



(51) International Patent Classification:

A23L 33/00 (2016.01) A23J 3/34 (2006.01)
A23L 33/17 (2016.01) A23K 10/00 (2016.01)
A23L 29/20 (2016.01) A23K 20/00 (2016.01)
A23L 29/256 (2016.01) A61K 38/01 (2006.01)
A23L 27/00 (2016.01) A61K 31/731 (2006.01)
A23J 3/30 (2006.01)

(21) International Application Number:

PCT/EP2015/080021

(22) International Filing Date:

16 December 2015 (16.12.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

14199645.4 22 December 2014 (22.12.2014) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))

(54) Title: ENTRAPMENT OF BITTER PEPTIDES BY A GEL COMPRISING KAPPA CARRAGEENANS AND/OR IOTA CARRAGEENANS

(57) Abstract: Encapsulated bitter peptides and methods of encapsulating bitter peptides are provided. The bitter peptides are encapsulated in a gel comprising kappa-carrageenan and/or iota-carrageenan.



WO 2016/102273 A1

TITLE

**ENTRAPMENT OF BITTER PEPTIDES BY A GEL COMPRISING KAPPA CARRAGEENANS
AND/OR IOTA CARRAGEENANS**

BACKGROUND

5 The present disclosure relates generally to entrapped bitter peptides, methods of 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 kappa carrageenans, iota carrageenans, or combinations thereof.

10 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
15 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 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.

20 Due to the hydrophobic fragments of proteins formed in protein hydrolysis, such nutritional compositions can 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
25 nutritional compositions having both hydrolyzed protein and tolerable physical and organoleptic properties.

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 carrageenans selected from the group consisting of kappa-carrageenans, iota-carrageenans, and combinations thereof.

In an embodiment, the present disclosure provides a method of reducing bitterness of a nutritional composition comprising bitter peptides, comprising the steps of: forming a
15 mixture comprising a protein hydrolysate and a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof; heating the mixture; and cooling the heated mixture to form a gel in which bitter peptides of the protein hydrolysate are encapsulated.

In an embodiment the mixture comprises about 0.1 to about 1.0 wt% of the
20 carrageenan and about 1 to about 30 wt% of protein hydrolysate, preferably about 5 to about 15 wt% of protein hydrolysate. In an embodiment the mixture comprises about 0.1 to about 0.6 wt% of the carrageenan 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
25 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
5 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
10 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 an additional component is added to further increase the gel strength, the additional component selected from the group consisting of a food acceptable salt (for example potassium salt, sodium salt, a calcium salt), an additional
15 hydrocolloid, and combinations thereof.

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 a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof, to an aqueous media; (b) heating the resulting
20 mixture; (c) cooling the heated mixture to form a gel; (d) 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, thereby selectively removing bitter peptides.

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
25 a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof, to an aqueous media; (b) heating the resulting mixture; (c) cooling the heated mixture to form a gel; (d) 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 (e) filtering to remove the gel particles.

In an embodiment an additional component may be added in step (a), the additional component selected from the group consisting of food acceptable salt (e.g. potassium salt, sodium salt, calcium salt), an additional hydrocolloid, and combinations thereof.

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 encapsulated to the non-bitter peptides that are not encapsulated.

In an embodiment the amount of carrageenan 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 wt%. Gel are prepared with an amount of carrageenan ranging from about 0.5% to about 10% (wt%), preferably about 1 % to about 5% (wt%). In an embodiment, the mixture of carrageenan in aqueous media comprises about 1 to about 4 wt% of the carrageenan in aqueous media.

In an embodiment, the method comprises adding an additional component to the mixture, the additional component selected from the group consisting of potassium, calcium, an additional hydrocolloid, and combinations thereof.

In an embodiment the present disclosure provides a nutritional composition 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 carrageenans selected from the group consisting of kappa-carrageenans, iota-carrageenans, and combinations thereof. 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 hydrolysate.

In an embodiment the present disclosure provides a nutritional composition
5 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 nutritional composition which has not been subjected to removal of bitter peptides.

In another embodiment the present disclosure provides a nutritional composition
10 comprising bitter peptides encapsulated in a gel comprising carrageenans selected from the group consisting of kappa-carrageenans, iota-carrageenans, and combinations thereof.

