (Marcor, Carlstadt, NJ), 25g *whey protein isolate* (Davisco, Eden Prairie, MN), 33g maltodextrin (Tate & Lyle, Decatur, IL) 10g inulin (Cargill, Minneapolis, MN), 2g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing L. rhamnosus sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 10.40 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample resulted 1.30 log unit/g loss after two weeks thus, failed the challenge test.
4. **d. A composition comprising lactose:** Another composition containing 50 g *pea protein hydrolysate* (Marcor, Carlstadt, NJ) 33g lactose (Foremost Farms, Rothschild, Wl) 10g inulin (Cargill, Minneapolis, MN), 2g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing *L. rhamnosus* sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 9.77 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample passed the first month stability but had 1.89 log unit/g loss after 2 months thus, failed the challenge test.
5. **e. The composition of the present invention:** A composition containing 65 g *pea protein hydrolysate* (Marcor, Carlstadt, NJ) 10g lactose (Foremost Farms, Rothschild,

WI) 17g inulin (Cargill, Minneapolis, MN), 3g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing Bifidobacterium sp. was prepared and dried as described above in Examples 1 and 2, except that the pH of pea protein hydrolysate solution adjusted to pH 8.5. The initial count of live bacteria in the dry composition was 10.87 log CFU/g. A sample of this product was placed under accelerated stability challenge. The sample lost only 0.70 log unit/g after 3 months, thus, passed the challenge test.

EXAMPLE 4. Effects of inulin on storage stability

[0079] US Standard codex 72 allows the use of oligosaccharides such as inulin and maltodextrin in infant formula. The effect of inulin levels from 0% to 30% in the composition was evaluated. Compositions containing 50 g pea protein hydrolysate (Marcor, Carlstadt, NJ), 43g lactose (Foremost Farms, Rothschild, WI), 2g Locust Bean gum (Tic gum, Belcamp, MD), 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w), and 0g, 10g, 15g or 30 g of inulin were prepared (the added amount of inulin was subtracted from the amount of lactose in the composition). The compositions containing *L. acidophilus* sp. were prepared and dried as described above in Examples 1 and 2. The initial counts of live bacteria in the dry compositions comprising 0g, 10g, 15g, and 30g inulin was 9.42, 9.57, 9.59 and 9.80 log CFU/g, respectively. A sample from each composition was placed under accelerated stability challenge. The sample comprising 0g inulin (no oligosaccharides) lost 1.14 log unit/g after 2 months and 2.25 log unit/g after 3 months while samples comprising 15g and higher inulin lost less than 2 log unit/g after 3 months, thus, it was determined that the minimal amount of inulin in the composition of the current invention must be higher than10%.

EXAMPLE 5. Effects of disaccharides on storage stability

[0080] US Standard codex 72 restricts the use of trehalose but allows the use of sucrose and lactose in infant formula. The following example demonstrates that trehalose was successfully replaced with an increased amount of hydrolyzed pea protein and only small amount of the disaccharide lactose according to the composition of the current invention. The following examples 5(a)-5(c) do not fall within the scope of the claims and are provided for information.

1. **a. A composition comprising trehalose:** A composition containing 25 g pea protein hydrolysate (Marcor, Carlstadt, NJ) *62g trehalose* (Cargill, Minneapolis, MN) 5g inulin (Cargill, Minneapolis, MN), 3 g Locust Bean gum (Tic gum, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing *L. acidophilus* sp. was prepared and dried as described above in Examples 1 and 2. The initial counts of live bacteria in the dry composition was 9.49 log CFU/g. A sample of the composition was placed under accelerated stability challenge. The sample

lost 0.83 log unit/g after 3 months thus, demonstrating a relatively good stability. Nevertheless, the use of trehalose is not allowed in infant formula.

