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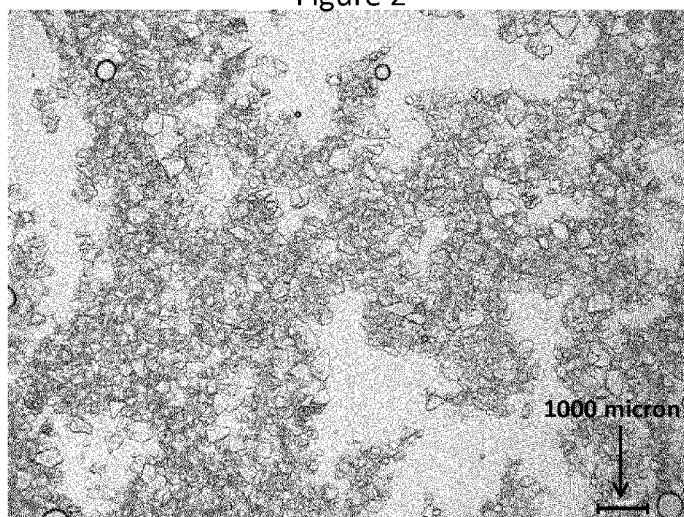
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(54) Title: INSTANT BEVERAGE POWDER BASED ON BLG

Figure 2



(57) Abstract: The present invention relates to an instant beverage powder product and a method for preparing the instant beverage powder product, a liquid food product produced from the instant beverage powder and a method for preparing the liquid food, use of the liquid food, and a kit comprising the instant beverage powder product.



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INSTANT BEVERAGE POWDER BASED ON BLG

FIELD OF THE INVENTION

The present invention relates to an instant beverage powder product and a method for preparing the instant beverage powder product, a liquid food product produced from the instant beverage powder and a method for preparing the liquid food, use of the liquid food, and a kit comprising the instant beverage powder product.

BACKGROUND

Nutritional supplements comprising milk serum proteins are commonly used for muscle synthesis, for weight control and for maintaining muscle and body weight. Nutritional supplements are targeted different kinds of consumers, e.g. sportsmen/women, athletes, children, elderly people and patients with or at risk of malnutrition, and/ or with increased protein needs. Thus, the consumer perception of the nutritional supplement is of great importance, as the consumer should feel for drinking the product.

Milk serum proteins can be isolated from milk serum or whey. Whey typically comprises a mixture of beta-lactoglobulin (BLG), alpha-lactalbumin (ALA), serum albumin and immunoglobulins, of which BLG is the most dominant. Whey protein concentrates (WPC) thus comprise a mixture of these proteins. Whey protein isolates (WPI) contain less fat and lactose than WPC.

Isolation of beta-lactoglobulin (BLG) from milk serum or whey is the subject of a number of publications and typically involves multiple separation steps and often chromatographic techniques to arrive at a purified beta-lactoglobulin product.

International patent application WO2002/056707 (Nestlé) concerns a balanced powder blend composition with at least one fat or oil source, at least one carbohydrate source, and at least one protein source, is described. This composition is advantageously added to a food to supplement the nutritional value of the food, but without substantially altering the taste of the food.

WO 2018/115520 A1 discloses a method of producing edible isolated beta-lactoglobulin compositions and/or compositions containing crystallised beta-lactoglobulin based on crystallisation of BLG in salting-in mode. The crystallised BLG may subsequently be separated from the remaining mother liquor.

WO 2011/112695 A1 discloses nutritional compositions and methods of making and using the nutritional compositions. The nutritional compositions comprise whey protein micelles and leu-

cine and provide a sufficient amount of leucine to improve protein synthesis in humans, while also maintaining a low-viscosity fluid matrix and acceptable organoleptic properties.

WO2011/051436 A1 discloses an at least partially transparent composition intended for human or animal consumption and relates to the packaging of such compositions. One embodiment of the present invention relates to an at least partially transparent container containing an at least partially transparent aqueous non-alcoholic composition. The container comprises at least one polarizer that makes liquid crystals present in the composition visible.

WO2004/049819 A2 discloses a method for improving the functional properties of globular proteins, comprising the steps of providing a solution of one or more globular proteins, in which solution the protein(s) is/are at least partially aggregated in fibrils; and performing one or more of the following steps in random order: increasing the pH; increasing the salt concentration; concentrating the solution; and changing the solvent quality of the solution. Preferably, the solution of the one or more globular protein is provided by heating at a low pH or the addition of a denaturing agent. Disclosed is also the protein additive thus obtained, the use thereof for food and non-food applications and to the food and non-food products containing the protein additive.

WO 2010/037736 A1 discloses isolation of whey proteins and the preparation of a whey product and a whey isolate. In particular the present invention relates to the isolation of a β -lactoglobulin product and the isolation of an α -enriched whey protein isolate from whey obtained from an animal. The α -enriched whey protein isolate provided by the present invention is besides from being low in β -lactoglobulin also high in α -lactalbumin and immunoglobulin G.

FR 2 296 428 discloses protein compositions for dietetic and therapeutic use based on lactoserum proteins obtained by any known separation process. The compositions can be used for the treatment or prophylaxis of digestive disorders in infants and adults (e.g. diarrhoea), to increase resistance to intestinal infections, and to treat certain metabolic disorders (e.g. hyperphylalaninaemia). They can also be used dermatologically or cosmetically, and can form part of a low-protein diet.

SUMMARY OF THE INVENTION

The inventors have provided instant beverage powder products with a high content of BLG. The products are shelf stable, while at the same time resulting in food products that are appetizing; i.e. the appearance and taste of the product is appealing to the customer.

Thus, an aspect of the invention pertains to an instant beverage powder comprising at least 1% w/w BLG, preferably at least 5%, wherein:

- i. the crystallinity of BLG is at least 20%, preferably at least 40%, and/or
 - ii. at least 85% w/w of the total amount of protein is comprised by BLG,
- and furthermore comprising at least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, minerals, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics, anti-foaming agents and non-whey protein.

Another aspect of the invention pertains to a method for preparing an instant beverage powder comprising BLG and at least one optional ingredient, said method comprising blending a dry BLG isolate with the least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, minerals, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics, antifoaming agents and non-whey protein to obtain an instant beverage powder.

Yet an aspect of the invention pertains to a liquid food product comprising a liquid and the powder according to the invention.

A further aspect of the invention pertains to a method for preparing a liquid food product according to the invention, said method comprising

- i. Adding an instant beverage powder according to the invention,
- ii. Optionally adding at least one further ingredient, and
- iii. Mixing the powder and liquid obtained to form a uniform mixture.

A further aspect of the invention pertains to an instant beverage powder according to the invention, for use as a nutritional supplement.

A further aspect of the invention pertains to a kit comprising the powder according to the invention,

- i. a tool for measuring said powder, and
 - ii. a container having a lid for opening and closing the container,
- wherein said container is for mixing said powder with a liquid to form a food product, and said container is adapted for drinking the food product directly from the container.

The present invention as claimed herein is described in the following items 1 to 21:

1. An instant beverage powder comprising at least 5% w/w beta-lactoglobulin (BLG), wherein:
 - i. at least 91 % w/w of the total amount of protein is comprised by BLG,and furthermore comprising at least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, sweeteners, antioxidants, food

acid, lipids, carbohydrate, prebiotics, probiotics, anti-foaming agents and non-whey protein.

2. The powder according to any of item 1, wherein said powder further comprises one or more of:

- i. a sweetener, e.g. a sugar sweetener and/or a non-sugar sweetener,
- ii. a flavoring agent,
- iii. at least one food acid, e.g. citric acid or other suitable food acids,
- iv. the sum of the amounts of Na, K, Mg, and Ca of the instant beverage is at most 10 mmol/g protein and

wherein a 10% w/w solution of the powder in demineralized water has a pH in the range of 2-8.

3. The powder according to any one of the preceding items, wherein said powder comprises at least 92% w/w BLG relative to total protein, at least 93% w/w BLG relative to total protein, at least 94% w/w BLG relative to total protein, at least 95% w/w BLG relative to total protein, at least 96% w/w BLG relative to total protein, at least 97% w/w BLG relative to total protein, at least 98% w/w BLG relative to total protein or at least 99% w/w BLG relative to total protein.

4. The powder according to any one of the preceding items wherein the powder comprises a water content in an amount of at most 6% w/w, such as at most 5% w/w, preferably at most 4% w/w, more preferably at most 3% w/w, and even more preferably at most 2% w/w.

5. The powder according to any one of the preceding items, wherein said powder comprises:

- i. at most 6% w/w water
- ii. at least 15% w/w total protein relative to total solids
- iii. at least 91% w/w BLG relative to total protein,

wherein said powder is a dry powder.

6. The powder according to any one of the preceding items, wherein said powder has a bulk density of at least 0.30 g/mL, preferably at least 0.4 g/mL, more preferably 0.5 g/mL or even more preferably 0.5 g/mL.

7. The powder according to any one of the preceding items, wherein the sum of ALA and caseinomacropeptide (CMP) comprises at least 40% w/w of the non-BLG protein of the powder, preferably at least 60% w/w, even more preferably at least 70% w/w, and most preferably at least 85% w/w of the non-BLG protein of the powder.

8. The powder according to any one of the preceding items, wherein the sum of the amounts of Na, K, Mg, and Ca is at most 10 mmol/g protein

9. The powder according to any one of the preceding items, wherein said powder has an energy content in the range of about 200-500 kcal/100 grams of powder.

10. The powder according to any one of the preceding items, wherein the energy amount of the protein is at least 7 E%, preferably at least 25 E%, more preferably at least 30 E%, even more preferably at least 40 E%.

11. The powder according to any one of the preceding items, wherein the contribution of energy from lipid is in the range of 0-60 E%.

12. The powder according to any one of the preceding items, wherein the contribution of energy from carbohydrate is in the range of 0-90 E%.

13. The powder according to any one of the preceding items, wherein said powder in a 10% w/w solution in demineralised water has a pH in the range of 2-8 at room temperature.

14. The powder according to any one of the preceding items, wherein said powder has a degree of protein denaturation of at most 10%, preferably at most 4%, more preferably at most 1 %, even more preferably at most 0.4% and even more preferably at most 0.1%.

15. The powder according to any one of the preceding items, wherein said powder has a crystallinity of BLG of at least 20%, preferably at least 40%.

16. The powder according to any one of the preceding items, for use in a method for the treatment of patients with or at risk of malnutrition.

17. The powder according to any one of the preceding items, for use in a method for the treatment of kidney disease.

18. A kit comprising the powder according to any one of items 1-17, said kit comprising

i. a tool for measuring said powder, and

ii. a container having a lid for opening and closing the container,

wherein said container is for mixing said powder with a liquid to form a food product, and said container is adapted for drinking the food product directly from the container.

19. A method for preparing a liquid food product according, comprising a liquid and the instant beverage powder according to any one of the items 1-17, said method comprising

- i. Adding a powder according to any one of items 1-17 to a liquid,
- ii. Optionally adding at least one further ingredient, and
- iii. Mixing the powder and liquid obtained to form a uniform mixture.

20. The method according to item 19, wherein the further ingredient is selected from fruits or vegetables.

21. The method according to any one of items 19-20, wherein the mixing is performed by shaking.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a microscope photo of the BLG crystals recovered from feed 3 of Example 3 of the PCT application PCT/EP2017/084553.

Figure 2 shows a microscope photo of the BLG crystals, both whole and fragmented, obtained from feed 2 of Example 3 of the PCT application PCT/EP2017/084553.

Figure 3 is a photo of test tubes containing sub-samples of the six low phosphorous beverages as prepared in example 5.

DEFINITIONS

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In the context of the present invention, the term "beta-lactoglobulin" or "BLG" pertains to beta-lactoglobulin from mammal species, e.g. in native, unfolded and/or glycosylated forms and includes the naturally occurring genetic variants. The term furthermore includes aggregated BLG, precipitated BLG and crystalline BLG. When referring to the amount of BLG reference is made to the total amount of BLG including aggregated BLG. The total amount of BLG is determined according to Example 1.31. The term "aggregated BLG" pertains to BLG which is at least partially unfolded and which furthermore has aggregated with other denatured BLG molecules and/or other denatured whey proteins, typically by means of hydrophobic interactions and/or covalent bonds.

15

BLG is the most predominant protein in bovine whey and milk serum and exists in several genetic variants, the main ones in cow milk being labelled A and B. BLG is a lipocalin protein, and can bind many hydrophobic molecules, suggesting a role in their transport. BLG has also been shown to be able to bind iron via siderophores and might have a role in combating pathogens. A homologue of BLG is lacking in human breast milk.

20

Bovine BLG is a relatively small protein of approx. 162 amino acid residues with a molecular weight of approx. 18.3-18.4 kDa. Under physiological conditions, it is predominantly dimeric, but dissociates to a monomer below about pH 3, preserving its native state as determined using Nuclear Magnetic Resonance spectroscopy. Conversely, BLG also occurs in tetrameric, octameric and other multimeric aggregation forms under a variety of natural conditions.

25

In the context of the present invention, the term "non-aggregated beta-lactoglobulin" or "non-aggregated BLG" also pertains to beta-lactoglobulin from mammal species, e.g. in native, unfolded and/or glycosylated forms and includes the naturally occurring genetic variants. However, the term does not include aggregated BLG, precipitated BLG or crystallised BLG. The amount or concentration of non-aggregated BLG is determined according to Example 1.6.

30

The percentage of non-aggregated BLG relative to total BLG is determined by calculate $(m_{\text{total BLG}} - m_{\text{non-aggregate BLG}})/m_{\text{total BLG}} * 100\%$. $m_{\text{total BLG}}$ is the concentration or amount of BLG determined according to Example 1.31 and $m_{\text{non-aggregated BLG}}$ is the concentration or amount of non-aggregated BLG determined according to Example 1.6.

35

In the context of the present invention, the term "crystal" pertains to a solid material whose constituents (such as atoms, molecules or ions) are arranged in a highly ordered microscopic structure, forming a crystal lattice that extends in all directions.

- 5 In the context of the present invention, the term "BLG crystal" pertains to protein crystals that primarily contain non-aggregated and preferably native BLG arranged in a highly ordered microscopic structure, forming a crystal lattice that extends in all directions. The BLG crystals may e.g. be monolithic or polycrystalline and may e.g. be intact crystals, fragments of crystals, or a combination thereof. Fragments of crystal are e.g. formed when intact crystals are subjected to
- 10 mechanical shear during processing. Fragments of crystals also have the highly ordered microscopic structure of crystal but may lack the even surface and/or even edges or corners of an intact crystal. See e.g. Figure 1 for an example of many intact BLG crystals and Figure 2 for an example of fragments of BLG crystals. In both cases, the BLG crystal or crystal fragments can be identified visually as well-defined, compact and coherent structures using light microscopy.
- 15 BLG crystal or crystal fragments are often at least partially transparent. Protein crystals are furthermore known to be birefringent and this optical property can be used to identify unknown particles having a crystal structure. Non-crystalline BLG aggregates, on the other hand, often appear as poorly defined, non-transparent, and as open or porous lumps of irregular size.
- 20 In the context of the present invention, the term "crystallise" pertains to the formation of protein crystals. Crystallisation may e.g. happen spontaneously or be initiated by the addition of crystallisation seeds.

In the context of the present invention, the term "edible composition" pertains to a composition

25 that is safe for human consumption and use as a food ingredient and that does not contain problematic amounts of toxic components, such as toluene or other unwanted organic solvents.

In the context of the present invention, the term "ALA" or "alpha-lactalbumin" pertains to alpha-lactalbumin from mammal species, e.g. in native and/or glycosylated forms and includes

30 the naturally occurring genetic variants. The term furthermore includes aggregated ALA and precipitated BLG. When referring to the amount of ALA reference is made to the total amount of ALA including e.g. aggregated ALA. The total amount of ALA is determined according to Example 1.31. The term "aggregated ALA" pertains to ALA which typically is at least partially unfolded and which furthermore has aggregated with other denatured ALA molecules and/or other

35 denatured whey proteins, typically by means of hydrophobic interactions and/or covalent bonds.

Alpha-lactalbumin (ALA) is a protein present in the milk of almost all mammalian species. ALA forms the regulatory subunit of the lactose synthase (LS) heterodimer and β -1,4-

galactosyltransferase (beta4Gal-T1) forms the catalytic component. Together, these proteins enable LS to produce lactose by transferring galactose moieties to glucose. One of the main structural differences with beta-lactoglobulin is that ALA does not have any free thiol group that can serve as the starting-point for a covalent aggregation reaction.

5

In the context of the present invention, the term "non-aggregated ALA" also pertains to ALA from mammal species, e.g. in native, unfolded and/or glycosylated forms and includes the naturally occurring genetic variants. However, the term does not include aggregated ALA or precipitated ALA. The amount or concentration of non-aggregated BLG is determined according to Example 1.6.

10

The percentage of non-aggregated ALA relative to total ALA is determined by calculate $(m_{\text{total ALA}} - m_{\text{non-aggregate ALA}})/m_{\text{total ALA}} * 100\%$. $m_{\text{total ALA}}$ is the concentration or amount of ALA determined according to Example 1.31 and $m_{\text{non-aggregated ALA}}$ is the concentration or amount of non-aggregated ALA determined according to Example 1.6.

