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(54) Title: ENCAPSULATED BITTER PEPTIDES, METHODS OF ENCAPSULATING BITTER PEPTIDES, AND NUTRITIONAL COMPOSITIONS INCLUDING ENCAPSULATED BITTER PEPTIDES

(57) Abrégé/Abstract:
Encapsulated bitter peptides, methods of encapsulating bitter peptides, and nutritional compositions including encapsulated bitter peptides are provided. The bitter peptides can be encapsulated in a hydrophobic matrix, such as an organogel, a solid lipid nanoparticle, a liposome or combinations thereof. The encapsulated bitter peptides can be bioavailable, can be selectively encapsulated such that peptides needed for emulsion stabilization are not encapsulated and can maintain encapsulation during further processing such as heat treatment and homogenization.

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(54) **Title**: ENCAPSULATED BITTER PEPTIDES, METHODS OF ENCAPSULATING BITTER PEPTIDES, AND NUTRITIONAL COMPOSITIONS INCLUDING ENCAPSULATED BITTER PEPTIDES

(57) **Abstract**: Encapsulated bitter peptides, methods of encapsulating bitter peptides, and nutritional compositions including encapsulated bitter peptides are provided. The bitter peptides can be encapsulated in a hydrophobic matrix, such as an organogel, a solid lipid nanoparticle, a liposome or combinations thereof. The encapsulated bitter peptides can be bioavailable, can be selectively encapsulated such that peptides needed for emulsion stabilization are not encapsulated and can maintain encapsulation during further processing such as heat treatment and homogenization.

TITLE

"ENCAPSULATED BITTER PEPTIDES, METHODS OF ENCAPSULATING BITTER PEPTIDES, AND NUTRITIONAL COMPOSITIONS INCLUDING ENCAPSULATED BITTER PEPTIDES"

BACKGROUND

[0001] The present disclosure relates generally to encapsulated bitter peptides, methods of encapsulating bitter peptides, and nutritional compositions including encapsulated bitter peptides. More specifically, the present disclosure is directed to bitter peptides encapsulated in a lipid matrix, such as an organogel, a solid lipid nanoparticle, a liposome or combinations thereof.

[0002] There is a growing need in the market for nutritional compositions that treat aging- and/or illness-related conditions and provide hydrolyzed protein. Protein hydrolyzates are commercially used specialized nutrition products for addressing specific consumers benefits and/or needs, such as for critical patients, and for prevention and management of allergy. Many such specialized nutrition products include proteins that are pre-digested by hydrolysis with an enzyme. However, hydrolysis may form hydrophobic fragments of proteins that have a bitter taste. See, e.g., Leksrisompong, P.P., "Characterization of flavor of whey protein hydrolysates," J. Agric. Food Chem., 58(10):6318-6327 (2010). Nevertheless, peptides not only contribute to the nutritional profile of the composition but also help stabilize the emulsion in the composition.

[0003] Due to the hydrophobic fragments of proteins formed in protein hydrolysis, such nutritional compositions have an undesirable taste from hydrolyzed protein that renders the composition unappealing for oral consumption. The desired biological result is not achieved when the consumer refuses to ingest the nutritional composition due to poor organoleptic properties of the composition. Thus, it would be beneficial to provide nutritional compositions having both hydrolyzed protein and tolerable physical and organoleptic properties, without impacting the emulsion stability and without compromising the bioavailability of the peptides.

SUMMARY

[0004] The present disclosure provides encapsulated bitter peptides, methods of selectively encapsulating bitter peptides, and nutritional compositions including encapsulated bitter peptides. In an embodiment, a nutritional composition is provided and includes bitter peptides encapsulated in a structure selected from the group consisting of organogels, liposomes, solid lipid nanoparticles and combinations thereof.

[0005] In an embodiment, the structure is an organogel comprising an oil and a gelator, for example distilled monoglycerides.

[0006] In an embodiment, the structure is a liposome comprising phospholipids extracted from at least one of soya, egg or milk.

[0007] In an embodiment, the structure is a solid lipid nanoparticle.

[0008] In another embodiment, a method of encapsulating bitter peptides is provided. The method includes the steps of forming an emulsion comprising a water phase containing protein hydrolysate and an oil phase comprising at least one of an oil or a melted fat; and adding a gelator to the emulsion to form an organogel after the bitter peptides from the protein hydrolysate migrate selectively into oil droplets in the oil phase, the addition of the gelator entrapping the bitter peptides in the organogel.

[0009] In an embodiment, the protein hydrolysate is a dairy based protein hydrolysate. In an embodiment, the dairy based protein hydrolysate protein is a whey protein hydrolysate. In another embodiment the protein hydrolysate is a vegetable based protein hydrolysate, such as pea and/or soy protein hydrolysate.

[0010] In an embodiment, the method includes hydrolysis of protein to form a solution comprising the protein hydrolysate in a water phase, the bitter peptides being selectively entrapped in the organogel in line such that the emulsion is formed after the hydrolysis directly in the solution comprising the protein hydrolysate.

[0011] In an embodiment, the hydrolysis forms non-bitter peptides, and at least a portion of the non-bitter peptides are not entrapped in the oil phase.

[0012] In an embodiment, the water phase contains about 5% to about 50% of the protein hydrolysate by weight, preferably about 5% to about 25%, such as about 5% to about 15% of the protein hydrolysate by weight.

[0013] In another embodiment, a method of encapsulating bitter peptides is provided. The method includes the steps of forming an emulsion comprising a water phase containing protein hydrolysate and an oil phase containing melted fat at a temperature above the melting point of the melted fat; and cooling the emulsion to generate solid lipid nanoparticles in which bitter peptides from the protein hydrolysate are entrapped.

[0014] In an embodiment, the method includes hydrolysis of protein to form a solution comprising the protein hydrolysate, the bitter peptides being entrapped in the solid lipid nanoparticles in line such that the emulsion is formed after the hydrolysis directly in the solution comprising the protein hydrolysate.