In an embodiment, the nutritional composition further comprises an additional ingredient selected from the group consisting of a lipid source, a protein source, a
15 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
20 formula comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof.

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
25 person, a critically ill individual, and combinations thereof. The method comprises administering to the patient a nutritional composition comprising bitter peptides from protein hydrolysate, the bitter peptides encapsulated in a gel comprising a carrageenan

selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof.

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

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
10 per 100ml of nutritional composition.

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

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

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

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
20 directly in the water phase after inactivation of the enzyme, or near line.

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
25 hydrophobic peptides in an aqueous solution containing a mixture of peptides having a different hydrophilic-lipophilic balance (HLB).

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.

5 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 kappa-carrageenan and/or iota-carrageenan.

10 **FIG. 2** shows results from the experimental example disclosed herein.

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.

5 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
10 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 “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
15 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” 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
20 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 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
25 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, 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.

5 The present disclosure is related encapsulation of bitter peptides in a gel comprising kappa carrageenans and/or iota carrageenans. A “gel” is a colloid in which the dispersed phase has combined with the dispersion medium to produce a semisolid material, such as a jelly.

10 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.

15 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.

20 The gel can be a physically cross-linked gel and can be formed by cooling a hot solution of kappa carrageenans and/or iota carrageenans. As shown in **FIG. 1**, the gel formation involves helix-formation, association of the helices, and formation of junction zones between the kappa carrageenans and/or the iota carrageenans. Carrageenan in hot solution above the melting transition temperature is present as random coil conformation. Upon cooling, the carrageenan transforms into rigid helical rods. In the presence of salt (K^+ , Na^+ , etc.), due to screening of repulsion of sulphat groups ($-OSO_3^-$), double helices of the carrageenan further aggregate to form stable gels.

25 The carrageenans 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 kappa carrageenans and/or the iota carrageenans form 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.

5 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
10 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.

15 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-macropptide ("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
20 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
25 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 kappa carrageenan and/or iota carrageenan 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 the gel comprising kappa carrageenan and/or iota carrageenan 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 kappa carrageenan and/or iota carrageenan at least partially prevents interaction between the T2Rs and the bitter peptides, possibly by establishing carrageenan-peptide interactions (e.g. ionic interactions such as coacervation) that mask the bitter peptides.

To produce the gel, an amount of kappa-carrageenan and/or iota-carrageenan able to form a gel can be mixed with the bitter peptides. In some embodiment, the kappa-carrageenan and/or iota-carrageenan are 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 kappa-carrageenan and/or iota-carrageenan can be mixed directly into the solution comprising the bitter 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 1% (wt%), preferably 0.1% to 1% (wt%), of the kappa-carrageenan and/or the iota-carrageenan, 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 kappa-carrageenan and/or the iota-carrageenan 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 kappa-carrageenan and/or the iota-carrageenan and about 2 to about 7% hydrolysed protein. Preferably, the resultant mixture contains less lambda-carrageenan than kappa-carrageenan and/or less lambda-carrageenan than iota-carrageenan, and more preferably the resultant mixture does not contain lambda-carrageenan.

In an embodiment the mixture is heated to a temperature of about 50-100 °C for about 30 minutes, and cooled to a temperature of about 5-25 °C. A gel is formed by the cooling; the inventors confirmed that two phases are observable by microscopy. In a preferred embodiment, the gel is formed using only food grade materials and without using any organic solvents.

In another embodiment to produce the gel, an amount of kappa-carrageenan and/or iota-carrageenan able to form a gel can be provided in an aqueous solution. The solution comprising the kappa-carrageenan and/or iota-carrageenan is then heated, for example to a temperature of about 50-100 °C for about 30 minutes, and then cooled to 5 to 25°C for the carrageenan gel. In some embodiments, the resultant mixture is a hard carrageenan gel containing 1 % to 6% (wt%), preferably 1% to 4% (wt%), of the kappa-carrageenan and/or the iota-carrageenan. In some embodiments, the resultant mixture contains 1% to 2% (wt%), of the kappa-carrageenan and/or the iota-carrageenan. Preferably, the resultant mixture contains less lambda-carrageenan than kappa-carrageenan and/or less lambda-carrageenan than iota-carrageenan, and more preferably the resultant mixture does not contain lambda-carrageenan.

