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(54) Title: ENTRAPMENT OF BITTER PEPTIDES BY A GEL COMPRISING ALGINATE

(57) Abstract: Encapsulated bitter peptides and methods of entrapping bitter peptides are provided. The bitter peptides are entrapped in a gel comprising alginate.



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ENTRAPMENT OF BITTER PEPTIDES BY A GEL COMPRISING ALGINATE

BACKGROUND

The present disclosure relates generally to entrapped bitter peptides, methods of
5 entrapping bitter peptides, and nutritional compositions including entrapped bitter peptides. More specifically, the present disclosure is directed to bitter peptides entrapped in a gel comprising alginate.

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
10 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., Leksrisonpong, P.P., "Characterization of
15 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.

Due to the hydrophobic fragments of proteins formed in protein hydrolysis, such
nutritional compositions can have an undesirable taste from hydrolyzed protein that
20 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.

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SUMMARY

The present disclosure provides encapsulated bitter peptides, and methods of selectively encapsulating bitter peptides. The encapsulated bitter peptides can be selectively encapsulated, can be generated in process directly in water phase, and can
5 maintain encapsulation during further processing such as heat treatment, homogenization, and/or spray-drying. The present disclosure also provides nutritional compositions including encapsulated bitter peptides, and/or provides nutritional compositions from which bitter peptides have been selectively removed.

Accordingly, in a general embodiment, the present disclosure provides a method of
10 reducing bitterness of a nutritional composition comprising bitter peptides, comprising entrapping bitter peptides in a gel comprising alginate.

In an embodiment, the present disclosure provides a method of reducing bitterness of a nutritional composition comprising bitter peptides, comprising the steps of: (i) providing a protein hydrolysate; (ii) adding di- or tri- valent ions to the protein
15 hydrolysate; (iii) adding alginate to form a gel in which bitter peptides of the protein hydrolysate are encapsulated. In an embodiment step (ii) is carried out before step (iii). In an alternative embodiment step (iii) may be carried out before step (ii), on other words, the alginate may be added to the protein hydrolysate, and then the di- or tri- valent ions added, to get the formation of a gel in which bitter peptides of the protein hydrolysate are
20 encapsulated.

In an embodiment the alginate may be sodium alginate or potassium alginate. In embodiment the di-valent or tri-valent ions are selected from Ca^{2+} , Zn^{2+} , Fe^{2+} and Fe^{3+} , or any combination thereof, preferably di-valent ions is Ca^{2+} .

In an embodiment the mixture comprises about 0.05 to about 0.6 wt% of the
25 alginate and about 1 to about 30 wt% of protein hydrolysate, preferably about 5 to about

15 wt% of protein hydrolyzate. In an embodiment the mixture comprises about 0.1 to about 0.4 wt% of the alginate and about 2 to about 7 wt% of protein hydrolysate. In an embodiment the bitter peptides are encapsulated in the gel in-process such that the gel is formed after the protein hydrolysis directly in the solution comprising the protein hydrolysate.

In an embodiment the gel encapsulating bitter peptides is formed directly in a nutritional composition comprising macronutrients (protein, fats and carbohydrates) and micronutrients. The nutritional compositions may be a source of complete nutrition.

In an embodiment the hydrolysis of the protein uses an enzyme, and the solution in which the gel is formed comprises the enzyme in an inactivated state.

In an embodiment the hydrolysis of the protein forms non-bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

In an embodiment the bitter peptides are selectively encapsulated such that a weight ratio of the bitter peptides that are encapsulated to the bitter peptides that are not encapsulated is greater than a weight ratio of the non-bitter peptides that are encapsulated to the non-bitter peptides that are not encapsulated.

In an embodiment the present disclosure provides a method of reducing bitterness of a nutritional composition comprising bitter peptides comprising the steps of: (a) providing a solution of di- or tri-valent ions in an aqueous media; (b) mixing a solution of alginate with the solution of di- or tri-valent ions in an aqueous media to form a gel; (c) contacting said gel with a protein hydrolysate in an aqueous media to form a gel in which bitter peptides of the protein hydrolysate are entrapped.

