

DESCRIPTION

PROBIOTIC STABILIZATION

TECHNICAL FIELD

[0001] The present disclosure relates to the stabilization of biological material for ingestion by an individual. More particularly, the present disclosure relates to a stabilization mixture comprising hydrolyzed protein, which provides improved stability to a probiotic organism when the probiotic is included in a nutritional composition. The disclosure also includes probiotic stabilization methods.

BACKGROUND ART

[0002] There are currently a variety of compositions for supplementing the nutrition of both humans and animals. These supplements may be provided to alter, reduce or increase the microflora within an individual's gut so as to cause a desired effect on digestion. Ideally, supplementation may cultivate an improved microflora for individuals, including humans, based upon the alteration of specific bacteria within the human's gastrointestinal (GI) tract. This style of supplementation may be conducted through the use of probiotics, which are understood to be live microorganisms, that when administered in effective amounts confer a health or nutritional benefit to the host. One of the more common types of probiotics is a lactic acid bacterium which is able to convert sugars and other carbohydrates into lactic acid. This conversion lowers the pH within the gut of the host and provides fewer opportunities for harmful organisms to grow and cause problems through gastrointestinal infections.

[0003] A common technological challenge is introducing probiotics into the host in an appropriate manner, both for the maintenance of the probiotics as well as for the health of an individual. Current technologies include the utilization of encapsulation and stabilization techniques for shielding the probiotics with a protective layer or matrix so that the protected microbe may be delivered to the appropriate location within the individual's GI tract. For example, in Batich et al. (U.S. Patent No. 5,286,495), a process for microencapsulating cells is provided so that oxalate-degrading enzymes and bacteria may be encapsulated for both enteric and intraperitoneal administration. According to Batich et al., bacteria and enzymes can

be successfully encapsulated in either alginate microcapsules or cellulose acetate phthalate microspheres. The model suggests that viability remains for the bacteria and enzymes so that the encapsulated cells reach the appropriate gastric region of the animal.

[0004] In U.S. Patent No. 5,733,568, issued to Ford, microencapsulated *Lactobacilli* bacteria are administered to the skin to treat or prevent recurrent skin infections. *Lactobacillus* species are mixed with a glucose saline solution and gently stirred with a sodium alginate solution prior to being forced through a needle and dried to create gelled droplets. Other methods of encapsulation may include the addition of bacteria to a suspension of polyvinyl povidone or hydroxypropyl methylcellulose for encapsulating the bacteria.

[0005] In U.S. Patent No. 6,465,706, issued to Rogers et al., encapsulation of microbes is described for use in biodecontamination. Rogers et al. asserts that suitable encapsulation materials include natural or synthetic polymeric binders that encompasses both gels and foams as well as gelatin polymers.

[0006] Although there have been developments concerning encapsulation and stabilization techniques for containing microorganisms for delivery into the digestive system of animals, there has been little development in encapsulation or stabilization techniques that protect the viability of probiotics during distribution and storage. There is a need for a stabilization technique that is useful where circumstances preclude refrigeration, and further where such formulations may be exposed to various environments, especially those associated with tropical climates. In addition, the inherent moisture of the product poses a challenge in that probiotics generally are sensitive to water, especially in combination with high temperature. There is a need to deliver sufficient protection to probiotics under intermediate moisture conditions (i.e. water activity of about 0.2 and higher, and up to about 0.4 or higher) and high temperatures during distribution and storage (i.e. temperatures of at least about 30°C, and up to and above 40°C) when incorporated into nutritional agents.

[0007] In particular, probiotics can provide a variety of benefits to a host, such as maintaining a healthy gastrointestinal flora, enhancing immunity, protecting against diarrhea, atopic dermatitis and other diseases, etc. As such, there is a need for probiotics to be administered in various geographic locations, including tropical climates, where the viability of the probiotic could be compromised. Conventional

encapsulation and stabilization techniques are generally considered suitable only for non-humans and possess a chemical makeup that is ill-suited for infant formulas and/or for use by children, or known techniques have poor stability characteristics that significantly limit commercial opportunities.

[0008] What is desired therefore, is a stabilization technique and a stabilized bacterial mixture using acceptable ingredients for either an infant formula or children's nutrition, the stabilized mixture allowing for improved stability properties so that probiotics may be distributed in a wide variety of geographical locations and climates while maintaining a useful shelf-life. Further desired is a stabilization technology for the protection of probiotics, such as *Lactobacillus rhamnosus*, for use in nutritional compositions, such as infant formulas, supplements and children's products. Indeed, a combination of characteristics, including improved stability combined with nutritional factors, provide an improved stabilization mixture applicable for prenatal, infant, and children's nutrition.

DISCLOSURE OF THE INVENTION

[0009] In some embodiments, the present disclosure is directed to a nutritional composition comprising a lipid or fat source, a protein source, and a probiotic stabilized in a protective matrix, the protective matrix includes (i) a hydrolyzed protein, (ii) a first carbohydrate selected from the group consisting of sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent ("DE") of about 2 to about 6, and any combination thereof, and (iii) a second carbohydrate selected from the group consisting of inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof. In such embodiments, the nutritional composition comprises viable microbial cells, such as viable *Lactobacillus rhamnosus* cells. Also, the matrix may additionally comprise (i) sodium alginate and/or pectin and/or (ii) a lipid chosen from lecithin, monoglycerides, diglycerides, and any combination thereof. At least 20% of the hydrolyzed protein of the matrix may contain protein having a molecular weight of less than 2000 Daltons. Moreover, the hydrolyzed protein may comprise between about 10 and about 20% (w/w) of the protective matrix on a dry basis. Further, the hydrolyzed protein may include or consist solely of hydrolyzed casein. And the nutritional composition may be a powdered formula, such as a powdered infant formula.

[0010] In other embodiments, the present disclosure is directed to a nutritional composition comprising a lipid or fat source, a protein source, and a probiotic stabilized in a protective matrix, wherein the protective matrix includes (i) hydrolyzed protein, (ii) a first carbohydrate selected from the group consisting of sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of about 2 to about 6, and any combination thereof, (iii) a second carbohydrate selected from the group consisting of inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof, and (iv) a compound binder selected from the group consisting of sodium alginate, pectin, and any combination thereof. In such embodiments, the nutritional composition may comprise viable microbial cells, such as viable *Lactobacillus rhamnosus* cells. Also, the matrix may additionally comprise a lipid component, such as lecithin, a monoglyceride and/or a diglyceride. At least 20% of the hydrolyzed protein of the matrix may contain protein having a molecular weight of less than 2000 Daltons. Moreover, the hydrolyzed protein may comprise between about 10 and about 20% (w/w) of the protective matrix on a dry basis. Further, the hydrolyzed protein may include or consist solely of hydrolyzed casein. And the nutritional composition may be a powdered formula, such as a powdered infant formula.

[0011] In another embodiment, the present disclosure is directed to a method for protecting a viable probiotic for use in a powdered nutritional composition, the method includes the steps of (i) providing a viable probiotic, (ii) preparing a protective matrix for the probiotic by blending together (a) hydrolyzed casein, (b) a first carbohydrate selected from the group consisting of sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of about 2 to about 6, and any combination thereof, (c) a second carbohydrate selected from the group consisting of inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof, and (d) a lipid selected from the group consisting of lecithin, monoglycerides, diglycerides, and any combination thereof, (iii) combining the viable probiotic, the protective matrix and water to produce a mixture, (iv) drying the mixture to a final moisture content of about 4% or less, and (v) adding the dried

mixture to a powdered nutritional product. In such an embodiment, the viable probiotic may be *Lactobacillus rhamnosus*.

[0012] These aspects and others that will become apparent to the skilled artisan upon review of the following description can be accomplished by providing a mixture including hydrolyzed mammalian protein for the stabilization of biological material, such as probiotics, to provide for increased stability of the biological material, resulting in the improved, long-term viability of the biological material. In an embodiment, the stabilization mixture advantageously provides for an extension of the shelf-life of probiotics such as *Lactobacillus rhamnosus* when compared to the use of non-hydrolyzed or hydrolyzed non-mammalian protein. The stabilization mixture may be combined with the probiotic in a variety of methods including freeze drying, air drying, vacuum drying, spray drying and any combination thereof for preserving the probiotic.

[0013] It is to be understood that both the foregoing general description and the following detailed description provide embodiments of the disclosure and are intended to provide an overview or framework of understanding to the nature and character of the disclosure as it is claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Fig. 1 is a graph that illustrates the stability of *Lactobacillus rhamnosus* GG (LGG) in a protective matrix (per the formulation presented in Table 1 hereinbelow) in a growing up milk having an available water (Aw) content of 0.28.

[0015] Fig. 2 is a graph that illustrates the stability of LGG without a protective matrix in a growing up milk having an available water (Aw) content of 0.28.

[0016] Fig. 3 presents scanning electron micrographs of stabilized LGG in a protective matrix (per the formulation presented in Table 1 hereinbelow).

BEST MODE FOR CARRYING OUT THE INVENTION

[0017] Reference now will be made in detail to the embodiments of the present disclosure, one or more examples of which are set forth hereinbelow. Each example is provided by way of explanation of the nutritional composition of the present disclosure and is not a limitation. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made to the teachings of the present disclosure without departing from the scope of the disclosure. For instance, features

illustrated or described as part of one embodiment can be used with another embodiment to yield a still further embodiment.

[0018] Thus, it is intended that the present disclosure covers such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features and aspects of the present disclosure are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only and is not intended as limiting the broader aspects of the present disclosure.

