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(71) Applicant(s):
Anabio Technologies Limited
(Incorporated in Ireland)
11 Herbert Street, Dublin, D02 RW27, Ireland

(72) Inventor(s):
Sinead Bleiel
Maja Severic Balunovic

(74) Agent and/or Address for Service:
PurdyLucey Intellectual Property
Forsyth House, Cromac Square, BELFAST, BT2 8LA, United Kingdom

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(56) Documents Cited:
WO 2019/014222 A1 **WO 2015/019307 A1**
CN 112220775 A **CN 111150068 A**
US 20210275461 A1 **US 20190099379 A1**

(58) Field of Search:
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Other: **WPI, EPODOC**

(54) Title of the Invention: **A method of forming a composition comprising a probiotic micro-encapsulated in a denatured plant protein matrix**
Abstract Title: **Method of forming a composition comprising a probiotic encapsulated in a denatured pea protein matrix**

(57) Method of forming a composition comprising probiotic encapsulated in denatured plant protein matrix (e.g. 10-15 % pea protein), comprising: preparing a protein suspension comprising denatured plant protein; preparing a suspension of hydrated probiotic; combining the protein suspension and the suspension of hydrated probiotic to encapsulate the probiotic in a denatured pea protein matrix; and polymerising the denatured plant protein matrix with a calcium salt. The protein suspension may comprise 0.5-5.0 % simple carbohydrate e.g. maltodextrin and glucose. The combining step may comprise extruding the suspensions to form microdroplets which are cured in a curing bath comprising a calcium salt and a calcium citrate buffer having a pH of 5-6.5 and a molarity of 0.05-0.15 M. Also disclosed is a method of forming a powder comprising agglomerated microparticles comprising fluidising a carrier material such as a native protein, an active agent, and a chelating salt on a fluidised bed of a fluidised bed drying chamber; spraying a suspension of denatured protein into the fluidised bed drying chamber; allowing the chelating salts to react with the denatured protein to polymerise the protein and form agglomerated microparticles having a polymerised denatured protein coat and a core comprising active agent and carrier material.