15

In the context of the present invention, the term "caseinomacropeptide" or "CMP" pertains to the hydrophilic peptide, residue 106–169, originated from the hydrolysis of "κ-CN" or "kappa-casein" from mammal species, e.g. in native and/or glycosylated forms and includes the naturally occurring genetic variants, by an aspartic proteinase, e.g. chymosin.

20

In the context of the present invention, the term "BLG isolate" means a composition that contains BLG in an amount of at least 85% w/w relative to total protein. A BLG isolate preferably has a total protein content of a least 30% w/w, and preferably at least 80% w/w relative to total solids.

25

In the context of the present invention, the term "BLG isolate powder" pertains to a BLG isolate in powder form and preferably a free-flowing powder.

30

In the context of the present invention, the term "BLG isolate liquid" pertains to a BLG isolate in liquid form and preferably an aqueous liquid.

The term "whey" pertains to the liquid phase that is left after the casein of milk has been precipitated and removed. Casein precipitation may e.g. be accomplished by acidification of milk and/or by use of rennet enzyme. Several types of whey exist, such as "sweet whey", which is the whey product produced by rennet-based precipitation of casein, and "acid whey" or "sour whey", which is the whey product produced by acid-based precipitation of casein. Acid-based precipitation of casein may e.g. be accomplished by addition of food acids or by means of bacterial cultures.

35

The term "milk serum" pertains to the liquid which remains when casein and milk fat globules have been removed from milk, e.g. by microfiltration or large pore ultrafiltration. Milk serum may also be referred to as "ideal whey".

5

The term "milk serum protein" or "serum protein" pertains to the protein which is present in the milk serum.

10 In the context of the present invention, the term "whey protein" pertains to protein that is found in whey or in milk serum. Whey protein may be a subset of the protein species found in whey or milk serum, and even a single whey protein species or it may be the complete set of protein species found in whey or/and in milk serum.

15 In the context of the present invention, the main non-BLG proteins of a standard whey protein concentrate from sweet whey are ALA, CMP, bovine serum albumin, immunoglobulin, osteopontin, lactoferrin, and lactoperoxidase. In the context of the present invention, the weight percentages of the main non-BLG whey proteins of a standard whey protein concentrate from sweet whey are:

ALA in an amount of 18% w/w relative to total protein,
20 CMP in an amount of 18% w/w relative to total protein,
BSA in an amount of 4% w/w relative to total protein,
Casein species in an amount of 5% w/w relative to total protein,
Immunoglobulin in an amount of 6% w/w relative to total protein,
Osteopontin in an amount of 0.5% w/w relative to total protein,
25 Lactoferrin in an amount of 0.1% w/w relative to total protein, and
Lactoperoxidase in an amount of 0.1% w/w relative to total protein.

The term casein pertains to casein protein found in milk and encompasses both native micellar casein as found in raw milk, the individual casein species, and caseinates.

30

In the context of the present invention, a liquid which is "supersaturated" or "supersaturated with respect to BLG" contains a concentration of dissolved, non-aggregated BLG which is above the saturation point of non-aggregated BLG in that liquid at the given physical and chemical conditions. The term "supersaturated" is well-known in the field of crystallisation (see e.g. Gérard Coquerela, "Crystallization of molecular systems from solution: phase diagrams, supersaturation and other basic concepts", Chemical Society Reviews, p. 2286-2300, Issue 7, 2014) and
35 supersaturation can be determined by a number of different measurement techniques (e.g. by spectroscopy or particle size analysis). In the context of the present invention, supersaturation with respect to BLG is determined by the following procedure.

Procedure for testing whether a liquid at a specific set of conditions is supersaturated with respect to BLG:

- a) Transfer a 50 ml sample of the liquid to be tested to a centrifuge tube (VWR Catalogue no. 525-0402) having a height of 115 mm, an inside diameter of 25 mm and a capacity of 50 mL. Care should be taken to keep the sample and subsequent fractions thereof at the original physical and chemical conditions of the liquid during steps a) – h).
- b) The sample is immediately centrifuged at 3000 g for 3.0 minutes with max. 30 seconds acceleration and max 30 seconds deceleration.
- c) Immediately after the centrifugation, transfer as much as possible of the supernatant (without disturbing the pellet if a pellet has formed) to a second centrifuge tube (same type as in step a)
- d) Take a 0.05 mL subsample of the supernatant (subsample A)
- e) Add 10 mg of BLG crystals (at least 98% pure, non-aggregated BLG relative to total solids) having a particle size of at most 200 micron to a second centrifuge tube and agitate the mixture.
- f) Allow the second centrifuge tube to stand for 60 minutes at the original temperature.
- g) Immediately after step f), centrifuge the second centrifuge tube at 500 g for 10 minutes and then take another 0.05 mL subsample of the supernatant (subsample B).
- h) Recover the centrifugation pellet of step g) if there is one, resuspend it in milliQ water and immediately inspect the suspension for presence of crystals that are visible by microscopy.
- i) Determine the concentration of non-aggregated BLG in subsamples A and B using the method outlined in Example 1.6 – the results are expressed as % BLG w/w relative to the total weight of the subsamples. The concentration of non-aggregated BLG of subsample A is referred to as $C_{\text{BLG, A}}$ and the concentration of non-aggregated BLG of subsample B is referred to as $C_{\text{BLG, B}}$.
- j) The liquid from which the sample of step a) was taken was supersaturated (at the specific conditions) if $C_{\text{BLG, B}}$ is lower than $C_{\text{BLG, A}}$ and if crystals are observed in step i).

In the context of the present invention, the terms "liquid" and "solution" encompass both compositions that are free of particulate matter and compositions that contain a combination of liquid and solid and/or semi-solid particles, such as e.g. protein crystals or other protein particles. A "liquid" or a "solution" may therefore be a suspension or even a slurry. However, a "liquid" and "solution" are preferably pumpable.

In the context of the present invention, the terms "whey protein concentrate" (WPC) and "serum protein concentrate" (SPC) pertain to dry or aqueous compositions which contain a total amount of protein of 20-89% w/w relative to total solids.

A WPC or an SPC preferably contains:

20-89% w/w protein relative to total solids,
15-70% w/w BLG relative to total protein,
8-50% w/w ALA relative to total protein, and
0-40% w/w CMP relative to protein.

5

Alternatively, but also preferred, a WPC or an SPC may contain:

20-89% w/w protein relative to total solids,
15-90% w/w BLG relative to total protein,
4-50% w/w ALA relative to total protein, and
0-40% w/w CMP relative to protein.

10

Preferably, a WPC or an SPC contains:

20-89% w/w protein relative to total solids,
15-80% w/w BLG relative to total protein,
4-50% w/w ALA relative to total protein, and
0-40% w/w CMP relative to protein.

15

More preferably a WPC or an SPC contains:

70-89% w/w protein relative to total solids,
30-90% w/w BLG relative to total protein,
4-35% w/w ALA relative to total protein, and
0-25% w/w CMP relative to protein.

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SPC typically contain no CMP or only traces of CMP.

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The terms "whey protein isolate" (WPI) and "serum protein isolate" (SPI) pertain to dry or aqueous compositions which contain a total amount of protein of 90-100% w/w relative to total solids.

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A WPI or an SPI preferably contains:

90-100% w/w protein relative to total solids,
15-70% w/w BLG relative to total protein,
8-50% w/w ALA relative to total protein, and
0-40% w/w CMP relative to total protein.

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Alternatively, but also preferred, a WPI or an SPI may contain:

90-100% w/w protein relative to total solids,
30-95% w/w BLG relative to total protein,
4-35% w/w ALA relative to total protein, and

0-25% w/w CMP relative to total protein.

More preferably a WPI or an SPI may contain:

90-100% w/w protein relative to total solids,

5 30-90% w/w BLG relative to total protein,

4-35% w/w ALA relative to total protein, and

0-25% w/w CMP relative to total protein.

SPI typically contain no CMP or only traces of CMP.

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In the context of the present invention, the term "additional protein" means a protein that is not BLG. The additional protein that is present in the whey protein solution typically comprises one or more of the non-BLG proteins that are found in milk serum or whey. Non-limiting examples of such proteins are alpha-lactalbumin, bovine serum albumin, immunoglobulines, caseino-

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The terms "consists essentially of" and "consisting essentially of" mean that the claim or feature in question encompasses the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

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In the context of the present invention, the phrase "Y and/or X" means "Y" or "X" or "Y and X". Along the same line of logic, the phrase " $n_1, n_2, \dots, n_{i-1}$, and/or n_i " means " n_1 " or " n_2 " or ... or " n_{i-1} " or " n_i " or any combination of the components : $n_1, n_2, \dots, n_{i-1}$, and n_i .

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In the context of the present invention, the term "dry" or "dried" means that the composition or product in question comprises at most 10% w/w water, preferably at most 6% w/w and more preferably even less.

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In the context of the present invention, the term "physical microbial reduction" pertains to physical interaction with a composition which results in reduction of the total amount of viable microorganisms of the composition. The term does not encompass addition of chemicals that result in killing of microorganisms. The term furthermore does not encompass the heat exposure to which the atomized droplets of liquid are exposed to during spray-drying but include possible pre-heating prior to spray-drying.

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In the context of the present invention, the pH of a powder refers to the pH of 10 g of the powder mixed into 90 g demineralised water and is measured according to Example 1.16.

In the context of the present invention, the weight percentage (% w/w) of a component of a certain composition, product, or material means the weight percentage of that component relative to the weight of the specific composition, product, or material unless another reference (e.g. total solids or total protein) is specifically mentioned.

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In the context of the present invention, the process step "concentration" and the verb "concentrate" pertain to concentration of protein and encompass both concentration of protein on total solids basis and concentration of protein on a total weight basis. This means e.g. that concentration does not necessarily require that the absolute concentration w/w of protein of a composition increases as long as the content of protein increases relative to total solids.

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In the context of the present invention, the term "weight ratio" between component X and component Y means the value obtained by the calculation m_x/m_y wherein m_x is the amount (weight) of components X and m_y is the amount (weight) of components Y.

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In the context of the present invention, the term "at least pasteurisation" pertains to a heat-treatment which has microbial killing effect equal to or higher than a heat-treatment of 70 degrees C for 10 seconds. The reference for determining the bacteria killing effect is E. coli O157:H7.

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In the context of the present invention, the term "whey protein feed" pertains to whey protein source from which the liquid BLG isolate is derived. The whey protein feed has a lower content of BLG relative to total protein than the liquid BLG isolate and is typically a WPC, a WPI, an SPC or an SPI.

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In the context of the present invention, the term "BLG-enriched composition" pertains to the BLG-enriched composition resulting from isolating BLG from the whey protein feed. The BLG-enriched composition typically comprises the same whey proteins as the whey protein feed but BLG is present in significantly higher concentration relative to total protein than in whey protein feed. The BLG-enriched composition may e.g. be prepared from the whey protein feed by chromatography, protein crystallisation and/or membrane-based protein fractionation. The BLG-enriched composition comprises BLG in an amount of at least 85% w/w relative to total protein, and preferably at least 90% w/w. In some cases the BLG-enriched composition can be used directly as the liquid BLG isolate. However, often additional processing is required to convert the BLG-enriched composition to the liquid BLG isolate.

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In the context of the present invention, the term "whey protein solution" is used to describe the special aqueous whey protein composition that is supersaturated with respect to BLG in salting-in mode and useful for preparing BLG crystals.

In the context of the present invention, the term "sterile" means that the sterile composition or product in question does not contain any viable microorganisms and therefore is devoid of microbial growth during storage at room temperature. A composition that has been sterilized is sterile.

When a liquid, such as a beverage preparation, is sterilized and packaged aseptically in a sterile container it typically has a shelf life of at least six months at room temperature. The sterilization treatment kills spores and microorganisms that could cause spoilage of the liquid.

In the context of the present invention the term "energy content" means the total content of energy contained in a food product. The energy content can be measured in kilojoule (kJ) or kilo calories (kcal) and are referred to as calories per amount of food product, e.g. kcal per 100 grams of the food product. One example is an instant beverage powder having an energy content of 350 kcal/100 grams of the instant beverage powder.

The total energy content of a food product includes the energy contribution from all the macronutrients present in the food product, e.g. energy from protein, lipid and carbohydrate. The distribution of energy from the macronutrients in the food product can be calculated based on the amount of the macronutrients in the food product and the contribution of the macronutrient to the total energy content of the food product. The energy distribution can be stated as energy percent (E%) of the total energy content of the food product. For example for an instant beverage powder comprising 20 E% protein, 50 E% carbohydrate and 30 E% lipid, this means that 20% of the total energy comes from protein, 50% of the total energy comes from carbohydrate and 30% of the total energy comes from fat (lipid).

In the context of the present invention the term "nutritional supplement" pertains to a food product comprising one or more macro nutrients such as protein, lipid and/or carbohydrate and optionally comprising vitamins and minerals. Nutritional supplements can be either complete or incomplete.

By the term "nutritionally complete nutritional supplement" is understood food products comprising protein, lipid and carbohydrate and further comprising vitamins, minerals and trace elements, where the food product has a nutrient profile matching a complete and healthy diet.

The term "nutritionally incomplete supplement" means food products comprising one or more macro nutrients and optionally further comprising vitamins, minerals and trace elements. A incomplete nutritionally supplement may comprise protein as the only nutrients or may comprise protein, lipid and a carbohydrate.

The term "food for special medical purposes (FSMP)" or "medical food" are food products for oral ingestion or tube feeding, which are used for specific medical disorders, diseases or conditions for which there are distinctive nutritional requirements and which are used under medical supervision. A medical food can be a nutritionally complete supplement or a nutritionally incomplete supplement.

The term "nutrient" means a substance used by an organism to survive, grow and reproduce. Nutrients can be either macronutrients or micronutrients. Macronutrients are nutrients that provide energy when consumed e.g. protein, lipid and carbohydrate. Micronutrients are nutrients are vitamins, minerals and trace elements.

By the term "instant beverage powder" or "instant beverage powder product" is meant a powder which can be converted to a liquid beverage by addition of a liquid, such as water.

In the context of the present invention the terms "beverage preparation" and "preparation" used as a substantive relate to any water-based liquid which can be ingested as a drink, e.g. by pouring, sipping or tube-feeding.

In the context of the present invention the term "protein fraction" relates to proteins of the composition in question e.g. the proteins of a powder or a beverage preparation.

In the context of the present invention the term "astringency" relates to a mouthfeeling. Astringency feels like a contraction of cheek muscles and results in increased saliva production. Thus, astringency is not a taste as such, but a physical mouthfeeling and time-dependent feeling in the mouth.

In the context of the present invention the term "drying mouthfeeling" relates to a feeling in the mouth, it feels like a drying of the mouth and teeth and results in minimization of the saliva production. Thus drying mouthfeeling is not a taste as such, but a physical mouthfeeling and time-dependent feeling in the mouth.

In the context of the present invention the term "minerals" as used herein, unless otherwise specified, refers to any one of major minerals, trace or minor minerals, other minerals, and combinations thereof. Major minerals include calcium, phosphorus, potassium, sulfur, sodium, chlorine, magnesium. Trace or minor minerals include iron, cobalt, copper, zinc, molybdenum, iodine, selenium, manganese and other minerals include chromium, fluorine, boron, lithium, and strontium.

In the context of the present invention the terms "lipid", "fat", and "oil" as used herein unless otherwise specified, are used interchangeably to refer to lipid materials derived or processed from plants or animals. These terms also include synthetic lipid materials so long as such synthetic materials are suitable for human consumption.

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In the context of the present invention the term "transparent" encompasses a beverage preparation having a visibly clear appearance and which allows light to pass and through which distinct images appear. A transparent beverage has a turbidity of at most 200 NTU.

10 In the context of the present invention the terms "opaque" encompasses a beverage preparation having a visibly unclear appearance and it has a turbidity of more than 200 NTU.

In the context of the present invention the term "mother liquor" pertains to the whey protein solution that remains after BLG has been crystallised and the BLG crystals have been at least partially removed. The mother liquor may still contain some BLG crystals but normally only small BLG crystals that have escaped the separation.

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By the term "instant beverage powder" or "instant beverage powder product" is meant a powder which can be converted to a liquid beverage by addition of a liquid, such as water.

20 **DETAILED DESCRIPTION**

The overall conception of the nutritional supplement is noticed by the consumer. The nutritional supplement should be appetizing in taste and appearance; otherwise it will be rejected by the consumer. Further, the consumer values natural products without additives. A further parameter of importance to the consumer is shelf-life of the product.

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An aspect of the invention pertains to an instant beverage powder comprising at least 1% w/w BLG, preferably at least 5%, wherein:

- i. the crystallinity of BLG is at least 20%, preferably at least 40%, and/or
- ii. at least 85% w/w of the total amount of protein is comprised by BLG,

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and furthermore comprising at least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, minerals, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics, anti-foaming agents and non-whey protein.

35 The BLG source used in the instant beverage powder can be the BLG isolate or BLG isolate powder as described in the present patent application. In some preferred embodiments of the invention the BLG source contributes with at least 90% w/w of the total protein of the instant

beverage powder, more preferably at least 95% w/w, even more preferred at least 98% w/w and most preferred all the protein of the instant beverage powder.

5 In some preferred embodiments of the invention the BLG source is the BLG isolate powder and it is the only source of protein in the instant beverage powder.