[0019] The present disclosure provides a stabilization technique and a stabilized mixture (also referred to herein as a “stabilization mixture” or a “protective matrix”) that may be used for improving the stability of a biological material (also referred to herein as a “substrate”). In embodiments of the disclosure, the stabilized substrate may be a probiotic, wherein the various health benefits associated with the stabilized probiotic may be conferred to an individual upon ingesting a nutritional composition containing the stabilized probiotic.

[0020] While probiotics have been recognized as nutritionally beneficial, it is thought that the beneficial effects of probiotics are maximized if a probiotic microorganism is ingested by a subject when the microorganism is still alive. Thus, it is desirable for a viable probiotic to survive the conditions of manufacturing and of placement into a consumable nutritional composition, such as a food or beverage, as well as to survive the subsequent shipping and storage time before the product is ingested and introduced to a subject’s gastrointestinal tract. Many conventional probiotic compositions utilize an extremely high count of viable cells, with the understanding that a significant number of cells ultimately lose viability during the manufacturing process, transport, and storage. Moreover, previously identified encapsulation and stabilization techniques provide some protection for probiotics, yet they do not provide a desired stability while simultaneously being functional for use in infant formula and/or children’s nutritional products.

[0021] By practice of the present disclosure, hydrolyzed mammalian protein is incorporated into a protective matrix. The hydrolyzed protein strengthens the protective matrix and increases the stability of probiotics that are protected by the matrix. As a result, infant- and children-compatible probiotics can be stabilized with

a matrix including hydrolyzed mammalian protein. The stabilized probiotics can be used in multiple environments, as the probiotics exhibit an improved viability. Advantageously, probiotics stabilized by the protective matrix of the present disclosure can be incorporated into nutritional compositions and shipped over extended distances, as the probiotics will maintain viability even after extended transportation and storage time, due to the improved stability of the stabilized mixture.

[0022] The present method of providing stabilization to probiotics may include the use of a matrix for stabilizing a biological material, wherein the matrix includes a hydrolyzed mammalian protein, one or more carbohydrates and a compound binder.

[0023] While the protective matrix may be utilized for a variety of substances, in an embodiment, it is utilized to protect probiotics, such as *Lactobacillus rhamnosus*. *Lactobacillus rhamnosus* is understood to possess relatively good bio-stability while having a high avidity for human intestinal mucosal cells. In use as a probiotic, *Lactobacillus rhamnosus* is thought to colonize the digestive tract and to balance intestinal microflora.

[0024] In creating the protective matrix for the probiotic, a hydrolyzed mammalian protein may be used to increase and strengthen the matrix around the probiotic. The hydrolyzed mammalian protein may include extensively hydrolyzed casein as well as other hydrolyzed mammalian proteins and is hypothesized to provide the increase in strength due to the characteristics associated with the short chain peptides comprising the hydrolyzed protein. In some embodiments, the hydrolyzed protein may be achieved by boiling mammalian protein in a strong acid or strong base or through an enzymatic degradation technique so as to break the protein down into shorter sequences of its component amino acids.

[0025] The protective matrix provides for improved stability of the probiotic, meaning that a greater percentage of the probiotic cells are viable after processing, transportation and storage conditions. Specifically, the shelf life of viable probiotics is improved when compared to encapsulation and stabilization techniques using intact protein, hydrolyzed non-mammalian protein or protein which is not as extensively hydrolyzed as that specified herein.

[0026] The protective matrix of the present disclosure may be used in a multiplicity of processes for forming a stabilized probiotic product. These processes

include freezing, flash-freezing, freeze-drying, ambient air drying, vacuum drying, spray drying, low temperature drying, high temperature drying and any combination thereof. The resulting stabilized probiotic, whether alone or integrated into a nutritional composition, possesses effective viability in a wide range of temperatures and conditions while displaying improved shelf-life. Furthermore, the stabilized probiotic may be incorporated into a variety of prenatal, infant and children's nutritional products for improving their gut microflora while simultaneously providing nutrition to the infant or child.

[0027] Accordingly, in one embodiment, the disclosure is directed to a method for stabilizing a biological material in a nutritional composition. Still another embodiment is a protective matrix for a probiotic. Yet another embodiment is a mixture for stabilizing a probiotic comprising one or more carbohydrates, a compound binder and hydrolyzed mammalian protein. A further embodiment is a method of increasing the shelf life of probiotics comprising stabilizing the probiotic with a stabilization mixture including hydrolyzed mammalian protein.

[0028] The present disclosure provides a novel stabilization mixture and method that provides stability and protection to biological materials, such as viable microorganisms. The present disclosure includes a stabilization mixture comprising a hydrolyzed mammalian protein, which in certain embodiments is combined with one or more carbohydrates and a compound binder, which together provide a protective matrix resulting in an increased shelf-life over other encapsulation and stabilization products utilizing non-hydrolyzed or hydrolyzed non-mammalian proteins.

DEFINITIONS

[0029] The terms "protective matrix" and "stabilization mixture" are used interchangeably throughout the present disclosure.

[0030] An "effective amount" as used herein is generally defined as an amount of an agent that provides an observable result within the subject administered thereto.

[0031] "Nutritional composition" means a substance or formulation that satisfies at least a portion of a subject's nutrient requirements. The terms "nutritional(s)", "nutritional formula(s)", "enteral nutritional(s)", and "nutritional supplement(s)" are used as non-limiting examples of nutritional composition(s) throughout the present disclosure. Moreover, "nutritional composition(s)" may refer

to liquids, powders, gels, pastes, solids, concentrates, suspensions, or ready-to-use forms of enteral formulas, oral formulas, formulas for infants, formulas for pediatric subjects, formulas for children, growing-up milks and/or formulas for adults.

[0032] The term “enteral” means deliverable through or within the gastrointestinal or digestive tract. “Enteral administration” includes oral feeding, intragastric feeding, transpyloric administration, or any other administration into the digestive tract. “Administration” is broader than “enteral administration” and includes parenteral administration or any other route of administration by which a substance is taken into a subject’s body.

[0033] “Pediatric subject” means a human less than 13 years of age. In some embodiments, a pediatric subject refers to a human subject that is less than 8 years old. In other embodiments, a pediatric subject refers to a human subject between 1 and 6 years of age. In still further embodiments, a pediatric subject refers to a human subject between 6 and 12 years of age.

[0034] “Infant” means a human subject ranging in age from birth to not more than one year and includes infants from 0 to 12 months corrected age. The phrase “corrected age” means an infant’s chronological age minus the amount of time that the infant was born premature. Therefore, the corrected age is the age of the infant if it had been carried to full term. The term infant includes low birth weight infants, very low birth weight infants, and preterm infants. A “pre-term infant” is an infant born after less than about 37 weeks gestation. A “full-term infant” as used herein means an infant born after at least about 37 weeks gestation.

[0035] “Child” means a subject ranging in age from 12 months to about 12 years. In some embodiments, a child is a subject between the ages of 1 and 12 years old. In other embodiments, the terms “children” or “child” refer to subjects that are between one and about six years old, or between about seven and about 12 years old. In other embodiments, the terms “children” or “child” refer to any range of ages between 12 months and about 12 years.

[0036] “Children’s nutritional product” refers to a composition that satisfies at least a portion of the nutrient requirements of a child. A growing-up milk (GUM) is an example of a children’s nutritional product.

[0037] As used herein, “hydrolyzed protein” means a product of protein hydrolysis. Within the present disclosure, hydrolyzed protein and protein hydrolysate

are used interchangeably to describe products of protein hydrolysis; extensively hydrolyzed protein is used to describe products of protein hydrolysis where at least 70%, more preferably at least about 90%, of the hydrolyzed protein has a molecular weight of less than 2000 Daltons.

[0038] The term “degree of hydrolysis” refers to the extent to which peptide bonds are broken by a hydrolysis method.

[0039] The term “protein-free” means containing no measurable amount of protein, as measured by standard protein detection methods such as sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or size exclusion chromatography. In some embodiments, the nutritional composition is substantially free of protein, wherein “substantially free” is defined hereinbelow.

[0040] “Infant formula” means a composition that satisfies at least a portion of the nutrient requirements of an infant. In the United States, the content of an infant formula is dictated by the federal regulations set forth at 21 C.F.R. Sections 100, 106, and 107. These regulations define macronutrient, vitamin, mineral, and other ingredient levels in an effort to simulate the nutritional and other properties of human breast milk.

[0041] The term “growing-up milk” refers to a broad category of nutritional compositions intended to be used as a part of a diverse diet in order to support the normal growth and development of a child between the ages of about 1 and about 6 years of age.

[0042] “Milk-based” means comprising at least one component that has been drawn or extracted from the mammary gland of a mammal. In some embodiments, a milk-based nutritional composition comprises components of milk that are derived from domesticated ungulates, ruminants or other mammals or any combination thereof. Moreover, in some embodiments, milk-based means comprising bovine casein, whey, lactose, or any combination thereof. Further, “milk-based nutritional composition” may refer to any composition comprising any milk-derived or milk-based product known in the art.

[0043] “Nutritionally complete” means a composition that may be used as the sole source of nutrition, which would supply essentially all of the required daily amounts of vitamins, minerals, and/or trace elements in combination with proteins, carbohydrates, and lipids. Indeed, “nutritionally complete” describes a nutritional

composition that provides adequate amounts of carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals and energy required to support normal growth and development of a subject.