In an embodiment the present disclosure provides a method of reducing bitterness of a nutritional composition comprising bitter peptides comprising the steps of: (a) adding alginate to an aqueous solution of di- or tri- valent ions to form a gel; (b) mixing particles of said gel and a protein hydrolysate in an aqueous media to form gel particles in which

bitter peptides of the protein hydrolysate are entrapped; and (c) filtering to remove the gel particles. In an embodiment the gel particles are gel beads/spheres and may be formed by adding alginate, in aqueous solution, dropwise into the aqueous solution of di- or tri- valent ions in step (a).

5 In an embodiment the protein hydrolysate comprises non-bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

In an embodiment the bitter peptides are selectively encapsulated such that a weight ratio of the bitter peptides that are encapsulated to the bitter peptides that are not encapsulated is greater than a weight ratio of the non-bitter peptides that are
10 encapsulated to the non-bitter peptides that are not encapsulated.

In an embodiment the amount of alginate gel contacted/mixed with the hydrolyzed protein solution (step (d)) is preferably 0.5 to 20 times in weight the amount of hydrolyzed protein. In a preferred embodiment the concentration of hydrolyzed protein in the aqueous media ranging from about 1 to about 30 wt%, preferably from about 5 to about
15 15 wt%. Gel are prepared with an amount of alginate ranging from about 0.5% to about 10% (wt%), preferably about 1 % to about 5% (wt%). In an embodiment, the mixture of alginate in aqueous media comprises about 1 to about 2 wt% of the alginate in aqueous media.

In an embodiment the present disclosure provides a nutritional composition
20 comprising a protein hydrolysate, wherein about 5 to about 20% (wt%) of the peptides from the protein hydrolysate have been removed by contacting the protein hydrolysate with a gel comprising alginate. In an embodiment the present disclosure provides a nutritional composition comprising a protein hydrolysate, wherein the nutritional composition contains about 80 to about 95% (wt%) of the peptides of the protein
25 hydrolysate.

In an embodiment the present disclosure provides a nutritional composition comprising a protein hydrolysate wherein a proportion of bitter peptides from the protein hydrolysate have been removed. The proportion of bitter peptides removed is sufficient to reduce bitterness of the nutritional composition, compared to a corresponding
5 nutritional composition which has not been subjected to removal of bitter peptides.

In another embodiment the present disclosure provides a nutritional composition comprising bitter peptides encapsulated in a gel comprising alginate.

In an embodiment, the nutritional composition further comprises an additional ingredient selected from the group consisting of a lipid source, a protein source, a
10 carbohydrate source, and combinations thereof.

In an embodiment, the bitter peptides are bioavailable upon ingestion of the nutritional composition.

In another embodiment, the present disclosure provides a method of providing nutrition to an infant. The method comprises administering to the infant an infant
15 formula comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising alginate.

In another embodiment, the present disclosure provides a method of providing nutrition to a patient selected from the group consisting of a child, an adult, an elderly person, a critically ill individual, and combinations thereof. The method comprises
20 administering to the patient a nutritional composition comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising alginate.

In an embodiment, the nutritional composition has a characteristic selected from the group consisting of treating an aging-related condition, treating an illness-related condition, providing hydrolyzed protein, and combinations thereof.

25 In an embodiment, the nutritional composition comprises a high level of protein hydrolyzate, preferably an amount of protein hydrolysate to provide from about 6 to

about 20g protein per 100ml of nutritional composition. In an embodiment the nutritional composition is a sports nutrition product comprising from about 6 to about 20g protein per 100ml of nutritional composition.

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

5 Another advantage of the present disclosure is to provide improved nutritional compositions.

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

10 Yet another advantage of the present disclosure is to hide bitter peptides to taste receptors by encapsulating the peptides in a gel.

Still another advantage of the present disclosure is to encapsulate bitter peptides in-process, such that due to specific interactions, the bitter peptides are encapsulated directly in the water phase after inactivation of the enzyme, or near line.

15 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.

Another advantage of the present disclosure is to selectively encapsulate the most hydrophobic peptides in an aqueous solution containing a mixture of peptides having a different hydrophilic-lipophilic balance (HLB).