In some preferred embodiments of the invention the instant beverage powder is prepared by dry-blending the BLG isolate powder and the other ingredients.

10 In other preferred embodiments of the invention the instant beverage powder is prepared by using at least one ingredient in dissolved form and subsequently performing a drying steps. The drying step may e.g. form part of a wet-granulation process or a spray-drying step.

15 The BLG of the instant beverage powder of the present invention preferably has a low degree of denaturation, such as at most 10%, preferably at most 4%, more preferably at most 1%, even more preferably at most 0.4% and even more preferably at most 0.1%. Most preferably, the BLG is not denatured at all. For instant beverage powders it is advantageous that BLG has a low degree of denaturation, as this reduces the tendency to foam when mixed with a liquid.

20 The instant beverage powder of preferably has a degree of protein denaturation of at most 10%, preferably at most 4%, more preferably at most 1%, even more preferably at most 0.4% and even more preferably at most 0.1%. Most preferably, the protein is not denatured at all.

25 In one embodiment of the invention the instant beverage powder comprises from 1-90 % w/w BLG. In a preferred embodiment of the invention, the instant beverage powder comprises from 30-90% w/w BLG, more preferably in the range of 40-90% w/w BLG or even more preferably in the range of 50-90% w/w BLG.

30 In other preferred embodiments of the invention the instant beverage powder comprises from 10-97 % w/w BLG. In a preferred embodiment of the invention, the instant beverage powder comprises from 30-96% w/w BLG, more preferably in the range of 40-95% w/w BLG or even more preferably in the range of 50-94% w/w BLG.

35 In one embodiment of the invention, the instant beverage powder comprises from 1-50 % w/w BLG. In a preferred embodiment of the invention, the instant beverage powder comprises from 2-45% w/w BLG, more preferably in the range of 3-40% w/w BLG or even more preferably in the range of 3-35% w/w BLG.

In a preferred embodiment of the invention, the instant beverage powder comprises at least 85% w/w of the total amount of protein is BLG.

In one embodiment of the invention, the instant beverage powder comprises at least 85% w/w BLG relative to total protein such as at least 86% w/w BLG relative to total protein, at least 87% w/w BLG relative to total protein, at least 88% w/w BLG relative to total protein, at least 89% w/w BLG relative to total protein.

In one embodiment of the invention, the instant beverage powder comprises at least 91% w/w BLG relative to total protein such as at least 92% w/w BLG relative to total protein, at least 93% w/w BLG relative to total protein, at least 94% w/w BLG relative to total protein, at least 95% w/w BLG relative to total protein, at least 96% w/w BLG relative to total protein, at least 97% w/w BLG relative to total protein, at least 98% w/w BLG relative to total protein or at least 99% w/w BLG relative to total protein.

In some preferred embodiments of the instant beverage powder of the invention, at least 85% w/w of the protein is BLG. Preferably, at least 88% w/w of the protein is BLG, more preferably at least 90% w/w, even more preferably at least 91% w/w, and most preferably at least 92% w/w of the protein is BLG.

Even higher relative amounts of BLG are both feasible and desirable thus in some preferred embodiments of the invention at least 94% w/w of the protein of the instant beverage powder is BLG, more preferably at least 96% w/w of the protein is BLG, even more preferably at least 98% w/w of the protein is BLG, and most preferably approx. 100% w/w of the protein is BLG.

For example, the instant beverage powder preferably comprises BLG in an amount of at least 97.5% w/w relative to total protein, preferably at least 98.0% w/w, more preferably at least 98.5% w/w, even more preferably at least 99.0%, and most preferably BLG in an amount of at least 99.5% w/w relative to total protein, such as approx. 100.0% w/w relative to total protein.

The protein of the instant beverage powder is preferably prepared from mammal milk, and preferably from ruminant milk such as e.g. milk from cow, sheep, goat, buffalo, camel, llama, horse and/or deer. Protein derived from bovine milk is particularly preferred. The protein of the instant beverage powder is therefore preferably bovine milk protein.

The protein of the instant beverage powder is preferably whey protein or milk serum protein and even more preferably bovine whey protein or milk serum protein.

The intrinsic tryptophan fluorescence emission ratio (I330nm/I350nm) is a measure of the degree of unfolding of BLG, and the inventors have found that at high BLG tryptophan fluores-

cence emission ratios, which correlate with low or no unfolding of BLG, the intrinsic tryptophan fluorescence emission ratio (I330nm/I350nm) is measured according to Example 1.1.

In some preferred embodiments of the invention, the instant beverage powder has an intrinsic tryptophan fluorescence emission ratio (I330nm/I350nm) of at least 1.11.

In some preferred embodiments of the invention, the instant beverage powder has an intrinsic tryptophan fluorescence emission ratio (I330nm/I350nm) of at least 1.12, preferably at least 1.13, more preferably at least 1.15, even more preferably at least 1.17, and most preferably at least 1.19.

If instant beverage powder contains considerable amounts of non-protein matter, it is preferred to isolate the protein fraction before measuring the intrinsic tryptophan fluorescence emission ratio. Thus in some preferred embodiments of the invention, the protein fraction of instant beverage powder has an intrinsic tryptophan fluorescence emission ratio of at least 1.11.

In some preferred embodiments of the invention, the protein fraction of the instant beverage powder has an intrinsic tryptophan fluorescence emission ratio (I330nm/I350nm) of at least 1.12, preferably at least 1.13, more preferably at least 1.15, even more preferably at least 1.17, and most preferably at least 1.19.

The protein fraction can e.g. be separated from the instant beverage powder by dissolving the instant beverage powder in demineralised water and subjecting the solution to dialysis or ultra-filtration-based diafiltration using a filter that retains the protein.

In some preferred embodiments of the invention the crystallinity of BLG of the instant beverage powder is at least 20%, preferably at least 40%, more preferably at least 60%, even more preferably at least 80%, and most preferably at least 90%. Having a crystallinity of BLG of at least 20% means that a significant amount of the BLG is present in the form of dried BLG crystals in the instant beverage powder.

The present inventors have found that a crystallinity of BLG of at least 20% is advantageous as it means that the protein is present in a form that has a higher density than traditional WPI.

This provides a higher overall bulk density to the instant beverage powder and makes it less dusty and easier to handle for the end user. The inventors have also observed a reduced tendency to particle segregation in dry-blended instant beverage powders that e.g. contain a carbohydrate powder and/or food acid powder in addition to a protein powder.

The present invention makes it possible to provide low carbohydrate instant beverage powder which have both sweetness and a high protein content.

Thus, in some preferred embodiments of the invention the instant beverage powder comprises:

- 5 - an energy content in the range of 320-380 kcal/100 grams of powder, and preferably in the range of 350-370 kcal/100 grams,
- a contribution of the energy from protein in the range of 90-100 E%, and preferably in the range of 95-100 E%,
- 10 - BLG in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w relative to total protein, and more preferably at least 94% w/w relative to total protein
- a contribution of the energy from carbohydrate in the range of 0-10 E%, and preferably in the range of 0-5 E%,
- a total amount of high intensity sweeteners in the range of 0.01-4% w/w, preferably in the range of 0.05-3%
- 15 - having a bulk density of at least 0.45 g/mL, preferably at least 0.50 g/mL, and more preferred at least 0.6 g/mL.

The protein of the instant beverage powder is preferably provided by a BLG isolate powder that: has a pH in the range of i) 2-4.9, ii) 6.1-8.5, or iii) 5.0-6.0 and comprises:

- 20 - total protein in an amount of at least 90% w/w, preferably at least 95% w/w,
- BLG in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w relative to total protein, and more preferably at least 94% w/w relative to total protein,
- said BLG isolate powder having:
- a bulk density of at least 0.45 g/mL, preferably at least 0.50 g/mL, and more preferred at
- 25 least 0.6 g/mL, and
- one or more of the following:
 - an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11, preferably at least 1.13, and more preferably at least 1.15
 - a degree of protein denaturation of at most 10%, preferably at least 5%
 - 30 - a heat-stability at pH 3.9 of at most 200 NTU, and
 - at most 1000 colony-forming units/g.

In one embodiment of the invention the instant beverage powder further comprises at least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, minerals, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics and non-whey protein.

The further ingredient ensures that the instant beverage powder contains the desired nutrients, i.e. nutrients specifically adapted to a patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or it can be used as a nutritional supplement, e.g. by sportsmen or athletes.

In a preferred embodiment of the invention, the instant beverage powder may include a vitamin selected from the group consisting of vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B7 (biotin),
5 vitamin B9 (folic acid), and vitamin B12 (cobalamine), vitamin C, vitamin D, vitamin E, vitamin K, choline, inositol, their salts, their derivatives and combinations thereof.

In an embodiment, the instant beverage powder may comprise a flavouring agent selected from the group consisting of salt, flavorings, flavor enhancers and/or spices. In a preferred embodi-
10 ment of the invention the flavor comprise chocolate, cocoa, lemon, orange, lime, strawberry, banana, forrest fruit flavor or combinations thereof.

In an embodiment, the instant beverage powder may include a mineral selected from the group consisting of boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molyb-
15 denum, nickel, phosphorus, potassium, sodium, selenium, silicon, tin, vanadium, zinc, or combinations thereof.

The instant beverage powder may furthermore contain salts and minerals which typically are present in whey or milk derived products. The mineral content of instant beverage powder are
20 typically represented as the ash content of the food ingredient or product. In a preferred embodiment, the instant beverage powder may comprise an antioxidant selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium, or combinations thereof.

In an embodiment of the invention, the instant beverage powder may comprise one or more
25 sweeteners, such as carbohydrate sweeteners, polyols and/or high intensity sweeteners. The instant beverage powder may e.g. comprise a total amount of carbohydrate sweetener in the range of 0.001-20% w/w relative to the total weight of the instant beverage powder. Alternatively, the instant beverage powder may comprise a total amount of carbohydrate sweetener in the range of 0.1-15% w/w relative to the total weight of the food product.

30 In one embodiment of the invention, the instant beverage powder comprises at least one high intensity sweetener. In one embodiment, the at least one high intensity sweetener is selected from the group consisting of aspartame, cyclamate, sucralose, acesulfame salt, neotame, saccharin, stevia extract, a steviol glycoside such as e.g. rebaudioside A, or a combination thereof.
35 In some embodiments of the invention, it is particularly preferred that the sweetener comprises or even consists of one or more high intensity sweeteners (HIS).

High intensity sweeteners are both found among both natural and artificial sweeteners and typically have a sweetening intensity of at least 10 times that of sucrose.

If used, the total amount of high intensity sweeteners is typically in the range of 0.01-4% w/w. For example, the total amount of high intensity sweeteners may be in the range of 0.05-3% w/w. Alternatively, the total amount of high intensity sweeteners may be in the range of 0.1-2.0% w/w.

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The choice of the sweetener may depend on the beverage to be produced, and the consumer of the product, e.g. it may be adjusted to a specific diagnosis of a patient. High-intensity sugar sweeteners (e.g. aspartame, acetsulfam-K or sucralose) may be used in beverage where no energy contribution from the sweetener is desired, whereas for beverages having a natural profile natural sweeteners (e.g. steviol glycosides, sorbitol or sucrose) may be used.

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It may furthermore be preferred that the sweetener comprises or even consists of one or more polyol sweetener(s). Non-limiting examples of useful polyol sweetener are maltitol, mannitol, lactitol, sorbitol, inositol, xylitol, threitol, galactitol or combinations thereof. If used, the total amount of polyol sweetener is typically in the range of 1-40% w/w. For example, the total amount of polyol sweetener may be in the range of 2-30% w/w. Alternatively, the total amount of polyol sweetener may be in the range of 4-20% w/w.

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In one embodiment of the invention the instant beverage powder may comprise one or more of:

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- i. a sweetener, e.g. a sugar sweetener and/or a non-sugar sweetener,
- ii. a flavoring agent,
- iii. at least one food acid, e.g. citric acid or other suitable food acids,
- iv. the sum of the amounts of Na, K, Mg, and Ca of the instant beverage is at most

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10 mmol/g protein and

wherein a 10% w/w solution of the powder in demineralized water has a pH in the range of 2-8.

The pH of the instant beverage powder can be measured by dissolving 10 gram of the instant beverage powder in 90 ml of demineralized water at room temperature, as described in example 1.16.

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The inventors have found that it is advantageous to use a low phosphorus/low potassium BLG isolate powder in the instant beverage powder, e.g. for instant beverage powders that are particularly useful to patients with kidney diseases.

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By adding sweetener, flavoring agents and/or food acids, the taste of the product can be designed so that the instant beverage powder is appealing to the consumer. In one embodiment of the invention the consumer can be a patient for which the flavor, sweetener and acidic profile of the instant beverage powder is adjusted to fit to the patients need and diagnosis.

In a preferred embodiment of the invention the instant beverage powder may comprise a high intensity sweetener and a flavoring agent. In an even more preferred embodiment of the invention, the instant beverage powder comprises 0.001-0.05 % w/w sucralose and 0.01-0.2% w/w and a flavor selected from chocolate, cocoa, lemon, orange, lime, strawberry, banana, forrest fruit flavor or combinations thereof.

In one embodiment of the invention, the instant beverage powder comprises an anti-foaming agent. The anti-foaming agent may be selected from anti-foaming agents suitable for food products. The anti-foaming agent may be selected from oil-based anti-foaming agents, water-based anti-foaming agents, silicone-based anti-foaming agents, EP/PO-based anti-foaming agents or a combination thereof.

The instant beverage powder has a water content of at most 6% w/w. In one embodiment of the invention, the instant beverage powder comprises at most 5% w/w water, preferably at most 4% w/w water, more preferably at most 3% w/w water, and even more preferably at most 2% w/w water.

The storage stability of the instant beverage powder may increase when lowering the water content of the powder.

The present inventors have found that it can be advantageous to control the mineral content in order to reach some of the desired properties of the instant beverage powder.

In some preferred embodiments of the invention, the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein. Preferably, the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 6 mmol/g protein, more preferably at most 4 mmol/g protein, even more preferably at most 2 mmol/g protein.

In other preferred embodiments of the invention the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 1 mmol/g protein. Preferably, the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 0.6 mmol/g protein, more preferably at most 0.4 mmol/g protein, even more preferably at most 0.2 mmol/g protein, and most preferably at most 0.1 mmol/g protein.

In one embodiment of the invention, the instant beverage powder comprises dry BLG crystals, e.g. obtainable by one or more methods described in PCT/EP2017/084553. The instant beverage powder containing BLG crystals may have a bulk density of at least 0.30 g/mL, preferably at least 0.4 g/mL.

The inventors have observed that instant beverage powder in which at least some of BLG is on crystal form has a higher density than comparable instant BLG compositions without BLG crystals. Thus, in some preferred embodiments of the invention the instant beverage powder has a bulk density of at least 0.30 g/mL, preferably at least 0.40 g/mL. Preferably the instant beverage powder has a bulk density of at least 0.45 g/mL. More preferably the instant beverage powder has a bulk density of at least 0.50 g/mL. It is even more preferred that the instant beverage powder has a bulk density of at least 0.6 g/mL. The instant beverage powder may e.g. have a bulk density of at least 0.7 g/mL.

The instant beverage powder of the present invention preferably has a bulk density in the range of 0.3-1.0 g/mL, preferably in the range of 0.40-0.9 g/mL, more preferably in the range of 0.45-0.8 g/mL, even more preferably in the range of 0.45-0.75 g/mL, even more preferably in the range of 0.50-0.75 g/mL, and most preferably in the range of 0.6-0.75 g/mL.

The bulk density of a powder can be measured according to Example 1.17.

The total protein content and the energy content in the instant beverage powder of the invention depend on the intended use of the instant beverage powder. The energy content of an instant beverage powder is in the range of 200-500 kcal/100 grams of powder.

For instant beverage powders, the contribution of the energy from protein may be at least 7 E%, preferably at least 25 E%, more preferably at least 30 E%, even more preferably at least 40 E%.

In a preferred embodiment of the invention the contribution of energy from protein is in the range of 10-30 E%, preferably in the range of 10-15 E% or even more preferably 11 E%. Alternatively, the contribution of energy from protein is in the range of 15-25 E%, preferably in the range of 18-22 E%.

In a preferred embodiment of the invention, the contribution of the energy from protein is in the range of 7-25 E%, preferably in the range of 10-25 E%, more preferably 15-20 E% or even more preferably the instant beverage powder contains 15 E% from protein or 20 E% from protein, or the contribution of the energy from protein is in the range of 8-15 E%.

In another preferred embodiment of the invention the contribution of the energy from protein is at least 50 E%, preferably at least 60 E% or at least 70 E% or even more preferred at least 80 E%. In a preferred embodiment of the invention, the contribution of the energy from protein is in the range of 80-100 E%, preferably in the range of 90-100 E% or even more preferably in the range of 95-100 E%.

In a preferred embodiment of the invention, the contribution of the energy from protein is in the range of 30-80 E%, preferably in the range of 60-80 E%. In another embodiment of the invention the contribution of the energy from protein is in the range of 30-40 E% or even more preferably the instant beverage powder contains 33 E% from protein.

The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or can be used as a nutritional supplement e.g. by sportsmen or athletes, before, during or after exercise.