[0044] The composition which is “nutritionally complete” for the preterm infant will, by definition, provide qualitatively and quantitatively adequate amounts of all carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of the preterm infant. The composition which is “nutritionally complete” for the term infant will, by definition, provide qualitatively and quantitatively adequate amounts of all carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of the term infant. The composition which is “nutritionally complete” for a child will, by definition, provide qualitatively and quantitatively adequate amounts of all carbohydrates, lipids, essential fatty acids, proteins, essential amino acids, conditionally essential amino acids, vitamins, minerals, and energy required for growth of a child.

[0045] As applied to nutrients, the term “essential” refers to any nutrient that cannot be synthesized by the body in amounts sufficient for normal growth and to maintain health and that, therefore, must be supplied by the diet. The term “conditionally essential” as applied to nutrients means that the nutrient must be supplied by the diet under conditions when adequate amounts of the precursor compound is unavailable to the body for endogenous synthesis to occur.

[0046] The term “probiotic” means a microorganism with low or no pathogenicity that exerts beneficial effects on the health of the host. A “viable probiotic” means a live or active microorganism that exerts beneficial effects on the health of the host.

[0047] The term “inactivated probiotic” or “inactivated LGG” means a probiotic wherein the metabolic activity or reproductive ability of the referenced probiotic or *Lactobacillus rhamnosus* GG (LGG) organism has been reduced or destroyed. The “inactivated probiotic” or “inactivated LGG” does, however, still retain, at the cellular level, at least a portion its biological glycol-protein and

DNA/RNA structure. As used herein, the term "inactivated" is synonymous with "non-viable". In some embodiments, the inactivated LGG is heat-inactivated LGG.

[0048] "Prebiotic" means a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the digestive tract that can improve the health of the host.

[0049] "Phytonutrient" means a chemical compound that occurs naturally in plants. Phytonutrients may be included in any plant-derived substance or extract. The term "phytonutrient(s)" encompasses several broad categories of compounds produced by plants, such as, for example, polyphenolic compounds, anthocyanins, proanthocyanidins, and flavan-3-ols (i.e. catechins, epicatechins), and may be derived from, for example, fruit, seed or tea extracts. Further, the term phytonutrient includes all carotenoids, phytosterols, thiols, and other plant-derived compounds.

[0050] " β -glucan" means all β -glucan, including specific types of β -glucan, such as β -1,3-glucan or β -1,3;1,6-glucan. Moreover, β -1,3;1,6-glucan is a type of β -1,3-glucan. Therefore, the term " β -1,3-glucan" includes β -1,3;1,6-glucan.

[0051] "Pectin" means any naturally-occurring oligosaccharide or polysaccharide that comprises galacturonic acid that may be found in the cell wall of a plant. Different varieties and grades of pectin having varied physical and chemical properties are known in the art. Indeed, the structure of pectin can vary significantly between plants, between tissues, and even within a single cell wall. Generally, pectin is made up of negatively charged acidic sugars (galacturonic acid), and some of the acidic groups are in the form of a methyl ester group. The degree of esterification of pectin is a measure of the percentage of the carboxyl groups attached to the galactopyranosyluronic acid units that are esterified with methanol.

[0052] Pectin having a degree of esterification of less than 50% (i.e., less than 50% of the carboxyl groups are methylated to form methyl ester groups) are classified as low-ester, low methoxyl, or low methylated ("LM") pectins, while those having a degree of esterification of 50% or greater than 50%, (i.e., more than 50% of the carboxyl groups are methylated) are classified as high-ester, high methoxyl or high methylated ("HM") pectins. Very low ("VL") pectins, a subset of low methylated pectins, have a degree of esterification that is less than approximately 15%.

[0053] All percentages, parts and ratios as used herein are by weight of the total nutritional composition, including the stabilized probiotic, unless otherwise specified.

[0054] All amounts specified as administered "per day" may be delivered in one unit dose, in a single serving or in two or more doses or servings administered over the course of a 24 hour period.

[0055] The nutritional composition of the present disclosure may be substantially free of any optional or selected ingredients described herein, provided that the remaining nutritional composition still contains all of the required ingredients or features described herein. In this context, and unless otherwise specified, the term "substantially free" means that the selected composition may contain less than a functional amount of the optional ingredient, typically less than 0.1% by weight, and also, including zero percent by weight of such optional or selected ingredient.

[0056] All references to singular characteristics or limitations of the present disclosure shall include the corresponding plural characteristic or limitation, and vice versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made.

[0057] All combinations of method or process steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[0058] The methods and compositions of the present disclosure, including components thereof, can comprise, consist of, or consist essentially of the essential elements and limitations of the embodiments described herein, as well as any additional or optional ingredients, components or limitations described herein or otherwise useful in nutritional compositions.

[0059] As used herein, the term "about" should be construed to refer to both of the numbers specified as the endpoint(s) of any range. Any reference to a range should be considered as providing support for any subset within that range.

THE PROTECTIVE MATRIX

[0060] In the practice of the present disclosure, hydrolyzed mammalian protein is utilized as a component of a protective matrix for stabilizing biological material.

[0061] Hydrolyzed mammalian protein can be created from a variety of mammalian protein sources, including milk products and animal products. It may be created through a process of acid hydrolysis where mammalian protein is subjected to a strong acid and heated until the desired size ranges of amino acid fragments are created. Further types of protein hydrolysis include the use of enzymatic agents which digest protein molecules in creating shorter chains of amino acids. Common processes for hydrolyzing protein are known in the art and described in U.S. Patent No. 4,377,601 issued to Conrad; U.S. Patent No. 4,443,540 issued to Chervan et al.; U.S. Patent No. 4,545,933 issued to Ernster; U.S. Patent No. 4,757,007 issued to Satoh et al.; U.S. Patent No. 4,873,108 issued to De Rooij et al.; U.S. Patent No. 5,401,527 issued to Brown et al.; U.S. Patent No. 6,214,585 issued to Kwong et al.; and U.S. Patent No. 6,221,423 issued to Cho et al., the disclosures each of which are hereby incorporated by reference in their entirety.

[0062] Mammalian proteins that may be hydrolyzed for use in the stabilization mixture/protective matrix of the present disclosure include egg proteins, animal proteins, poultry, meat, serum albumen, glycol proteins, collagen, gelatin, milk proteins, casein, whey protein, albumen and others. In an embodiment, the protective matrix includes mammalian protein, such as a bovine protein. As previously defined, the hydrolyzed mammalian protein of any of the above types is, in a preferred embodiment, extensively hydrolyzed, meaning at least about 70% of the hydrolyzed protein yielding peptides having a molecular weight of less than about 2,000 Daltons.

[0063] In one embodiment, the hydrolyzed mammalian protein comprises hydrolyzed casein having over about 80%, advantageously over about 90%, of the peptides with a molecular weight of less than about 2,000 Daltons. Casein is understood to be a phospho-protein which comprises almost 80% of the total protein in bovine milk. The protein includes no disulfide bridges and, as a result, has little secondary or tertiary structure. Non-hydrolyzed casein includes casein variants having a molecular weight in the range of from about 19,000 Daltons to about 68,000 Daltons. One embodiment of a mammalian protein hydrolysate, such as casein hydrolysate, which may be used in practice of the present disclosure is one in which over 90% of the peptides have a molecular weight of less than 1,000 Daltons, with over 97% having a molecular weight of less than 2,000 Daltons. Less than 0.3% of

the mammalian protein hydrolysate in certain embodiments is over 5,000 Daltons, illustrating that virtually all of the protein was hydrolyzed.

[0064] The use of the hydrolyzed mammalian protein(s) in the protective matrix of the present disclosure, including the aforementioned hydrolyzed casein, provides superior protection to probiotics, including *Lactobacillus rhamnosus*, beyond the protection observed through the use of larger peptide fragments or whole proteins. While not being bound by any theory, one possibility for the increased protection to the probiotics against both moisture and heat may involve the increase in the zeta potential of the surface resulting from the hydrolyzed mammalian protein. The zeta potential is a value which indicates the degree of repulsion between adjacent similarly charged particles within dispersion. Smaller compounds and molecules possess a high zeta potential which confers stability as the solution or dispersion will resist aggregation. Conversely, when the zeta potential is low attraction exceeds repulsion and the dispersion may break and flocculate. While hydrophobicity decreases, the magnitude of the zeta potential increases with an increasing degree of hydrolysis. The high degree of hydrolysis providing for many short peptide sequences may increase the zeta potential of the protein interface in contact with the probiotic and thus increase the stability of the biological agent to both heat and humidity. Of course, this does not explain the differences between hydrolyzed mammalian and non-mammalian proteins.

[0065] In some embodiments, the stabilization mixture may comprise between about 5 and about 25 grams of a hydrolyzed protein per 100 grams of the mixture on a dry basis. In certain embodiments, the stabilization mixture comprises between about 10 and about 20 grams of a hydrolyzed protein per 100 grams of the mixture on a dry basis. And in an embodiment, the stabilization mixture comprises about 15 grams of a hydrolyzed protein per 100 grams of the mixture on a dry basis. In some embodiments, the hydrolyzed protein comprises hydrolyzed casein.

[0066] In certain embodiments, the majority component of the stabilization mixture, based on a dry weight basis, is one or more carbohydrates, which may include polysaccharides, disaccharides and monosaccharides. Indeed, the protective matrix may include lactulose, lactosucrose, raffinose, gluco-oligosaccharide, trehalose, inulin, polydextrose, galacto-oligosaccharide, fructo-oligosaccharide, isomalto-oligosaccharide, soybean oligosaccharides, lactosucrose, xylo-

oligosaccharide, chito-oligosaccharide, manno-oligosaccharide, aribino-oligosaccharide, sialyl-oligosaccharide, fuco-oligosaccharide, gentio-oligosaccharides, and/or any combination thereof. In some embodiments, the protective matrix includes a first carbohydrate chosen from: sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of about 2 to about 6, and any combination thereof. In certain embodiments, the protective matrix includes a second carbohydrate chosen from: inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof.