2. **b. Effect of trehalose replacement with lactose:** Compositions containing 50 g pea protein hydrolysate (Marcor, Carlstadt, NJ), 2 g Locust Bean gum (Tic gum, Belcamp, MD), 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w)and *33g lactose* (Foremost Farms, Rothschild, WI), 10g inulin (Cargill, Minneapolis, MN) or 13g and 30 g inulin were prepared. The compositions containing *L. acidophilus* sp. were prepared and dried as described above in Examples 1 and 2. The initial counts of live bacteria in the dry compositions comprising 33g or 13 g lactose were 9.57 log CFU/g and 9.80 log CFU/g, respectively. A sample from each composition was placed under accelerated stability challenge. The sample comprising 33 g lactose lost 1.25 log unit/g after 1 month while the sample comprising 13g lactose last for 2 months, thus, it was determined that 13% of lactose and accompanied with 50% of hydrolyzed pea protein in the stabilizing composition can effectively replace trehalose.
3. **c. Stability of compositions without trehalose and alginate:** To evaluate the effect of replacement of ingredients (e.g., trehalose and alginate) not desirable for an infant formula in compositions having over 50% disaccharides, three (3) compositions containing 25 g pea protein hydrolysate (Marcor, Carlstadt, NJ), 5g inulin (Cargill, Minneapolis, MN), 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w), 3 g alginate (ISP Corp., Wayne, NJ) or 3 g Locust Bean gum (Tic gum, Belcamp, MD), and 62 g trehalose (Cargill, Minneapolis, MN) or 62 g *lactose* (Foremost Farms, Rothschild, WI) were prepared (Table 1). Compositions containing *L. rhamnosus* sp. were prepared and dried as described above in Examples 1 and 2. The initial counts of live bacteria in the three dry compositions were about 10 log CFU/g (Table 1). A sample from each composition was subject to accelerated stability challenge. Only the composition comprising both alginate and trehalose demonstrated less than 1 log unit/g loss after 84 days, while the other two compositions, in which trehalose and alginate were replaced with lactose and locust bean gum, respectively, lost more than a log unit/g after only 28 days. Thus, trehalose and alginate are essential ingredients in compositions, which typically have over 50% disaccharides, for achieving the stability requirement of less than 1 log unit/g loss after 3 months at 40 °C and 33% relative humidity.

Table 1. Effect of trehalose and alginate replacement on probiotic stability

Composition	1	2	3
Pea protein hydrolysate	25g	25g	25g
Trehalose	62g	0	0
Lactose	0	62g	62g
Inulin	5g	5g	5g
Alginate	3g	3g	0
Locust bean gum	0	0	3g
Vitamin mixture	5g	5g	5g
Initial viability (log CFU/g)	10.08	9.84	10.16

Composition	1	2	3
Viability loss (log CFU/g/days)	0.96/84	1.24/28	1.62/28

EXAMPLE 6. Effects of polysaccharides on storage stability

[0081] Polysaccharide provides structural support that is essential in the probiotic composition. US Standard codex 72 restricts the use of several polysaccharides such as alginate but allows the use of guar gum, locust bean gum and starch in infant formula. Sodium alginate, guar gum, locust bean gum, and starch are commercially available polysaccharides and their effect in the stabilizing composition of the present invention was evaluated. Examples 6(a)-6(c) do not fall within the scope of the claims and are provided for information.

1. **a. A composition comprising guar gum:** A composition containing 36 g casein hydrolysate (DMV International Nutritionals, Delhi, NY) 25g inulin (Cargill, Minneapolis, MN) *36g sucrose* (Sigma), and 3g guar gum (Tic gum, Belcamp, MD) was prepared. A composition containing L. rhamnosus sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 9.90 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample passed the first month stability but had 1.10 log unit/g loss after 2 months thus, failed the challenge test.
2. **b. A composition comprising sodium alginate:** A composition containing 17 g casein hydrolysate (DMV International Nutritionals, Delhi, NY) 5g inulin (Cargill, Minneapolis, MN) 75g sucrose (Sigma), and 3g sodium alginate (ISP Corp., Wayne, NJ) was prepared. A composition containing *L. rhamnosus* sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 9.90 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample passed the first month stability but had 1.25 log unit/g loss after 2 months thus, failed the challenge test.
3. **c. A composition comprising locust bean gum:** A composition containing 25g pea protein hydrolysate (Marcor, Carlstadt, NJ), 5g inulin (Cargill, Minneapolis, MN), 62g trehalose (Cargill, Minneapolis, MN), 3g locust bean gum (Tic Gums, Belcamp, MD) and 5 g mixture of vitamin E and sodium ascorbate (4:1 w/w) was prepared. A composition containing *L. rhamnosus* sp. was prepared and dried as described above in Examples 1 and 2. The initial count of live bacteria in the dry composition was 9.93 log CFU/g. A sample of this product was placed under accelerated stability challenge as described in Example 1. The sample passed the 2 month stability with 0.81 log unit/g loss. It was determined that locust bean gum can replace the alginate functionality in stabilized probiotic compositions.