Applicants surprisingly found that by contacting a solution comprising hydrolysed protein with a gel comprising kappa carrageenan and/or iota carrageenan, bitter peptides from the protein hydrolysate may be selectively entrapped in the gel comprising kappa carrageenan and/or iota carrageenan. 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 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
5 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 an improved taste and reduced bitterness.

An additional component can be used to strengthen the gel. For example,
10 potassium can be used with kappa-carrageenan to strengthen the gel. As another example, calcium can be used with iota-carrageenan to strengthen the gel. As yet another example, an additional hydrocolloid, such as xanthan gum, konjac glucomannan, or locust bean gum, can be used with kappa-carrageenan and/or iota-carrageenan to strengthen the gel. Moreover, processing techniques can be used to strengthen the gel, for example
15 freeze-thawing the gel to form microcrystal junctions.

The gel can be used to produce a nutritional composition. In some embodiments, the kappa-carrageenan and/or iota-carrageenan are 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
20 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 kappa and/or iota carrageenan per 100 ml of the
25 formula, in the form of the kappa and/or iota carrageenan 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 kappa and/or iota carrageenan. 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 kappa-carrageenan and/or iota-carrageenan.

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 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 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 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.

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 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.

5 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
10 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.

Nutritional compositions of the present disclosure may contain a carbohydrate source. Any carbohydrate source conventionally found in nutritional compositions such as
15 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.

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
20 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
25 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
5 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
10 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
15 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
20 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.
25 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.,
5 “Probiotics: how should they be defined?,” Trends Food Sci. Technol., 10, 107-110 (1999).
10 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*,
15 *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.

20 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.

25 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.

In an embodiment, the nutritional compositions further include a vitamin selected from the group consisting of vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin),
5 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.

10 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
15 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
20 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
25 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).

5 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, 10 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 15 intestine.

 By way of example and not limitation, the following Examples are illustrative of 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 20 from protein hydrolysate with a gel comprising kappa carrageenans and/or iota carrageenans.

EXAMPLE 1

 Hyprol™ 3315 from Kerry Group was used as the hydrolyzed protein. Hyprol™ 3315 was dispersed in 5 mL water containing 1% w/w kappa-carrageenan to obtain a 10%w/w 25 solution of the hydrolyzed protein, and homogenized via mixing. Then the mixture was heated at a temperature of 80 °C in a water bath for 30 minutes. The heated mixture was poured into 5 mL of water having a temperature of 20 °C.

As shown in **FIG. 2**, a phase separation occurred during the heating step at 80 °C and flakes of thermogel were formed. These gelled particles were easily disrupted into smaller entities by mixing. The phase separation was found to remain upon cooling of the mixture to room temperature.

EXAMPLE 2

Solutions comprising hydrolysed protein (5% protein w/w) containing gelled particles of kappa-carrageenan was prepared according to Example 1 (here using a protein solution having protein concentration 5% w/w). Sample solutions were prepared with kappa-carrageenan concentration of 0.10%, 0.20%, 0.30%, 0.40%, 0.50%, 0.60%, 0.70%, 0.80% w/w. Sensory profile of the solutions was evaluated by a trained panel composed of 12 people. The solutions with kappa-carrageen gel were compared to a solution comprising hydrolysed protein (5% protein w/w) without the carrageenan. At 0.5% kappa-carrageenan, the bitterness is masked. Lowering the kappa-carrageenan concentration to 0.20% and 0.10% still allowed masking of bitterness with less impact on viscosity.

EXAMPLE 3

Gels of kappa/carrageenan and iota/carrageenan were prepared.

Kappa-carrageenan: the gel is prepared using 2 % (w/w) of kappa-carrageen dispersed in a KCl water solution (0.2 % w/w). The solution is heated at 80 °C for 30 minutes, and then is poured in plastic molds and allowed to gel at room temperature. The gel obtained is cut in small cubes (edge length: 0.5 cm) and added to the solution of peptides (Hyprol™ 3315 at 5% w/w dispersed in water).