20 Yet another advantage of the present disclosure is to encapsulate bitter peptides with a food-grade structure.

Yet another advantage of the present disclosure is to remove bitter peptides from nutritional compositions comprising protein hydrolysate.

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

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows a schematic diagram of formation of a gel by alginate.

DETAILED DESCRIPTION

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. The term “and/or” used in the context of “X and/or Y” should be interpreted as “X,” or “Y,” or “X and Y.”

As used herein, “about” is understood to refer to numbers in a range of numerals, for example the range of -10% to +10% of the referenced number, preferably within -5% to +5% of the referenced number, more preferably within -1% to +1% of the referenced number, most preferably within -0.1% to +0.1% of the referenced number. Furthermore, all numerical ranges herein should be understood to include all integers, whole or fractions, within the range. Moreover, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 1 to 8, from 3 to 7, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

All percentages expressed herein are by weight of the total weight of the composition unless expressed otherwise. When reference is made to the pH, values correspond to pH measured at 25 °C with standard equipment.

“Prevention” includes reduction of risk and/or severity of a condition or disorder. The terms “treatment,” “treat” and “to alleviate” include both prophylactic or preventive treatment (that prevent and/or slow the development of a targeted pathologic condition or disorder) and curative, therapeutic or disease-modifying treatment, including therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression

of a diagnosed pathologic condition or disorder; and treatment of patients at risk of contracting a disease or suspected to have contracted a disease, as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition. The term does not necessarily imply that a subject is treated until total recovery. The terms

5 “treatment” and “treat” also refer to the maintenance and/or promotion of health in an individual not suffering from a disease but who may be susceptible to the development of an unhealthy condition. The terms “treatment,” “treat” and “to alleviate” are also intended to include the potentiation or otherwise enhancement of one or more primary prophylactic or therapeutic measure. The terms “treatment,” “treat” and “to alleviate”

10 are further intended to include the dietary management of a disease or condition or the dietary management for prophylaxis or prevention a disease or condition. A treatment can be patient- or doctor-related.

The terms “food,” “food product” and “food composition” mean a product or composition that is intended for ingestion by an animal, including but not limited to a

15 human, and provides at least one nutrient to the animal. The compositions disclosed herein may lack any element that is not specifically disclosed herein. Thus, a disclosure of an embodiment using the term “comprising” includes a disclosure of embodiments “consisting essentially of” and “consisting of” the components identified.

As used herein, an “effective amount” is an amount that prevents a deficiency,

20 treats a disease or medical condition in an individual or, more generally, reduces symptoms, manages progression of the diseases or provides a nutritional, physiological, or medical benefit to the individual.

The present disclosure is related encapsulation of bitter peptides in a gel comprising alginate. A “gel” is a colloid in which the dispersed phase has combined with

25 the dispersion medium to produce a semisolid material, such as a jelly.

Bitter peptides, as used herein, include peptides that bind to the human bitter receptors, known as 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.

5 Hydrolysed proteins, also referred to as “protein hydrolysates”, comprise mixtures of peptides and amino acids formed by the hydrolysis of a protein. In the present disclosure reference to “bitter peptides” formed by hydrolysis of a protein encompasses also free “amino acids” formed by the hydrolysis of the protein.

10 The gel can be an ionotropic gel and can be formed by addition of di- or tri-valent counter ions to a solution of alginate. The alginate can be sodium alginate or potassium alginate. As shown in **FIG. 1**, the gel formation involves cross-linking of ionic polymers in the presence of di- or tri- valent ions. Alginate in solution is present as random coil conformation. On addition of di- or tri valent ions (in Fig. 1 di-valent ions Ca^{2+}) the alginate transforms into a cross-linked “egg-box” structure around the Ca^{2+} ions in the
15 cavities.

The di- or tri- valent ions may be selected from Zn, Fe Co, Cu or Ca ions. Preferably the di- valent ions is Ca^{2+} .