[0015] In another embodiment, a method of encapsulating bitter peptides is provided. The method includes the steps of adding phospholipids to protein hydrolysate; and homogenizing the protein hydrolysate and the phospholipids to form liposomes that entrap selectively bitter peptides from the protein hydrolysate.

[0016] In an embodiment, the homogenization is performed at about 50 to about 600 bars, preferably about 50 to 400 bars, more preferably about 100 to about 300 bars.

[0017] In an embodiment, the method includes hydrolysis of at least one protein, preferably a mixture of proteins, to form a solution comprising the protein hydrolysate, the bitter peptides being entrapped in the liposomes in line such that the phospholipids are added after the hydrolysis directly in the solution comprising the protein hydrolysate.

[0018] In another embodiment, bitter peptides encapsulated in a structure selected from the group consisting of organogels, liposomes, solid lipid nanoparticles and combinations thereof are provided.

[0019] In another embodiment, a method of providing nutrition is provided. The method includes administering a nutritional composition comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a structure

selected from the group consisting of organogels, liposomes, solid lipid nanoparticles and combinations thereof.

[0020] An advantage of the present disclosure is to provide encapsulated bitter peptides.

[0021] Another advantage of the present disclosure is to provide improved nutritional compositions.

[0022] Still another advantage of the present disclosure is to provide nutritional compositions that have acceptable organoleptic properties.

[0023] Yet another advantage of the present disclosure is to hide bitter peptides to taste receptors by encapsulating the peptides.

[0024] Still another advantage of embodiments of the present disclosure is to encapsulate bitter peptides in line, such that hydrophobic interactions encapsulate the bitter peptides directly in the water phase after inactivation of the enzyme, or near line.

[0025] Yet another advantage of the present disclosure is to selectively encapsulate bitter peptides such that at least a portion of the peptides that assist emulsion stability are not encapsulated.

[0026] Additional features and advantages are described herein, and will be apparent from the following Detailed Description and the figures.

BRIEF DESCRIPTION OF THE FIGURES

[0027] FIG. 1 shows microscopy pictures of liposomes encapsulating bitter peptides in accordance with embodiments of the present disclosure, stained by Nile red.

[0028] FIG. 2 is a graph showing results from tastings of liposomes encapsulating bitter peptides in accordance with embodiments of the present disclosure.

[0029] FIG. 3 is a graph showing results from tastings of encapsulated bitter peptides in accordance with embodiments of the present disclosure.

[0030] FIG. 4 shows bitter peptides encapsulated in solid lipid nanoparticles in accordance with embodiments of the present disclosure.

DETAILED DESCRIPTION

[0031] All dosage ranges contained within this application are intended to include all numbers, whole or fractions, contained within said range.

[0032] As used in this disclosure and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a mixture of two or more polypeptides, and the like.

[0033] As used herein, "about" is understood to refer to numbers in a range of numerals. Moreover, all numerical ranges herein should be understood to include all integer, whole or fractions, within the range.

[0034] The present disclosure is related to bitter peptides encapsulated in a hydrophobic matrix. As used herein, "bitter peptides" are any hydrophobic peptide formed by hydrolysis of protein. In some embodiments, the bitter peptides include peptides that bind to the human bitter receptors (for examples T2Rs). The bitter peptides can be formed by hydrolysis of a dietary protein. Pre-hydrolysed proteins are generally more easily digested and absorbed by the gastro-intestinal tract, and have reduced allergenic potential.

[0035] The hydrolysate of any suitable dietary protein may be used, for example animal proteins, such as milk protein, meat protein and egg protein; or vegetable proteins, such as soy protein, wheat protein, rice protein, pea protein, corn protein, canola protein, oat protein, potato protein, peanut protein, and any proteins derived from beans, buckwheat or lentils. Milk proteins, such as casein and whey, and soy proteins may be preferred for some applications. If the protein is a milk protein or a milk protein fraction, the protein may be, for example, sweet whey, acid whey, α-lactalbumin, β-lactoglobulin, bovine serum albumin, acid casein, caseinates, α-casein, β-casein and/or γ-casein. Of course, hydrolysate from a combination of different proteins may be used.

[0036] Protein hydrolysates can be obtained as a commercial raw material powder or can be produced by hydrolysis of protein, e.g. enzymatic hydrolysis of protein. For example, according to an embodiment, protein hydrolysates may be produced by hydrolysis of whey protein in water using, proteolitic enzymes. Processes and enzymes for the preparation of protein hydrolysates for food applications are well-

known in the art. One example of a process for preparation of protein hydrolysates is described in US5,039,532.

[0037] The peptides may be produced by hydrolyzing protein as desired and as known in the art. For example, protein hydrolysate, e.g. a whey protein hydrolysate, may be prepared by enzymatically hydrolysing the protein in one or more steps. In some embodiments, the protein can be obtained already hydrolyzed.

[0038] Applicants surprisingly found that encapsulating bitter peptides masked the bitterness relative to un-encapsulated bitter peptides from protein hydrolysates. Without wishing to be bound to any theory, it is believed that the encapsulated bitter peptides reduce undesirable taste of a composition in which they are included because the hydrophobic matrix in which they are encapsulated blocks the bitter peptides from taste receptors.

[0039] In some embodiments, at least a portion of the bitter peptides from the protein hydrolysate can be encapsulated in an organogel. An organogel is a substantially dilute system which exhibits no flow when in the steady-state and is a non-crystalline, non-glassy solid material. These solid materials are composed of a liquid organic phase (e.g vegetable oil) entrapped in and/or physically attached to a three-dimensional network. For example, an organogel can comprise macromolecules interconnected by either strong chemical bonds, such as the bonds of cross-linked polymers, or weaker bonds, such as non-covalent interactions. These systems can be based on self-assembly of structurant molecules called gelators (e.g. monoglyceride). Immobilization of the liquid component within the networked structure of the solid component has been attributed to the interfacial tension between the solid and liquid components.