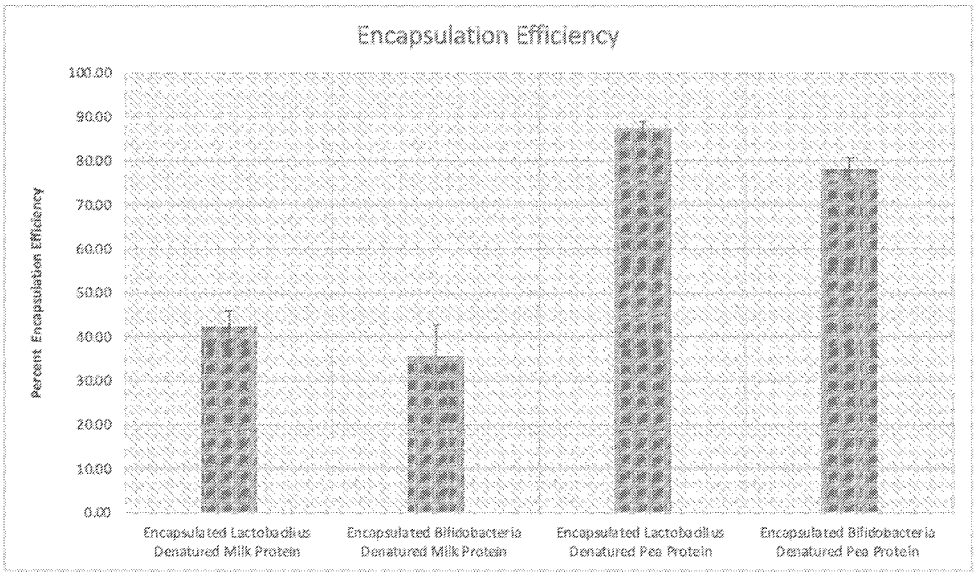


FIG. 3

GB 2615103 A

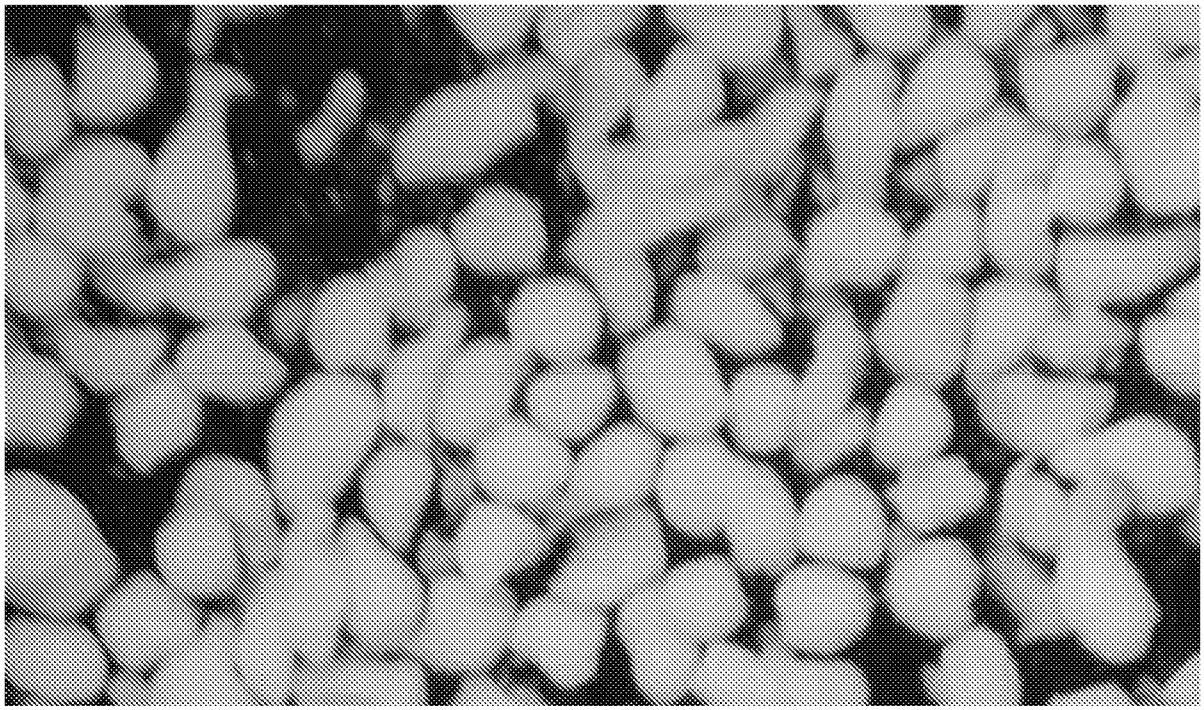


FIG. 1

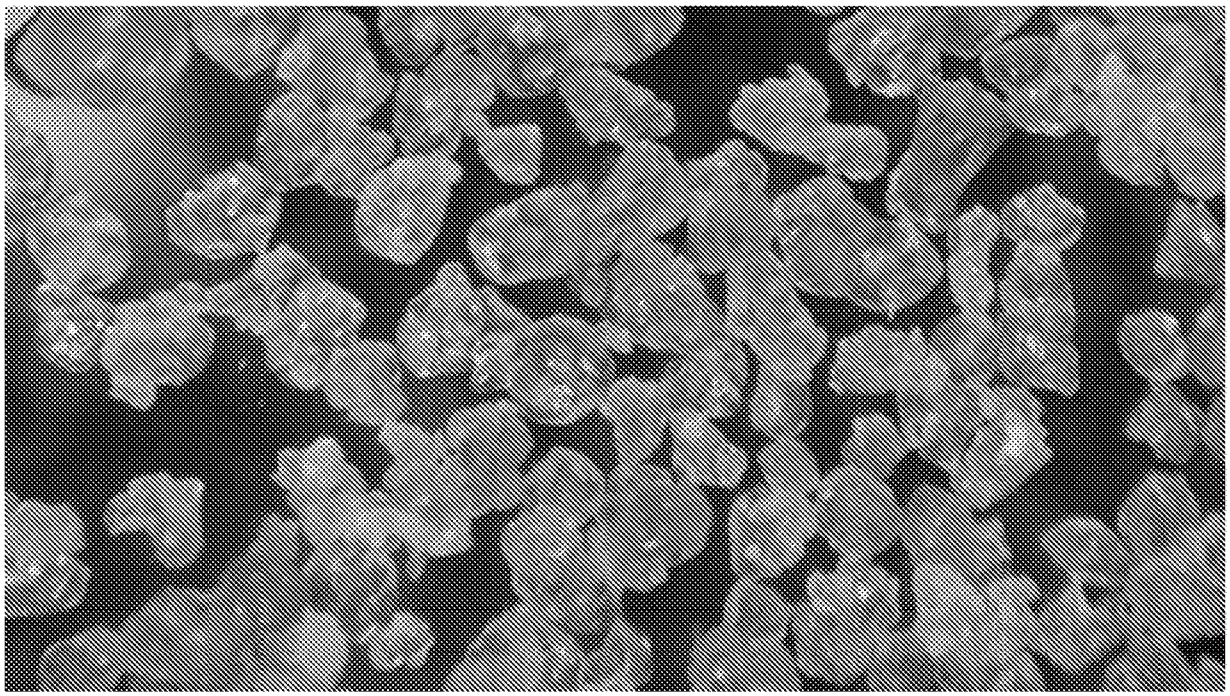


FIG. 2

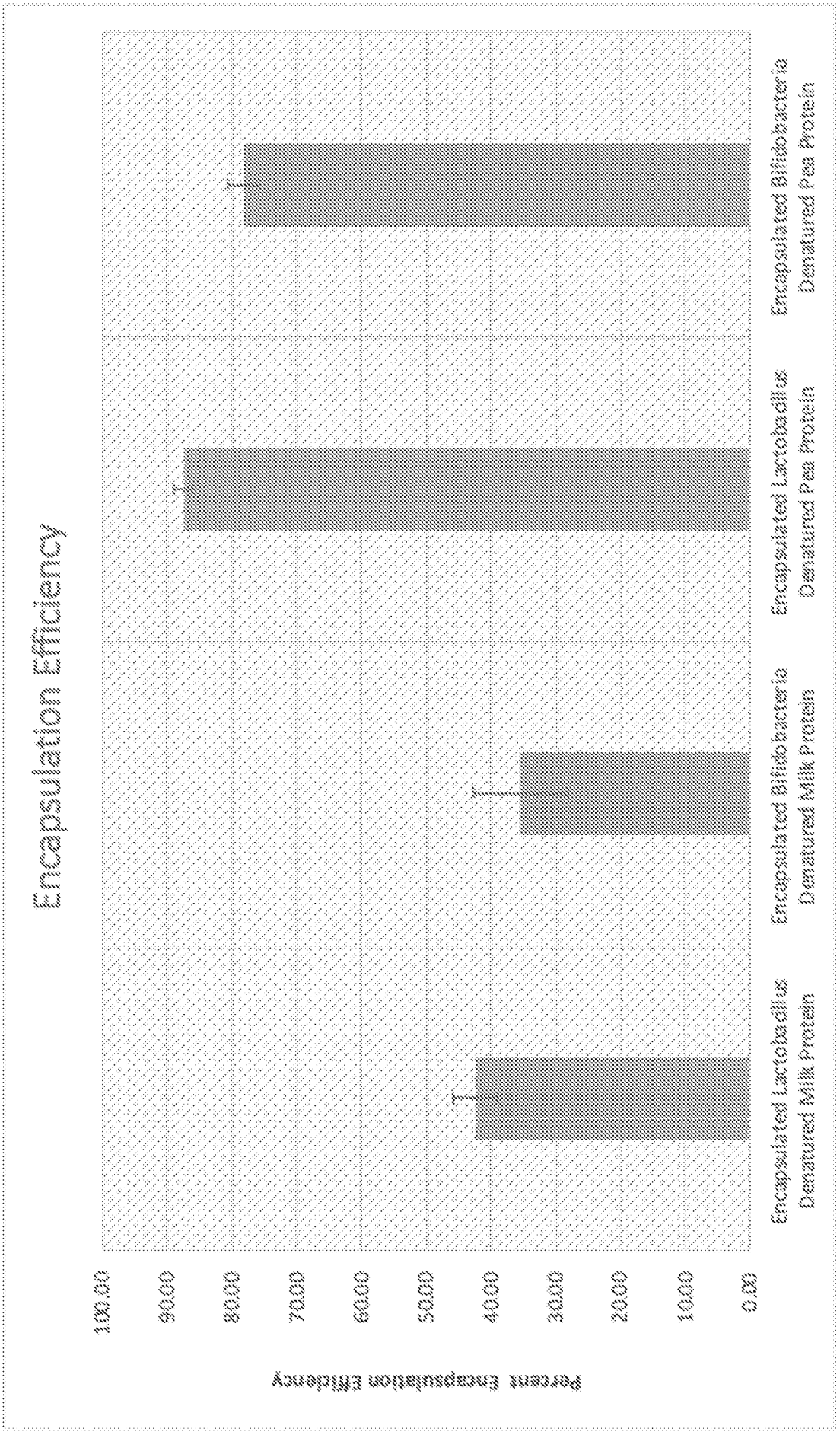

Encapsulation Efficiency
Percent Encapsulation Efficiency
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Encapsulated Lactobacillus Denatured Milk Protein
Encapsulated Bifidobacteria Denatured Milk Protein
Encapsulated Lactobacillus Denatured Pea Protein
Encapsulated Bifidobacteria Denatured Pea Protein