The instant beverage powder of the present invention may comprise other macronutrients than protein. The instant beverage powder can comprise carbohydrates and/or lipids in addition to the protein. The total lipid content in the instant beverage powder of the invention depends on the intended use of the instant beverage powder. In one embodiment of the invention the contribution of energy from lipid is in the range of 0-60 E%

In a preferred embodiment of the invention the contribution of energy from lipid is in the range of 0-5 E%, preferably in the range of 0-3 E% or more preferred in the range of 0-2 E% from lipid.

Even less lipid may be preferred, thus in a preferred embodiment of the invention the contribution of energy from lipid is in the range of 0-1 E%, preferably in the range of 0-0.1 E% or more preferred in the range of 0-0.01 E% from lipid.

In a preferred embodiment of the invention the contribution of the energy from lipid is in the range of 30-60 E%, preferably in the range of 30-50 E% or even more preferably the instant beverage powder contains 35 E% from lipid, 45 E% from lipid or 50 E% from lipid. Alternatively the contribution of the energy from lipid is in the range of 25-45 E%.

In a preferred embodiment of the invention, the contribution of the energy from lipid is in the range of 15-20 E%, preferably in the range of 16-18 E% or even more preferably the instant beverage powder contains 16 E% from lipid.

The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or can be used as a nutritional supplement e.g. by sportsmen or athletes, before, during or after exercise.

In one embodiment of the invention, the instant beverage powder may comprise a carbohydrate in addition to protein. The energy contribution of the carbohydrate to the total energy of the instant beverage powder may be in the range of 0-90 E%.

5 The carbohydrate can be selected from sugars, oligosaccharides or polysaccharides. Examples of sugars are mono-, disaccharides and polyols. Examples of oligosaccharides are malto-oligosaccharides, such as maltodextrins or other oligosaccharides, such as raffinose, stachyose or fructo-oligosaccharides. Examples of polysaccharides are starches such as amylose, amylo-pectins, or modified starches and non-starch polysaccharides, such as dietary fibers, cellulose,
10 pectins and hydrocolloids. In a preferred embodiment of the invention, the carbohydrate is selected from maltodextrine, saccharose or glucose syrup.

The total carbohydrate content in the instant beverage powder of the invention depends on the intended use of the instant beverage powder. For instant beverage powders used for sportsmen
15 or athletes, carbohydrate in the form of sugars may be added in order to boost immediate energy for the sportsman or carbohydrate in the form of slow carbohydrates or dietary fiber may be added in order to prolong satiety.

In a preferred embodiment of the invention, the contribution of energy from carbohydrate is in
20 the range of 70-90 E%, preferably in the range of 75-85 E% or more preferably the instant beverage powder contains 89 E% from carbohydrate

In a preferred embodiment of the invention, the contribution of the energy from carbohydrate is in the range of 30-50 E%, preferably in the range of 35-45 E% or even more preferably the
25 instant beverage powder contains 35 E% from carbohydrate, 45 E% from carbohydrate or 50 E% from carbohydrate. Alternatively, the contribution of the energy from carbohydrate is in the range of 40-60 E%, such as in the range of 45-55 E%.

In another preferred embodiment of the invention, the contribution of the energy from carbohydrate is in the range of 0-20 E%. In a preferred embodiment of the invention the contribution
30 of the energy from carbohydrate is in the range of 0-10 E%, preferably in the range of 0-5 E%.

In yet a preferred embodiment of the invention, the contribution of the energy from carbohydrate is in the range of 0-4 E%, more preferably 0-1 E%, and even more preferably 0-0.2 E%.

35 In another preferred embodiment of the invention the contribution of the energy from carbohydrate is in the range of 3-20 E%. In a preferred embodiment of the invention, the contribution of the energy from carbohydrate is in the range of 4-15 E%. In another embodiment of the invention the contribution of the energy from carbohydrate is in the range of 45-55 E%.

The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or can be used as a nutritional supplement e.g. by sportsmen or athletes, before, during or after exercise.

The instant beverage powder of the present invention comprises protein and may in addition to the protein comprise lipid and/or carbohydrate depending on the intended use of the instant beverage powder.

In a preferred embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements. The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or it can be used as a nutritional supplement e.g. by sportsmen or athletes.

The energy content of an instant beverage powder can be in the range of 200-400 kcal/100 grams of powder. In a preferred embodiment of the invention, the energy content of the instant beverage powder is in the range of 300-400 kcal/100 grams of powder, even more preferred the energy content of the instant beverage powder is in the range of 320-380 kcal/100 grams of powder, or most preferred in the range of 350-370 kcal/100 grams of powder.

In a preferred embodiment of the invention, the instant beverage supplement has an energy distribution as follows 10-30 E% protein, 70-90 E% carbohydrates and 0-5 E% lipid. In a more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 10-15 E% protein, 75-85 E% carbohydrates and 0-1 E% lipid. In a preferred embodiment of the invention the instant beverage supplement has an energy distribution of 11 E% protein, 89 E% carbohydrates and 0 E% lipid.

In one embodiment of the invention the instant beverage powder comprises protein, carbohydrate and lipid and optionally comprising vitamins, minerals and trace elements. The instant beverage powder can be designed so that the recommended daily intake of the beverage powder supplies the recommended daily intake of the vitamins, minerals and trace elements. However this is not a requirement.

Such instant beverage powder may be useful as nutritional supplement where the consumer is interested in a nutritional supplement with a proportion of macronutrients reflecting a healthy diet with respect to energy distribution, macronutrients and micronutrients, e.g. where the nutritional supplement is given under supervision of a health care professional.

The energy content of an instant beverage powder is in the range of 400-500 kcal/100 grams of powder. In a preferred embodiment of the invention, the energy content of the instant beverage powder is in the range of 410-490 kcal/100 grams of powder, even more preferably the energy content of the instant beverage powder is in the range of 420-480 kcal/100 grams of powder or most preferred in the range of 440-460 kcal/100 grams of powder.

In a preferred embodiment of the invention, the instant beverage supplement has an energy distribution as follows; 7-25 E% protein, 30-50 E% carbohydrates and 30-55 E% lipid. In a more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 10-25 E% protein, 30-50 E% carbohydrates and 30-55 E% lipid, or even more preferably 15-20 E% protein, 35-45 E% carbohydrates and 35-50 E% lipid. In an even more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 15 E% protein, 35 E% carbohydrates and 50 E% lipid, has an energy distribution of 20 E% protein, 45 E% carbohydrates and 35 E% lipid or has an energy distribution of 8-15 E% protein, 40-47 E% carbohydrates and 45 E% lipid.

In a preferred embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements.

The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or can be used as a nutritional supplement e.g. by sportsmen or athletes, before, during or after exercise.

In one embodiment of the invention, the instant beverage powder comprises protein, carbohydrate and lipid. Such instant beverage powder may be useful as nutritional supplement where intake of protein is of highest priority of the consumer, e.g. where the consumer would like to supplement the regular meals. The energy content of an instant beverage powder is in the range of 200-500 kcal/100 grams of powder. In a preferred embodiment of the invention, the energy content of the instant beverage powder is in the range of 200-350 kcal/100 grams of powder, or even more preferably the energy content of the instant beverage powder is in the range of 200-300 kcal/100 grams of powder.

In a preferred embodiment of the invention, the instant beverage supplement has an energy distribution as follows 80-98 E% protein, 0-20 E% carbohydrates and 0-5 E% lipid. In a more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 90-98 E% protein, 0-10 E% carbohydrates and 0-3 E% lipid. In an even more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 95-98 E% protein, 0-5 E% carbohydrates and 0-2 E% lipid.

In an embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements. In a preferred embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements. The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or can be used as a nutritional supplement e.g. by sportsmen or athletes.

In one embodiment of the invention the instant beverage powder comprises protein, carbohydrate and lipid. The energy content of an instant beverage powder is in the range of 200-420 kcal/100 grams of powder. In a preferred embodiment of the invention, the energy content of the instant beverage powder is in the range of 300-420 kcal/100 grams of powder, or more preferred the energy content of the instant beverage powder is in the range of 320-380 kcal/100 grams of powder, or even more preferred the energy content of the instant beverage powder is in the range of 350-370 kcal/100 grams of powder.

In a preferred embodiment of the invention, the instant beverage supplement has an energy distribution as follows; 30-80 E% protein, 3-20 E% carbohydrates and 15-20 E% lipid. In a more preferred embodiment of the invention, the instant beverage supplement has an energy distribution of 60-80 E% protein, 4-15 E% carbohydrates and 16-18 E% lipid. In an even more preferred embodiment of the invention the instant beverage supplement has an energy distribution of 30-40 E% protein, 45-55 E% carbohydrates and 12-18 E% lipid, such as eg. 33 E% protein, 46 E% carbohydrates and 15 E% lipid.

Alternatively, the energy content of an instant beverage powder can be in the range of 150-250 kcal/100 grams of powder with an energy distribution as follows; 10-30 E% protein, 40-60 E% carbohydrates and 25-45 E% lipid, or preferably 15-25 E% protein, 45-55 E% carbohydrates and 30-40 E% lipid.

In an embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements.

In a preferred embodiment of the invention, the instant powder further comprises vitamins, minerals and trace elements. The instant beverage powder can be used as a nutritional supplement e.g. for treating patients with or at risk of malnutrition, for patients suffering from kidney disease, for weight gain or it can be used as a nutritional supplement e.g. by sportsmen or athletes.

In a preferred embodiment of the invention, the instant beverage powder may further comprise a flavouring agent, colouring agent, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics or non-whey protein.

5 For reasons of convenience the instant beverage powder may be sold in a kit comprising the instant powder of the invention, a tool for measuring said powder, and a container having a lid for opening and closing the container, wherein said container is for mixing said powder with a liquid to form a food product, and said container is adapted for drinking the food product directly from the container. Examples of useful containers are e.g. bottles, cartons, bricks, pouches
10 and/or bags.

The consumer buying the kit will obtain all items for readily preparing a liquid food product according to the invention. The measuring tool ensures that the consumer weights out the correct amount of instant powder for the amount of water in the container.

15 In one embodiment of the invention, the tool for measuring the instant powder is a spoon and the container is a drinking bottle. In one embodiment of the invention the container has an inside indication of how much liquid to fill in the container. In one embodiment of the invention the lid has an opening adapted for drinking the liquid food product directly from the container
20 and for closing while mixing the liquid food product.

The pH of the instant beverage powder is important because the taste of the product prepared from the instant beverage powder depend on the pH of the product. The pH of the powder can be determined by measuring the pH in a 10% w/w solution of the instant beverage powder in
25 demineralised water at 25°C, as described in example 1.16. In one embodiment of the invention, the pH of the instant beverage powder in a 10% w/w solution in demineralised water is in the range of 2-8 at 25°C.

30 In one embodiment of the invention, the pH is in the range of 2.0-4.9, such as in the range of in the range of 2.5-4.7, more preferably 2.8-4.3, even more preferably 3.2-4.0, and most preferably 3.4-3.9. Alternatively, but also preferred, the instant beverage powder may have a pH in the range of 3.6-4.3.

35 Alternatively, the pH of the instant beverage powder in a 10% w/w solution in demineralised water is a pH in the range of 5.0-6.0 at 25°C, preferably, the powder has a pH in the range of 5.1-5.9, more preferably 5.2-5.8, even more preferably 5.3-5.7, and most preferably 5.4-5.6.

Alternatively, the pH of the instant beverage powder is in the range of 6.1-8.5, more preferably 6.2-8.0, even more preferably 6.3-7.7, and most preferably 6.5-7.5.

Yet an aspect of the invention pertains to the use of the instant beverage as defined herein as a food ingredient.

- 5 An aspect of the invention relates to a packaged instant beverage powder product comprising a container containing the instant beverage powder product as described herein.

In an embodiment of the invention, the instant beverage powder product is hermetically sealed in the container, optionally packaged with an inert gas.

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A wide range of different containers may be used to store the instant beverage powder product. For example, the container may be a container selected from the group consisting of a bottle, a can, a bag, a pouch, and a sachet.

- 15 In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

- 20 In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2-8.

- 25 In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2.0-4.9.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 5.0-6.0.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at

least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 6.1-8.5.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 2-8 and the instant beverage powder further comprises vitamins, minerals and trace elements

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 2.0-4.9 and the instant beverage powder further comprises vitamins, minerals and trace elements.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 5.0-6.0 and the instant beverage powder further comprises vitamins, minerals and trace elements.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 6.1-8.5 and the instant beverage powder further comprises vitamins, minerals and trace elements.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2-8, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2.0-4.9, the instant beverage powder further

comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 5.0-6.0, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-500 kcal/100 grams of powder and the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 6.1-8.5, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2-8.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2.0-4.9.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder

comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 5.0-6.0.

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- 5 In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 6.1-8.5.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 2-8 and the instant beverage powder further comprises vitamins, minerals and trace elements

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, and the pH of the powder is in the range of 2.0-4.9 and the instant beverage powder further comprises vitamins, minerals and trace elements.

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25 In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 5.0-6.0 and the instant beverage powder further comprises vitamins, minerals and trace elements.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 6.1-8.5 and the instant beverage powder further comprises vitamins, minerals and trace elements.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2-8, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2.0-4.9, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 5.0-6.0, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 400-500 kcal/100 grams of powder and the energy distribution is in the range of 7-25 E% protein, 30-50 E% carbohydrate and 30-55 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 6.1-8.5, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-400 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 70-90 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-400 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 70-90 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2-8.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-400 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 70-90 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2-8, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-400 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 70-90 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2.0-4.9, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-400 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 70-90 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 6.1-8.5, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-350 kcal/100 grams of powder and the energy distribution is in the range of 80-98 E% protein, 0-20 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-350 kcal/100 grams of powder and the energy distribution is in the range of 80-98 E% protein, 0-20 E% carbohydrate and 0-5 E% lipid wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2-8.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-350 kcal/100 grams of powder and the energy distribution is in the range of 80-98 E% protein, 0-20 E% carbohydrate and 0-5 E% lipid, wherein the instant beverage powder comprises at least 95% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2.0-4.9.

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comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2.0-4.9, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

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relative to total protein, the pH of the powder is in the range of 5.0-6.0, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 200-420 kcal/100 grams of powder and the energy distribution is in the range of 30-80 E% protein, 3-20 E% carbohydrate and 15-20 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 150-250 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 40-60 E% carbohydrate and 25-45 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein.

In one embodiment of the invention, the instant beverage powder has an energy content in the range of 150-250 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 40-60 E% carbohydrate and 25-45 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein and the pH of the powder is in the range of 2-8.

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In one embodiment of the invention, the instant beverage powder has an energy content in the range of 150-250 kcal/100 grams of powder and the energy distribution is in the range of 10-30 E% protein, 40-60 E% carbohydrate and 25-45 E% lipid, wherein the instant beverage powder comprises at least 85% w/w BLG relative to total protein, preferably at least 90% w/w BLG relative to total protein, the pH of the powder is in the range of 2-8, the instant beverage powder further comprises vitamins, minerals and trace elements and wherein the sum of the amounts of Na, K, Mg, and Ca of the instant beverage powder is at most 10 mmol/g protein.

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In some preferred embodiments of the invention the food product is a dry food product, e.g. a bar or an instant beverage powder, comprising carbohydrate and protein, said dry food product comprising at least 1% w/w BLG, preferably at least 5%, wherein:

- i) the crystallinity of BLG is at least 20%, preferably at least 40%, and/or
- ii) at least 90% w/w of the total amount of protein is comprised by BLG.

In some particularly preferred embodiments of the invention the food product is a low phosphorus food product comprising at most 40 mg phosphorus per 100 g protein.

Non-limiting examples of the food product are e.g. a dairy product, a candy, a beverage, an instant beverage, a protein bar, an enteral nutritional composition, a bakery product.

In other preferred embodiments of the invention the food product is an instant beverage powder comprising, or even essentially consisting of:

- an edible BLG composition as defined herein in the form of a powder to provide at total amount of BLG of at least 1% w/w, preferably at least 5% w/w, said edible BLG composition having a crystallinity of BLG of at least 20%,
- a sweetener in the form of a powder, e.g. a sugar sweetener and/or a non-sugar sweetener,
- optionally, a flavouring agent
- at least one food acid in the form of a powder, e.g. citric acid or other suitable food acids, and
- at most 80 mg phosphorus/100 g protein, and

wherein a 10% solution of the instant beverage powder in demineralised water has a pH in the range of 2.5-4.0.

In some preferred embodiments of the invention, the edible BLG composition comprises:

- At most 6% w/w water
- At least 80% total protein relative to total solids
- At least 95% BLG relative to total protein, and

said edible BLG composition:

- Is a dry powder, and
- Has a bulk density of at least 0.50 g/mL, and preferably at least 0.60 g/mL.

In other preferred embodiments of the invention, the edible BLG composition comprises:

- At most 6% w/w water
- At least 80% total protein relative to total solids

5 - At least 95% BLG relative to total protein, and
said edible BLG composition:

- Is a dry powder,
- Has a bulk density of at least 0.50 g/mL, and preferably at least 0.60 g/mL, and
- Has a crystallinity of BLG of at least 20% and preferably at least 40%.

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In further preferred embodiments of the invention, the edible BLG composition comprises:

- At most 6% w/w water
- At least 80% total protein relative to total solids,
- At least 95% BLG relative to total protein,

15 - at most 80 mg phosphorus per 100 g protein.

said edible BLG composition:

- Is a dry powder.