[0040] To produce an organogel, an emulsion can be formed. In some embodiments, the emulsion is formed after protein hydrolysis directly in the water phase after inactivation of the enzyme. The emulsion can comprise a water phase containing the protein hydrolysate and also comprise an oil phase containing oil and/or melted fat. For example, protein hydrolysate, such as whey protein hydrolysate, can be dispersed in vegetable oil, such as sunflower oil. In an embodiment, the water phase can comprise about 5% to about 50% of the protein hydrolysate by weight, preferably

about 5% to about 25%, such as about 5% to about 15% of the protein hydrolysate by weight.

[0041] The bitter peptides can initially be located in the water phase but then can migrate inside the oil droplets by hydrophobic interactions. For example, stirring, such as for about 30 minutes, can assist the bitter peptides in migrating inside the oil droplets. After the emulsion is formed, gelator molecules, such as, for example, phytosterols, phospholipids, monoglycerides and combinations thereof, can be added to the emulsion to gel the oil droplets in which the bitter peptides will be entrapped. Examples of suitable gelators include low molecular weight gelators (LMWGs), such as phospholipids, lecithin, monoglyceride, amphiphilic peptides, sorbitan monostearate, mono-, di- and/or triacyglycerols, fatty acids, fatty alcohol, wax and/or phytosterol. For example, a gelator can be added to the emulsion to gel the oil droplets. In a preferred embodiment, the organogel is formed using only food grade materials and without using any organic solvents. In a particular embodiment the gelator may be distilled monoglycerides.

[0042] The organogel can be used to produce a nutritional composition. In some embodiments, the oil and/or melted fat are standard ingredients in a nutritional composition in which the organogel that encapsulates the bitter peptides is used. For example, the formulation of the nutritional composition may not need to be altered to include the organogel. In some embodiments, at least a portion of the peptides from the protein hydrolysate that are not bitter and/or not hydrophobic are not encapsulated by the organogel. In some embodiments, the solid lipid nanoparticles are stabilized by a surfactant.

[0043] In some embodiments, at least a portion of the bitter peptides from the protein hydrolysate can be encapsulated in solid lipid nanoparticles. Solid lipid nanoparticles are a solid lipid matrix that can solubilize lipophilic molecules such as bitter peptides. The lipid matrix can be a core that is stabilized by emulsifiers that surround the lipid matrix core and act as surfactants.

[0044] To produce the solid lipid nanoparticles, the oil in a water-in-oil emulsion can be replaced by a solid lipid or a blend of solid lipids. In an embodiment, the resulting solid lipid nanoparticles are composed of 0.1 to 30% w/w of solid lipid dispersed in an aqueous medium. The solid lipid nanoparticles can be formed in line

after hydrolysis directly in the water phase after inactivation of the enzyme, or after additional process steps (such as membrane filtration, and/or chromatographic techniques). In some embodiments, the solid lipid nanoparticles are stabilized by a surfactant. The mean particle size of the solid lipid nanoparticles can be from about 40 nm to about 100 microns.

[0045] For example, an emulsion can be formed with the emulsion comprising a water phase containing the protein hydrolysate and an oil phase containing melted fat, at temperatures above the melting point of the lipid. In an embodiment, the water phase can comprise about 5% to about 50% of the protein hydrolysate by weight, preferably about 5% to about 25%, such as about 5% to about 15% of the protein hydrolysate by weight. The bitter peptides in the protein hydrolysate can migrate inside the fat droplets by hydrophobic interactions. Then, the emulsion can be cooled to generate solid lipid nanoparticles in which the bitter peptides are entrapped. In a preferred embodiment, the solid lipid nanoparticles are formed using only food grade materials and without using any organic solvents.

[0046] The solid lipid nanoparticles can be used to produce a nutritional composition. In some embodiments, the solid lipid or the blend of solid lipids are standard ingredients in a nutritional composition in which the solid lipid nanoparticles that encapsulate the bitter peptides are used. For example, the formulation of the nutritional composition may not need to be altered to include the solid lipid nanoparticles. In some embodiments, at least a portion of the peptides from the protein hydrolysate that are not bitter and/or not hydrophobic are not encapsulated by the solid lipid nanoparticles.

[0047] In some embodiments, at least a portion of the bitter peptides from the protein hydrolysate can be encapsulated in liposomes. Phospholipids are characterized by having a lipophilic tail and a hydrophilic head on the same molecule. Upon interaction with water, phospholipids self-assemble and form self-organized colloidal particles. In general terms in a liposome the hydrophilic heads of the phospholipids are oriented toward the water compartment while the lipophilic tails orient away from the water toward the center of the vesicle, thus forming a bilayer. Consequently, lipid-soluble compounds aggregate in the lipid bilayers.

[0048] Upon liposome formation, the hydrophobicity of the bitter peptides can cause the bitter peptides to migrate inside the lipid bilayers of the liposomes, and hydrophobic interactions can stabilize the positioning of the bitter peptides within the lipid bilayers. As a result, the bitter peptides can be entrapped within the liposomes.

[0049] For example, liposomes entrapping bitter peptides can be formed by adding phospholipids, such as phospholipids extracted from soya or egg, to water that contains the protein hydrolysate. In an embodiment, the water can comprise about 5% to about 50% of the protein hydrolysate by weight, preferably about 5% to about 25%, such as about 5% to about 15% of the protein hydrolysate by weight. Homogenization can then be performed, either directly or after additional processing setps, to form the liposomes that entrap the bitter peptides from the protein hydrolysate. For example, the homogenization can be performed about about 50 to 600 bars, such as about 100 to about 300bars. In a preferred embodiment, the liposomes are formed using only food grade materials and without using any organic solvents.

[0050] The liposomes can be used to produce a nutritional composition. In some embodiments, the phospholipids are standard ingredients in a nutritional composition in which the liposomes that encapsulate the bitter peptides are used. For example, the formulation of the nutritional composition may not need to be altered to include the liposomes. In some embodiments, at least a portion of the peptides that are not bitter and/or not hydrophobic are not encapsulated by the liposomes.