20 The alginate can encapsulate the bitter peptides by (i) entrapping the bitter peptides in the gel and/or (ii) interacting with the bitter peptides through polysaccharide-peptide interactions, e.g. ionic-based interactions such as in a coacervation system. In a preferred embodiment, at least a portion of the alginate forms a complex with the bitter peptides in a selective fashion such that peptides which are not bitter peptides are not encapsulated or are encapsulated to a lesser extent than the bitter peptides.

25 The hydrolysate of any suitable dietary protein may be used, for example artificial proteins; 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, and a combination of different hydrolysates may be used.

Sweet whey is a readily available by-product of cheese making and is frequently used in the manufacture of infant formulas based on cows' milk. However, sweet whey includes a component that is undesirably rich in threonine and poor in tryptophan called caseino-glyco-macropeptide ("CGMP"). Removal of the CGMP from sweet whey results in a protein with a threonine content closer to that of human milk. This modified sweet whey can then be supplemented with those amino acids in respect of which it has a low content (principally histidine and tryptophan).

Protein hydrolysate can be obtained as a commercial raw material powder or can be produced by hydrolysis of protein, e.g. enzymatic hydrolysis of protein. In an embodiment, whey protein hydrolysate can be produced in-process during a hydrolysis of whey protein in water using proteolytic enzymes. Processes and enzymes for the preparation of protein hydrolysates for food applications are well-known. One example of a process for preparation of protein hydrolysates is disclosed in U.S. Patent No. 5,039,532, incorporated herein by reference in its entirety.

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

Applicants surprisingly found that encapsulating bitter peptides with a gel comprising alginate masked the bitterness relative to un-encapsulated bitter peptides. 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
5 the gel comprising alginate blocks the bitter peptides from taste receptors. Humans perceive bitterness using 25 human bitter receptors, known as T2Rs, and Applicants believe that the gel comprising alginate at least partially prevents interaction between the T2Rs and the bitter peptides, possibly by establishing alginate-peptide interactions (e.g. ionic interactions such as coacervation) that mask the bitter peptides.

10 To produce the gel, an amount of alginate able to form a gel can be mixed with the bitter peptides in the presence of di- or tri-valent ions. In some embodiment, the alginate is provided in an aqueous solution or powder, and/or the bitter peptides are provided with other non-bitter peptides in an aqueous solution such as a hydrolysate. The solution comprising the alginate can be mixed directly into the solution comprising the bitter
15 peptides, or vice versa, or the solutions can be added to a third solution, for example water. In some embodiments, the resultant mixture contains about 0.05% to about 0.6% (wt%), preferably 0.1% to 0.4% (wt%), of the alginate, and about 1 to about 30% (wt%) hydrolyzed protein, preferably about 5 to about 15% hydrolysed protein. In some embodiments, the resultant mixture contains about 0.1% to about 0.5% (wt%) of the
20 alginate and about 2 to about 15% (wt%) of hydrolyzed protein. In an embodiment, the resultant mixture contains 0.1% to 0.4% (wt%), of the alginate and about 2 to about 7% hydrolysed protein.

In an embodiment the mixture of the protein hydrolysate and di- or tri-valent ions may be heated before addition of the alginate, and subsequently cooled after addition of
25 the alginate. Advantageously this can enable better dissolution and dispersion of the alginate. For example the mixture of the protein hydrolysate and di- or tri-valent ions may

be heated to about 50°C to about 100°C, before addition of the alginate, and subsequently allowed to cool to room temperature after addition of the alginate.

In a preferred embodiment, the gel is formed using only food grade materials and without using any organic solvents.

5 In another embodiment to produce the gel, an amount of alginate able to form a gel can be added to a solution of di- or tri- valent ions in an aqueous solution to form the alginate gel. In some embodiments, the resultant mixture is a hard alginate gel containing 1 % to 6% (wt%), preferably 1% to 4% (wt%), of the alginate. In some embodiments, the resultant mixture contains 1% to 2% (wt%), of the alginate.