FIG. 3

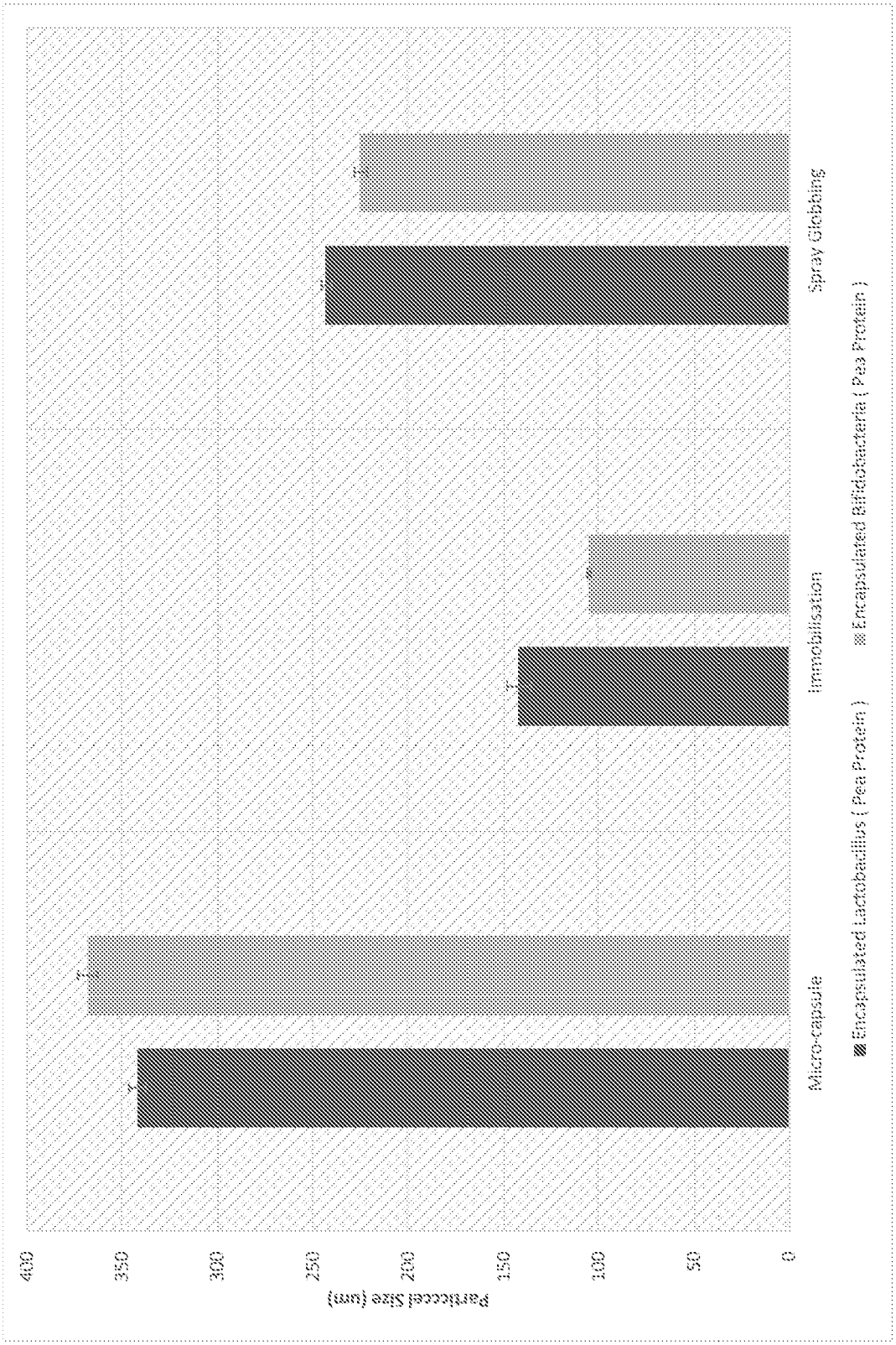

400
350
300
250
200
150
100
50
0
Particeel Size (um)
Micro-capsule
Immobilisation
Spray Globbing
Encapsulated Lactobacillus (Pea Protein)
Encapsulated Bifidobacteria (Pea Protein)