Iota-carrageenan: the gel is prepared using 4 % (w/w) of iota-carrageen dispersed in a CaCl₂ water solution (0.3 % w/w). The solution is heated at 80 °C for 30 minutes, and then is poured in plastic molds and allowed to gel at room temperature. The gel obtained is cut in small cubes (edge length: 0.5 cm) and added to the solution of peptides (Hyprol™ 3315 at 5% w/w dispersed in water).

The gelled carrageenan particles (made of kappa and/or iota-carrageenan) were added to a solution comprising the protein hydrolysate in water to provide a concentration of 1% carrageenan w/w, and the mixture subjected to agitation at 500rpm for 1 to 2 hours, sufficient time to entrap bitter peptides in the carrageenan gel particles.

5 The resultant mixture was then filtered through a sieve to remove the gelled particles, and then spray-dried to obtain a powder.

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
10 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 kappa-carrageenan or iota-carrageenan particles. No change in product thickness was observed. The results are thereby seen to demonstrate the selective removal of bitter peptides from the system

15 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
20 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 carrageenans
5 selected from the group consisting of kappa-carrageenans, iota-carrageenans, and combinations thereof.
2. The method of claim 1 comprising the steps of: forming a mixture comprising a protein hydrolysate and a carrageenan selected from the group consisting of
10 kappa-carrageenan, iota-carrageenan, and a combination thereof; heating the mixture; and cooling the heated mixture to form a gel in which bitter peptides of the protein hydrolysate are encapsulated.
3. The method of Claim 2, wherein the mixture comprises 0.1 to 0.5 wt% of the
15 carrageenan.
4. 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
20 the protein hydrolysate.
5. The method of Claim 4, 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
6. The method of Claim 4, wherein the hydrolysis forms non-bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

7. The method of Claim 6 comprising adding an additional component to the mixture, the additional component selected from the group consisting of salts such as a potassium salt, a calcium salt, an additional hydrocolloid, and combinations thereof.

5

8. The method of claim 1 comprising the steps of: (a) adding a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof, to an aqueous media; (b) heating the resulting mixture; (c) cooling the heated mixture to form a gel; (d) 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

9. The method of claim 8, comprising adding an additional component in step (a), the additional component selected from the group consisting of salt such as a potassium salt, a calcium salt, an additional hydrocolloid, and combinations thereof.

15

10. The method of claim 8, wherein the protein hydrolysate comprises non-bitter peptides, and at least a portion of the non-bitter peptides are not encapsulated by the gel.

20

11. A method of encapsulating bitter peptides comprising the steps of: forming a mixture comprising a protein hydrolysate and a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof; heating the mixture; and cooling the heated mixture to form a gel in which bitter peptides of the protein hydrolysate are encapsulated.

25

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

13. A nutritional composition comprising bitter peptides encapsulated in a gel comprising carrageenans selected from the group consisting of kappa-carrageenans, iota-carrageenans, and combinations thereof.

5 14. The nutritional composition of Claim 13 or 14, further comprising an additional ingredient selected from the group consisting of a lipid source, a protein source, a carbohydrate source, and combinations thereof.

10 15. 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 a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof.

15 16. 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 a carrageenan selected from the group consisting of kappa-carrageenan, iota-carrageenan, and a combination thereof.