[0051] The present disclosure provides nutritional compositions comprising encapsulated bitter peptides produced using any method disclosed herein. The present disclosure also provides a method of administering bitter peptides to an individual comprising the steps of administering to the individual a nutritional composition comprising the bitter peptides in encapsulated form. The present disclosure also provides a method of treating or preventing a condition in an individual in need thereof comprising the steps of administering to the individual a nutritional composition comprising an effective amount of encapsulated bitter peptides.

[0052] For example, in an embodiment, a method of providing nutrition to a person comprises administering to the person a nutritional formulation comprising bitter peptides from protein hydrolysate. The bitter peptides are encapsulated in a

structure selected from the group consisting of organogels, liposomes, solid lipid nanoparticles and combinations thereof.

[0053] The inventive encapsulation system advantageously liberates the encapsulated peptides during digestion so that the bitter taste of the peptides is masked on consumption/administration of the nutritional composition, whilst maintaining the biological properties of the hydrolysate, such that the hydrophobic peptides are still bioavailable.

[0054] Nutritional compositions comprising the encapsulated bitter peptides may be pharmaceutical formulations, nutritional formulations, dietary supplements, functional foods, beverage products and combinations thereof. The nutritional composition can be for long-term administration (continuous administrations for more than 6 weeks and/or short-term administration (continuous administrations for less than 6 weeks).

[0055] In an embodiment, the nutritional compositions are in a form selected from the group consisting of tablets, capsules, liquids, chewables, soft gels, sachets, powders, syrups, liquid suspensions, emulsions, solutions, or combinations thereof. In an embodiment, the nutritional compositions are oral nutritional supplements.

[0056] The nutritional compositions may also be a source of complete nutrition. Complete nutrition provides types and levels of macronutrients (protein, fats and carbohydrates) and micronutrients to be sufficient to be a sole source of nutrition for the animal to which it is being administered. Patients can receive 100% of their nutritional requirements from such complete nutritional compositions. Alternatively, the nutritional compositions may be a source of incomplete nutrition. Incomplete nutrition does not provide levels of macronutrients (protein, fats and carbohydrates) or micronutrients to be sufficient to be a sole source of nutrition for the animal to which it is being administered. Partial or incomplete nutritional compositions can be used as a nutritional supplement. According to another embodiment the nutritional composition can be an infant formula.

[0057] "Nutritional products," or "nutritional compositions," as used herein, are understood to include any number of optional additional ingredients, including conventional food additives (synthetic or natural), for example one or more acidulants, additional thickeners, buffers or agents for pH adjustment, chelating agents, colorants,

emulsifies, excipient, flavor agent, mineral, osmotic agents, a pharmaceutically acceptable carrier, preservatives, stabilizers, sugar, sweeteners, texturizers, and/or vitamins. The optional ingredients can be added in any suitable amount. The nutritional products or compositions may be a source of complete nutrition or may be a source of incomplete nutrition.

[0058] The nutritional compositions can include any number of optional additional ingredients, including conventional food additives (synthetic or natural), for example one or more acidulants, additional thickeners, buffers or agents for pH adjustment, chelating agents, colorants, emulsifiers, excipients, flavor agents, osmotic agents, pharmaceutically acceptable carriers, preservatives, stabilizers, sugar, sweeteners, texturizers, and/or vitamins. For example, the nutritional compositions may contain emulsifiers and stabilizers such as soy lecithin, citric acid esters of mono- and di-glycerides, and the like. The optional ingredients can be added in any suitable amount.

[0059] Nutritional compositions of the present disclosure may contain a carbohydrate source. Any carbohydrate source conventionally found in nutritional compositions such as lactose, saccharose, maltodextrin, starch and mixtures thereof may be used although the preferred source of carbohydrates is lactose. In an embodiment, the carbohydrate source contributes between 35% and 60% of the total energy of the nutritional compositions.

[0060] Nutritional compositions of the present disclosure may also contain a source of lipids in addition to the lipids from the encapsulated bitter peptides. The lipid source may be any lipid or fat which is suitable for use in nutritional compositions. Sources of fat include, but are not limited to, high oleic sunflower oil and high oleic safflower oil. The essential fatty acids linoleic and α-linolenic acid may also be added as may small amounts of oils containing high quantities of preformed arachidonic acid and docosahexaenoic acid such as fish oils or microbial oils. In total, the fat content is preferably such as to contribute between about 10% and about 10% of the total energy of the nutritional compositions.

[0061] In an embodiment, the nutritional compositions further include a source of ω-3 fatty acids and/or a source of ω-6 fatty acids. The source of ω-3 fatty acids may be selected from the group consisting of fish oil, krill, plant sources containing ω-3

fatty acids, flaxseed, walnut, algae, or combinations thereof. The ω-3 fatty acids may be selected from the group consisting of α-linolenic acid ("ALA"), docosahexaenoic acid ("DHA"), eicosapentaenoic acid ("EPA"), or combinations thereof. The source of ω-6 fatty acids may be selected from the group consisting of vegetable oils (e.g. sunflower, sassflower, soybean, corn, sesame, cottonseed, grapeseed, palm, primerose, borage and walnut), nuts (e.g. walnuts, almonds and cashews), and seeds (e.g. flax, hemp, sunflower, sesame, pine nuts, black current and pumpkin).

[0062] In an embodiment, the nutritional compositions can include at least one nucleotide selected from the group consisting of a subunit of deoxyribonucleic acid ("DNA"), a subunit of ribonucleic acid ("RNA"), polymeric forms of DNA and RNA, yeast RNA, or combinations thereof. In an embodiment, the at least one nucleotide is an exogenous nucleotide.

[0063] In an embodiment, the nutritional compositions further include a phytonutrient selected from the group consisting of flavanoids, allied phenolic compounds, polyphenolic compounds, terpenoids, alkaloids, sulphur-containing compounds, or combinations thereof. The phytonutrient may be selected from the group consisting of carotenoids, plant sterols, quercetin, curcumin, limonin, or combinations thereof.