[0067] In some embodiments, the stabilization mixture may comprise between about 50 and about 80 grams of a first carbohydrate per 100 grams of the mixture on a dry basis; between about 60 and about 70 grams of a first carbohydrate per 100 grams of the mixture on a dry basis; or between about 65 and about 70 grams of a first carbohydrate per 100 grams of the mixture on a dry basis. The first carbohydrate may be chosen from: sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of about 2 to about 6, and any combination thereof.

[0068] The stabilization mixture may also comprise between about 1 and about 10 grams of a second carbohydrate per 100 grams of the mixture on a dry basis; between about 4 and about 6 grams of a second carbohydrate per 100 grams of the mixture on a dry basis; or about 5 grams of a second carbohydrate per 100 grams of the mixture on a dry basis. In some embodiments, the second carbohydrate is chosen from: inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof.

[0069] A further component of the stabilization mixture can be a compound binder (also referred to as a gelling agent), which may act as a thickener and produce a gel-like consistency. Compound binders that may be included in the protective matrix of the present disclosure include alginates, such as sodium alginate, pectin, chitosan, carboxymethylcellulose, and mixtures thereof, among others. The incorporation of the compound binder provides for the formation of a viscous consistency providing for efficient matrix formation and a structural quality suitable for subsequent drying.

[0070] A compound binder can, in some embodiments, form a gum-like material and increase the viscosity of mixtures to which it is added. Additionally, the compound binder may also provide for greater ease in mixing of the components together. For instance, sodium alginate may also possess emulsifier characteristics.

[0071] In some embodiments, the stabilization mixture may comprise LM pectin, HM pectin, VL pectin, or any mixture thereof. The included pectin may be soluble in water.

[0072] Pectins for use herein typically have a peak molecular weight of 8,000 Daltons or greater. The pectins of the present disclosure have a preferred peak molecular weight of between 8,000 and about 500,000, more preferred is between about 10,000 and about 200,000 and most preferred is between about 15,000 and about 100,000 Daltons. In some embodiments, the pectin of the present disclosure may be hydrolyzed pectin. In certain embodiments, the protective matrix comprises hydrolyzed pectin having a molecular weight less than that of intact or unmodified pectin. The hydrolyzed pectin of the present disclosure can be prepared by any means known in the art to reduce molecular weight. Examples of said means are chemical hydrolysis, enzymatic hydrolysis and mechanical shear. A preferred means of reducing the molecular weight is by alkaline or neutral hydrolysis at elevated temperature. In some embodiments, the protective matrix comprises partially hydrolyzed pectin. In certain embodiments, the partially hydrolyzed pectin has a molecular weight that is less than that of intact or unmodified pectin but more than 3,300 Daltons.

[0073] The stabilization mixture may comprise between about 0.5 and about 5 grams of a compound binder, such as sodium alginate and/or pectin, per 100 grams of the mixture on a dry basis. In certain embodiments, the stabilization mixture comprises between about 1 and about 3 grams of a compound binder per 100 grams of the mixture on a dry basis. And in an embodiment, the stabilization mixture comprises about 2 grams of a compound binder per 100 grams of the mixture on a dry basis.

[0074] Moreover, the stabilization mixture may also comprise at least one starch, source of starch and/or starch component. In some embodiments, the stabilization mixture may comprise native or modified starches, such as, for example,

waxy corn starch, waxy rice starch, waxy potato starch, waxy tapioca starch, corn starch, rice starch, potato starch, tapioca starch, wheat starch or any mixture thereof.

[0075] Furthermore, the stabilization mixture may comprise a lipid component. In some embodiments, the stabilization mixture comprises between about 0.5 and about 2 grams of a lipid component per 100 grams of the mixture on a dry basis. In certain embodiments, the stabilization mixture comprises between about 0.75 and about 1.25 grams of a lipid component per 100 grams of the mixture on a dry basis. And in an embodiment, the stabilization mixture comprises about 1 gram of a lipid component per 100 grams of the mixture on a dry basis. In some embodiments, the lipid component is chosen from: lecithin, monoglycerides, diglycerides, and any combination thereof.

[0076] The stabilization mixture may also include additional ingredients that provide further benefits to either the probiotic or the individual ingesting the stabilized probiotic. These ingredients may comprise minerals, vitamins, antioxidants, trace elements, sterols, antioxidants, fatty acids, functional molecules, and any combination thereof. Other ingredients may include resistant starches, high amylose starches, guar, and locust bean gum, agar, xanthan, carrageenans, glucans, and any combination thereof.

[0077] In some embodiments, the stabilization mixture may comprise between about 5 and about 20 grams of probiotic and/or other biological material per 100 grams of the mixture on a dry basis. In certain embodiments, the stabilization mixture comprises between about 9 and about 12 grams of probiotic and/or other biological material per 100 grams of the mixture on a dry basis. And in an embodiment, the stabilization mixture comprises about 11 grams of probiotic and/or other biological material per 100 grams of the mixture on a dry basis. In another embodiment, the concentration of the probiotic, for instance LGG, in the protective matrix is from about 1×10^6 to about 1×10^{14} cfu per gram of the protective matrix, more preferably from about 1×10^9 to about 1×10^{11} cfu per gram of the protective matrix.

[0078] The stabilization mixture may be used to provide stability to a probiotic organism which may exert a beneficial effect on the health and welfare of individuals. Examples of suitable probiotics include but are not limited to yeasts such as *Saccharomyces cerevisiae*, molds such as *Aspergillus*, *Rhizopus*, *Mucor*, and bacteria

such as *Lactobacillus*. Specific examples of suitable probiotic micro-organisms are: *Aspergillus niger*, *A. oryzae*, *Bacillus coagulans*, *B. lentus*, *B. licheniformis*, *B. mesentericus*, *B. pumilus*, *B. subtilis*, *B. natto*, *Bifidobacterium adolescentis*, *B. animalis*, *B. breve*, *B. bifidum*, *B. infantis*, *B. lactis*, *B. longum*, *B. longum* BB536, *B. longum* AH1206 (NCIMB: 41382), *B. breve* AH1205 (NCIMB: 41387), *B. infantis* 35624 (NCIMB: 41003), *B. longum* AH1714 (NCIMB 41676), *B. animalis* subsp. *lactis* BB-12 (DSM No. 10140), *B. pseudolongum*, *B. thermophilum*, *Candida pintolepesii*, *Clostridium butyricum*, *Enterococcus cremoris*, *E. diacetylactis*, *E. faecium*, *E. intermedius*, *E. lactis*, *E. muntidi*, *E. thermophilus*, *Lactobacillus acidophilus*, *L. alimentarius*, *L. amylovorus*, *L. crispatus*, *L. brevis*, *L. casei*, *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*, *Lactobacillus rhamnosus* GG (ATCC number 53103), *L. sakei*, *L. salivarius* and any combination thereof. In an embodiment, the stabilized probiotic(s) may be viable or non-viable. The stabilized probiotics useful in the present disclosure may be naturally-occurring, synthetic or developed through the genetic manipulation of organisms, whether such new source is now known or later developed.

[0079] In an embodiment of the present disclosure *Lactobacillus rhamnosus* GG is utilized as a probiotic that may be stabilized by the protective matrix of the present disclosure. *Lactobacillus rhamnosus* GG is described in U.S. Patent Application 4,839,281, issued to Sharwood et al., which is hereby incorporated by reference in its entirety. Notably, Sharwood et al. describes *Lactobacillus rhamnosus* GG as being a species in which the bacteria have avid adherence to intestinal cells while being simultaneously able to survive at low pHs and produce large amounts of lactic acid.

[0080] The selected probiotic is preferably concentrated to a wet paste-like consistency prior to combining with the stabilization mixture of the present disclosure. Starting with probiotics in dry form is also an alternative. Concentration levels of selected probiotics include concentrations of from about 3X to about 20X though may include lesser or greater concentrations depending upon the specific probiotic biomass and subsequent processing steps.

[0081] Generally, the preparation of a stabilized probiotic includes the steps of concentrating the selected probiotic or probiotics; providing components of the

stabilization mixture in desired quantities; mixing the stabilization mixture with the concentrated probiotic; drying the stabilized probiotic and either packaging or combining the stabilized probiotic into a nutritional product, such as an infant formula.

[0082] In some embodiments, the present disclosure is directed to a method for protecting a viable probiotic for use in a nutritional composition, the method may include the steps of providing a viable probiotic, preparing a protective matrix for the probiotic by blending (i) hydrolyzed casein, (ii) a first carbohydrate selected from the group consisting of sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of about 2 to about 6, and any combination thereof, (iii) a second carbohydrate selected from the group consisting of inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than about 8, and any combination thereof, and (iv) a lipid selected from the group consisting of lecithin, monoglycerides, diglycerides, and any combination thereof, then combining the viable probiotic, the protective matrix and water to produce a mixture and drying the mixture to a final moisture content of about 4% or less, and further adding the dried mixture to a powdered nutritional product.