In yet preferred embodiments of the invention, the edible BLG composition comprises:

- 20 - At most 6% w/w water
- At least 90% total protein relative to total solids,
- At least 97% BLG relative to total protein,
- at most 50 mg phosphorus per 100 g protein.

said edible BLG composition:

25 - Is a dry powder.

In other preferred embodiments of the invention, the edible BLG composition comprises:

- At most 6% w/w water
 - At least 80% total protein relative to total solids, and preferably at least 90% total protein
- 30 relative to total solids,
- 30-70% BLG relative to total protein,
 - 8-25% w/w ALA relative to total protein,

said edible BLG composition:

- Is a dry powder, and
- 35 - Has a crystallinity of BLG of at least 20% and preferably at least 40%.

In one aspect of the invention, a liquid food product is prepared from the instant beverage powder. By use of the instant beverage powder according to the invention, it is possible to obtain a liquid food product within a very short time.

Thus, in one aspect of the invention pertains to a method for preparing a liquid food product according to the invention, said method comprising

- i. Adding an instant beverage powder according to the invention,
- 5 ii. Optionally adding at least one further ingredient, and
- iii. Mixing the powder and liquid obtained to form a uniform mixture.

In one embodiment the liquid is selected from the group consisting of water, milk products, fruit juice, vegetable juice, beverages and combinations thereof. In one embodiment, the further ingredient is selected from fruits or vegetables.

When mixing the powder and liquid, the mixing can be performed by shaking. After shaking, the liquid food product, the instant beverage powder may be allowed to stand for ½-2 minutes in order to fully dissolve. One advantage of the instant beverage powder is that the instant beverage powder easily dissolves, forms a uniform solution and remains a uniform solution, i.e. substantially no segregation occurs.

A problem usually associated with instant beverage powders is that when preparing a liquid food product from the powder, foam is developed. The instant beverage powder of the invention has a tendency not to foam when preparing a liquid food product from the powder.

A liquid food product comprising a liquid and the powder according to the invention may be prepared by mixing the instant beverage powder of the invention with the liquid. In one embodiment of the invention, the instant beverage powder may comprise at most 40 gram of said powder per 100 grams of said liquid, such as at most 30 gram of said powder per 100 grams of said liquid. In a preferred embodiment of the invention, the liquid food product comprises 1-30 gram of said powder per 100 grams of said liquid, more preferably 1-20 gram of said powder, even more preferably 1-10 gram of said powder, or preferably 1-5 gram of said powder or even more preferably 2.5-5 gram of said powder.

In one embodiment of the invention the liquid food product comprises 5-25 gram of said powder per 100 gram of the liquid, preferably 5-25 gram of said powder per 100 gram of the liquid, more preferably 10-15 gram of said powder, even more preferably 11-14 gram of said powder or more preferably 11-12 gram of said powder.

In one embodiment of the invention the food product has energy content in the range of 30-300 kcal/100 grams of food product, preferably in the range of 30-100 kcal/100 grams of food product, more preferably in the range of 40-90 kcal/100 grams of food product, or even more preferably in the range of 40-70 kcal/100 grams of food product.

Alternatively, the food product has energy content in the range of 100-300 kcal/100 grams of food product, preferably in the range of 100-250 kcal/100 grams of food product, or more preferably in the range of 125-225 kcal/100 grams of food product.

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The liquid food product may comprise a liquid selected from the group consisting of water, milk products, fruit juice, vegetable juice, beverages and combinations thereof.

10 The appearance of liquid food products e.g. a beverage prepared from the instant beverage powder, is of great importance to the consumer. Transparency is a parameter that the consumer uses to evaluate the product. One way of determining the transparency of the liquid food product is by measuring the turbidity of the product as described in example 1.7.

15 In some embodiments of the beverage prepared from the instant beverage powder, it is beneficial that the beverage is transparent. This may for example be advantageous when the beverage is used as a sport beverage or in "protein water", in which case it is beneficial that the beverage resemble water in appearance.

20 In a preferred embodiment of the present invention, the beverage prepared from the instant beverage powder has a turbidity of at most 200 NTU, and such a beverage is transparent and/or translucent.

25 In some preferred embodiment of the present invention, the beverage prepared from the instant beverage powder have a turbidity of at most 150 NTU, or preferably a turbidity of at most 100 NTU, or preferably a turbidity of at most 80 NTU, or preferably a turbidity of at most 60 NTU or more preferably a turbidity of at most 40 NTU, or preferably a turbidity of at most 30 NTU, preferably a turbidity of at most 20 NTU, more preferably a turbidity of at most 10 NTU, and more preferably a turbidity of at most 5 NTU, even more preferably, it has a turbidity of at most 2 NTU.

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In a preferred embodiment of the present invention the beverage prepared from the instant beverage powder have a turbidity of more than 200 NTU, such a beverage is opaque.

35 In some embodiments of the beverage prepared from the instant beverage powder, it is beneficial that the beverage is opaque. This is for example advantageous when the beverage should resemble milk and have a milky appearance. The appearance of nutritionally complete nutritional supplements is typically opaque.

In some preferred embodiments of the invention, the beverage prepared from the instant beverage powder have a turbidity of more than 250 NTU. Preferably the beverage have a turbidity of more than 300 NTU, more preferably, it has a turbidity of more than 500 NTU, more preferably it has a turbidity of more than 1000, preferably a turbidity of more than 1500 NTU, even more preferably it has a turbidity of more than 2000 NTU.

The colour of the product is of great importance to the consumer. Instant beverage powder products comprising whey proteins have a slightly yellow colour. However, when using instant beverage powder comprising BLG having a crystallinity of at least 20% or instant beverage powder, where at least 90% of the protein is comprised by BLG, the colour of the product is substantially less yellow and the product appears more white. Thus, addition of colour to the instant beverage powder in order to mask the yellow colour is not necessary.

One way of measuring the colour of a product is by using the CIELAB colour space, which expresses colour as three numerical values; L^* for lightness and a^* and b^* for the green-red and blue-yellow colour components. Example 1.9 describes how to measure the L^* , a^* and b^* values for the liquid food product.

In one embodiment of the invention the protein fraction of the liquid food product has a colour value Δb^* in the range of -0.10 to +0.51 at the CIELAB color scale, wherein $\Delta b^* = b_{\text{sample standardized to 6.0 w/w\% protein}}^* - b_{\text{demin. water}}^*$, measured at room temperature.

Another aspect of the invention pertains to a method for preparing an instant beverage powder comprising BLG and at least one optional ingredient, said method comprising blending a dry BLG isolate, with at least one additional ingredient selected from the group consisting of vitamins, flavouring agent, colouring agent, minerals, sweeteners, antioxidants, food acid, lipids, carbohydrate, prebiotics, probiotics, anti-foaming agents and non-whey protein to obtain an instant beverage powder.

In one embodiment of the invention, the BLG of the BLG source is coated with an organic acid. If the BLG source e.g. is a powder, this means that the powder is coated with the organic acid. When preparing instant beverage powders comprising protein, lecithin is commonly used for improving the solubility of the protein. However, lecithin is a source of phosphorus. It is therefore desirable to find another way of improving the solubility of instant beverage powders comprising protein.

The inventors have found that by coating the BLG crystals or powder particles of the BLG source with one or more organic acids, the solubility of the instant beverage powder improves. The organic acid or salt of organic acid can be selected from the group consisting of pyruvate,

aconitate, citrate, iso-citrate, ketoglutarate, succinyl-CoA, succinate, fumarate, malate, oxaloacetate, tartrate, acetate, tannic acid, benzoic acid, maleic acid and lactate. In a preferred embodiment of the invention, the BLG crystals are coated with organic acid or salt of organic acid selected from the group consisting of pyruvate, citrate, iso-citrate, ketoglutarate, succinate, fumarate, malate, oxaloacetate, tartrate, acetate, maleic acid and lactate and salts thereof

In a preferred embodiment of the invention, the BLG crystals of the BLG source are coated with citrate, e.g. a citrate selected from the group consisting of trisodium citrate, potassium citrate and calcium citrate.

In preferred embodiment of the invention, the BLG of the BLG source is coated with organic acid by use of spray-drying or fluid bed. It is particularly preferred that dried BLG crystals are are coated with the organic acid or salts thereof using e.g. spray-drying or fluid bed.

The BLG source can be obtained from a whey protein feed, from which the BLG is isolated as crystals. One method of preparing the BLG isolate is described in international patent application No. PCT/EP2017/084553, which is hereby incorporated by reference. The BLG isolate can be prepared as described on page 6, line 23-32 of PCT/EP2017/084553 as filed, where the edible composition comprising BLG in crystallised and/or isolated form corresponds to the BLG isolate of the present invention. In a preferred embodiment, the BLG isolate is prepared by the method described on page 39, line 15-34 of PCT/EP2017/084553 as filed. In another preferred embodiment the BLG isolate is prepared by the method described on page 41, line 1-24 of PCT/EP2017/084553 as filed.

In some preferred embodiments of the invention the instant beverage powder comprises, or even consists of, a BLG isolate powder comprising dried BLG crystals, said BLG isolate powder is coated with an organic acid and/or a salt of an organic acid. The weight ratio between the weight of the BLG isolate and the total weight of the sum of organic acids and deprotonated organic acids is preferably 5-100, more preferably 8-60, even more preferably 10-40, and most preferably 12-30.

An aspect of the invention pertains to a method of producing a BLG isolate powder coated with organic acid and/or a salt of an organic acid, the method comprises the following steps

- Providing a BLG isolate powder to be coated, preferably comprising, or even consisting of, dried BLG crystals, preferably obtained by the BLG crystallization process described herein, and
- Applying organic acid and/or salt of an organic acid to the BLG isolate powder to be coated, preferably in an amount sufficient to coat the BLG isolate powder but avoiding that it is dissolved,
- Optionally evaporating residual moisture from the coated BLG isolate powder.

The BLG isolate powder to be coated, preferably has both a high protein content and a high BLG purity. The BLG isolate powder to be coated preferably has a crystallinity of BLG of at least 20%, preferably at least 40%, more preferably at least 60%, and even more preferably at least 80%.

The organic acid and/or salt of an organic acid to the BLG isolate powder to be coated in an amount sufficient to provide a weight ratio between the weight of the BLG isolate powder and the total weight of the sum of organic acids and deprotonated organic acids is preferably 5-100, more preferably 8-60, even more preferably 10-40, and most preferably 12-30.

The organic acid and/or salt of a organic acid is preferably applied to the BLG isolate powder in a fluid bed system by spraying organic acid and/or salt of an organic acid, preferably in dissolved form into the fluid bed to coat the BLG isolate powder. The temperature during operation is preferably in the range of 5-70 degrees C more preferably in the range of 50-65 degrees C such as preferably approx. 60 degrees C.

After application of the organic acid and/or salt of an organic acid the coated BLG isolate may be processed to evaporate additional moisture, preferably until the water content is at most 6% w/w and more preferably at most 5% w/w.

The organic acids are preferably edible organic acids, i.e. so-called food acids.

In some preferred embodiments of the invention the BLG source used for preparing the instant beverage powder has a solids content of at least 20% w/w. Preferably, the BLG source has a solids content of at least 30% w/w, more preferably, the BLG source has a solids content of at least 40% w/w, even more preferably, the BLG source has a solids content of at least 50% w/w, such as e.g. at least 60% w/w.

In other preferred embodiments of the invention the BLG source used for preparing the instant beverage powder has a solid content of in the range of 20-80% w/w. Preferably, the BLG source has a solid content in the range of 30-70% w/w. More preferably, the BLG source has a solid content in the range of 40-65% w/w. Even more preferably, the BLG source has a solid content in the range of 50-65% w/w, such as e.g. approx. 60% w/w.

The BLG source is preferably a BLG isolate powder or a liquid BLG isolate contain water and the solids of the BLG isolate powder in an amount in the range from 1-50% w/w. It is particularly preferred that the BLG source is a BLG isolate powder.

The beta-lactoglobulin (BLG) isolate powder, preferably prepared by spray-drying, has a pH in the range of i) 2-4.9, ii) 6.1-8.5, or iii) 5.0-6.0 and comprises:

- total protein in an amount of at least 30% w/w,
- BLG in an amount of at least 85% w/w relative to total protein, and
- 5 - water in an amount of at most 10% w/w.

The BLG isolate powder preferably has one or more of the following:

- a bulk density of at least 0.2 g/cm³,
- an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11,
- 10 - a degree of protein denaturation of at most 10%,
- a heat-stability at pH 3.9 of at most 200 NTU, and
- at most 1000 colony-forming units/g.

15 The BLG isolate powder is preferably an edible composition. In some preferred embodiments of the invention the BLG isolate powder is an edible BLG composition as defined herein.

In some preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 2-4.9. Such powders are particularly useful for acidic food products and particularly acidic beverages.

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In other preferred embodiments of the invention, BLG isolate powder has a pH in the range of 6.1-8.5.

25 In some preferred embodiments of the invention, the BLG isolate powder comprises total protein in an amount of at least 40% w/w, preferably at least 50% w/w, at least 60% w/w, more preferably at least 70% w/w, even more preferably at least 80% w/w.

30 Even higher protein contents may be required and in some preferred embodiments of the invention, the BLG isolate powder comprises total protein in an amount of at least 85% w/w, preferably at least 90% w/w, at least 92% w/w, more preferably at least 94% w/w, and even more preferably at least 95% w/w.

Total protein is measured according to Example 1.5.

35 In some preferred embodiments of the invention, the BLG isolate powder comprises BLG in an amount of at least 92% w/w relative to total protein, preferably at least 95% w/w, more preferably at least 97% w/w, even more preferably at least 98%, and most preferably BLG in an amount of at least 99.5% w/w relative to total protein.

In some preferred embodiments of the invention, the sum of alpha-lactalbumin (ALA) and caseinomacropptide (CMP) comprises at least 40% w/w of the non-BLG protein of the powder, preferably at least 60% w/w, even more preferably at least 70% w/w, and most preferably at least 90% w/w of the non-BLG protein of the powder.

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In other preferred embodiments of the invention, each main non-BLG whey protein is present in a weight percentage relative to total protein which is at most 25% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 20%, more preferably at most 15%, even more preferably at most 10%, most preferably at most 6%.

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Even lower concentrations of the main non-BLG whey proteins may be desirable. Thus, in additional preferred embodiments of the invention, each main non-BLG whey protein is present in a weight percentage relative to total protein which is at most 4% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 3%, more preferably at most 2%, even more preferably at most 1%.

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The inventors have seen indications that reduction of lactoferrin and/or lactoperoxidase is particularly advantageous for obtaining a colour-neutral whey protein product.

20

Thus in some preferred embodiments of the invention, lactoferrin is present in a weight percentage relative to total protein which is at most 25% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 20%, more preferably at most 15%, even more preferably at most 10%, most preferably at most 6%. Even lower concentrations of lactoferrin may be desirable. Thus, in additional preferred embodiments of the invention, lactoferrin is present in a weight percentage relative to total protein which is at most 4% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 3%, more preferably at most 2%, even more preferably at most 1%.

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Similarly, in some preferred embodiments of the invention, lactoperoxidase is present in a weight percentage relative to total protein which is at most 25% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 20%, more preferably at most 15%, even more preferably at most 10%, most preferably at most 6%. Even lower concentrations of lactoperoxidase may be desirable. Thus, in additional preferred embodiments of the invention, lactoperoxidase is present in a weight percentage relative to total protein which is at most 4% of its weight percentage relative to total protein in a standard whey protein concentrate from sweet whey, preferably at most 3%, more preferably at most 2%, even more preferably at most 1%.

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Lactoferrin and lactoperoxidase are quantified according to Example 1.29.

5 In some preferred embodiments of the invention, the BLG isolate powder has a water content in an amount of at most 10% w/w, preferably at most 7% w/w, more preferably at most 6% w/w, even more preferably at most 4% w/w, and most preferred at most 2% w/w.

10 In some preferred embodiments of the invention the BLG isolate powder comprises carbohydrate in an amount of at most 60% w/w, preferably at most 50% w/w, more preferably at most 20% w/w, even more preferably at most 10% w/w, even more preferably at most 1% w/w, and most preferably at most 0.1%. The BLG isolate powder may for example contain carbohydrates, such as e.g. lactose, oligosaccharides and/or hydrolysis products of lactose (i.e. glucose and galactose), sucrose, and/or maltodextrin.

15 In some preferred embodiments of the invention, the BLG isolate powder comprises lipid in an amount of at most 10% w/w, preferably at most 5% w/w, more preferably at most 2% w/w, and even more preferably at most 0.1% w/w.

20 The present inventors have found that it can be advantageous to control the mineral content to reach some of the desired properties of the BLG isolate powder.

In some preferred embodiments of the invention, the sum of the amounts of Na, K, Mg, and Ca of the BLG isolate powder is at most 10 mmol/g protein. Preferably, the sum of the amounts of Na, K, Mg, and Ca of the BLG isolate powder is at most 6 mmol/g protein, more preferably at
25 most 4 mmol/g protein, even more preferably at most 2 mmol/g protein.

In other preferred embodiments of the invention, the the sum of the amounts of Na, K, Mg, and Ca of the BLG isolate powder is at most 1 mmol/g protein. Preferably, the sum of the amounts of Na, K, Mg, and Ca of the BLG isolate powder is at most 0.6 mmol/g protein, more preferably
30 at most 0.4 mmol/g protein, even more preferably at most 0.2 mmol/g protein, and most preferably at most 0.1 mmol/g protein.