[0064] The nutritional compositions can further include a source of protein in addition to the encapsulated bitter peptides and/or the remainder of the hydrolysate. Any suitable dietary protein may be used, for example animal proteins, such as dairy protein, meat protein and egg protein; or vegetable proteins, such as soy protein, wheat protein, rice protein, pea protein, corn protein, canola protein, oat protein, potato protein, peanut protein, and any proteins derived from beans, buckwheat or lentils. The dairy based proteins may be casein, caseinates, casein hydrolysate, whey, whey hydrolysates, whey concentrates, whey isolates, milk protein concentrate, milk protein isolate, or combinations thereof. Dairy proteins, such as casein and whey, and/or soy proteins may be preferred for some applications. In an embodiment, the protein source contributes between 15% and 35% of the total energy of the nutritional compositions.

[0065] The nutritional compositions can further include a probiotic. Probiotics are food-grade microorganisms (alive, including semi-viable or weakened, and/or non-

replicating), metabolites, microbial cell preparations or components of microbial cells that could confer health benefits on the host when administered in adequate amounts, more specifically, that beneficially affect a host by improving its intestinal microbial balance, leading to effects on the health or well-being of the host. See Salminen S., et al., "Probiotics: how should they be defined?," Trends Food Sci. Technol., 10, 107-10 (1999). In general, it is believed that these micro-organisms inhibit or influence the growth and/or metabolism of pathogenic bacteria in the intestinal tract. The probiotics may also activate the immune function of the host.

[0066] Non-limiting examples of probiotics include *Aerococcus*, *Aspergillus*, *Bacteroides*, *Bifidobacterium*, *Candida*, *Clostridium*, *Debaromyces*, *Enterococcus*, *Fusobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Melissococcus*, *Micrococcus*, *Mucor*, *Oenococcus*, *Pediococcus*, *Penicillium*, *Peptostrepococcus*, *Pichia*, *Propionibacterium*, *Pseudocatenulatum*, *Rhizopus*, *Saccharomyces*, *Staphylococcus*, *Streptococcus*, *Torulopsis*, *Weissella*, or combinations thereof.

[0067] In an embodiment, the nutritional compositions further include an amino acid selected from the group consisting of alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, hydroxyproline, hydroxyserine, hydroxytyrosine, hydroxylysine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine, or combinations thereof.

[0068] In an embodiment, the nutritional compositions further include an antioxidant. Non-limiting examples of antioxidants include astaxanthin, carotenoids, coenzyme Q10 ("CoQ10"), flavonoids, glutathione, Goji (wolfberry), hesperidin, lactowolfberry, lignan, lutein, lycopene, polyphenols, selenium, vitamin A, vitamin C, vitamin E, zeaxanthin, and combinations thereof.

[0069] In an embodiment, the nutritional compositions further include a vitamin selected from the group consisting of vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), vitamin C,

vitamin D, vitamin E, vitamin K, K1 and K2 (i.e., MK-4, MK-7), folic acid, biotin, or combinations thereof.

[0070] In an embodiment, the nutritional compositions further include a mineral selected from the group consisting of boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, tin, vanadium, zinc, or combinations thereof. Minerals may be added in salt form. The presence and amounts of specific minerals and other vitamins will vary depending on the intended population.

[0071] In an embodiment, the nutritional composition includes branched chain fatty acids that are present in the nutritional composition in an amount from about 6.25 mg to about 12.5 mg/100 g nutritional composition and assuming the nutritional composition includes 1,600 grams and is a complete feeding for a day for an adult. Alternatively, the nutritional compositions may be provided in an amount to provide from about 100 mg to about 1,500 mg of branched chain fatty acids per day. Alternatively, the nutritional compositions may include from about 0.5% to about 5% branched chain fatty acids by weight of total fatty acids.

[0072] In an embodiment, the nutritional compositions also include a prebiotic. A prebiotic is a food substance that selectively promotes the growth of beneficial bacteria or inhibits the growth or mucosal adhesion of pathogenic bacteria in the intestines. They are not inactivated in the stomach and/or upper intestine or absorbed in the gastrointestinal tract of the person ingesting them, but they are fermented by the gastrointestinal microflora and/or by probiotics. Prebiotics are, for example, defined by Glenn Gibson et al., "Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics," J. Nutr., 125: 1401-1412 (1995).

[0073] Non-limiting examples of prebiotics include acacia gum, alpha glucan, arabinogalactans, beta glucan, dextrans, fructooligosaccharides, fucosyllactose, galactooligosaccharides, galactomannans, gentiooligosaccharides, glucooligosaccharides, guar gum, inulin, isomaltooligosaccharides, lactoneotetraose, lactosucrose, lactulose, levan, maltodextrins, milk oligosaccharides, partially hydrolyzed guar gum, pecticoligosaccharides, resistant starches, retrograded starch, sialooligosaccharides, sialyllactose, soyoligosaccharides, sugar alcohols, xylooligosaccharides, or their hydrolysates, or combinations thereof.

[0074] In some embodiments, the nutritional composition can be a synbiotic that contains both a prebiotic and a probiotic that work together to improve the microflora of the intestine.

[0075] By way of example and not limitation, the following Examples are illustrative of embodiments of the present disclosure.

[0076] **EXAMPLES**

[0077] The following examples present scientific data developing and supporting the concept of encapsulating bitter peptides from protein hydrolysate.

[0078] Example 1

[0079] Liposomes were formed by adding 1% of phospholipids extracted from soya or egg to water containing 10% hydrolyzed protein by weight, followed by homogenization. Hyprol™ 3315 from Kerry Group was used as the hydrolyzed protein. The assays are detailed in Table 1.