[0083] In optimizing the stabilization for probiotic, the multiple constituents may be varied in some embodiments. In some embodiments, carbohydrates may comprise from about 70% to about 85% of the stabilization mixture on a dry basis; the hydrolyzed mammalian protein may comprise from about 10% to about 20% of the stabilization mixture on a dry basis, and the compound binder may comprise from about 0% to about 10% (i.e., up to about 10%) of the stabilization mixture on a dry basis.

[0084] The stabilized probiotic, with over 70% of the matrix protein having a molecular weight of less than 2,000 Daltons, may be packaged and sold commercially or may be instead combined with a variety of nutritional products. Such nutritional products may include both infant formulas and children's products useful for applications where one desires to incorporate a probiotic into a nutritional product that necessitates an improved shelf-life and stability.

[0085] Table 1 presents a sample embodiment of a stabilized probiotic mixture/protective matrix according to the present disclosure.

TABLE 1: An embodiment of the protective matrix

Ingredient	Grams per 100g (dry basis)
Probiotic (LGG, AH1206 or 35624)	11
Sucrose, maltose, lactose, trehalose, maltotriose, maltotriose enriched maltodextrin or low DE maltodextrin (preferably 2-6 DE)	69
Hydrolyzed casein	15
Inulin, PDX, GOS, FOS, starch (including modified versions) and/or maltodextrin (>8 DE)	5

[0086] Fig. 1 illustrates the stability of LGG in a protective matrix prepared according to the embodiment presented in Table 1, wherein the stabilized LGG is provided in a growing up milk having an available water (Aw) content of 0.28. Fig. 2 is a graph that illustrates the relative stability of LGG without a protective matrix in a growing up milk having an available water (Aw) content of 0.28. Fig. 3 presents scanning electron micrographs of stabilized LGG in a protective matrix per the formulation presented in Table 1.

[0087] Table 2 provides another example embodiment of a stabilized probiotic mixture/protective matrix according to the present disclosure.

TABLE 2: An embodiment of the protective matrix

Ingredient	Grams per 100g (dry basis)
Probiotic (LGG, AH1206 or 35624)	11
Sucrose, maltose, lactose, trehalose, maltotriose, maltotriose enriched maltodextrin or low DE maltodextrin (preferably 2-6 DE)	67
Hydrolyzed casein	15
Inulin, PDX, GOS, FOS, starch (including modified versions) and/or maltodextrin (>8 DE)	5
Sodium alginate or pectin	2

[0088] Table 3 presents yet another example embodiment of a stabilized probiotic mixture/protective matrix according to the present disclosure.

TABLE 3: An embodiment of the protective matrix

Ingredient	Grams per 100g (dry basis)
Probiotic (LGG, AH1206 or 35624)	11
Sucrose, maltose, lactose, trehalose, maltotriose, maltotriose enriched maltodextrin or low DE maltodextrin (preferably 2-6 DE)	68
Hydrolyzed casein	15
Inulin, PDX, GOS, FOS, starch (including modified versions) and/or maltodextrin (>8 DE)	5
Lecithin, mono- or di-glyceride(s)	1

[0089] Table 4 also provides an example embodiment of a stabilized probiotic mixture/protective matrix according to the present disclosure.

TABLE 4: An embodiment of the protective matrix

Ingredient	Grams per 100g (dry basis)
Probiotic (LGG, AH1206 or 35624)	11
Sucrose, maltose, lactose, trehalose, maltotriose, maltotriose enriched maltodextrin or low DE maltodextrin (preferably 2-6 DE)	65
Hydrolyzed casein	15
Inulin, PDX, GOS, FOS, starch (including modified versions) and/or maltodextrin (>8 DE)	5
Sodium alginate or pectin	2
Lecithin, mono- or di-glyceride(s)	1

[0090] In order to further illustrate the principles and operations of the present disclosure, the following example is provided. However, this example should not be taken as limiting in any regard.

EXAMPLE

[0091] *Lactobacillus rhamnosus* GG (LGG) is grown in a fermenter. The biomass is subsequently washed with buffer and centrifuged to obtain a LGG moist pellet. A stabilization mixture is pre-blended comprising on a dry weight basis approximately 75% trehalose, about 16.7% hydrolyzed casein, 5.2% inulin and approximately 3.1% sodium alginate. At a temperature of 37°C, the LGG moist pellet is mixed with the stabilization mixture and enough water to yield a total solids content of approximately 55%. The slurry is mixed under vacuum to yield a density of around 1.1 g/cm³.

[0092] The combination of stabilization mixture and LGG is then either vacuum-dried or freeze-dried to a final moisture content of approximately 3%. It is preferred for the mixture to be spread in a tray at a load ranging from 100g/ft² to 150g/ft². The mixture is dried ensuring product temperature is below 30°C during removal of 80% of the total water. Temperature during removal of the remaining 17% moisture

should not exceed 50°C. The dried, stabilized LGG may subsequently be ground and size selected through the use of sieves to obtain a product having a desirable size.

NUTRITIONAL PRODUCTS FOR COMBINATION WITH A STABILIZED PROBIOTIC

[0093] A stabilized probiotic prepared as described hereinabove may be combined with a nutritional product to form a novel nutritional composition.

[0094] For example, the stabilized probiotic may be combined with a nutritional product, such as an infant formula or children's nutritional product, to form a stabilized nutritional composition. In another embodiment, the stabilized probiotic may be combined with a human milk fortifier, which is added to human milk in order to enhance the nutritional value of human milk.

[0095] Further, the stabilized probiotic of the disclosure may be combined with a nutritional product that provides minimal, partial, or total nutritional support. Such nutritional product(s) may be nutritional supplements or meal replacements. Indeed, the stabilized probiotic can be intermixed with food or other nutritional products prior to ingestion by a subject.

[0096] The nutritional product for combination with the stabilized probiotic may, but need not, be nutritionally complete. Likewise, the combination of the stabilized probiotic with a nutritional product may produce a nutritional composition that is nutritionally complete. In an embodiment, the nutritional composition of the disclosure is nutritionally complete and contains suitable types and amounts of lipid, carbohydrate, protein, vitamins and minerals.

[0097] The stabilized probiotic created by the present disclosure may be combined with a nutritional product provided in any form known in the art, including a powder, a gel, a suspension, a paste, a solid, a liquid, a liquid concentrate, or a ready-to-use product. In one combination, the nutritional product is an infant formula, especially an infant formula adapted for use as sole source nutrition for an infant.

[0098] The nutritional products described for combining with the stabilized probiotic may be administered enterally.

NUTRITIONAL COMPOSITIONS COMPRISING STABILIZED PROBIOTICS

[0099] Again, a stabilized/protected probiotic prepared as described above may be combined with a nutritional product to form a novel nutritional composition.

[0100] The nutritional composition may comprise any fat or lipid source that is known or used in the art, including but not limited to, animal sources, e.g., milk fat, butter, butter fat, egg yolk lipid; marine sources, such as fish oils, marine oils, single cell oils; vegetable and plant oils, such as corn oil, canola oil, sunflower oil, soybean oil, palmolein, coconut oil, high oleic sunflower oil, evening primrose oil, rapeseed oil, olive oil, flaxseed (linseed) oil, cottonseed oil, high oleic safflower oil, palm stearin, palm kernel oil, wheat germ oil; medium chain triglyceride oils and emulsions and esters of fatty acids; and any combinations thereof. The amount of lipid or fat in the nutritional composition typically varies from about 1 to about 7 g/100 kcal.

[0101] Further, the nutritional composition may comprise a source of bovine milk protein. The source of bovine milk protein may include, but is not limited to, milk protein powders, milk protein concentrates, milk protein isolates, nonfat milk solids, nonfat milk, nonfat dry milk, whey protein, whey protein isolates, whey protein concentrates, sweet whey, acid whey, casein, acid casein, caseinate (e.g. sodium caseinate, sodium calcium caseinate, calcium caseinate) and any combination thereof.

[0102] In certain embodiments, the nutritional composition may comprise intact protein. In other embodiments, the proteins of the nutritional composition are provided as a combination of both intact proteins and partially hydrolyzed proteins, with a degree of hydrolysis of between about 4% and 10%. Certain of these embodiments can be extremely hypoallergenic, as both the stabilizer and the protein of the nutritional product contain only hydrolyzed protein. In yet another embodiment, the nutritional composition may be supplemented with glutamine-containing peptides.

[0103] The whey:casein ratio of the protein source of the nutritional composition may be similar to that found in human breast milk. In an embodiment, the protein source of the nutritional composition comprises from about 40% to about 80% whey protein. In another embodiment, the protein source may comprise from about 20% to about 60% caseins. The amount of protein in a nutritional composition typically varies from about 1 to about 7 g/100 kcal.

[0104] In other embodiments the nutritional composition comprises lactoferrin, which retains its stability and activity in the human gut against certain undesirable bacterial pathogens.

[0105] The nutritional composition described herein can, in some embodiments, also comprise non-human lactoferrin, non-human lactoferrin produced by a genetically modified organism and/or human lactoferrin produced by a genetically modified organism. Lactoferrin is generally described as an 80 kilodalton glycoprotein having a structure of two nearly identical lobes, both of which include iron binding sites. As described in "*Perspectives on Interactions Between Lactoferrin and Bacteria*" which appeared in the publication BIOCHEMISTRY AND CELL BIOLOGY, pp 275-281 (2006), lactoferrin from different host species may vary in an amino acid sequence though commonly possesses a relatively high isoelectric point with positively charged amino acids at the end terminal region of the internal lobe. Lactoferrin has been recognized as having bactericidal and antimicrobial activities. In at least one embodiment, the lactoferrin is bovine lactoferrin.