FIG. 4

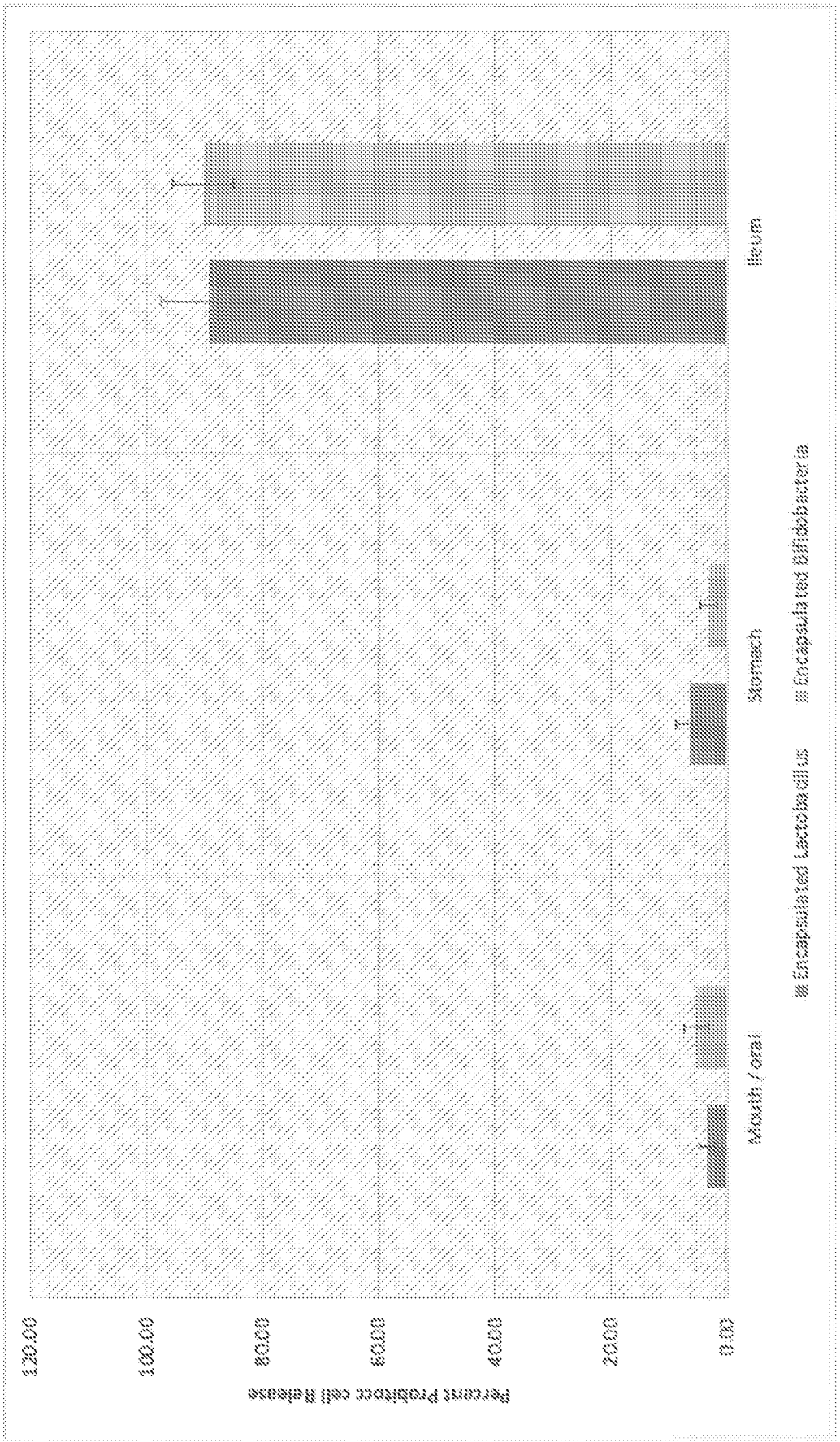

120.00
100.00
80.00
60.00
40.00
20.00
0.00
Percent Probiotic cell Release
Mouth / oral
Stomach
Ileum
Encapsulated Lactobacillus
Encapsulated Bifidobacteria

FIG. 5

TITLE

A method of forming a composition comprising a probiotic micro-encapsulated in a denatured plant protein matrix

Field of the Invention

The present invention relates to a method of forming a composition comprising a probiotic micro-encapsulated in a denatured plant (pea) protein matrix. The invention also relates to method of forming a denatured plant protein suspension. The invention also provides products and compositions formed according to the methods of the invention.

Background to the Invention

Manufacturing microencapsulated active agents such as probiotic bacteria that are robust, protect the encapsulated probiotic bacteria, and are storage stable, requires the use of protein suspensions that have a high solids content and a high proportion of the protein in solution. WO2008/056344 describes the encapsulation of probiotic bacteria in a denatured whey protein matrix using a cold gelation process in which the probiotic bacteria are mixed with a suspension of denatured whey, extruded into microdroplets, which are cured in an acidic bath at a pH of 4.6. Depending on the probiotic, there is often an adverse reaction when probiotics are added to whey protein via the cold gelation method, which leads to solidification and loss of cell counts. Furthermore, the pH required to create cold gelated whey protein is often deleterious for probiotic survival i.e. pH 4.6.

It is an objective of the invention to overcome at least one of the above-referenced problems.

Summary of the Invention

The objective is met by the provision of a method for encapsulating probiotics that employs plant protein as the encapsulating matrix. The Applicant has discovered that using a plant protein such as denatured pea protein as a micro-encapsulating matrix for probiotic bacteria results in a greater number of bacteria surviving the encapsulation process, for example >75-80 % survival of cells compared to 40 - 50% with dairy based encapsulation systems, such as WPI or MPC. Without being bound by theory, it is believed that the increase in survival is due to the plant protein polymerising at a pH of 6-7 as opposed to a pH of 4.6 for whey protein. A further advantage of the invention is that the use of plant protein as an encapsulation matrix makes the product suitable for use by vegans and vegetarians. The disclosure describes a number of methods of encapsulating probiotics with denatured pea protein (as an example of a plant protein), all of which employ a calcium salt chelating agent that is capable of polymerising denatured plant protein at a weakly acidic pH, a pH which does not have a detrimental effect on probiotics survival or metabolism. Examples provided herein show encapsulated probiotics made by extrusion into a gelling bath, and by gel immobilisation and freeze or vacuum drying. The invention also describes a method of making a suspension of plant protein (generally denatured pea protein) that is obtained by hydration of the protein at pH 7-8, resting the protein suspension, and a two-stage heating step carried under elevated pressure. The process has been found to produce suspensions of plant protein that are high in solids (e.g. up to 15% solids), have a high proportion of soluble protein (e.g. 90% of more), and do not have a highly acidic or alkaline pH, which makes the suspension ideal for micro-encapsulating pH sensitive agents such as probiotics.

In a first aspect, the invention provides a method of forming a composition (e.g. a microparticle) comprising a probiotic encapsulated in a denatured plant protein (generally denatured pea protein) matrix, comprising the steps of

preparing a protein suspension comprising denatured plant protein;

preparing a suspension of a hydrated probiotic;

combining the protein suspension and the suspension of hydrated probiotic to encapsulate the probiotic in a denatured plant protein matrix; and
polymerising the denatured plant protein matrix with a suitable chelating agent such as a calcium salt.

In any embodiment, the plant protein is or comprises a plant protein isolate.

In any embodiment, the plant protein is or comprises pea protein.

In any embodiment, a simple carbohydrate is added to the protein suspension. Examples include monosaccharides and/or disaccharides. A simple carbohydrate is added to the suspension pre-encapsulation to create a charged slurry to enhance compatibility of the denatured plant protein suspension with the probiotic cells for stable equilibrium of cells in protein matrix during encapsulation and create a charged slurry that interacts with the respective charged cell membrane of the probiotic. The simple carbohydrate also helps generate an instantaneous chelation / polymerisation due to the interaction between charged simple carbohydrate and denatured plant protein after heat treatment. This charge interaction creates a robust lattice for entrapment and encapsulation of sensitive bateria.

In any embodiment, the combining step comprises extruding the protein suspension and the suspension of hydrated probiotic to form microdroplets, in which the polymerisation step comprises curing the extruded microdroplets in a curing bath comprising a chelating agent such as a calcium salt to form microcapsules.

In any embodiment, the curing bath comprises a calcium citrate buffer.

In any embodiment, the curing bath comprises a calcium citrate buffer having a pH of 5 to 6.5

In any embodiment, the curing bath comprises a calcium citrate buffer having a molarity of 0.05 to 0.15 M.

In any embodiment, the curing bath comprises a calcium citrate buffer having a pH of 5.2 to 6.1.