Phospholipid used	Supplier	Main components	Origin	Abbreviation
Topcithin: fluid lecithin	Cargill	13% phosphatidylcholine; 10% phosphatidylinositol	soy	Top.
Emulfluid F30: tailored fluid lecithin	Cargill	30% Phosphatidylcholine	soy	Emul.
Metarin: de-oiled lecithin	Cargill	30% phosphatidylcholine; 12% phosphatidylethanolamine; 13% phosphatidylinositol	soy	Met.
Epikuron 200	Cargill	92% phosphatidylcholine	soy	Epi.
Egg PC	TLC	30% Phosphatidylcholine	egg	Egg

[0080] Table 1: Phospholipids used in the trials

[0081] Size distribution analysis demonstrates that liposomes were formed (see Table 2). Each sample homogenized at 200 bars resulted in a population of aggregates having a size distribution ranging from 200 to 300 nm, characteristic of liposome formation.

Sample Name	REF	Top.	Emul.	Met.	Epi.	Egg

Z-Average (d.nm)	288.8	276.6	328.9	284.2	283.1	293.9
PdI	0.254	0.233	0.29	0.251	0.289	0.253

[0082] Table 2: Size distribution analysis of trials

[0083] As shown in Figure 1, fluorescence microscopy using Nile Red to stain the lipids also demonstrated formation of aggregates.

[0084] To ensure that the bitter peptides migrated into the lipid bilayers of the liposomes by hydrophobic interaction, tastings of the different solutions were performed. As shown in Figure 2, the bitterness of the samples was ranked by seven trained panelists, from the least bitter (rank 1) to the most bitter (rank 6). The results indicate that bitter peptides encapsulated in Egg PC liposomes had the bitterness reduced the most relative to the control containing only 10% of Hyprol™ 3315 in water, and bitter peptides encapsulated in Topcithin™ extracted from soya were second most reduced in bitterness. The decreased bitterness relative to the control for most of the assays indicates that the bitter peptides did migrate into the liposomes.

[0100] Example 2

[0101] To ensure that bitter peptides migrate into the oil phase of organogels and solid lipid nanoparticles, an emulsion was created containing oil or melted fat and a water phase in which 10% of Hyprol™ 3315 was dissolved. After 30 minutes of stirring, the water phase and the oil phase were separated by decantation. The extracted water phase was then tasted by a trained panel. It is important to note that the extraction step is used for evaluation and would not be used in most embodiments of the encapsulated bitter peptides that are used in nutritional compositions.

[0102] Samples were prepared with milk fat, cocoa fat or a mixture of medium chain triglycerides (MCT) and soy lecithin. Each of the oil/fats were added at an amount of 4% by weight to the 10% Hyprol™ 3315 solution before extraction. Thirteen trained panelists ranked the samples from the least bitter (rank 1) to the most bitter (rank 4). As shown in Figure 3, the cocoa fat sample was slightly less bitter than the reference, and the hydrolysates extracted with milk fat and the mixture of MCT/soy lecithin were perceived as significantly less bitter than the reference. A

Friedman test was performed on the results (global risk 5%) and concluded that the perceived decrease in bitterness relative to the control for the milk fat and MCT/soy fat extracts was statistically significant.

[0103] Example 3

[0104] To further examine encapsulation of bitter peptides in solid lipid nanoparticles, fat coating of the Hyprol™ 3315 was performed using a fluidizing bed coating (Glatt, bench scale). The resulting solid lipid nanoparticles are depicted in Figure 4. After the fat coating was performed, reconstituted solutions of 10% Hyprol™ 3315 were no longer bitter, meaning that the hydrophobic bitter peptides were encapsulated in the solid lipid nanoparticles and were not released in water.

[0105] Example 4

[0106] To further examine encapsulation of bitter peptides in an organogel, organogels were formed by dispersing Hyprol™ 3315 in sunflower oil and then adding a monoglyceride gelator (Dimodan™). After gellation, the bitterness of reconstituted solutions of 10% Hyprol™ 3315 were significantly reduced, meaning that the hydrophobic bitter peptides were encapsulated in organogel and were not released from organogel in water.

[0107] It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present subject matter and without diminishing its intended advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

CLAIMS:

1. A nutritional composition comprising bitter peptides encapsulated in a structure, wherein said structure is an organogel comprising an oil or melted fat and a gelator selected from phospholipids, lecithin, monoglyceride, amphiphilic peptides, sorbitan monostearate, mono-glycerols, di-glycerols, triacyglycerols, fatty acids, fatty alcohol, wax, phytosterol, or combinations thereof.

2. A method of encapsulating bitter peptides, the method comprising the steps of:

forming an emulsion comprising a water phase containing protein hydrolysate and an oil phase comprising at least one of an oil or a melted fat; and

adding a gelator to the emulsion to form an organogel after bitter peptides from the protein hydrolysate migrate into oil droplets in the oil phase, the addition of the gelator entrapping the bitter peptides in the organogel.

3. The method of Claim 2 comprising hydrolysis of a protein to form a solution comprising the protein hydrolysate, the bitter peptides being entrapped in the organogel in line such that the emulsion is formed after the hydrolysis directly in the solution comprising the protein hydrolysate.

4. The method of any one of Claims 2 or 3, wherein the protein hydrolysate is a dairy protein hydrolysate.

5. The method of any one of Claims 2 or 3, wherein the hydrolysis forms non-bitter peptides, and at least a portion of the non-bitter peptides are not entrapped in the oil phase.

6. Bitter peptides encapsulated in a structure, wherein said structure is an organogel.

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7. A method of providing nutrition to a person, comprising administering to the person a nutritional composition comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a structure, wherein said structure is an organogel.