10 Applicants surprisingly found that by contacting a solution comprising hydrolysed protein with a gel comprising alginate, bitter peptides from the protein hydrolysate may be selectively entrapped in the gel comprising alginate. As non-limiting examples, the gel may be contacted with the solution comprising hydrolysed protein for example by passing the solution comprising hydrolysed protein through a column comprising the gel, or
15 through a membrane comprising the gel, or for example by mixing a multiplicity of solid forms of the gel, e.g. particles of the gel, with the solution comprising hydrolysed protein followed by filtering to remove the gel particles. Subsequent removal of the gel advantageously permits selective removal of bitter peptides from the protein hydrolysate and permits the provision of nutritional compositions based on protein hydrolysate have
20 an improved taste and reduced bitterness.

 An additional hydrocolloid, such as xanthan gum, konjac glucomannan, or locust bean gum, can be used with alginate to strengthen the gel. Moreover, processing techniques can be used to strengthen the gel, for example freeze-thawing the gel to form microcrystal junctions.

25 The gel can be used to produce a nutritional composition. In some embodiments, alginate is a standard ingredients in a nutritional composition in which the gel that

encapsulates the bitter peptides is used. For example, the formulation of the nutritional composition may not need to be altered to include the gel. 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 gel. As a non-limiting example, the nutritional composition can be a nutritional composition that is milk-protein based and/or soy-based and is formulated to contain about 3g to 5g hydrolyzed protein per 100ml and about 0.3 g of alginate per 100 ml of the formula, in the form of the alginate gel encapsulating the bitter peptides. The present disclosure provides nutritional compositions having pleasant non-bitter taste.

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 a therapeutically effective amount of encapsulated bitter peptides. The encapsulation system can advantageously liberate the encapsulated peptides during digestion so that the bitter taste of the peptides is masked during administration or consumption of the nutritional composition, while maintaining the biological properties of the hydrolysate, such that the bitter peptides are still bioavailable.

The present disclosure provides nutritional compositions comprising hydrolysed protein from which at least a proportion of bitter peptides from the protein hydrolysate have been removed. According to one embodiment bitter peptides may be removed by entrapping bitter peptides in a gel comprising alginate. In some embodiments the gel

encapsulating bitter peptides is formed directly in a nutritional composition comprising macronutrients (protein, fats and carbohydrates) and micronutrients.

Bitter peptides may be selectively removed from the protein hydrolysate by entrapping the bitter peptides in a gel comprising alginate.

5 Nutritional compositions according to the invention may be pharmaceutical formulations, nutritional formulations, infant 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
10 6 weeks). The composition is preferably administered daily or at least twice a week for short-term administration, for example daily or at least twice a week for at least 30 days, or daily or at least twice a week for long-term administration.

The above examples of administration do not require continuous daily administration with no interruptions. Instead, there may be some short breaks in the
15 administration, such as a break of two to four days during the period of administration. The ideal duration of the administration of the composition can be determined by those of skill in the art.

The nutritional compositions according to the invention may be intended for health care; for example, the nutritional compositions according to the invention may be
20 administered to a patient who is at least one of a child, an adult, an elderly person (an individual at least 65 years old) or a critically ill individual. In an embodiment, the patient is a hospital patient. The nutritional compositions according to the invention can treat one or more aging- and/or illness-related conditions and/or can provide hydrolyzed protein.

25 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. Alternatively, the nutritional compositions may be tube feedings.

The nutritional compositions may also be a source of complete nutrition. Complete
5 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
10 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.

The nutritional compositions can include any number of optional additional ingredients, including conventional food additives (synthetic or natural), for example one
15 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.
20 The optional ingredients can be added in any suitable amount.

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
25 contributes between 35% and 60% of the total energy of the nutritional compositions.

Nutritional compositions of the present disclosure may also contain a source of lipids. 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 55% of the total energy of the nutritional compositions.

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, safflower, soybean, corn, sesame, cottonseed, grapeseed, palm, primrose, borage and walnut), nuts (e.g. walnuts, almonds and cashews), and seeds (e.g. flax, hemp, sunflower, sesame, pine nuts, black current and pumpkin).

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.

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.