In any embodiment, the curing bath comprises a calcium citrate buffer having a molarity of about 0.1 M.

In any embodiment, the curing bath comprises a calcium chloride buffer.

In any embodiment, the curing bath comprises a calcium chloride buffer having a pH of 4.3 to 5.

In any embodiment, the curing bath comprises a calcium chloride buffer having a molarity of 0.05 to 0.15 M, or about 01.M.

In any embodiment, the curing bath comprises a surfactant, especially a non-ionic surfactant, especially a polysorbate-type non-ionic surfactant. In any embodiment, the curing bath comprises 0.01 to 0.1, especially about 0.05%, surfactant.

In any embodiment, the curing bath comprises chitosan In any embodiment, the curing bath comprises 0.01 to 0.1, especially about 0.05%, chitosan.

In any embodiment, the combining step comprising mixing the protein suspension and probiotic suspension to form a mixture, adding a calcium salt buffer to the mixture to gel the mixture, and drying the gelled mixture by freeze-drying or vacuum drying.

In any embodiment, the calcium salt buffer comprises a calcium citrate buffer having a pH of 5 to 6.5.

In any embodiment, the calcium salt buffer comprises a calcium citrate buffer having a molarity of 0.05 to 0.15 M.

In any embodiment, the calcium salt buffer is added to the mixture at a volumetric ratio of 1:100 to 1:300.

In any embodiment, the protein suspension comprises 10 to 15%, 11 to 15%, 12 to 15%, 13 to 15% or 14 to 15% denatured plant protein (by weight).

In any embodiment, at least 90%, 91%, 92%, 93%, 94% or 95% of the protein in the protein suspension is solubilised.

In any embodiment, the protein suspension comprises 0.5 to 5.0%, 1-3% or about 2% simple sugar (w/v)

In any embodiment, the combining step comprises combining the protein suspension and the suspension of hydrated probiotic at a denatured plant protein to probiotic dry weight ratio of 1:5 to 1:25 or 1:8 to 1:20.

In any embodiment, the suspension of hydrated probiotic is suspended in a 0.05 to 0.15 M, 0.08 to 0.12 M, or about 0.1 M phosphate buffer.

In any embodiment, the simple carbohydrate comprises maltodextrin and glucose. GLUCIDEX is a commercial product containing maltodextrin and glucose.

In any embodiment, the protein suspension is formed by a process comprising the steps of:
hydrating plant protein in an aqueous solvent at pH 7 to 8 with agitation and optionally heating;
resting the aqueous suspension of plant protein;
adjusting the pH of the aqueous suspension to 7 to 8 if necessary;
heating the rested aqueous suspension under pressure; and

cooling the heated aqueous suspension.

In any embodiment, the aqueous suspension of plant protein is rested for at least 30, 60 or 90 minutes.

In any embodiment, the hydration step is carried out at a temperature of 25 to 40°C, 30 to 40°C, and ideally about 35°C.

In any embodiment, the heating step comprises:
pre-heating the rested aqueous suspension under pressure at a first heating temperature of 60 to 70°C (e.g. about 65°C) for a first heating time; and
heating the pre-heated aqueous suspension under pressure at a second heating temperature of 90 to 100°C (e.g. about 95°C) for a second heating time.

In any embodiment, the aqueous solvent has a pH of about pH 7.5 (e.g. 7.3 to 7.7).

In any embodiment, the heating step is performed at a pressure of 1.3 to 1.6 Bar.

In any embodiment, the pea protein is or comprises pea protein isolate.

In any embodiment, the cooled protein suspension is allowed to settle for at least 2 hours at room temperature.

In any embodiment, the method includes a step of adding a simple carbohydrate to the protein suspension to provide a protein suspension comprising 0.5 to 5.0%, 1-3% or about 2% simple carbohydrate (w/v).

In any embodiment, the method has a % probiotic survival of greater than 50%, 55%, 60%, 65%, 70% or 75%. % probiotic survival as applied to the method of the invention means the % of the probiotic bacteria that survive the encapsulation process. A method of calculating % probiotic survival is provided below.

In another aspect, the invention provides a composition comprising a probiotic encapsulated in a denatured pea protein matrix, formed according to the method of the invention. The composition is generally particulate, for example microparticulate.

In any embodiment, the microparticles may have an average dimension of (in microns, μm) 50-500, 50-400, 50-300, 50-200, 50-150, 50-100, 20-100, 20-50, 100-500, 100-400, 100-300, or 100-200. When formed by extrusion into a bath, the micropartciles typically have an average dimension of 150 to 400 μm. When a single nozzle is employed, the microparticles have a continuous denatured protein matrix and probiotic bacteria disdributed throughout the matrix (these are term microbeads). When a concentric nozzle is employed, the microparticles have a core-shell morphology, with a shell comprising denatured protein and a core comprising probiotic bacteria (these are term microcapsules). When formed by gel immobilisation, the microparticle typically have an average dimension of 50 to 150 μm.

In any embodiment, the microparticles are gastric resistant and ileal sensitive. This means that the micropartciles can pass through a human stomach intact and break up in the ileum releasing the probiotic payload for subsequent colonisation.

In another aspect, the invention provides a method of forming a denatured plant protein suspension comprising the steps of:
hydrating a plant protein in an aqueous solvent at pH 6 to 8 or 7 to 8 with agitation and optional heatng;
resting the aqueous suspension of plant protein;
adjusting the pH of the aqueous suspension to 6 to 8 or 7 to 8 if necessary;
heating the rested aqueous suspension under pressure; and
cooling the heated aqueous suspension.

In any embodiment, the aqueous suspension of plant protein is rested for at least 30, 60 or 90 minutes.

In any embodiment, the hydration step is carried out at a temperature of 25 to 40°C, 30 to 40°C, and ideally about 35°C.

In any embodiment, the heating step comprises:
pre-heating the rested aqueous suspension under pressure at a first heating temperature of 60 to 70 °C (e.g. about 65C) for a first heating time; and
heating the pre-heated aqueous suspension under pressure at a second heating temperature of 90 to 100 °C (e.g. about 95C) for a second heating time.

In any embodiment, the aqueous solvent has a pH of about pH 7.5 (e.g. 7.3 to 7.7).

In any embodiment, the heating step is performed at a pressure of 1.3 to 1.6 Bar.

In any embodiment, the plant protein is a plant protein isolate.

In any embodiment, the plant protein is pea protein.

In any embodiment, the cooled protein suspension is allowed to settle for at least 1, 2, 3, 4 or 5 hours at room temperature.