Date Reçue/Date Received 2020-11-02

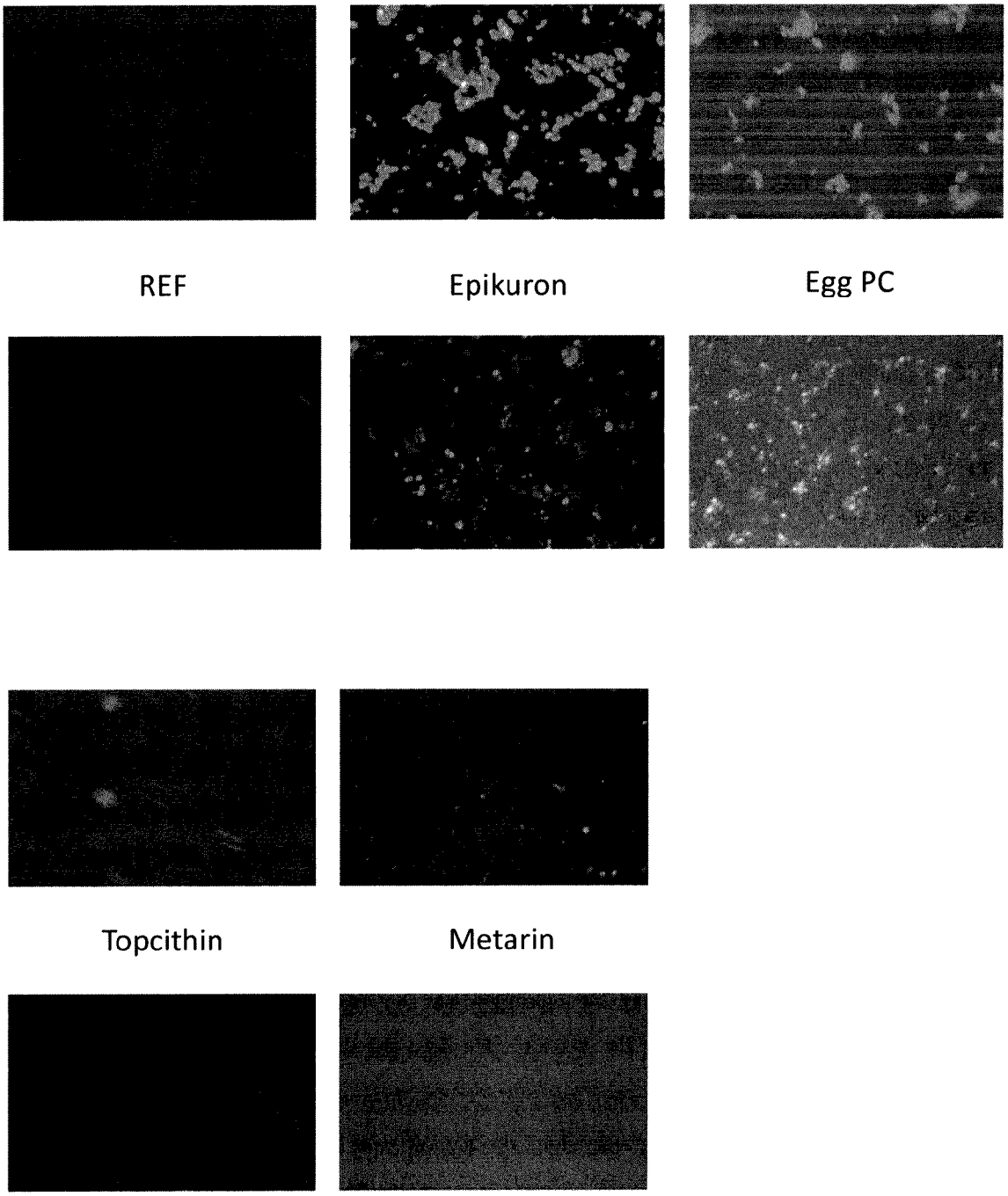


FIG. 1

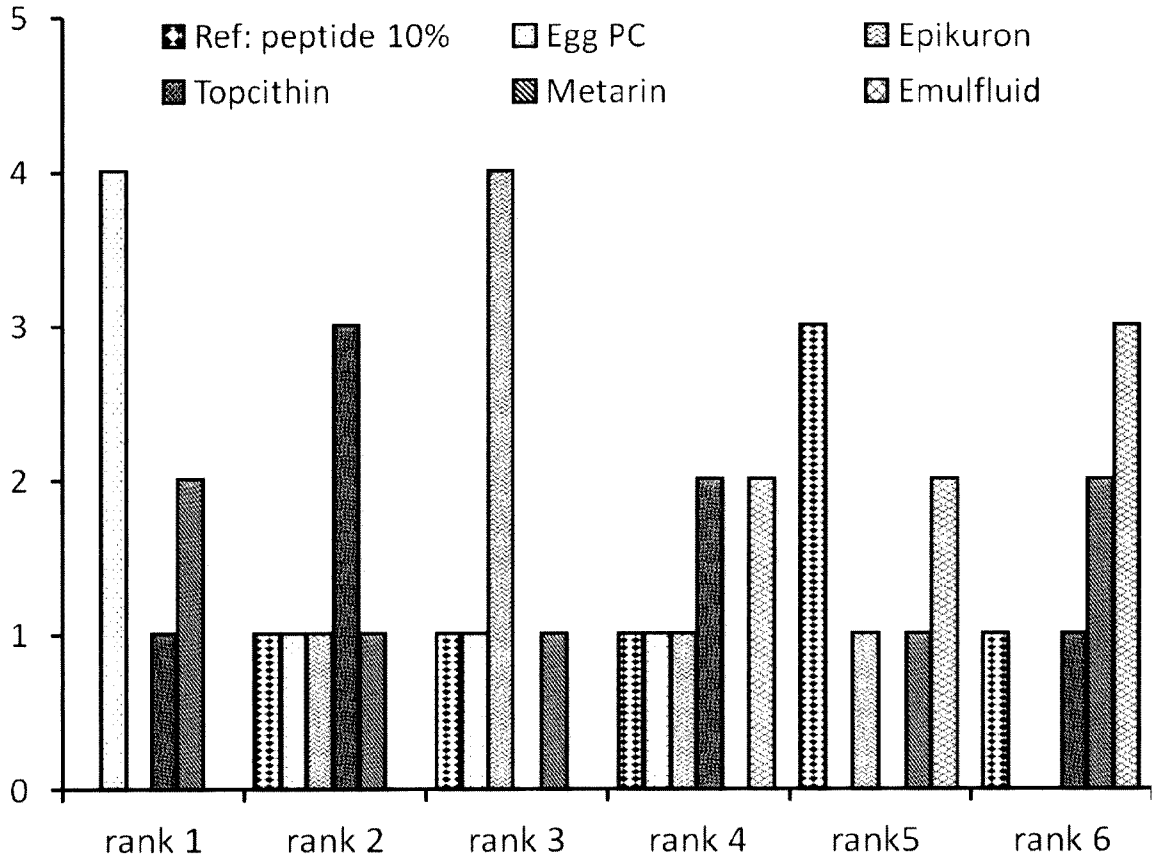

5
4
3
2
1
0
Ref: peptide 10%
Egg PC
Epikuron
Topcithin
Metarin
Emulfluid
rank 1
rank 2
rank 3
rank 4
rank5
rank 6

FIG. 2

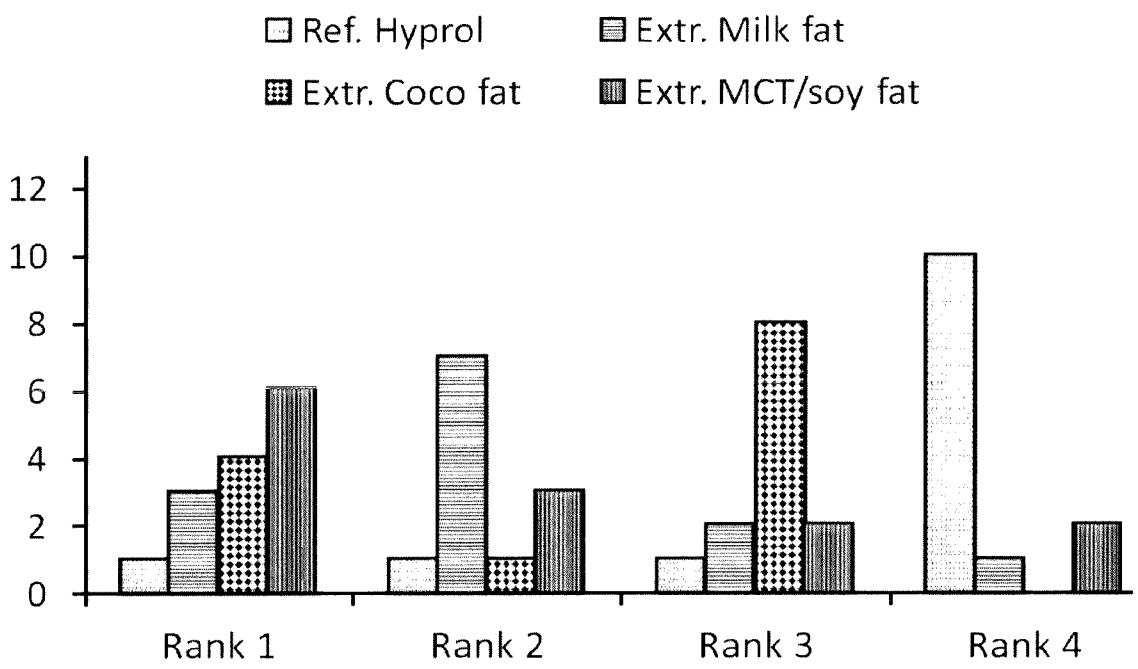