EXAMPLE 7. Infant formula

[0082] A stable dry composition comprising *Bifidobacterium sp.* was prepared according to Example 1 followed by sieving into two particle size groups (above 50 µm and below 250 µm). An infant formula comprising probiotic bacteria was prepared by mixing 99.9 g of Gerber Good Start (Nestle Infant Nutrition, Florham Park, NJ.) with 0.1 g of the dry composition particles in the size range between 50 µm and 250 µm). The final product contains about 10^8 CFU of *Lactobacillus* GG per 100 g infant formula. The probiotic infant formula were packed into 180 cc HDPE bottles of and exposed to controlled temperature/humidity of 40°C/33%RH. The product is subjected to monthly microbiological stability testing over a period of 12 months or until a reduction in the assay count below 5 x 10^7/ unit dose is observed.

EXAMPLE 8. Probiotic supplement

[0083] A stable dry composition comprising *Lactobacillus acidophilus* is prepared according to Example 1 and formulated into oral dosage forms, such as tablets, caplets, or capsules. Orange flavored tablets containing 99.9 g of a compression agent (dextrose) and 0.1 g of the dry formulation particles in the size range between 50 µm and 250 µm are prepared by direct compression on a rotary machine using a 1/2" round standard concave tooling. The final product contains about 10^8 CFU/unit dose. Hardness of the tablets is in the range of 8-10 kp and disintegration times is approximately 20 second. The compressed tablets are packaged into 180 cc HDPE bottles of 100 tablets each and exposed to controlled temperature/humidity of 40°C/33%RH. The product is subjected to monthly microbiological stability testing over a period of 12 months or until a reduction in the assay count below 1 x 10^6/ unit dose is observed.

EXAMPLE 9. A functional beverage drink

[0084] A stable dry composition comprising *Lactobacillus acidophilus* is prepared according to Example 1 and formulated into a dry mix containing (% by weight) 71% sucrose, 14% maltodextrin, 10% inulin, 2% dextrose, 1% citric acid anhydrous, 0.3% gum acacia, 0.3% flavors, 0.3% Tricalcium phosphate and 0.1% dry probiotic composition particles (*L. acidophilus*) in the size range between 50 µm and 250 µm. The final product contains about 10^9cfu/unit dose (30g dry mix). The product is packaged in small aluminum foil bags (30g unit dose/bag) for drinking by stirring in 340 mil water. The stability of the probiotic bacteria in the beverage dry mix is subjected to monthly microbiological stability testing over a period of 12 months or until a reduction in the assay count below 1 x 10^7/ unit dose is observed.

EXAMPLE 10. Multivitamins/probiotic tablets

[0085] Ten (10) g of dry powder composition is produced as described in Example 1. For tableting, the dry and stable probiotic composition (100 mg) is mixed with 400 mg of commercially available multivitamins powder (Centrum®, Pfizer) containing 2% w/w magnesium stearate and 2% w/w hydrophilic fumed silica (AEROSIL® 200, Evonik Industries) and compressed in hand held pill press equipment (using a ½" tablet diameter housing). Each tablet contains about 10^7cfu/tablet). The tablets are packaged into 180 cc HDPE bottles of 100 tablets each and exposed to controlled temperature/humidity of 40°C/33%RH. The bottles are subjected to monthly microbiological stability testing over a period of 12 months or until a reduction in the assay count below 1 x 10^6/ tablet is observed.