In any embodiment, the method includes a step of adding a simple carbohydrate to the protein suspension to provide a protein suspension typically comprising 0.5 to 5.0%, 1-3% or about 2% simple carbohydrate (w/v).

In another aspect, the invention provides a denatured plant protein suspension formed according to the method of the invention.

In any embodiment, the denatured plant protein suspension comprises 10 to 15%, 11 to 15%, 12 to 15%, 13 to 15% or 14 to 15% denatured plant protein (by weight).

In any embodiment, at least 90%, 91%, 92%, 93%, 94% or 95% of the protein in the denatured plant protein suspension is solubilised.

In another aspect, the invention provides a denatured plant protein suspension obtained by a method of the invention.

In another aspect, the invention provides a method of forming a powder comprising agglomerated microparticles, in which the agglomerated particles comprise an active agent encapsulated in a denatured protein matrix, the method comprising the steps of:

fluidising a carrier material such as native protein, an active agent, and a chelating salt on a fluidised bed of a fluidised bed drying chamber; and

spraying a suspension of denatured protein into the fluidised bed drying chamber,

whereby the chelating salt reacts with the denatured protein to polymerise the protein and form agglomerated microparticles having a polymerised denatured protein coat and core comprising active agent and carrier material.

In any embodiment, the carrier material is added first to the fluidised bed, the active agent is added to the bed after the carrier material, and the chelating salt is added to the fluidised bed after the active agent.

In any embodiment, the carrier material is native protein typically having a moisture content of about or less than 5%, 4%, 3%, or 2%.

In any embodment, an oil is sprayed into the fluidised bed drying chamber before the suspension of denatured protein. Typically the oil is an edible oil, such as a high oleic oil.

In any embodiment, the bed is fluidised with air at 30 to 40 °C and a high airflow rate (approximately 30 to 55 RPM**).**

In any embodiment, the native protein and denatured protein are plant protein, for example pea protein. In any embodiment, the native protein and denatured protein are protein isolates.

In any embodiment, the suspension of denatured plant protein is a denatured plant protein suspension of the invention.

In any embodiment, the method has a % probiotic survival of greater than 60%, 65%, 70%, 75%, 80%, 85% or 90%.

In any embodiment, the agglomerated microparticles are gastric resistant and ileal sensitive. This means that the microparticles can pass through a human stomach intact and break up in the ileum releasing the probiotic payload.

Other aspects and preferred embodiments of the invention are defined and described in the other claims set out below.

Brief Description of the Figures

FIG. 1: An image of probiotic-denatured pea protein microcapsules formed according to the method of Example 4 and imaged before the drying stage.

FIG. 2: An image of probiotic-denatured pea protein microcapsules formed according to the method of Example 4 and imaged after the drying stage.

FIG. 3: Summary of % probiotic survival of probiotic encapsulation using pea protein vs whey protein and a single nozzle encapsulator.

FIG.4: Comparison of particle size of probiotic encapsulation systems made using pea protein and a single nozzle encapsulator.

FIG. 5: Release profile of encapsulated probiotic cells during in vitro human digestion

Detailed Description of the Invention

All publications, patents, patent applications and other references mentioned herein are hereby incorporated by reference in their entireties for all purposes as if each individual publication, patent or patent application were specifically and individually indicated to be incorporated by reference and the content thereof recited in full.

Where used herein and unless specifically indicated otherwise, the following terms are intended to have the following meanings in addition to any broader (or narrower) meanings the terms might enjoy in the art:

Unless otherwise required by context, the use herein of the singular is to be read to include the plural and *vice versa*. The term "a" or "an" used in relation to an entity is to be read to refer to one or more of that entity. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein.

As used herein, the term "comprise," or variations thereof such as "comprises" or "comprising," are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, element, characteristics, properties, method/process steps or limitations) but not the exclusion of any other integer or group of integers. Thus, as used herein the term "comprising" is inclusive or open-ended and does not exclude additional, unrecited integers or method/process steps.

As used herein, the term “plant protein” refers to a protein preparation obtained from a plant source. Examples include plant protein powders, plant protein concentrates and plant protein isolates. Plant protein preparations can be obtained from pea, zein, barley, spelt, soya, seaweed, hemp, seiten (wheat gluten),

chickpea, tofu, lentil, quinoa and other plant sources. Plant proteins having an ioelectric point above pH 5, 5.5 or 6 are preferred.

As used herein, the term "simple carbohydrate" includes monosaccharides such as glucose, fructose and sucrose and disaccharides such as sucrose, lactose and maltose, a mixtures thereof. In one embodiment, the simple carbohydrate comprises GLUCIDEX.

As used herein, the term "% probiotic survival" as applied to the compositions made according to the method of the invention means the % of live cells that survive the encapsulation process (including the drying process where the process includes a dryng step). % probiotic survival can be measured by determining the live cell input into the process and comparing with the number of cells in the output product.

"Gastric-resistant": means that the microparticles can survive intact for at least 60 minutes in the simulated stomach digestion model described in Minekus et al., 1999 and 2014 (A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation product, Minekus, M., Smeets-Peeters M, Bernalier A, Marol-Bonnin S, Havenaar R, Marteau P, Alric M, Fonty G, Huis in't Veld JH, Applied Microbiology Biotechnology. 1999 Dec;53 (1):108-14) and (Minekus et al., 2014, A standardised static in vitro digestion method suitable for food – an international consensus, Minekus, A. et al., Food Function, 2014, 5, 1113).

"Ileal-sensitive": means that the microparticles are capable of releasing their contents in vivo in the ileum of a mammal.

Exemplification

The invention will now be described with reference to specific Examples. These are merely exemplary and for illustrative purposes only: they are not intended to be limiting in any way to the scope of the monopoly claimed or to the invention

described. These examples constitute the best mode currently contemplated for practicing the invention.

EXAMPLE 1

Preparation of Pea Protein Solution for Denaturation

- Hydrate 14 % (w/v) pea protein isolate in water or phosphate buffer 0.1M pH 7.5
- Hydrate at 30 DegC for 45 minutes
- Agitate at 450 RPM using jacketed vessel or hot plate at 30DegC
- Leave for minimum 90 min as the request resting step
- Check pH pf water and verify pH 7.5 – if not, adjust using 1 N HCl / NaOH
- Heat treat as follows
  - Pre heat 65 DegC
  - Final heat 95.5 DegC
  - Cooling temperature 22- 25 DEgC
  - Flow Rate 1.5L – 20 L per minute
  - Pressure 1.3 – 1.6 bar
- Allow material to settle for 4 H at room temperature and it is ready for use
- This material is referred to as "dPPI".