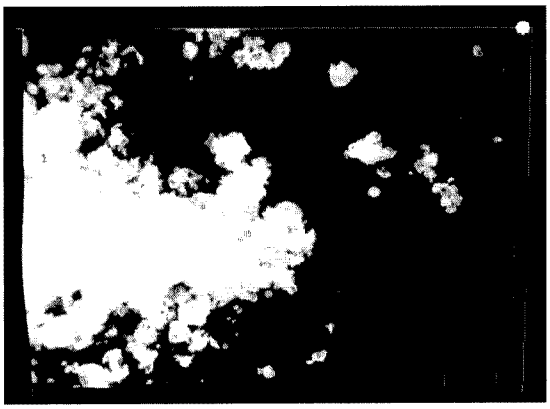
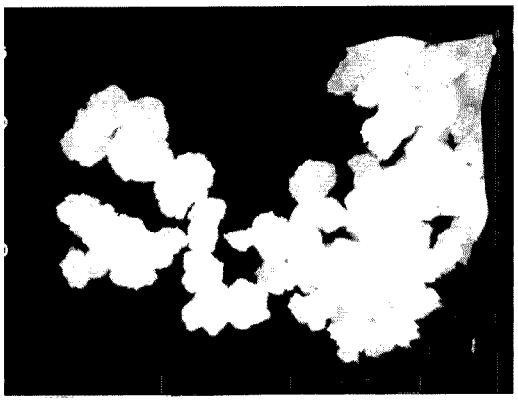

Ref. Hyprol
Extr. Milk fat
Extr. Coco fat
Extr. MCT/soy fat
12
10
8
6
4
2
0
Rank 1
Rank 2
Rank 3
Rank 4

FIG. 3

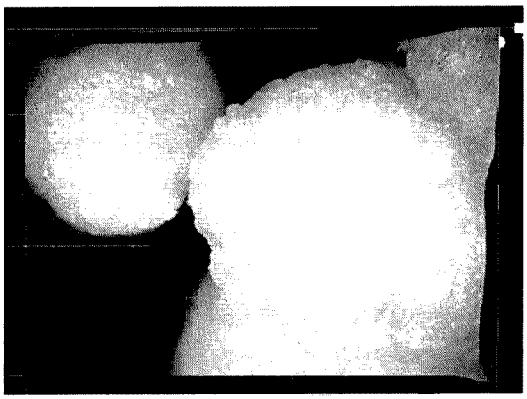


REF

4% fat



20% fat



25 % fat

FIG. 4