The nutritional compositions can further include a source of protein in addition to the encapsulated bitter peptides and/or the remainder of the hydrolysate. The source of protein may be selected from the group consisting of dairy based proteins, plant based proteins, animal based proteins, or combinations thereof. 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. The plant based proteins may be soy protein, pea protein, canola protein, wheat and fractionated wheat proteins, corn proteins, zein proteins, rice proteins, oat proteins, potato proteins, peanut proteins, green pea powder, green bean powder, spirulina, proteins derived from vegetables, beans, buckwheat, lentils, pulses, single cell proteins, or combinations thereof. In an embodiment, the protein source contributes between 15% and 35% of the total energy of the nutritional compositions.

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, 107-110 (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.

Non-limiting examples of probiotics include *Aerococcus*, *Aspergillus*, *Bacteroides*, *Bifidobacterium*, *Candida*, *Clostridium*, *Debaromyces*, *Enterococcus*, *Fusobacterium*,

Lactobacillus, Lactococcus, Leuconostoc, Melissococcus, Micrococcus, Mucor, Oenococcus, Pediococcus, Penicillium, Peptostreptococcus, Pichia, Propionibacterium, Pseudocatenulatum, Rhizopus, Saccharomyces, Staphylococcus, Streptococcus, Torulopsis, Weissella, or combinations thereof.

5 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.

10 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.

15 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
20 supplements), vitamin C, vitamin D, vitamin E, vitamin K, K1 and K2 (i.e., MK-4, MK-7), folic acid, biotin, or combinations thereof.

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,
25 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.

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.

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).

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.

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.

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

The following non-limiting example, for illustrative purposes only, presents scientific data developing and supporting the concept of encapsulating bitter peptides from protein hydrolysate with a gel comprising alginate.

EXAMPLE 1

10 Hyprol™ 3315 from Kerry Group was used as the hydrolyzed protein. 300 mg of sodium alginate was added to 10 ml of a water solution containing 10 % w/w of Hyprol™ 3315 and 0.05 % of CaCl₂ at 80°C, with vigorous stirring during addition of the alginate, and particles of alginate gel were formed. The solution was left to cool to room temperature.

EXAMPLE 2

15 Solutions comprising hydrolysed protein (5% protein w/w) containing gelled particles of alginate was prepared according to Example 1 (here using a protein solution having protein concentration 5% w/w). Sample solutions were prepared with alginate concentration of 0.10%, 0.20%, 0.30%, 0.40%, w/w. Sensory profile the solutions was
20 evaluated by a trained panel composed of 12 people. This solution was compared to a solution comprising hydrolysed protein (5% protein w/w) without the alginate. At 0.3% alginate, the bitterness is masked. Lowering the alginate concentration to 0.20% and 0.10% still allowed masking of bitterness with less impact on viscosity.

EXAMPLE 3

25 Alginate gel beads are prepared using a solution of sodium alginate in water (1 % (w/w)). The solution is poured dropwise into a solution of CaCl₂ in water (0.5% w/w) at

room temperature, whereby beads of alginate gel are formed. The gel beads are left in the CaCl₂ solution for 30 minutes, then they are removed from the solution by filtration and added to the solution of peptides (Hyprol™ 3315 at 5% dispersed in water).

5 The gelled alginate particles were added to a solution comprising the protein hydrolysate in water to provide a concentration of 0.5% alginate w/w, and the mixture subjected to agitation at 500rpm for 1 to 2 hours, sufficient time to entrap bitter peptides in the alginate gel particles. The resultant mixture was then filtered through a sieve to remove the gelled particles, and then spray-dried to obtain a powder.

10 This powder was reconstituted at 5% w/w in water and the sensory profile compared to a solution comprising hydrolysed protein (5% protein w/w) was evaluated by a trained panel composed of 9 people. When reconstituted, all solutions had thus the same quantity of peptide, only the peptidic profile was changed. It was observed that the bitter taste of the peptide solution was significantly reduced after filtration of the gelled alginate particles. No change in product thickness was observed. The results are thereby
15 seen to demonstrate the selective removal of bitter peptides from the system.

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
20 therefore intended that such changes and modifications be covered by the appended claims.

CLAIMS

The invention is claimed as follows:

1. A method of reducing bitterness of a nutritional composition comprising bitter peptides, comprising entrapping bitter peptides in a gel comprising alginate.