EXAMPLE 2

Hydration of probiotic

- Make stock solution of probiotic – hydrate in phosphate buffer pH 0.1M
- Add probiotic (liquid or powder form) to phosphate buffer
- Agitate at 200 – 450 RPM for 10 -45 minutes at RT for resuscitation of the cells
- Ultraturrax cell suspension if required (9,000 – 11,000 RPM for 30 – 90 seconds)

EXAMPLE 3

Immobilisation of probiotics in PPI using denatured PPi

- Using dPPI prepared as per Example 1 and hydrated probiotic prepared as per Example 2, add probiotic suspension to dPPI solution at 410RPM to provide a suspension of PPI and probiotic in 9:1 or 18:1 ratio.
- Add 2% glucidex / simple carbohydrate to the protein solution prior to encapsulation (this adds charge to the denatured protein solution the create a compatable matrix for probiotics)
- Hydrate at RT for 2 hours using mild agitation to avoid creation of air pockets
- Once all probiotic is added, after 2 minutes add 0.1M/ pH 6.1 CalciumCitrate or Calcium Chloride
- Add the CaCitrate until the buffer / suspension ratio is 1:200.
- During pH adjustment, maintain high level of agitation at 450 RPM RT
- Maintain agitation for 15 minutes min and transfer to drying chamber
- The solution will visibly appear more viscous.
- Drying (Freeze-drying or Vacum drying) can be conducted as follows:
- Freeze Drying (See Table 1)

Table 1: Two Freeze Drying Runs (48 and 72 H runs)

- Temperature	Time	Temperature	Time
-25°C	23 Hours	-25°C	18 Hours
-10°C	25 Hours	-10°C	12 Hours
0°C	8 Hours	0°C	2 Hours
10°C	8 Hours	10°C	2 Hours
20°C	4 Hours	25°C	2 Hours
25°C	4 Hours		
Total Drying Time = 72 Hours		**Total Drying Time = 48 Hours**	

- Vacuum Drying:

  Set product temperature to 25DegC

  Create vacuum in product chamber

  Dry for 24H once full vacuum is achieved i.e. < 10mBar

After drying the dry product can be size reduced into a particulate form, using a high shear solid separator.

EXAMPLE 4

Microbeads comprising probiotic encapsulated in denatured PPI – single nozzle extrusion

- Using dPPI prepared as per Example 1, prepare a suspension of dPPI and agitate at 250 RPM at RTemp
- Add 2% glucidex / simple carbohydrate to the protein solution prior to encapsulation (this adds charge to the denatured protein solution the create a compatable matrix for probiotics)
- Hydrate at room temperature for 2 hours using mild agitation to avoid creation of air pockets
- Add the hydrated probiotic suspension of Example 2 to dPPI solution, that is agitating at RT
- Add probiotic suspension to protein at 9:1 or 18:1 (protein: probiotic ratio)
- Agitate the Protein – Probiotic Suspension at 400 – 450 RPM for 10 minutes

PREPARATION OF CURING BATH

- Prepare Calcium citrate buffer 0.1M; adjust pH to 5.2 (range is 5.2 – 6.1). Add 0.05% Polysorbate 20% as a surfactant
- Alternative curing bath is Calcium Chloride buffer 0.1M; adjust pH to 4.8 (range is 4.3 – 5.0). Add 0.05% Polysorbate 20% as a surfactant
- Alternative curing bath is Calcium Citrate buffer 0.1M; with 0.05% chitosan calcium chelator) and 0.05% Polysorbate 20% (surfactant)
- Once probiotic is fully hydrated, extrude the protein- probiotic solution through a single nozzle exruder at Room Temperature for polymerisation in the calcium curing bath
- Polymerisation will occur in the calcium buffer bath at Room Temperature
- Microcapsules are collected and washed in neutralising solution at pH 6.1 (prevents sticking of micro- capsules during drying – creating optimised yield

- Micro-capsules are loaded to the relevant drying chamber
- Drying can be conducted as follows:
    - Freeze Drying for 48H (See Table 1)
    - Fluidised Bed Drying: 37DegC for 4- 6 Hours
    - Vacuum Drying: Less than 10mBar for 24 - 48 hours min at 37DegC

EXAMPLE 5

Microbeads comprising probiotic encapsulated in denatured PPI – double (concentric) nozzle extrusion

- Using dPPI prepared as per Example 1, prepare a suspension of dPPI agitate at 250 RPM at RTemp
- Add 2% glucidex / simple carbohydrate to the protein solution prior to encapsulation (this adds charge to the denatured protein solution the create a compatable matrix for probiotics)
- Hydrate at room temperature for 2 hours using mild agitation to avoid creation of air pockets
    - Prepare a calcium bath according to Example 3.
- Simultaneously extrude the suspension of dPPI and the hydrated probiotic through the concentric nozzle into the curing bath, with the dPPI/glucidex suspension extruded through the outer nozzle and the probiotic suspension extruded through the inner nozzle
- Polymerisation will occur in the calcium buffer bath at Room Temperature
- Micro- capsules are collected and washed in neutralising solution at pH 6.1 (prevents sticking of micro- capsules during drying – creating optimised yield
- Micro-capsules are loaded to the relevant drying chamber
- Drying can be conducted as follows:
    - Freeze Drying for 48H (See Table 1)
    - Fluidised Bed Drying: 37DegC for 4- 6 Hours
    - Vacuum Drying: Less than 10mBar for 24 - 48 hours min at 37DegC

EXAMPLE 6

Spray englobing of probiotics, bioactives in denature PPI

- Using dPPI prepared as per Example 1, prepare a suspension of dPPI and agitate at 250 RPM at RTemp
- Add 2% glucidex / glucose / simple carbohydrate to the protein solution prior to encapsulation (this adds charge to the denatured protein solution the create a compatible matrix for probiotics)
- Hydrate at room temperature for 2 hours using mild agitation to avoid creation of air pockets
- Load fluidised bed chamber with Dry pea protein powder (native powder; approx. 2% moisture).
- Fluidise at 37DegC at high airflow
- Added probiotic culture to the powder at 5:1 or 10:1 ratio
- Add 15 CaCitrate or Calcium Chloride to the chamber and fludise
- Spray 10% glucidex solution onto the material at high flow rate (20-30 RPM) high airflow(>150 RPM) and low nozzle pressure (<1.5 bar) □ promotes agglomeration
- Spray coconut oil / sunflower oil / high oleic oil onto the powder at low flow rate (18-22 RPM), high airflow(>150 RPM) and high nozzle pressure (> 2.5 bar)
- Spray dPPI – glucidex solution into chamber at high flow rate (25-30 RPM), high airflow(>150 RPM) and high nozzle pressure (> 2.5 bar)
- Post dry the material to achieve Aw < 0.15

Equivalents

The foregoing description details presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are intended to be encompassed within the claims appended hereto.