[0106] Surprisingly, the forms of lactoferrin included herein maintain relevant activity even if exposed to a low pH (i.e., below about 7, and even as low as about 4.6 or lower) and/or high temperatures (i.e., above about 65°C, and as high as about 120°C, conditions which would be expected to destroy or severely limit the stability or activity of human lactoferrin or recombinant human lactoferrin. These low pH and/or high temperature conditions can be expected during certain processing regimen for nutritional compositions of the types described herein, such as pasteurization.

[0107] In one embodiment, lactoferrin is present in the nutritional composition in an amount of from about 5 mg/100 kcal to about 16 mg/100 kcal. In another embodiment, lactoferrin is present in an amount of about 9 mg/100 kcal to about 14 mg/100 kcal. In still further embodiments, the nutritional composition may comprise between about 75 mg and about 200 mg lactoferrin per 100 kcal. And in certain embodiments, the nutritional composition may comprise between about 90 mg and about 148 mg lactoferrin per 100 kcal.

[0108] The nutritional composition may also contain TGF- β . In some embodiments, the level of TGF- β may be from about 0.0150 (pg/ μ g) ppm to about 0.1000 (pg/ μ g) ppm. In other embodiments, the level of TGF- β in final composition including a stabilized probiotic is from about 0.0225 (pg/ μ g) ppm to about 0.0750 (pg/ μ g) ppm.

[0109] In some embodiments of the nutritional composition, the level of TGF- β is from about 2500 pg/mL to about 10,000 pg/mL, more preferably from about 3000 pg/mL to about 8000 pg/mL. In an embodiment, the ratio of TGF- β 1: TGF- β 2 is in the range of about 1:1 to about 1:20, or, more particularly, in the range of about 1:5 to about 1:15.

[0110] In some embodiments, the bioactivity of TGF- β in a nutritional composition is enhanced by the addition of a bioactive whey fraction. Any bioactive whey fraction known in the art may be used in such embodiments provided it achieves the intended result. In an embodiment, this bioactive whey fraction may be a whey protein concentrate. In a particular embodiment, the whey protein concentrate may be Salibra® 800, available from Glanbia Nutritionals.

[0111] The nutritional composition may comprise an amount of probiotic in addition to the stabilized probiotic. When the stabilized probiotic is combined with the nutritional product, the resulting nutritional composition may include a total amount of probiotics effective to provide from about 1×10^4 to about 1×10^{10} colony forming units (cfu) per kg body weight per day to a subject. In other embodiments, the amount of the probiotic may vary from about 1×10^6 to about 1×10^9 cfu per kg body weight per day. In even further embodiments, the nutritional composition may include an amount of probiotics effective to provide about 1×10^6 cfu per kg body weight per day.

[0112] In certain embodiments, the nutritional composition of the present disclosure comprises between about 1×10^6 cfu probiotic and about 1×10^{10} cfu per 100 kcal of the composition. In some embodiments, the amount of probiotic may be in the range of about 1×10^6 cfu to about 1×10^9 cfu per 100 kcal of the composition. Additionally, the nutritional composition may include non-stabilized probiotics, with the final composition including some stabilized probiotics and some non-stabilized probiotics.

[0113] The nutritional composition may further comprise at least one prebiotic. The term "prebiotic" as used herein refers to indigestible food ingredients that exert health benefits upon the host. Such health benefits may include, but are not limited to, selective stimulation of the growth and/or activity of one or a limited number of beneficial gut bacteria, stimulation of the growth and/or activity of ingested probiotic (stabilized or not) microorganisms, selective reduction in gut pathogens, and

favorable influence on gut short chain fatty acid profile. Such prebiotics may be naturally-occurring, synthetic, or developed through the genetic manipulation of organisms and/or plants, whether such new source is now known or developed later. Prebiotics may include oligosaccharides, polysaccharides, and other prebiotics that contain fructose, xylose, soya, galactose, glucose and mannose. More specifically, prebiotics useful in the present disclosure may include lactulose, lactosucrose, raffinose, gluco-oligosaccharide, inulin, polydextrose, polydextrose powder, galacto-oligosaccharide, fructo-oligosaccharide, isomalto-oligosaccharide, soybean oligosaccharides, lactosucrose, xylo-oligosaccharide, chito-oligosaccharide, manno-oligosaccharide, aribino-oligosaccharide, sialyl-oligosaccharide, fuco-oligosaccharide, and gentio-oligosaccharides, and combinations thereof.

[0114] In some embodiments, the total amount of prebiotics present in the nutritional composition may be from about 1.0 g/L to about 10.0 g/L of the composition (in the liquid form). In certain embodiments, the total amount of prebiotics present in the nutritional composition may be from about 2.0 g/L and about 8.0 g/L of the composition.

[0115] The nutritional composition may comprise polydextrose (PDX). If polydextrose is used as a prebiotic, the amount of polydextrose in the nutritional composition may, in an embodiment, be within the range of from about 1.0 g/L to about 4.0 g/L. If polydextrose is used as a prebiotic, the amount of polydextrose in the nutritional product may, in an embodiment of the composition including stabilized probiotics, be within the range of from about 0.1 mg/100 kcal to about 0.5 mg/100 kcal. In another composition, the amount of polydextrose may be about 0.3 mg/100 kcal. At least 20% of the prebiotics should, in a preferred embodiment, comprise polydextrose (PDX).

[0116] In certain embodiments, the nutritional composition comprises galacto-oligosaccharide. The amount of galacto-oligosaccharide in the nutritional composition may be from about 0.2 mg/100 kcal to about 1.0 mg/100 kcal. In other embodiments, the amount of galacto-oligosaccharide in the nutritional composition may be from about 0.1 mg/100 kcal to about 0.5 mg/100 kcal. Galacto-oligosaccharide and polydextrose may also be supplemented into the nutritional composition in a total amount of about 0.6 mg/100 kcal.

[0117] In some embodiments, the nutritional composition comprises an additional carbohydrate source, that is, a carbohydrate source provided in addition to the other carbohydrates described throughout the present disclosure. Suitable additional carbohydrate sources can be any used in the art, e.g., lactose, glucose, fructose, corn syrup solids, maltodextrins, sucrose, starch, rice syrup solids, and the like. The amount of additional carbohydrate in the nutritional composition typically can vary from between about 5 g and about 25 g/100 kcal. In some embodiments, the amount of carbohydrate is between about 6 g and about 22 g/100 kcal. In other embodiments, the amount of carbohydrate is between about 12 g and about 14 g/100 kcal.

[0118] The nutritional composition may contain a source of long chain polyunsaturated fatty acids (LCPUFAs) which comprise docosahexanoic acid (DHA). Other suitable LCPUFAs include, but are not limited to, α -linoleic acid, γ -linoleic acid, linoleic acid, linolenic acid, eicosapentaenoic acid (EPA) and arachidonic acid (ARA).

[0119] In some embodiments, the nutritional composition may be supplemented with both DHA and ARA, and the weight ratio of ARA:DHA may be from about 1:3 to about 9:1. In certain embodiments the ARA:DHA ratio is from about 1:2 to about 4:1.

[0120] The amount of long chain polyunsaturated fatty acids in the nutritional composition may vary from about 5 mg/100 kcal to about 100 mg/100 kcal, more preferably from about 10 mg/100 kcal to about 50 mg/100 kcal.

[0121] Moreover, a nutritional composition may be supplemented with oils containing DHA and ARA using standard techniques known in the art. As an example, the oils containing DHA and ARA may be added to a nutritional composition by replacing an equivalent amount of the rest of the overall fat blend normally present in the nutritional composition.

[0122] If utilized, the source of DHA and ARA may be any source known in the art such as marine oil, fish oil, single cell oil, egg yolk lipid, and brain lipid. In some compositions, the DHA and ARA are sourced from the single cell Martek oil, DHASCO®, or variations thereof. The DHA and ARA can be in natural form, provided that the remainder of the LCPUFA source does not result in any substantial deleterious effect on the infant. Alternatively, the DHA and ARA can be used in refined form.

[0123] In an embodiment of the nutritional composition, sources of DHA and ARA are single cell oils as taught in U.S. Patent Nos. 5,374,567; 5,550,156; and 5,397,591, the disclosures of which are incorporated herein in their entirety by reference.

[0124] In certain embodiments, the nutritional composition may be a milk-based nutritional composition that provides physiochemical and physiological benefits. As is known in the art, bovine milk protein comprises two major components: acid soluble whey protein and acid insoluble casein, with the latter representing about 80% of the total protein content of bovine milk. Upon entering the acidic environment of the stomach, casein precipitates and complexes with minerals forming semi-solid curds of varying size and firmness. Softer, smaller curds are easier for the body to digest than larger, harder curds. Curd formation may be an important consideration in the development of nutritional compositions, including, but not limited to infant formulas, medical foods, and premature infant formulas. As such, stabilized probiotics may be combined with compositions that include softer and smaller curds than standard infant formulas.

[0125] One or more vitamins and/or minerals may also be added in to the nutritional composition in amounts sufficient to supply the daily nutritional requirements of a subject. It is to be understood by one of ordinary skill in the art that vitamin and mineral requirements will vary, for example, based on the age of the child. For instance, an infant may have different vitamin and mineral requirements than a child between the ages of one and thirteen years. Thus, the embodiments are not intended to limit the nutritional composition to a particular age group but, rather, to provide a range of acceptable vitamin and mineral components.