[0086] The term "about" as used herein when referring to a measurable value such as an amount, a percentage, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- US2013296165A1 **[0006]**
- CA2420095A1 **[0007]**

Patentkrav

1. Tør sammensætning der omfatter en eller flere levedygtige probiotiske mikroorganismer, et eller flere hydrolyserede proteiner, et eller flere disaccharider, et eller flere oligosaccharider og et eller flere polysaccharider,

hvilken sammensætning omfatter mindst 40 % af det ene eller de nævnte flere hydrolyserede proteiner, baseret på sammensætningens samlede tørvægt, og hvor det ene eller de nævnte flere hydrolyserede proteiner er valgt fra gruppen, der består af: hydrolyseret kasein, hydrolyseret valleprotein, hydrolyseret ærteprotein , hydrolyseret sojaprotein og kombinationer deraf,

hvilken sammensætning omfatter mindre end 30 % af det ene eller de nævnte flere disaccharider, baseret på sammensætningens samlede tørvægt, og hvor det ene eller de nævnte flere disaccharider er valgt fra gruppen, der består af: saccharose, lactose og kombinationer deraf,

hvilken sammensætningen omfatter 1-30 % af det ene eller de nævnte flere oligosaccharider, baseret på sammensætningens samlede tørvægt, og hvor det ene eller de nævnte flere oligosaccharider er valgt fra gruppen, der består af: inulin, maltodextriner, dextrans, fructo-oligosaccharider (FOS) , galacto-oligosaccharider (GOS), mannan-oligosaccharider (MOS) og kombinationer deraf,

hvilken sammensætning omfatter 0,1-40 % af det ene eller de nævnte flere polysaccharider, baseret på sammensætningens samlede tørvægt, og hvor det ene eller de nævnte flere polysaccharider er valgt fra gruppen, der består af: carrageenan, guargummi, gummiacacia, johannesbrødgummi, stivelse, modificeret stivelse og kombinationer deraf,

hvilken sammensætning har levedygtighed på mindst $1x10^{10}$ CFU/g, og hvilken sammensætning har et levedygtighedstab på mindre end 1 log-enhed/g efter 3 måneder ved en temperatur på 40 °C og en relativ fugtighed på 33 %.

2. Sammensætningen ifølge krav 1, hvilken sammensætning omfatter en effektiv mængde af den ene eller de nævnte flere levedygtige probiotiske mikroorganismer for at tilvejebringe en probiotisk fordel til en vært i et særligt kostprodukt.

3. Sammensætningen ifølge krav 1, hvor den levedygtige probiotiske mikroorganisme er valgt fra gruppen, der består af: levende probiotiske bakterier, svampe og gær.

4. Sammensætningen ifølge krav 1, hvilken sammensætning omfatter mindst 50 % af det ene eller de nævnte flere hydrolyserede proteiner, baseret på sammensætningens samlede tørvægt.

5. Sammensætningen ifølge krav 1, hvilken sammensætning omfatter mindre end 20 % af det ene eller de nævnte flere disaccharider, baseret på sammensætningens samlede tørvægt.

6. Sammensætningen ifølge krav 1, hvilken sammensætning omfatter 5-30 % af det ene eller de nævnte flere oligosaccharider, baseret på sammensætningens samlede tørvægt.

7. Sammensætningen ifølge krav 1, hvilken sammensætning omfatter 1-10 % af det ene eller de nævnte flere polysaccharider, baseret på sammensætningens samlede tørvægt.

8. Sammensætningen ifølge krav 1 der yderligere omfatter et eller flere yderligere midler.

9. Sammensætningen ifølge krav 8, hvilken sammensætning omfatter 0,5-10 % af det ene eller de nævnte flere yderligere midler, baseret på sammensætningens samlede vægt.

10. Sammensætningen ifølge krav 8, hvor det ene eller de nævnte flere yderligere midler er valgt fra gruppen, der består af: carboxylsyresalte, tocopheroler og kombinationer deraf.

11. Sammensætningen ifølge krav 10, hvor carboxylsyresaltene er valgt fra gruppen, der består af: ascorbinsyresalte og citronsyresalte.

12. Sammensætningen ifølge krav 8, hvor det ene eller de nævnte flere yderligere midler omfatter et eller flere tocopheroler og et eller flere carboxylsyresalte i et vægtforhold fra 1: 4 til 4: 1.

13. Sammensætningen ifølge krav 8, hvor det ene eller de nævnte flere yderligere midler omfatter E -vitamin og natriumascorbat i et vægtforhold på 4: 1.