In other preferred embodiments of the invention, the sum of the amounts of Mg and Ca of the BLG isolate powder is at most 5 mmol/g protein. Preferably, the sum of the amounts of Mg and
35 Ca of the BLG isolate powder is at most 3 mmol/g protein, more preferably at most 1.0 mmol/g protein, even more preferably at most 0.5 mmol/g protein.

In other preferred embodiments of the invention, the sum of the amounts of Mg and Ca of the BLG isolate powder is at most 0.3 mmol/g protein. Preferably, the sum of the amounts of Mg

and Ca of the BLG isolate powder is at most 0.2 mmol/g protein, more preferably at most 0.1 mmol/g protein, even more preferably at most 0.03 mmol/g protein, and most preferably at most 0.01 mmol/g protein.

- 5 The inventors have found that it is possible to use low phosphorus/low potassium variants of the BLG isolate powder that are particularly useful to patients with kidney diseases. To make such a product, the BLG isolate powder has to have an equally low content of phosphorus and potassium.
- 10 Thus, in some preferred embodiments of the invention, the BLG isolate powder has a total content of phosphorus of at most 100 mg phosphorus per 100 g protein. Preferably, the BLG isolate powder has a total content of at most 80 mg phosphorus per 100 g protein. More preferably, the BLG isolate powder has a total content of at most 50 mg phosphorus per 100 g protein. Even more preferably, the BLG isolate powder has a total content of phosphorus of at most 20
- 15 mg phosphorus per 100 g protein. The BLG isolate powder has a total content of phosphorus of at most 5 mg phosphorus per 100 g protein.

- In some preferred embodiments of the invention, the BLG isolate powder comprises at most 600 mg potassium per 100 g protein. More preferably, the BLG isolate powder comprises at most
- 20 500 mg potassium per 100 g protein. More preferably, the BLG isolate powder comprises at most 400 mg potassium per 100 g protein. More preferably, the BLG isolate powder comprises at most 300 mg potassium per 100 g protein. Even more preferably, the BLG isolate powder comprises at most 200 mg potassium per 100 g protein. Even more preferably, the BLG isolate powder comprises at most 100 mg potassium per 100 g protein. Even more preferably, the BLG isolate
- 25 powder comprises at most 50 mg potassium per 100 g protein and even more preferably, the BLG isolate powder comprises at most 10 mg potassium per 100 g protein.

- The content of phosphorus relates to the total amount of elemental phosphorus of the composition in question and is determined according to Example 1.19. Similarly, the content of potassium
- 30 um relates to the total amount of elemental potassium of the composition in question and is determined according to Example 1.19.

- In some preferred embodiments of the invention, the BLG isolate powder comprises at most 100 mg phosphorus/100 g protein and at most 700 mg potassium/100g protein, preferably at
- 35 most 80mg phosphorus/100 g protein and at most 600mg potassium/ 100g protein, more preferably at most 60mg phosphorus/100 g protein and at most 500mg potassium/ 100g protein, more preferably at most 50mg phosphorus/100 g protein and at most 400mg potassium/ 100g protein, or more preferably at most 20mg phosphorus/100 g protein and at most 200mg potassium/100g protein, or even more preferably at most 10mg phosphorus/100 g protein and at

most 50mg potassium/ 100g protein. In some preferred embodiments of the invention the BLG isolate powder comprises at most 100mg phosphor/100 g protein and at most 340mg potassium/100g protein.

- 5 The low phosphorus and/or low potassium compositions according to the present invention may be used as a food ingredient for the production of a food product for patients groups that have a reduced kidney function.

10 The present inventors have found that for some applications, e.g. acidic food products and particularly acidic beverages, it is particularly advantageous to have an acidic BLG isolate powder having a pH of at most 4.9 and even more preferably at most 4.3. This is especially true for high protein, transparent acidic beverages.

15 In the context of the present invention, a transparent liquid has a turbidity of at most 200 NTU measured according to Example 1.7.

Thus, in some preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 2-4.9. Preferably, the BLG isolate powder has a pH in the range of 2.5-4.7, more preferably 2.8-4.3, even more preferably 3.2-4.0, and most preferably 3.4-3.9. Alternatively, but
20 also preferred, the BLG isolate powder may have a pH in the range of 3.6-4.3.

The present inventors have found that for some applications, e.g. pH-neutral food products and particularly pH-neutral beverages, it is particularly advantageous to have a pH-neutral BLG isolate powder. This is especially true for high protein, transparent or opaque pH-neutral beverages.
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Thus, in some preferred embodiments of the invention, BLG isolate powder has a pH in the range of 6.1-8.5. Preferably, the powder has a pH in the range of 6.1-8.5, more preferably 6.2-8.0, even more preferably 6.3-7.7, and most preferably 6.5-7.5.
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In other preferred embodiments of the invention, BLG isolate powder has a pH in the range of 5.0-6.0. Preferably, the powder has a pH in the range of 5.1-5.9, more preferably 5.2-5.8, even more preferably 5.3-5.7, and most preferably 5.4-5.6.

35 Advantageously, the BLG isolate powder used in the present invention may have bulk density of at least 0.20 g/cm³, preferably at least 0.30 g/cm³, more preferably at least 0.40 g/cm³, even more preferably at least 0.45 g/cm³, even more preferably at least 0.50 g/cm³, and most preferably at least 0.6 g/cm³.

Low density powders such as freeze-dried BLG isolates are fluffy and easily drawn into the air of the production site during use. This is problematic as it increases the risk of cross-contamination of the freeze-dried powder to other foods products and a dusty environment is known to be a cause of hygiene issues. In extreme cases, a dusty environment also increases the risk of dust explosions.

The high density variants of the present invention are easier to handle and less prone to flow into the surrounding air.

An additional advantage of the high density variants of the present invention is that they take up less space during transportation and thereby increase weight of BLG isolate powder that can be transported in one volume unit.

Yet an advantage of the high density variants of the present invention is that they are less prone to segregation when used in powder mixtures with other powdered food ingredients, such as e.g. powdered sugar (bulk density of approx. 0.56 g/cm^3), granulated sugar (bulk density of approx. 0.71 g/cm^3), powdered citric acid (bulk density of approx. 0.77 g/cm^3).

The BLG isolate powder of the present invention may have bulk density in the range of $0.2\text{-}1.0 \text{ g/cm}^3$, preferably in the range of $0.30\text{-}0.9 \text{ g/cm}^3$, more preferably in the range of $0.40\text{-}0.8 \text{ g/cm}^3$, even more preferably in the range of $0.45\text{-}0.75 \text{ g/cm}^3$, even more preferably in the range of $0.50\text{-}0.75 \text{ g/cm}^3$, and most preferably in the range of $0.6\text{-}0.75 \text{ g/cm}^3$.

The bulk density of a powder is measured according to Example 1.17.

The present inventors have found that it is advantageous to maintain the native conformation of BLG and have seen indications that increased unfolding of BLG gives rise to an increased level of drying mouthfeel when the BLG is used for acidic beverages.

The intrinsic tryptophan fluorescence emission ratio (I330/I350) is a measure of degree of unfolding of BLG and the inventors have found that at high intrinsic tryptophan fluorescence emission ratios, which correlate with low or no unfolding of BLG, less drying mouthfeel was observed. The intrinsic tryptophan fluorescence emission ratio (I330/I350) is measured according to Example 1.1.

In some preferred embodiments of the invention, the BLG isolate powder has an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11.

In some preferred embodiments of the invention, the BLG isolate powder has an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.12, preferably at least 1.13, more preferably at least 1.15, even more preferably at least 1.17, and most preferably at least 1.19.

- 5 If BLG isolate powder contains considerable amounts of non-protein matter it is preferred to isolate the protein fraction before measuring the intrinsic tryptophan fluorescence emission ratio. Thus in some preferred embodiments of the invention, the protein fraction of the BLG isolate powder has an intrinsic tryptophan fluorescence emission ratio of at least 1.11.
- 10 In some preferred embodiments of the invention, the protein fraction of the BLG isolate powder has an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.12, preferably at least 1.13, more preferably at least 1.15, even more preferably at least 1.17, and most preferably at least 1.19.
- 15 The protein fraction can e.g. be separated from the BLG isolate powder by dissolving the BLG isolate powder in demineralised water and subjecting the solution to dialysis or ultrafiltration-based diafiltration using a filter that retains the protein. If the BLG isolate powder contains interfering levels of lipid such lipid can e.g. be removed by microfiltration. Steps of microfiltration and ultrafiltration/diafiltration can be combined to remove both lipid and small molecules
- 20 from the protein fraction.

- It is often preferred that a substantial amount of the BLG of the BLG isolate powder is non-aggregated BLG. Preferably at least 50% of the BLG is non-aggregated BLG. More preferably at least at least 80% of the BLG is non-aggregated BLG. Even more preferred at least 90% of the
- 25 BLG is non-aggregated BLG. Most preferred, at least 95% of the BLG is non-aggregated BLG. Even more preferred approx. 100% of the BLG of the BLG isolate powder is non-aggregated BLG.

- In some preferred embodiments of the invention, the BLG isolate powder has a degree of protein denaturation of at most 10%, preferably at most 8%, more preferably at most 6%, even
- 30 more preferably at most 3%, even more preferably at most 1%, and most preferably at most 0.2%.

- However, it may also be preferred that the BLG isolate powder has a significant level of protein denaturation, e.g. if an opaque beverage is desired. Thus, in other preferred embodiments of
- 35 the invention, the BLG isolate powder has a degree of protein denaturation of at least 11%, preferably at least 20%, more preferably at least 40%, even more preferably at least 50%, even more preferably at least 75%, and most preferably at least 90%.

If BLG isolate powder has a significant level of protein denaturation it is often preferred to keep a low level of insoluble protein matter, i.e. precipitated protein matter that would settle in a beverage during storage. The level of insoluble matter is measure according to Example 1.10.

5 In some preferred embodiments of the invention the BLG isolate powder comprises at most 20% w/w insoluble protein matter, preferably at most 10% w/w insoluble protein matter, more preferably at most 5% w/w insoluble protein matter, even more preferred at most 3% w/w insoluble protein matter, and most preferred at most 1% w/w insoluble protein matter. It may even be preferred that the BLG isolate powder does not contain any insoluble protein matter at
10 all.

In some preferred embodiments of the invention the BLG isolate powder has a crystallinity of BLG of at most 19%, preferably at most 10%, more preferably at most 5%, and most preferably 0%.

15 In other preferred embodiments of the invention the BLG isolate powder has a crystallinity of BLG of at least 20%, preferably at least 40%, more preferably at least 60%, and most preferably at least 80%. These embodiments contain a significant amount of dried BLG crystals and provide the benefits of having the protein source present in solid, compact form.

20 The present inventors have found that the heat-stability at pH 3.9 of a BLG isolate powder is a good indicator for its usefulness for transparent high protein beverages. The heat-stability at pH 3.9 is measured according to Example 1.2.

25 It is particularly preferred that the BLG isolate powder has a heat-stability at pH 3.9 of at most 200 NTU, preferably at most 100 NTU, more preferred at most 60 NTU, even more preferred at most 40 NTU, and most preferred at most 20 NTU. Even better heat-stabilities are possible and the BLG isolate powder preferably has a heat-stability at pH 3.9 of at most 10 NTU, preferably at most 8 NTU, more preferred at most 4 NTU, even more preferred at most 2 NTU.

30 The content of microorganisms of the BLG isolate powder is preferably kept to a minimum. However, it is a challenge to obtain both a high degree of protein nativeness and a low content of microorganism as processes for microbial reduction tend to lead to protein unfolding and denaturation. The present invention makes it possible to obtain a very low content of microor-
35 ganism while at the same time maintain a high level of the nativeness of BLG.

Thus, in some preferred embodiments of the invention, the BLG isolate powder contains at most 15000 colony-forming units (CFU)/g. Preferably, the BLG isolate powder contains at most 10000 CFU/g. More preferably, the BLG isolate powder contains at most 5000 CFU/g. Even

more preferably, the BLG isolate powder contains at most 1000 CFU/g. Even more preferably, the BLG isolate powder contains at most 300 CFU/g. Most preferably, the BLG isolate powder contains at most 100 CFU/g such as e.g. at most 10 CFU/g. In a particularly preferred embodiment the powder is sterile. A sterile BLG isolate powder may e.g. be prepared by combining several physical microbial reduction processes during the production of the BLG isolate powder, such as e.g. microfiltration and heat-treatment at acidic pH.

In some preferred embodiments of the invention, the BLG isolate powder has a pH in the range of i) 2-4.9, ii) 6.1-8.5, or iii) 5.0-6.0 and comprises:

- total protein in an amount of at least 30% w/w, preferably at least 80% w/w, and even more preferably at least 90% w/w
 - beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w,
 - water in an amount of at most 6% w/w,
 - lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,
- said BLG isolate powder having:
- an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11,
 - a degree of protein denaturation of at most 10%, and
 - a heat-stability at pH 3.9 of at most 200 NTU.

In some preferred embodiments of the invention, the BLG isolate powder has a pH in the range of i) 2-4.9 or ii) 6.1-8.5 and comprises:

- total protein in an amount of at least 30% w/w, preferably at least 80% w/w, and even more preferably at least 90% w/w
 - beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w, and more preferably at least 94% w/w relative to total protein
 - water in an amount of at most 6% w/w,
 - lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,
- said BLG isolate powder having:
- an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11,
 - a degree of protein denaturation of at most 10%, preferably at most 5%, and
 - a heat-stability at pH 3.9 of at most 70 NTU, preferably at most 50 NTU and even more preferably at most 40 NTU.

In some preferred embodiments of the invention the BLG isolate powder has a pH in the range of i) 2-4.9 or ii) 6.1-8.5 and comprises:

- total protein in an amount of at least 30% w/w,
- beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w,

- water in an amount of at most 6% w/w,

said BLG isolate powder having:

- a bulk density of at least 0.2 g/cm³,

- an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11,

5 - a degree of protein denaturation of at most 10%, and

- a heat-stability at pH 3.9 of at most 200 NTU.

In other preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 2-4.9 and comprises:

10 - total protein in an amount of at least 80% w/w, preferably at least 90% w/w, and even more preferably at least 94% w/w

- beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w, and even more preferably at least 94% w/w relative to total protein,

- water in an amount of at most 6% w/w,

15 - lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,

said BLG isolate powder having:

- a bulk density of at least 0.2 g/cm³, preferably at least 0.3 g/cm³, and more preferably at least 0.4 g/cm³,

- an intrinsic tryptophan fluorescence emission ratio (I330/I350) of at least 1.11,

20 - a degree of protein denaturation of at most 10%, preferably at most 5%, and more preferably at most 2%, and

- a heat-stability at pH 3.9 of at most 50 NTU, preferably at most 30 NTU and even more preferably at most 10 NTU.

25 In yet other preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 6.1-8.5 and comprises:

- total protein in an amount of at least 80% w/w, preferably at least 90% w/w, and even more preferably at least 94% w/w

- beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w, and even more preferably at least 94% w/w relative to total protein,

30 - water in an amount of at most 6% w/w,

- lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,

said BLG isolate powder having:

- a bulk density of at least 0.2 g/cm³, preferably at least 0.3 g/cm³, and more preferably at least 0.4 g/cm³,

35 - a degree of protein denaturation of at most 10%, preferably at most 5%, and more preferably at most 2%, and

- a heat-stability at pH 3.9 of at most 50 NTU, preferably at most 30 NTU, and even more preferably at most 10 NTU.

In further preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 6.1-8.5 and comprises:

- total protein in an amount of at least 80% w/w, preferably at least 90% w/w, and even more preferably at least 94% w/w
- beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w, and even more preferably at least 94% w/w relative to total protein,
- water in an amount of at most 6% w/w,
- lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,

said BLG isolate powder having:

- a bulk density of at least 0.2 g/cm³, preferably at least 0.3 g/cm³, and more preferably at least 0.4 g/cm³,
- a degree of protein denaturation of at most 10%, preferably at most 5%, and more preferably at most 2%, and
- a heat-stability at pH 3.9 of at most 50 NTU, preferably at most 30 NTU, and even more preferably at most 10 NTU.

In further preferred embodiments of the invention, the BLG isolate powder has a pH in the range of 5.0-6.0 and comprises:

- total protein in an amount of at least 80% w/w, preferably at least 90% w/w, and even more preferably at least 94% w/w,
- beta-lactoglobulin (BLG) in an amount of at least 85% w/w relative to total protein, preferably at least 90% w/w, and even more preferably at least 94% w/w relative to total protein,
- water in an amount of at most 6% w/w,
- lipid in an amount of at most 2% w/w, preferably at most 0.5% w/w,

said BLG isolate powder having:

- a bulk density of at least 0.2 g/cm³, preferably at least 0.3 g/cm³, and more preferably at least 0.4 g/cm³,
- a degree of protein denaturation of at most 10%, preferably at most 5%, and more preferably at most 2%,
- a heat-stability at pH 3.9 of at most 50 NTU, preferably at most 30 NTU, and even more preferably at most 10 NTU, and
- preferably, a BLG crystallinity of less than 10%.