5

2. The method of claim 1 comprising the steps of: (i) providing a protein hydrolysate; (ii) adding di- or tri- valent ions to the protein hydrolysate in an aqueous media; (iii) adding alginate to form a gel in which bitter peptides of the protein hydrolysate are encapsulated.

10

3. The method of claim 2 in which step (ii) is carried out before step (iii).

4. The method of claim 2, wherein the di-valent or tri-valent ions are selected from Ca^{2+} , Zn^{2+} , Fe^{2+} and Fe^{3+} , and any combination thereof, preferably Ca^{2+} .

15

5. The method of Claim 2, wherein the mixture comprises 0.1 to 0.5 wt% of the alginate.

6. The method of Claim 2 comprising hydrolysis of a protein to form a solution comprising the protein hydrolysate, the bitter peptides are encapsulated in the gel in-process such that the gel is formed after the hydrolysis directly in the solution comprising the protein hydrolysate.

7. The method of Claim 6, wherein the hydrolysis of the protein uses an enzyme, and the solution in which the gel is formed comprises the enzyme in an inactivated state.

25

8. The method of Claim 7, wherein the hydrolysis forms non-bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

5 9. The method of claim 1 comprising the steps of: (a) providing a solution of di- or tri-valent ions in an aqueous media; (b) mixing a solution of alginate with the solution of di- or tri-valent ions in an aqueous media to form a gel; (c) contacting said gel with a protein hydrolysate in an aqueous media to form a gel in which bitter peptides of the protein hydrolysate are entrapped.

10 10. The method of claim 9, wherein the di-valent or tri-valent ions are selected from Ca^{2+} , Zn^{2+} , Fe^{2+} and Fe^{3+} , and any combination thereof, preferably Ca^{2+} .

11. The method of claim 9, wherein the protein hydrolysate comprises non-
15 bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

12. A method of encapsulating bitter peptides comprising the steps of: forming a mixture comprising a protein hydrolysate and di- or tri-valent ions, adding alginate to
20 the mixture to form a gel in which bitter peptides of the protein hydrolysate are encapsulated.

13. A method according to claim 11 wherein the di-valent or tri-valent ions are selected from Ca^{2+} , Zn^{2+} , Fe^{2+} and Fe^{3+} , and any combination thereof, preferably Ca^{2+} .

25 14. A nutritional composition obtainable according to the method of claim 1.

15. A nutritional composition comprising bitter peptides encapsulated in a gel comprising alginate.

16. The nutritional composition of Claim 14 or 15, further comprising an additional ingredient selected from the group consisting of a lipid source, a protein source, a carbohydrate source, and combinations thereof.

5

17. A method of providing nutrition to an infant, comprising administering to the infant an infant formula comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising alginate.