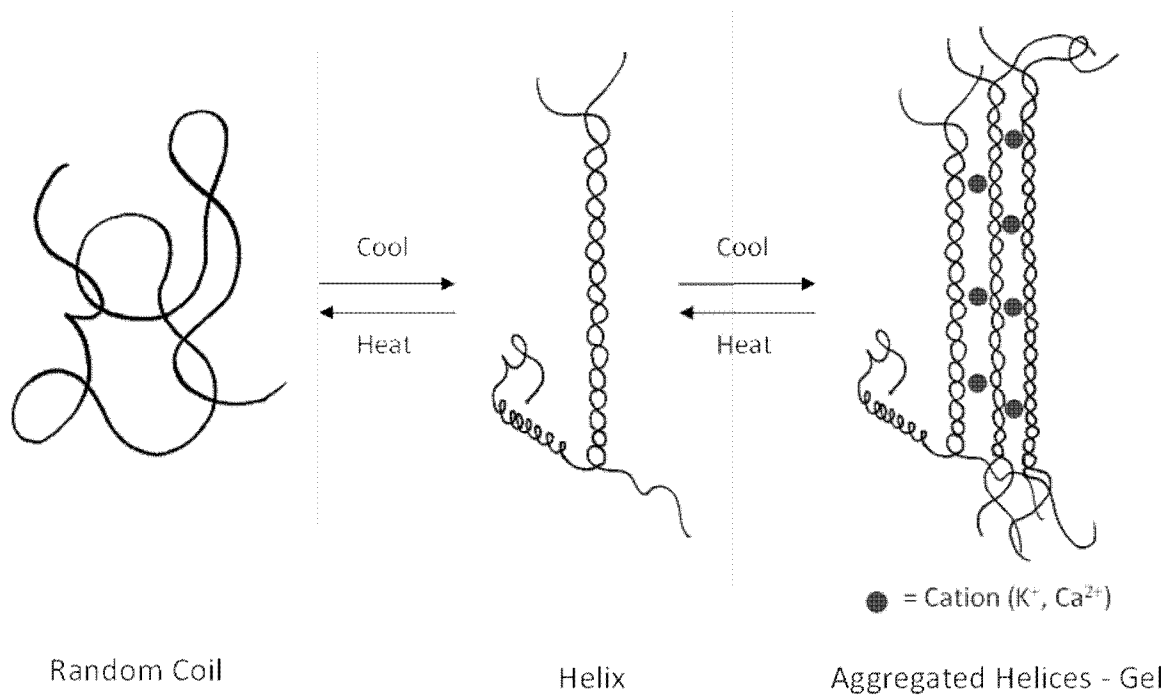
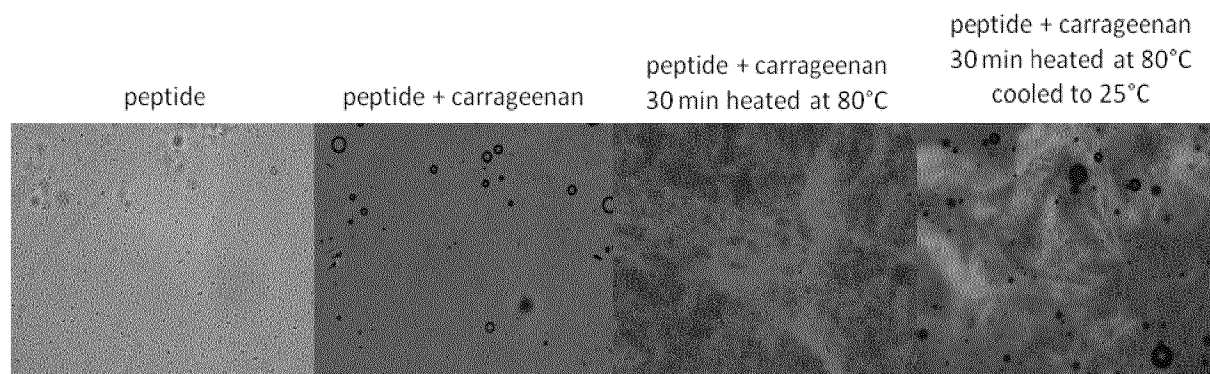
20 17. The method of Claim 16 wherein the nutritional composition has a characteristic selected from the group consisting of treating an aging-related condition, treating an illness-related condition, providing hydrolyzed protein, and combinations thereof.

25 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 protein hydrolysate obtained according to claim 1.

19. The method of Claim 18 wherein the nutritional composition has a
5 characteristic selected from the group consisting of treating an aging-related condition, treating an illness-related condition, providing hydrolyzed protein, and combinations thereof.

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**FIG. 1****FIG. 2**

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/080021

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L33/00 A23L33/17 A23L29/20 A23L29/256 A23L27/00
A23J3/30 A23J3/34 A23K10/00 A23K20/00 A61K38/01
A61K31/731

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, BIOSIS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

9 March 2016

Date of mailing of the international search report

17/03/2016

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/080021

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

International application No

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