CLAIMS:

1. A method of forming a micoparticle comprising a probiotic encapsulated in a denatured plant protein matrix, comprising the steps of

preparing a protein suspension comprising denatured plant protein;

preparing a suspension of hydrated probiotic;

combining the protein suspension and the suspension of hydrated probiotic to form a microparticle comprising probiotic encapsulated in a denatured plant protein matrix;

polymerising the denatured plant protein matrix with a calcium salt; and

drying the microparticle.

2. A method according to Claim 1, in which the protein suspension comprises a simple carbohydrate.

3. A method according to Claim 1 or 2, in which the plant protein is pea protein.

4. A method according to any preceding Claim, in which the combining step comprises extruding the protein suspension and the suspension of hydrated probiotic to form microdroplets, in which the polymerisation step comprises curing the extruded microdroplets in a curing bath comprising the calcium salt to form the microparticles.

5. A method according to Claim 4, in which the curing bath comprises a calcium citrate buffer having a pH of 5 to 6.5 and a molarity of 0.05 to 0.15 M.

6. A method according to any of Claims 1 to 3, in which the combining step comprising mixing the protein suspension and probiotic suspension to form a mixture, adding a calcium salt buffer to the mixture to gel the mixture, drying the gelled mixture by freeze-drying or vacuum drying to form a dry solid, and size reducing the dry solid to provide the microparticles.

7. A method according to Claim 6, in which the calcium salt buffer comprises a calcium citrate buffer having a pH of 5 to 6.5 and a molarity of 0.05 to 0.15 M.

8. A method according to Claim 6 or 7, in which the calcium salt buffer is added to the mixture at a volumetric ratio of 1:100 to 1:300.

9. A method according to any preceding Claim, in which the protein suspension comprises 10 to 15% denatured plant protein (by weight).

10. A method according to any preceding Claim, in which the protein suspension comprises 0.5 to 5.0% simple carbohydrate..

11. A method according to any preceding Claim, in which the combining step comprises combining the protein suspension and the suspension of hydrated probiotic at a denatured plant protein to probiotic dry weight ratio of 1:5 to 1:25.

12. A method according to any preceding Claim, in which the suspension of hydrated probiotic is suspended in a 0.05 to 0.15 M phosphate buffer.

13. A method according to any preceding Claim, in which the simple carbohydrate comprises maltodextrin and glucose.

14. A method according to any preceding Claim, in which the protein suspension is formed by a process comprising the steps of:
hydrating plant protein in an aqueous solvent at pH 7 to 8 with heating and agitation;
resting the aqueous suspension of plant protein;
adjusting the pH of the aqueous suspension to 7 to 8 if necessary;
heating the rested aqueous suspension under pressure; and
cooling the heated aqueous suspension.

15. A method according to Claim 14, in which the heating step comprises:

pre-heating the rested aqueous suspension under pressure at a first heating temperature of 60 to 70 °C for a first heating time;
heating the pre-heated aqueous suspension under pressure at a second heating temperature of 90 to 100 °C for a second heating time.

16. A method according to Claim 14 or 15, in which the aqueous solvent has a pH of about pH 7.5.

17. A method according to any of Claims 14 to 16, in which the heating step is performed at a pressure of 1.3 to 1.6 mBar.

18. A method according to any of Claims 14 to 17, in which the plant protein is pea protein.

19. A method according to any of Claims 14 to 18, in which the cooled protein suspension is allowed to settle for at least 2 hours at room temperature.

20. A method according to any of Claims 14 to 19, including a step of adding a simple carbohydrate to the protein suspension to provide a protein suspension comprising 1 to 5% simple carbohydrate (w/v).

21. A microparticle obtained by a method of any of Claims 1 to 20, in which the microparticle is gastric resistant and ileal sensitive.

22. A method of forming a powder comprising agglomerated microparticles, in which the agglomerated particles comprise an active agent encapsulated in a denatured protein matrix, the method comprising the steps of:

fluidising a carrier material such as native protein, an active agent, and a chelating salt on a fluidised bed of a fluidised bed drying chamber; and

spraying a suspension of denatured protein into the fluidised bed drying chamber,

whereby the chelating salt reacts with the denatured protein to polymerise the protein and form agglomerated microparticles having a polymerised denatured protein coat and core comprising active agent and carrier material.

23. A method according to Claim 22, in which the chelating salt is a calcium salt.

24. A method according to Claim 22 or 23, in which the active agent is a probiotic bacteria.

25. A method according to any of Claims 22 to 24, in which the protein is plant protein and the carrier material is native plant protein.

26. A method according to any of Claims 22 to 25, in which the carrier material is added first to the fluidised bed, the active agent is added to the bed after the carrier material, and the chelating salt is added to the fluidised bed after the active agent.

27. A method according to any of Claims 22 to 26, in which a high oleic oil is sprayed into the fluidised bed drying chamber before the suspension of denatured protein.

28. A method according to any of Claims 22 to 27, in which the bed is fluidised with air at 30 to 40 °C and an airflow rate of 30 to 55 RPM.

Intellectual Property Office

Application No:	GB2201083.9	**Examiner:**	Natasha Riley
Claims searched:	1-21	**Date of search:**	30 June 2022

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-4, 6, 13, and 21	WO 2019/014222 A1 (MIRABLUE BIO LLC) Example 3 and paragraph [0062]
X	1-4, 6, 8-11, 13, and 21	CN 111150068 A (INNER MONGOLIA MENGNIU DAIRY (GROUP) CO LTD) Examples. WPI abstract accession number 2020-44317J
X	1, 3, 4,6, 9, 11, and 21	WO 2015/019307 A1 (UNIV SASKATCHEWAN) Examples

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

A23L; A61K; B01J

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

International Classification:

Subclass	Subgroup	Valid From
A61K	0047/42	01/01/2017
A23L	0033/135	01/01/2016
A61K	0009/50	01/01/2006
B01J	0013/04	01/01/2006

Intellectual Property Office

Application No: GB2201083.9 **Examiner:** Natasha Riley

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Patents Act 1977
Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	22-28	US 2021/0275461 A1 (MOASERI) Paragraphs [0031], [0054], [0102], and [0175]
X	22-28	CN 112220775 A (CHANGSHA JINGYI MEDICAL TECH CO LTD) Example 1
X	22-28	CN 111150068 A (INNER MONGOLIA MENGNIU DAIRY (GROUP) CO LTD) Examples
X	22-28	US 2019/0099379 A1 (MCNALLY et al.) Examples

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&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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A61K	0009/50	01/01/2006

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Subclass	Subgroup	Valid From
B01J	0013/04	01/01/2006