[0126] The nutritional composition may optionally include, but is not limited to, one or more of the following vitamins or derivations thereof: vitamin B₁ (thiamin, thiamin pyrophosphate, TPP, thiamin triphosphate, TTP, thiamin hydrochloride, thiamin mononitrate), vitamin B₂ (riboflavin, flavin mononucleotide, FMN, flavin adenine dinucleotide, FAD, lactoflavin, ovoflavin), vitamin B₃ (niacin, nicotinic acid, nicotinamide, niacinamide, nicotinamide adenine dinucleotide, NAD, nicotinic acid mononucleotide, NicMN, pyridine-3-carboxylic acid), vitamin B₃-precursor tryptophan, vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine, pyridoxine hydrochloride), pantothenic acid (pantothenate, panthenol), folate (folic acid, folacin,

pteroylglutamic acid), vitamin B₁₂ (cobalamin, methylcobalamin, deoxyadenosylcobalamin, cyanocobalamin, hydroxycobalamin, adenosylcobalamin), biotin, vitamin C (ascorbic acid), vitamin A (retinol, retinyl acetate, retinyl palmitate, retinyl esters with other long-chain fatty acids, retinal, retinoic acid, retinol esters), vitamin D (calciferol, cholecalciferol, vitamin D₃, 1,25,-dihydroxyvitamin D), vitamin E (α-tocopherol, α-tocopherol acetate, α-tocopherol succinate, α-tocopherol nicotinate, α-tocopherol), vitamin K (vitamin K₁, phyloquinone, naphthoquinone, vitamin K₂, menaquinone-7, vitamin K₃, menaquinone-4, menadione, menaquinone-8, menaquinone-8H, menaquinone-9, menaquinone-9H, menaquinone-10, menaquinone-11, menaquinone-12, menaquinone-13), choline, inositol, β-carotene and any combinations thereof.

[0127] Further, the nutritional composition may optionally include, but is not limited to, one or more of the following minerals or derivations thereof: boron, calcium, calcium acetate, calcium gluconate, calcium chloride, calcium lactate, calcium phosphate, calcium sulfate, chloride, chromium, chromium chloride, chromium picolinate, copper, copper sulfate, copper gluconate, cupric sulfate, fluoride, iron, carbonyl iron, ferric iron, ferrous fumarate, ferric orthophosphate, iron trituration, polysaccharide iron, iodide, iodine, magnesium, magnesium carbonate, magnesium hydroxide, magnesium oxide, magnesium stearate, magnesium sulfate, manganese, molybdenum, phosphorus, potassium, potassium phosphate, potassium iodide, potassium chloride, potassium acetate, selenium, sulfur, sodium, docusate sodium, sodium chloride, sodium selenate, sodium molybdate, zinc, zinc oxide, zinc sulfate and mixtures thereof. Non-limiting exemplary derivatives of mineral compounds include salts, alkaline salts, esters and chelates of any mineral compound.

[0128] The minerals can be added to nutritional compositions in the form of salts such as calcium phosphate, calcium glycerol phosphate, sodium citrate, potassium chloride, potassium phosphate, magnesium phosphate, ferrous sulfate, zinc sulfate, cupric sulfate, manganese sulfate, and sodium selenite. Additional vitamins and minerals can be added as known within the art.

[0129] The nutritional composition of the present disclosure may optionally include one or more of the following flavoring agents, including, but not limited to, flavored extracts, volatile oils, cocoa or chocolate flavorings, peanut butter flavoring, cookie crumbs, vanilla or any commercially available flavoring. Examples of useful

flavorings include, but are not limited to, pure anise extract, imitation banana extract, imitation cherry extract, chocolate extract, pure lemon extract, pure orange extract, pure peppermint extract, honey, imitation pineapple extract, imitation rum extract, imitation strawberry extract, or vanilla extract; or volatile oils, such as balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, cinnamon oil, clove oil, or peppermint oil; peanut butter, chocolate flavoring, vanilla cookie crumb, butterscotch, toffee, and mixtures thereof. The amounts of flavoring agent can vary greatly depending upon the flavoring agent used. The type and amount of flavoring agent can be selected as is known in the art.

[0130] The nutritional composition of the present disclosure may optionally include one or more emulsifiers that may be added for stability of the final product. Examples of suitable emulsifiers include, but are not limited to, lecithin (*e.g.*, from egg or soy), alpha lactalbumin and/or mono- and di-glycerides, and mixtures thereof. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product. In some embodiments, nutritional compositions of the present disclosure may comprise emulsifiers such as citric acid esters of mono- and/or diglycerides, diacetyl tartaric acid esters of mono- and/or diglycerides, and/or octenyl succinic anhydride modified starches.

[0131] The nutritional composition of the present disclosure may optionally include one or more preservatives that may also be added to extend product shelf life. Suitable preservatives include, but are not limited to, potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate, calcium disodium EDTA, and mixtures thereof.

[0132] The nutritional composition of the present disclosure may optionally include one or more stabilizers. Suitable stabilizers for use in practicing the nutritional composition of the present disclosure include, but are not limited to, gum arabic, gum ghatti, gum karaya, gum tragacanth, agar, furcellaran, guar gum, gellan gum, locust bean gum, pectin, low methoxyl pectin, gelatin, microcrystalline cellulose, CMC (sodium carboxymethylcellulose), methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, DATEM (diacetyl tartaric acid esters of mono- and diglycerides), dextran, carrageenans, and mixtures thereof.

[0133] The nutritional composition of the present disclosure may further include at least one additional phytonutrient, that is, another phytonutrient component in addition to the pectin, starch or other phytonutrient components described herein. Phytonutrients, or their derivatives, conjugated forms or precursors, that are identified in human milk are preferred for inclusion in the nutritional composition. For example, in some embodiments, the nutritional composition of the present disclosure may comprise, in an 8 fl. oz. (236.6 mL) serving, between about 80 and about 300 mg anthocyanins, between about 100 and about 600 mg proanthocyanidins, between about 50 and about 500 mg flavan-3-ols, or any combination or mixture thereof. In other embodiments, the nutritional composition comprises apple extract, grape seed extract, or a combination or mixture thereof. Further, the at least one phytonutrient of the nutritional composition may be derived from any single or blend of fruit, grape seed and/or apple or tea extract(s).

[0134] Examples of additional phytonutrients suitable for the nutritional composition include, but are not limited to, anthocyanins, proanthocyanidins, flavan-3-ols (i.e., catechins, epicatechins, etc.), flavanones, flavonoids, isoflavonoids, stilbenoids (i.e. resveratrol, etc.) proanthocyanidins, anthocyanins, resveratrol, quercetin, curcumin, and/or any mixture thereof, as well as any possible combination of phytonutrients in a purified or natural form. Certain components, especially plant-based components of the nutritional compositions may provide a source of phytonutrients.

[0135] The phytonutrient component of the nutritional composition may also comprise naringenin, hesperetin, anthocyanins, quercetin, kaempferol, epicatechin, epigallocatechin, epicatechin-gallate, epigallocatechin-gallate or any combination thereof. In certain embodiments, the nutritional composition comprises between about 50 and about 2000 nmol/L epicatechin, between about 40 and about 2000 nmol/L epicatechin gallate, between about 100 and about 4000 nmol/L epigallocatechin gallate, between about 50 and about 2000 nmol/L naringenin, between about 5 and about 500 nmol/L kaempferol, between about 40 and about 4000 nmol/L hesperetin, between about 25 and about 2000 nmol/L anthocyanins, between about 25 and about 500 nmol/L quercetin, or a mixture thereof. Furthermore, the nutritional composition may comprise the metabolite(s) of a phytonutrient or of its parent compound, or it may comprise other classes of dietary

phytonutrients, such as glucosinolate or sulforaphane. In certain embodiments, the nutritional composition comprises carotenoids, such as lutein, zeaxanthin, astaxanthin, lycopene, beta-carotene, alpha-carotene, gamma-carotene, and/or beta-cryptoxanthin.

[0136] The nutritional composition may also comprise isoflavonoids and/or isoflavones. Examples include, but are not limited to, genistein (genistin), daidzein (daidzin), glycitein, biochanin A, formononetin, coumestrol, irilone, orobol, pseudobaptigenin, anagyroidisoflavone A and B, calycosin, glycitein, irigenin, 5-O-methylgenistein, pratensein, prunetin, psi-tectorigenin, retusin, tectorigenin, iridin, ononin, puerarin, tectoridin, derrubone, luteone, wighteone, alpinumisoflavone, barbigerone, di-O-methylalpinumisoflavone, and 4'-methyl-alpinumisoflavone. Plant sources rich in isoflavonoids, include, but are not limited to, soybeans, psoralea, kudzu, lupine, fava, chick pea, alfalfa, legumes and peanuts.

[0137] In an embodiment, the nutritional composition of the present disclosure comprises an effective amount of choline. An effective amount of choline is between about 20 mg choline per 8 fl. oz. (236.6 mL) serving to about 100 mg per 8 fl. oz. (236.6 mL) serving.

[0138] The disclosed nutritional composition may additionally comprise a source of β -glucan. Glucans are polysaccharides, specifically polymers of glucose, which are naturally occurring and may be found in cell walls of bacteria, yeast, fungi, and plants. Beta glucans (β -glucans) are themselves a diverse subset of glucose polymers, which are made up of chains of glucose monomers linked together via beta-type glycosidic bonds to form complex carbohydrates.