The BLG isolate powder containing BLG in an amount of at least 85% w/w relative to total protein, is typically provided by a method comprising the steps of:

- a) providing a liquid BLG isolate having

- i) a pH in the range of 2-4.9,
- ii) a pH of in the range of 6.1-8.5, or
- iii) a pH of in the range of 5.0-6.0

said liquid BLG isolate containing BLG in an amount of at least 85 w/w relative to total protein,

- b) optionally, subjecting the liquid BLG isolate to a physical microbial reduction,
- c) drying the liquid BLG isolate, preferably by spray-drying.

The BLG isolate is preferably prepared from mammal milk, and preferably from ruminant milk such as e.g. milk from cow, sheep, goat, buffalo, camel, llama, mare and/or deer. Protein derived from bovine milk is particularly preferred. The BLG is therefore preferably bovine BLG.

The liquid BLG isolate may be provided in a number of different ways.

Typically, the provision of the liquid BLG isolate involves, or even consists of, isolating BLG from a whey protein feed to provide a BLG-enriched composition by one or more of the following methods:

- crystallisation or precipitation of BLG by salting-in,
- crystallisation or precipitation of BLG of BLG by salting-out,
- ion exchange chromatography, and
- fractionation of whey proteins by ultrafiltration.

A particularly preferred way of providing the BLG-enriched composition is by crystallisation of BLG, preferably by salting-in or alternatively by salting-out.

The whey protein feed is preferably a WPC, a WPI, an SPC, an SPI, or a combination thereof.

The term "whey protein feed" pertains to the composition from which the BLG-enriched composition and subsequently the liquid BLG isolate are derived.

In some embodiments of the invention, the preparation of the BLG-enriched composition includes, or even consist of, high salt BLG crystallisation in the pH range 3.6-4.0 according to US 2,790,790 A1.

In other embodiments of the invention the preparation of the BLG-enriched composition includes, or even consists of, the method described by de Jongh *et al* (Mild Isolation Procedure Discloses New Protein Structural Properties of β -Lactoglobulin, J Dairy Sci., vol. 84(3), 2001, pages 562-571) or by Vyas *et al* (Scale-Up of Native β -Lactoglobulin Affinity Separation Process, J. Dairy Sci. 85:1639-1645, 2002).

However, in particularly preferred embodiments of the invention, the BLG-enriched composition is prepared by crystallisation at pH 5-6 under salting-in conditions as described in the PCT application PCT/EP2017/084553, which is incorporated herein by reference for all purposes.

5

In some preferred embodiments of the invention, the BLG-enriched composition is an edible BLG composition according to PCT/EP2017/084553 containing at least 90% BLG relative to total protein and preferably containing BLG crystals.

10 If it does not already have the required characteristics to be used as liquid BLG isolate, the BLG-enriched composition which has been isolated from whey protein feed may be subjected to one or more steps selected from the group of:

- demineralisation,
- addition of minerals

15

- dilution,
- concentration,
- physical microbial reduction, and
- pH adjustment

as part of providing the liquid BLG isolate.

20

Non-limiting examples of demineralisation include e.g. dialysis, gel filtration, UF/diafiltration, NF/diafiltration, and ion exchange chromatography.

25 Non-limiting examples of addition of minerals include addition of soluble, food acceptable salts, such as e.g. salts of Na, K, Ca, and/or Mg. Such salts may e.g. be phosphate-salts, chloride salts or salts of food acids, such as e.g. citrate salt or lactate salt. The minerals may be added in solid, suspended, or dissolved form.

30 Non-limiting examples of dilution include e.g. addition of liquid diluent such as water, demineralised water, or aqueous solutions of minerals, acids or bases.

Non-limiting examples of concentration include e.g. evaporation, reverse osmosis, nanofiltration, ultrafiltration and combinations thereof.

35 If the concentration has to increase the concentration of protein relative to total solids, it is preferred to use concentration steps such as ultrafiltration or alternatively dialysis. If the concentration does not have to increase the concentration of protein relative to total solids, methods such as e.g. evaporation, nanofiltration and/or reverse osmosis can be useful.

Non-limiting examples of physical microbial reduction include e.g. heat-treatment, germ filtration, UV radiation, high pressure treatment, pulsed electric field treatment, and ultrasound. These methods are well-known to the person skilled in the art.

- 5 Non-limiting examples of pH adjustment include e.g. addition of bases and/or acids, and preferably food acceptable bases and/or acids. It is particularly preferred to employ acids and/or bases that are capable of chelating divalent metal cations. Examples of such acids and/or bases are citric acid, citrate salt, EDTA, lactic acid, lactate salt, phosphoric acid, phosphate salt, and combinations thereof.

10

In the following a number of preferred embodiments of the provision of the liquid BLG isolate of step a) from BLG-enriched composition. The process steps mentioned in this context are applied to the BLG-containing product stream following the BLG-enriched composition.

- 15 The present invention has been described above with reference to specific embodiments. However, other embodiments than the above described are equally possible within the scope of the invention. The different features and steps of various embodiments and aspects of the invention may be combined in other ways than those described herein unless it is stated otherwise.

EXAMPLES

20 EXAMPLE 1: METHODS OF ANALYSIS

EXAMPLE 1.1: DETERMINATION OF PROTEIN NATIVENESS BY INTRINSIC TRYPTOPHAN FLUORESCENCE

- 25 Tryptophan (Trp) fluorescence spectroscopy is a well-described tool to monitor protein folding and unfolding. Trp residues buried within native proteins typically display highest fluorescence emission around 330nm than when present in more solvent exposed positions such as unfolded proteins. In unfolded proteins, the wavelengths for Trp fluorescence emission typically shift to higher wavelengths and are often measured around 350nm. We here exploit this transition to monitor thermally induced unfolding by calculating the ratio between fluorescence emission at 330nm and 350nm to investigate the influence of heating temperature.

30

The analysis comprises the following steps:

- Beverage compositions were diluted to 0.6mg/ml in MQ water.
- 300µl sample was transferred to white 96-well plate avoiding bubbles or 3mL was transferred to 10mm quartz cuvette.
- 35 • The tryptophan fluorescence emission intensity between 310 and 400nm was recorded from the top by excitation at 295 using 5nm slits.

- Samples were measured at 22 degrees C using a Cary Eclipse fluorescence spectrophotometer equipped with a plate reader accessory (G9810A) or single cuvette holder.
- The emission intensity ratio was calculated by dividing the measured fluorescence emission intensity at 330nm with the emission intensity at 350nm, $R = I_{330}/I_{350}$, and used as a measure of protein nativity.
 - R of at least 1.11 describes a predominant native BLG conformation and
 - R of less than 1.11 reports on at least partial unfolding and aggregation.

EXAMPLE 1.2: HEAT-STABILITY AT PH 3.9

Heat-stability at pH 3.9:

The heat-stability at pH 3.9 is a measure of the ability of protein composition to stay clear upon prolonged pasteurization at pH 3.9.

The heat-stability at pH 3.9 is determined by forming an aqueous solution having a pH of 3.9 and comprising 6.0% w/w protein by mixing a sample of the powder or liquid to be tested with water (or alternatively concentrating it by low temperature evaporation if it is a dilute liquid) and adjusting the pH to 3.9 with the minimum amount of 0.1 M NaOH or 0.1 M HCl required.

The pH-adjusted mixture is allowed to rest for 30 minutes after which 25 mL of the mixture is transferred to a 30 mL thin-walled glass test tube. It is heated to 75.0 degrees C for 300 seconds by immersion into a water-bath having a temperature of 75.0 degrees C. Immediately after the heating, the glass test tube is cooled to 1-5 degrees C by transferring it to an ice bath and the turbidity of the heat-treated sample is measured according to Example 1.7.

EXAMPLE 1.3: DETERMINATION OF THE DEGREE OF PROTEIN DENATURATION OF A WHEY PROTEIN COMPOSITION

Denatured whey protein is known to have a lower solubility at pH 4.6 than at pH values below or above pH 4.6, therefore the degree of denaturation of a whey protein composition is determined by measuring the amount of soluble protein at pH 4.6 relative to the total amount of protein at a pH where the proteins in the solution are stable.

More specifically for whey proteins, the whey protein composition to be analysed (e.g. a powder or an aqueous solution) is converted to:

- a first aqueous solution containing 5.0% (w/w) total protein and having a pH of 7.0 or 3.0 , and
- a second aqueous solution containing 5.0% (w/w) total protein and having a pH of 4.6.

pH adjustments are made using 3% (w/w) NaOH (aq) or 5% (w/w) HCl (aq).

The total protein content ($P_{\text{pH } 7.0 \text{ or } 3.0}$) of the first aqueous solution is determined according to example 1.5.

The second aqueous solution is stored for 2 h at room temperature and subsequently centrifuged at 3000 g for 5 minutes. A sample of the supernatant is recovered and analysed according to Example 1.5 to give the protein concentration in the supernatant ($S_{\text{pH } 4.6}$).

The degree of protein denaturation, D, of the whey protein composition is calculated as:

$$D = ((P_{\text{pH } 7.0 \text{ or } 3.0} - S_{\text{pH } 4.6}) / P_{\text{pH } 7.0 \text{ or } 3.0}) * 100\%$$

EXAMPLE 1.4 DETERMINATION OF PROTEIN DENATURATION (WITH PH 4.6 ACID PRECIPITATION) USING REVERSE PHASE UPLC ANALYSIS.

BLG samples (such as non-heated reference and heated BLG beverage compositions) were diluted to 2% in MQ water. 5mL protein solution, 10mL Milli-Q, 4mL 10% acetic acid and 6mL 1.0M NaOAc are mixed and stirred for 20 minutes to allow precipitation agglomeration of denatured protein around pH 4.6. The solution is filtered through 0.22µm filter to remove agglomerates and non-native proteins.

All samples were subjected to the same degree of dilution by adding polished water.

For each sample, the same volume was loaded on an UPLC system with a UPLC column (Protein BEH C4; 300Å; 1.7 µm; 150 x 2.1 mm) and detected at 214nm.

The samples were run using the following conditions:

Buffer A: Milli-Q water, 0.1%w/w TFA

Buffer B: HPLC grade acetonitrile, 0.1%w/w TFA

Flow: 0.4ml/min

Gradient: 0-6.00 minutes 24-45%B; 6.00-6.50 minutes 45-90%B; 6.50-7.00 minutes 90%B; 7.00-7.50 minutes 90-24%B and 7.50-10.00 minutes 24%B.

The area of BLG peaks against a protein standard (Sigma L0130) was used to determine the concentration of native bLG in samples (5 level calibration curve)

Samples were diluted further and reinjected if outside linear range.

EXAMPLE 1.5: DETERMINATION TOTAL PROTEIN

The total protein content (true protein) of a sample is determined by:

1) Determining the total nitrogen of the sample following ISO 8968-1/2|IDF 020-1/2- Milk - Determination of nitrogen content - Part 1/2: Determination of nitrogen content using the Kjeldahl method.

2) Determining the non-protein nitrogen of the sample following ISO 8968-4|IDF 020-4- Milk - Determination of nitrogen content - Part 4: Determination of non-protein-nitrogen content.

3) Calculating the total amount protein as $(m_{\text{total nitrogen}} - m_{\text{non-protein-nitrogen}}) \cdot 6.38$.

5

EXAMPLE 1.6: DETERMINATION OF NON-AGGREGATED BLG, ALA, AND CMP

The content of non-aggregated alpha-lactalbumin (ALA), beta-lactoglobulin (BLG) and caseinomacropptide (CMP), respectively was analysed by HPLC analysis at 0.4mL/min. 25 microL filtered sample is injected onto 2 TSKgel3000PWxl (7.8 mm 30 cm, Tosohass, Japan) columns
10 connected in series with attached pre-column PWxl (6 mm x 4 cm, Tosohass, Japan) equilibrated in the eluent (consisting of 465g Milli-Q water, 417.3 g acetonitrile and 1mL trifluoroacetic acid) and using a UV detector at 210nm.

Quantitative determination of the contents of native alpha-lactalbumin (C_{α}), beta-
15 lactoglobulin (C_{β}), and caseinomacropptide (C_{CMP}) was performed by comparing the peak areas obtained for the corresponding standard proteins with those of the samples.

The total amount of additional protein (non-BLG protein) was determined by subtracting the amount of BLG from the amount of total protein (determined according to Example 1.5)

20

EXAMPLE 1.7: DETERMINATION OF TURBIDITY

Turbidity is the cloudiness or haziness of a fluid caused by large number of particles that are generally invisible to the naked eye, similar to smoke in air.

Turbidity is measured in nephelometric turbidity units (NTU).

25

20mL beverages/samples were added to NTU-glass and placed in the Turbiquant® 3000 IR Turbidimeter. The NTU-value was measured after stabilisation and repeated twice.

EXAMPLE 1.8: DETERMINATION OF VISCOSITY

30 The viscosity of beverage preparations was measured using a Rheometer (Anton Paar, Physica MCR301).

3.8 mL sample was added to cup DG26.7. Samples were equilibrated to 22 °C, then pre-sheared for 30 sec. at 50 s^{-1} , followed by a 30 sec. equilibrium time and shear rate sweeps between 1 s^{-1} and 200 s^{-1} and 1 s^{-1} were performed.

35 The viscosity is presented in the unit centipoise (cP) at a shear rate of 100 s^{-1} unless otherwise stated. The higher the measured cP values, the higher the viscosity.

Alternatively, the viscosity was estimated using a Viscoman by Gilson and reported at a shear rate of about 300s^{-1}

EXAMPLE 1.9: DETERMINATION OF COLOUR

- 5 The colour was measured using a Chroma Meter (Konica Minolta, CR-400). 15 g sample was added to a small petri dish (55x14.2mm, VWR Cat# 391-0895) avoiding bubble formation. The protein content of the samples was standardised to 6.0w/w% protein or less.
- The Chroma Meter was calibrated to a white calibration plate (No. 19033177). The illuminant was set to D65 and the observer to 2 degree. The color (CIELAB color space, a^* -, b^* -, L^* -value)
- 10 was measured with lids covering the suspension, as the average of three individual readings in different places of the petri dish.

Demineralised water reference has the following values:

$L^* 39.97 \pm 0.3$

$a^* 0.00 \pm 0.06$

- 15 $b^* -0.22 \pm 0.09$

The measurements were converted to delta/difference values based on demineralised water measurement.

$\Delta L^* = L^*_{\text{sample standardised to 6.0 w/w\% protein}} - L^*_{\text{demin. water}}$, measured at room temperature.

$\Delta a^* = a^*_{\text{sample standardised to 6.0 w/w\% protein}} - a^*_{\text{demin. water}}$, measured at room temperature.

- 20 $\Delta b^* = b^*_{\text{sample standardised to 6.0 w/w\% protein}} - b^*_{\text{demin. water}}$, measured at room temperature.

The samples is standardized to 6.0w/w% protein or below.

- The $L^*a^*b^*$ colour space (also referred to as the CIELAB space) is one of the uniform colour spaces defined by the International Commission on Illumination (CIE) in 1976 and
- 25 was used to quantitatively report lightness and hue (ISO 11664-4:2008(E)/CIE S 014-4/E:2007).

In this space, L^* indicates lightness (value from 0-100), the darkest black at $L^* = 0$, and the brightest white at $L^* = 100$.

- The colour channels a^* and b^* , represent true neutral grey values at $a^* = 0$ and $b^* = 0$. The
- 30 a^* axis represents the green-red component, with green in the negative direction and red in the positive direction. The b^* axis represents the blue-yellow component, with blue in the negative direction and yellow in the positive direction.

EXAMPLE 1.10 BEVERAGE STABILITY TEST/INSOLUBLE PROTEIN MATTER

- 35 Whey protein beverage compositions were considered stable if less than 15% of total protein in heated samples precipitated upon centrifugation at 3000 g for 5 minutes:

- Approx. 20 g samples were added to centrifuge tubes and centrifugated at 3000 g 5 min.

- Kjeldahl analysis of protein before centrifugation and the supernatant after centrifugation were used to quantify protein recovery See example 1.5

The loss of protein is calculated:

$$\text{Denaturation}\% = \left(\frac{P_{\text{total}} - P_{3000\text{g}}}{P_{\text{total}}} \right) * 100\%$$

5

This parameter is also sometimes referred to as the level of insoluble protein matter and can be used for analyzing both liquid and powder samples. If the sample is a powder, 10 g of the powder is suspended in 90 g demineralized water and allowed to hydrate at 22 degrees C under gentle stirring for 1 hours. Approx. 20 g of sample (e.g. liquid sample or the suspended powder sample) to centrifuge tubes and centrifugated at 3000 g 5 min. Kjeldahl analysis of protein before centrifugation (P_{total}) and the supernatant after centrifugation ($P_{3000\text{g}}$) were used to quantify protein recovery according to Example 1.5.

10

The amount of insoluble protein matter is calculated:

$$\text{percentage of insoluble protein matter} = \left(\frac{P_{\text{total}} - P_{3000\text{g}}}{P_{\text{total}}} \right) * 100\%$$

15

EXAMPLE 1.11: SENSORY EVALUATION

The heat-treated beverage preparations underwent a descriptive sensory evaluation. The beverage preparations had been subjected to heat using plate heat exchangers.

20

1 volume sample was mixed with 1 volume water and compared to non-heated whey protein isolate, lactic acid and citric acid are also used to form an attribute list prior to the final tasting session:

Category	Attributes:
Aroma	Whey, acidic (sour milk product)
Basic taste	Acid, bitter
Flavour	Whey, citric acid, lactic acid
Mouth feeling	Drying, astringency

Crackers, white tea, melon and water were used to cleanse the mouth of participants between each sample.