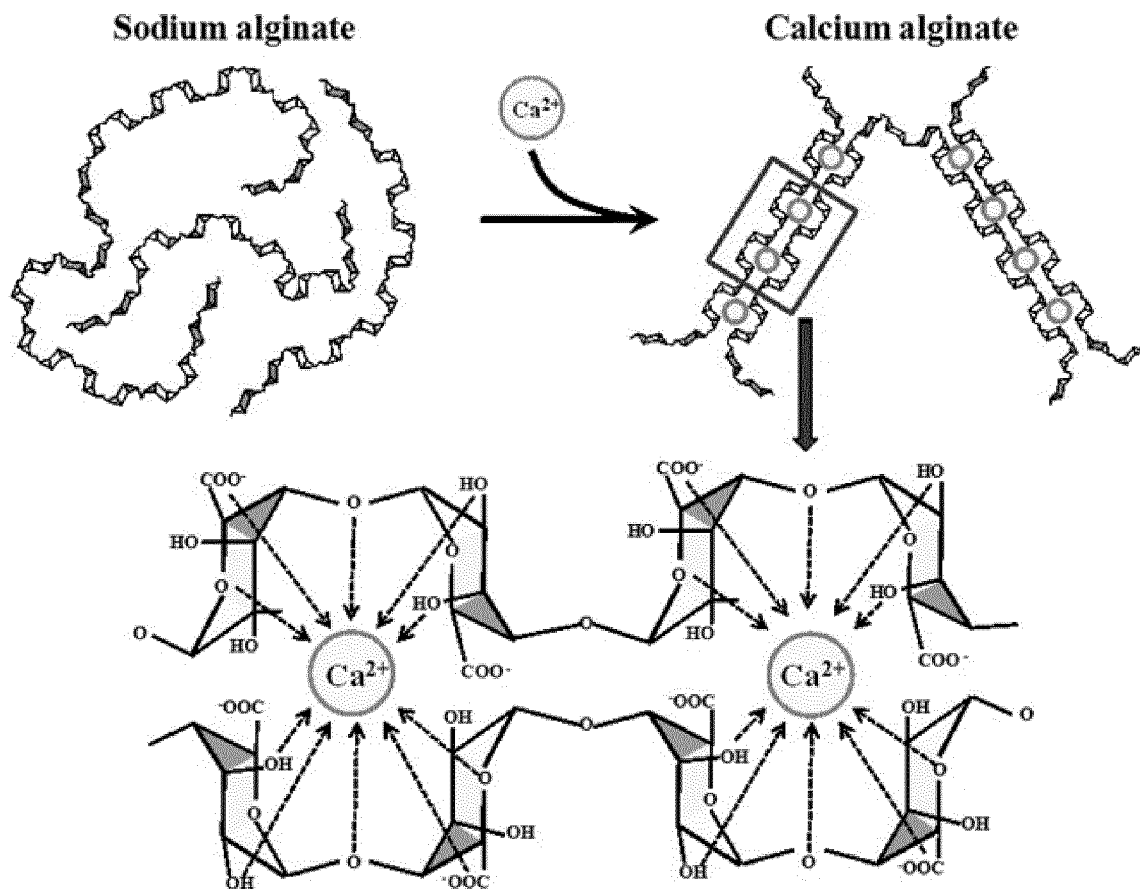
10

18. A method of providing nutrition to a patient selected from the group consisting of a child, an adult, an elderly person, a critically ill individual, and combinations thereof, the method comprising administering to the patient a nutritional composition comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising alginate.

15

19. A method of providing nutrition to a patient selected from the group consisting of a child, an adult, an elderly person, a critically ill individual, and combinations thereof, the method comprising administering to the patient a nutritional composition protein hydrolysate obtained according to claim 1.

20

**FIG. 1**

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/080027

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L33/17 A23L29/20 A23L29/256 A23L27/00 A23L33/00
A23J3/30 A23J3/34 A23K10/00 A23K20/00 A61K38/01
A61K31/734

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23L A23J A23K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2013 031453 A (ROHTO PHARMA) 14 February 2013 (2013-02-14)	1-14, 16
Y	paragraph [0001] - paragraph [0004] paragraph [0026] paragraph [0028] paragraph [0032]; claims 1-4; tables 5, No.43 paragraph [0009] - paragraph [0011] & DATABASE WPI Week 201315 Thomson Scientific, London, GB; AN 2013-C07483 & JP 2013 031453 A (ROHTO SEIYAKU KK) 14 February 2013 (2013-02-14) abstract ----- -/-	17-19



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/080027

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	JING-JIAO MA ET AL: "Effect of spray drying and freeze drying on the immunomodulatory activity, bitter taste and hygroscopicity of hydrolysate derived from whey protein concentrate", LWT - FOOD SCIENCE AND TECHNOLOGY, vol. 56, no. 2, 1 May 2014 (2014-05-01), pages 296-302, XP055193116, ISSN: 0023-6438, DOI: 10.1016/j.lwt.2013.12.019 abstract page 297, left-hand column, paragraph 2 - right-hand column, paragraph 2 page 298, left-hand column, paragraph 2 page 301, right-hand column, paragraph 1 -----	1,14-19
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Y	US 2014/213762 A1 (FITZGERALD DICK [IE] ET AL) 31 July 2014 (2014-07-31) paragraph [0001] - paragraph [0004] paragraph [0011] paragraph [0027] paragraph [0040] paragraph [0069]; claims 16,28 -----	17-19

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International application No

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