[0139] β -1,3-glucans are carbohydrate polymers purified from, for example, yeast, mushroom, bacteria, algae, or cereals. (Stone BA, Clarke AE. Chemistry and Biology of (1-3)-Beta-Glucans. London:Portland Press Ltd; 1993.) The chemical structure of β -1,3-glucan depends on the source of the β -1,3-glucan. Moreover, various physiochemical parameters, such as solubility, primary structure, molecular weight, and branching, play a role in biological activities of β -1,3-glucans. (Yadomae T., Structure and biological activities of fungal beta-1,3-glucans. Yakugaku Zasshi. 2000;120:413-431.)

[0140] β -1,3-glucans are naturally occurring polysaccharides, with or without β -1,6-glucose side chains that are found in the cell walls of a variety of plants, yeasts,

fungi and bacteria. β -1,3;1,6-glucans are those containing glucose units with (1,3) links having side chains attached at the (1,6) position(s). β -1,3;1,6 glucans are a heterogeneous group of glucose polymers that share structural commonalities, including a backbone of straight chain glucose units linked by a β -1,3 bond with β -1,6-linked glucose branches extending from this backbone. While this is the basic structure for the presently described class of β -glucans, some variations may exist. For example, certain yeast β -glucans have additional regions of β (1,3) branching extending from the β (1,6) branches, which add further complexity to their respective structures.

[0141] β -glucans derived from baker's yeast, *Saccharomyces cerevisiae*, are made up of chains of D-glucose molecules connected at the 1 and 3 positions, having side chains of glucose attached at the 1 and 6 positions. Yeast-derived β -glucan is an insoluble, fiber-like, complex sugar having the general structure of a linear chain of glucose units with a β -1,3 backbone interspersed with β -1,6 side chains that are generally 6-8 glucose units in length. More specifically, β -glucan derived from baker's yeast is poly-(1,6)- β -D-glucopyranosyl-(1,3)- β -D-glucopyranose.

[0142] Furthermore, β -glucans are well tolerated and do not produce or cause excess gas, abdominal distension, bloating or diarrhea in pediatric subjects. Addition of β -glucan to a nutritional composition for a pediatric subject, such as an infant formula, a growing-up milk or another children's nutritional product, will improve the subject's immune response by increasing resistance against invading pathogens and therefore maintaining or improving overall health.

[0143] The nutritional composition of the present disclosure may comprise β -glucan. In some embodiments, the β -glucan is β -1,3;1,6-glucan. In some embodiments, the β -1,3;1,6-glucan is derived from baker's yeast. The nutritional composition may comprise whole glucan particle β -glucan, particulate β -glucan, microparticulate β -glucan, PGG-glucan (poly-1,6- β -D-glucopyranosyl-1,3- β -D-glucopyranose) or any mixture thereof. In some embodiments, microparticulate β -glucan comprises β -glucan particles having a diameter of less than 2 μ m.

[0144] In some embodiments, the amount of β -glucan present in the composition is at between about 0.010 and about 0.080 g per 100g of the nutritional composition. In other embodiments, the nutritional composition comprises between

about 10 and about 30 mg β -glucan per serving. In another embodiment, the nutritional composition comprises between about 5 and about 30 mg β -glucan per 8 fl. oz. (236.6 mL) serving. In other embodiments, the nutritional composition comprises an amount of β -glucan sufficient to provide between about 15 mg and about 90 mg β -glucan per day. In some embodiments, the nutritional composition may be delivered in multiple doses to reach a target amount of β -glucan delivered to the subject throughout the day.

[0145] In some embodiments, the amount of β -glucan in the nutritional composition is between about 3 mg and about 17 mg per 100 kcal. In another embodiment the amount of β -glucan is between about 6 mg and about 17 mg per 100 kcal.

[0146] The nutritional composition may be expelled directly into a subject's intestinal tract. In some embodiments, the nutritional composition is expelled directly into the gut. In some embodiments, the composition may be formulated to be consumed or administered enterally under the supervision of a physician and may be intended for the specific dietary management of a disease or condition, such as celiac disease and/or food allergy, for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.

[0147] The nutritional composition of the present disclosure is not limited to compositions comprising nutrients specifically listed herein. Any nutrients may be delivered as part of the composition for the purpose of meeting nutritional needs and/or in order to optimize the nutritional status in a subject.

[0148] The nutritional composition of the present disclosure may be standardized to a specific caloric content, it may be provided as a ready-to-use product, or it may be provided in a concentrated form.

[0149] In some embodiments, the nutritional composition of the present disclosure is a growing-up milk. Growing-up milks are fortified milk-based beverages intended for children over 1 year of age (typically from 1-3 years of age, from 4-6 years of age or from 1-6 years of age). Growing-up milks are designed with the intent to serve as a complement to a diverse diet to provide additional insurance that a child achieves continual, daily intake of all essential vitamins and minerals, macronutrients plus additional functional dietary components, such as non-essential nutrients that have purported health-promoting properties.

[0150] The exact composition of a nutritional composition according to the present disclosure can vary from market-to-market, depending on local regulations and dietary intake information of the population of interest. In some embodiments, nutritional compositions according to the disclosure include a milk protein source, such as whole or skim milk, plus added sugar and sweeteners to achieve desired sensory properties, and added vitamins and minerals. The fat composition is typically derived from the milk raw materials. Total protein can be targeted to match that of human milk, cow milk or a lower value. Total carbohydrate is usually targeted to provide as little added sugar, such as sucrose or fructose, as possible to achieve an acceptable taste. Typically, Vitamin A, calcium and Vitamin D are added at levels to match the nutrient contribution of regional cow milk. Otherwise, in some embodiments, vitamins and minerals can be added at levels that provide approximately 20% of the dietary reference intake (DRI) or 20% of the Daily Value (DV) per serving. Moreover, nutrient values can vary between markets depending on the identified nutritional needs of the intended population, raw material contributions and regional regulations.

[0151] In certain embodiments, the nutritional composition is hypoallergenic. In other embodiments, the nutritional composition is kosher. In still further embodiments, the nutritional composition is a non-genetically modified product. In an embodiment, the nutritional formulation is sucrose-free. The nutritional composition may also be lactose-free. In other embodiments, the nutritional composition does not contain any medium-chain triglyceride oil. In some embodiments, no carrageenan is present in the composition. In other embodiments, the nutritional composition is free of all gums.

[0152] Accordingly, by the practice of the present disclosure, stabilized probiotics having heretofore unrecognized stability are prepared. The stabilized bacterial mixture exhibits exceptionally high stability through the use of hydrolyzed mammalian protein, especially hydrolyzed mammalian protein with over 70% of the peptides having a molecular weight of less than 2,000 Daltons. The stabilized probiotics are uniquely effective for nutritional applications with intermediate moisture levels (such as water activity as high as 0.4) where increased shelf life and stability in hot and humid environments are desired. The stabilized probiotics may be

packed separately or be combined with any of the embodiments of nutritional compositions described herein.

[0153] All references cited in this specification, including without limitation, all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

[0154] Although preferred embodiments of the disclosure have been described using specific terms, devices, and methods, such description is for illustrative purposes only. The words used are words of description rather than of limitation. It is to be understood that changes and variations may be made by those of ordinary skill in the art without departing from the spirit or the scope of the present disclosure, which is set forth in the following claims. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part. For example, while methods for the production of a commercially sterile liquid nutritional supplement made according to those methods have been exemplified, other uses are contemplated. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred versions contained therein.

CLAIMS

What is claimed is:

1. A nutritional composition comprising
 - a lipid or fat source;
 - a protein source; and
 - a probiotic stabilized in a protective matrix, the protective matrix comprising,
 - a. a hydrolyzed protein;
 - b. a first carbohydrate chosen from sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of 2 to 6, and any combination thereof; and
 - c. a second carbohydrate chosen from inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than 8, and any combination thereof ; and
 - d. a lipid selected from the group consisting of lecithin, monoglycerides, diglycerides, and any combination thereof.
2. The composition of claim 1, wherein the probiotic comprises viable microbial cells.
3. The composition of claim 2, wherein the viable microbial cells comprise *Lactobacillus rhamnosus*.
4. The composition of claim 1, wherein the matrix further comprises sodium alginate.
5. The composition of claim 1, wherein the matrix further comprises pectin.
6. The composition of claim 1, wherein at least 20% of the total hydrolyzed protein is comprised of protein having a molecular weight of less than 2000 Daltons.
7. The composition of claim 1, wherein the hydrolyzed protein comprises hydrolyzed casein.
8. The composition of claim 1, wherein the hydrolyzed protein consists of hydrolyzed casein.
9. The composition of claim 1, wherein the nutritional composition is a powdered infant formula.

10. A method for protecting a viable probiotic for use in a powdered nutritional composition, the method comprising:
- a. providing a viable probiotic;
 - b. preparing a protective matrix for the probiotic by blending
 - (i) hydrolyzed casein,
 - (ii) a first carbohydrate chosen from sucrose, maltose, lactose, trehalose, maltotriose, maltodextrin having a dextrose equivalent of 2 to 6, and any combination thereof,
 - (iii) a second carbohydrate chosen from inulin, polydextrose, galactooligosaccharide, fructooligosaccharide, starch, maltodextrin having a dextrose equivalent of greater than 8, and any combination thereof and
 - (iv) a lipid chosen from lecithin, monoglycerides, diglycerides, and any combination thereof;
 - c. combining the viable probiotic, the protective matrix and water to produce a mixture;
 - d. drying the mixture of step (c) to a final moisture content of 4% or less; and
 - e. adding the dried mixture of step (d) to a powdered nutritional product.
11. The method of claim 10 , wherein the viable probiotic comprises *Lactobacillus rhamnosus*.