14. Fremgangsmåde til fremstilling af sammensætningen ifølge krav 1 der omfatter:

(a) at kombinere den ene eller de nævnte flere levedygtige probiotiske mikroorganismer, det ene eller de nævnte flere hydrolyserede proteiner, det ene eller de nævnte flere disaccharider, det ene eller de nævnte flere oligosaccharider og det ene eller de nævnte flere polysaccharider i et alkalisk vandigt opløsningsmiddel til dannelse af en opslæmning;

(b) at hurtigfryse opslæmningen i flydende nitrogen til dannelse af faste frosne partikler i form af perler, dråber eller strenge;

(c) et primært tørretrin af de faste frosne partikler ved fordampning under vakuum, mens partiklernes temperatur holdes over deres frysetemperatur, hvorved der dannes en primær tørret formulering; og

(d) en sekundær tørring af den primære tørrede formulering ved vakuum med fuld styrke og en varmekildetemperatur på 20 °C eller højere i et tidsrum, der er tilstrækkeligt til at reducere vandaktiviteten af den primære tørrede formulering til 0,3 Aw eller lavere, hvorved sammensætningen ifølge krav 1 fremstilles.

15. Fremgangsmåden ifølge krav 14 der yderligere omfatter at sterilisere det ene eller de nævnte flere hydrolyserede proteiner, det ene eller de nævnte flere disaccharider, det ene eller de nævnte flere oligosaccharider og det eller de nævnte flere polysaccharider før trin (a).

16. Fremgangsmåden ifølge krav 9 der yderligere omfatter at skære, knuse, formale eller pulverisere sammensætningen til et fritflydende pulver.

17. Fremgangsmåden ifølge krav 16, hvor partikelstørrelsen af pulveret er mindre end ca. 1000 µm.

18. Fremgangsmåden ifølge krav 14, hvor sammensætningen omfatter en effektiv mængde af en eller flere levedygtige probiotiske mikroorganismer for at tilvejebringe en probiotisk fordel til en vært i et særligt kostprodukt.

19. Fremgangsmåden ifølge krav 14 der yderligere omfatter fremstilling af det særlige kostprodukt med sammensætningen.

20. Fremgangsmåden ifølge krav 14, hvor det særlige kostprodukt er valgt fra gruppen, der består af: en modermælkserstatning, en tilskudsformel, forarbejdet mad der er baseret på korn, babymad på dåse og særlig mad til et medicinsk formål.

21. Fremgangsmåden ifølge krav 19, hvor det særlige kostprodukt er en modermælkserstatning.

22. Fremgangsmåde til fremstilling af en modermælkserstatning med sammensætningen ifølge krav 1 der omfatter:

(a) at sterilisere det ene eller de nævnte flere hydrolyserede proteiner, det ene eller de nævnte flere disaccharider, det ene eller de nævnte flere oligosaccharider og det eller de nævnte flere polysaccharider;

(b) at kombinere det steriliserede ene eller de nævnte flere levedygtige probiotiske mikroorganismer, det steriliserede ene eller de nævnte flere hydrolyserede proteiner, det steriliserede ene eller de nævnte flere disaccharider, det steriliserede ene eller de nævnte flere oligosaccharider og det steriliserede ene eller de nævnte flere polysaccharider i et alkalisk vandigt opløsningsmiddel til dannelse af en opslæmning;

(c) at hurtigfryse opslæmningen i flydende nitrogen til dannelse af faste frosne partikler i form af perler, dråber eller strenge;

(d) et primært tørretrin af de faste frosne partikler ved fordampning under vakuum, mens partiklernes temperatur holdes over deres frysetemperatur, hvorved der dannes en primær tørret formulering;

(e) en sekundær tørring af den primære tørrede formulering ved vakuum med fuld styrke og en varmekildetemperatur på 20 °C eller højere i et tidsrum, der er tilstrækkeligt til at reducere vandaktiviteten af den primære tørrede formulering til 0,3 Aw eller lavere, hvorved sammensætningen ifølge krav 1 fremstilles;

(f) at skære, knuse, formale eller pulverisere sammensætningen ifølge krav 1 til et fritflydende pulver, hvor partikelstørrelsen af pulveret er mindre end ca. 1000 µm, og hvor pulveret omfatter en effektiv mængde af den ene eller de nævnte flere levedygtige probiotiske mikroorganismer for at tilvejebringe en probiotisk fordel til en vært i en modermælkserstatning; og

g) at fremstille modermælkserstatningen med pulveret.

DRAWINGS

Figure 1