25

15mL test sample at ambient temperature (20-25 degrees C) was served in small cups.

Test samples were each served to 10 individuals three times in three different blocks in randomised order.

The attributes (see table above) were rated on a 15cm scale with 0 = low intensity and 15 = high intensity.

The statistical analysis was conducted in 'Panelcheck' software using a 3-way ANOVA test for multiple replicates. Samples were fixed and panel was set to random.

Bonferroni correction implying least significance difference values (pairwise comparisons of groups associated to a letter) was used to evaluate significant differences between samples.

5

EXAMPLE 1.12: DETERMINATION OF TRANSPARENCY BY IMAGING

Photographs of beverage preparations were conducted by placing samples in turbidity NTU measuring vials touching a piece of paper with 'lorem ipsum' text. Vials were photographed using a smartphone and the inventors evaluated whether the text could be clearly observed through the vial.

10

EXAMPLE 1.13: DETERMINATION OF ASH CONTENT

The ash content of a food product is determined according to NMKL 173:2005 "Ash, gravimetric determination in foods".

15

EXAMPLE 1.14: DETERMINATION OF CONDUCTIVITY

The "conductivity" (sometimes referred to as the "specific conductance") of an aqueous solution is a measure of the ability of the solution to conduct electricity. The conductivity may e.g. be determined by measuring the AC resistance of the solution between two electrodes and the result is typically given in the unit milliSiemens per cm (mS/cm). The conductivity may for example be measured according to the EPA (the US Environmental Protection Agency) Method No. 120.1.

20

Conductivity values mentioned herein have been normalised to 25 degrees C unless it is specified otherwise.

25

The conductivity is measured on a Conductivity meter (WTW Cond 3210 with a tetracon 325 electrode).

The system is calibrated as described in the manual before use. The electrode is rinsed thoroughly in the same type of medium as the measurement is conducted on, in order to avoid local dilutions. The electrode is lowered into the medium so that the area where the measurement occurs is completely submerged. The electrode is then agitated so that any air trapped on the electrode is removed. The electrode is then kept still until a stable value can be obtained and recorded from the display.

30

EXAMPLE 1.15: DETERMINATION OF THE TOTAL SOLIDS OF A SOLUTION

The total solids of a solution may be determined according NMKL 110 2nd Edition, 2005 (Total solids (Water) - Gravimetric determination in milk and milk products). NMKL is an abbreviation for "Nordisk Metodikkomité for Næringsmidler".

35

The water content of the solution can be calculated as 100% minus the relative amount of total solids (% w/w).

5 **EXAMPLE 1.16: DETERMINATION OF PH**

All pH values are measured using a pH glass electrode and are normalised to 25 degrees C.

The pH glass electrode (having temperature compensation) is rinsed carefully before and calibrated before use.

10 When the sample is in liquid form, then pH is measured directly in the liquid solution at 25 degrees C.

When the sample is a powder, 10 gram of a powder is dissolved in 90 ml of demineralised water at room temperature while stirring vigorously. The pH of the solution is then measured at 25 degrees C.

15 **EXAMPLE 1.17: DETERMINATION OF LOOSE DENSITY AND BULK DENSITY**

The density of a dry powder is defined as the relation between weight and volume of the powder which is analysed using a special Stampf volumeter (i.e. a measuring cylinder) under specified conditions. The density is typically expressed in g/ml or kg/L.

20 In this method, a sample of dried powder is tamped in a measuring cylinder. After a specified number of tappings, the volume of the product is read and the density is calculated.

Three types of densities can be defined by this method:

- 25
- Poured density, which is the mass divided with the volume of powder after it has been transferred to the specified measuring cylinder.
 - Loose density, which is the mass divided with the volume of powder after 100 tappings according to the specified conditions in this standard.
- 30
- Bulk density, which is the mass divided with the volume of powder after 625 tappings according to the specified conditions in this standard.

The method uses a special measuring cylinder, 250 ml, graduated 0-250 ml, weight 190 ± 15 g
35 (J. Engelsmann A. G. 67059 Ludwigshafen/Rh) and a Stampf volumeter, e.g. J. Engelsmann A. G.

The loose density and the bulk density of the dried product are determined by the following procedure.

Pre-treatment:

- 5 The sample to be measured is stored at room temperature.

The sample is then thoroughly mixed by repeatedly rotating and turning the container (avoid crushing particles). The container is not filled more than 2/3.

10 Procedure:

Weigh 100.0 ± 0.1 gram of powder and transfer it to the measuring cylinder. The volume V_0 is read in ml.

If 100 g powder does not fit into the cylinder, the amount should be reduced to 50 or 25 gram.

15

Fix the measuring cylinder to the Stampf volumeter and let it tap 100 taps. Level the surface with the spatula and read the volume V_{100} in ml.

Change the number of tabs to 625 (incl. the 100 taps). After tapping, level the surface and read the volume V_{625} in ml.

20

Calculation of densities:

Calculate the loose and the bulk densities expressed in g/ml according to the following formula:

25

$$\text{Bulk density} = M/V$$

where M designates weighed sample in grams and V designates volume after 625 tappings in ml.

30

EXAMPLE 1.18: DETERMINATION OF THE WATER CONTENT OF A POWDER

The water content of a food product is determined according to ISO 5537:2004 (Dried milk - Determination of moisture content (Reference method)). NMKL is an abbreviation for "Nordisk Metodikkomité for Næringsmidler".

EXAMPLE 1.19: DETERMINATION OF THE AMOUNTS OF CALCIUM, MAGNESIUM, SODIUM, POTASSIUM, PHOSPHORUS (ICP-MS METHOD)

The total amounts of calcium, magnesium, sodium, potassium, and phosphorus are determined using a procedure in which the samples are first decomposed using microwave digestion, and then the total amount of mineral(s) is determined using an ICP apparatus.

Apparatus:

The microwave is from Anton Paar and the ICP is an Optima 2000DV from PerkinElmer Inc.

Materials:

1 M HNO₃

Yttrium in 2% HNO₃

Suitable standards for calcium, magnesium, sodium, potassium, and phosphorus in 5% HNO₃

Pre-treatment:

Weigh out a certain amount of powder and transfer the powder to a microwave digestion tube. Add 5 mL 1M HNO₃. Digest the samples in the microwave in accordance with microwave instructions. Place the digested tubes in a fume cupboard, remove the lid and let volatile fumes evaporate.

Measurement procedure:

Transfer pre-treated sample to DigiTUBE using a known amount of Milli-Q water. Add a solution of yttrium in 2% HNO₃ to the digestion tube (about 0.25 mL per 50 mL diluted sample) and dilute to known volume using Milli-Q water. Analyse the samples on the ICP using the procedure described by the manufacturer.

A blind sample is prepared by diluting a mixture of 10 mL 1M HNO₃ and 0.5 mL solution of yttrium in 2% HNO₃ to a final volume of 100 mL using Milli-Q water.

At least 3 standard samples are prepared having concentrations which bracket the expected sample concentrations.

EXAMPLE 1.20: DETERMINATION OF THE FUROSINE-VALUE:

The furosine value is determined as described in "Maillard Reaction Evaluation by Furosine Determination During Infant Cereal Processing", Guerra-Hernandez et al, Journal of Cereal Science 29 (1999) 171–176, and the total amount of protein is determined according to Example 1.5. The furosine value is reported in the unit mg furosine per 100 g protein.

EXAMPLE 1.21: DETERMINATION OF THE CRYSTALLINITY OF BLG IN A LIQUID

The following method is used to determine the crystallinity of BLG in a liquid having a pH in the range of 5-6.

- 5 a) Transfer a 10 mL sample of the liquid in question to a Maxi-Spin filter with a 0.45 micron pore size CA membrane.
- b) Immediately spin the filter at 1500 g for 5 min. keeping the centrifuge at 2 degrees C
- c) Add 2 mL cold Milli-Q water (2 degrees C) to the retentate side of the spin filter and immediately, spin the filter at 1500 g for 5 min while keeping the centrifuge cooled at 2 degrees C,
- 10 collect the permeate (permeate A), measure the volume and determine BLG concentration via HPLC using the method outlined in Example 1.31.
- d) Add 4 mL 2M NaCl to the retentate side of the filter, agitate quickly and allow the mixture to stand for 15 minutes at 25 degrees C.
- e) Immediately spin the filter at 1500 g for 5 min and collect the permeate (permeate B)
- 15 f) Determine the total weight of BLG in permeate A and permeate B using the method outlined in Example 1.31 and convert the results to total weight of BLG instead of weight percent. The weight of BLG in permeate A is referred to as $m_{\text{Permeate A}}$ and the weight of BLG in permeate B is referred to as $m_{\text{Permeate B}}$.
- g) The crystallinity of the liquid with respect to BLG is determined as:
- 20
$$\text{crystallinity} = m_{\text{Permeate B}} / (m_{\text{Permeate A}} + m_{\text{Permeate B}}) * 100\%$$

EXAMPLE 1.22: DETERMINATION OF THE CRYSTALLINITY OF BLG IN A DRY POWDER

This method is used to determine the crystallinity of BLG in a dry powder.

- 25 a) 5.0 gram of the powder sample is mixed with 20.0 gram of cold Milli-Q water (2 degrees C) and allowed to stand for 5 minute at 2 degrees C.
- b) Transfer the sample of the liquid in question to a Maxi-Spin filter with a 0.45 micron CA membrane.
- 30 c) Immediately spin the filter at 1500 g for 5 min. keeping the centrifuge at 2 degrees C
- d) Add 2 mL cold Milli-Q water (2 degrees C) to the retentate side of the spin filter and immediately, spin the filter at 1500 g for 5 min, collect the permeate (permeate A), measure the volume and determine BLG concentration via HPLC using the method outlined in Example 1.6 and convert the results to total weight of BLG instead of weight percent. The weight of BLG in permeate A is referred to as $m_{\text{permeate A}}$
- 35 f) The crystallinity of BLG in the powder is then calculated using the following formula:

$$\text{crystallinity} = \frac{m_{\text{BLG total}} - m_{\text{permeate A}}}{m_{\text{BLG total}}} * 100\%$$

where $m_{\text{BLG total}}$ is the total amount of BLG in the powder sample of step a).

If the total amount of BLG of powder sample is unknown, this may be determined by suspending another 5 g powder sample (from the same powder source) in 20.0 gram of Milli-Q water, adjusting the pH to 7.0 by addition of aqueous NaOH, allowing the mixture to stand for 1 hour at 25 degrees C under stirring, and finally determining the total amount of BLG of the powder sample using Example 1.31.

EXAMPLE 1.23: DETERMINATION OF UF PERMEATE CONDUCTIVITY

15 mL of sample is transferred to an Amicon Ultra-15 Centrifugal Filter Units with a 3 kDa cut off (3000 NMWL) and centrifugated at 4000 g for 20-30 minutes or until a sufficient volume of UF permeate for measuring conductivity is accumulated in the bottom part of the filter units. The conductivity is measured immediately after centrifugation. The sample handling and centrifugation are performed at the temperature of the source of the sample.

EXAMPLE 1.24: DETECTION OF DRIED BLG CRYSTALS IN A POWDER

The presence of dried BLG crystals in a powder can be identified the following way:

A sample of the powder to be analysed is re-suspended and gently mixed in demineralised water having a temperature of 4 degrees C in a weight ratio of 2 parts water to 1 part powder, and allowed to rehydrate for 1 hour at 4 degrees C.

The rehydrated sample is inspected by microscopy to identify presence of crystals, preferably using plan polarised light to detect birefringence.

Crystal-like matter is separated and subjected to x-ray crystallography in order verify the existence of crystal structure, and preferably also verifying that the crystal lattice (space group and unit cell dimensions) corresponds to those of a BLG crystal.

The chemical composition of the separated crystal-like matter is analysed to verify that its solids primarily consists of BLG.

EXAMPLE 1.25: DETERMINATION OF THE TOTAL AMOUNT OF LACTOSE

The total amount of lactose is determined according to ISO 5765-2:2002 (IDF 79-2: 2002)

"Dried milk, dried ice-mixes and processed cheese – Determination of lactose content – Part 2: Enzymatic method utilizing the galactose moiety of the lactose".

EXAMPLE 1.26: DETERMINATION OF THE TOTAL AMOUNT OF CARBOHYDRATE:

The amount of carbohydrate is determined by use of Sigma Aldrich Total Carbohydrate Assay Kit (Cat MAK104-1KT) in which carbohydrates are hydrolysed and converted to furfural and hydroxyfurfurals which are converted to a chromagen that is monitored spectrophotometrically at 490nm.

EXAMPLE 1.27: DETERMINATION OF THE TOTAL AMOUNT OF LIPIDS

The amount of lipid is determined according to ISO 1211:2010 (Determination of Fat Content - Röse-Gottlieb Gravimetric Method).

EXAMPLE 1.28: DETERMINATION OF BRIX

Brix measurements were conducted using a PAL- α digital hand-held refractometer (Atago) calibrated against polished water (water filtered by reverse osmosis to obtain a conductivity of at most 0.05 mS/cm).

Approx. 500 μ l of sample was transferred to the prism surface of the instrument and the measurement was started. The measured value was read and recorded

The Brix of a whey protein solution is proportional to the content of total solids (TS) and TS (%w/w) is approx. Brix * 0.85.

EXAMPLE 1.29 DETERMINATION OF LACTOFERRIN AND LACTOPEROXIDASE

The concentration of lactoferrin is determined by an ELISA immunoassay as outlined by Soyeurt 2012 (Soyeurt et al; Mid-infrared prediction of lactoferrin content in bovine milk: potential indicator of mastitis; Animal (2012), 6:11, pp 1830–1838)

The concentration of lactoperoxidase is determined using a commercially available bovine lactoperoxidase kit.

EXAMPLE 1.30: DETERMINATION THE NUMBER OF COLONY-FORMING UNITS

The determination of the number of colony-forming units per gram sample is performed according to ISO 4833-1:2013(E): Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of microorganisms - Colony-count technique at 30°C.

EXAMPLE 1.31: DETERMINATION OF THE TOTAL AMOUNT OF BLG, ALA, AND CMP

This procedure is a liquid chromatographic (HPLC) method for the quantitative analysis of proteins such as ALA, BLG and CMP and optionally also other protein species in a composition.

Contrary to the method of Example 1.6 the present method also measures proteins that are present in aggregated and therefore provides a measure of the total amount of the protein species in the composition in question.

- 5 The mode of separation is Size Exclusion Chromatography (SEC) and the method uses 6M Guanidine HCl buffer as both sample solvent and HPLC mobile phase. Mercaptoethanol is used as a reducing agent to reduce the disulphide (S-S) in the proteins or protein aggregates to create unfolded monomeric structures.
- 10 The sample preparation is easily achieved by dissolving 10mg protein equivalent in the mobile phase.

Two TSK-GEL G3000SWXL (7.7mm x 30.0cm) columns (GPC columns) and a guard column are placed in series to achieve adequate separation of the major proteins in raw materials.

- 15 The eluted analytes are detected and quantified by UV detection (280nm).

Equipment /Materials :

1. HPLC Pump 515 with manual seal wash (Waters)
- 20 2. HPLC Pump Controller Module II (Waters)
3. Autosampler 717 (Waters)
4. Dual Absorbance Detector 2487 (Waters)
5. Computer software capable of generating quantitative reports (Empower 3, Waters)
6. Analytical column: Two TSK-GEL G3000SWXL (7.8 x 300mm, P/N: 08541).
- 25 Guard Column: TSK- Guard Column SWxL (6.0 x 40mm, P/N: 08543) .
7. Ultrasonic Bath (Branson 5200)
8. 25mm Syringe filter with 0.2 µm Cellulose Acetate membrane. (514-0060, VWR)

Procedure :

30 Mobile Phase :

A. Stock buffer solution.

1. Weigh 56.6g of Na₂HPO₄, 3.5g of NaH₂PO₄, and 2.9g of EDTA in to a 1000mL beaker.
Dissolve in 800mL of water.

2. Measure pH and adjust to 7.5 ± 0.1, if necessary, with HCl (decrease pH) or NaOH (increase pH).

- 35 3. Transfer to a 1000mL volumetric flask and dilute to volume with water.

B. 6M Guanidine HCl Mobile Phase.

1. Weigh 1146 g of Guanidine HCl in to a 2000mL beaker, and add 200mL of the stock buffer solution(A)
2. Dilute this solution to about 1600mL with water while mixing with a magnetic stir bar (50°C)
3. Adjust the pH to 7.5 ± 0.1 with NaOH.
- 5 4. Transfer into a 2000mL volumetric flask and dilute to volume with water.
5. Filter using the solvent filtration apparatus with the 0.22µm membranefilter.

Calibration Standards.

- 10 Calibration standards of each protein to be quantified are prepared the following way:
 1. Weigh accurately (to 0.01mg) about 25mg of the protein reference standard into a 10mL volumetric flask and dissolve in 10mL of water.
This is the protein stock standard solution (S1) of the protein
 - 15 2. Pipette 200 µl of S1 into a 20ml volumetric flask and dilute to volume with mobile phase.
This is the low working standard solution WS1.
 3. Pipette 500 µL of S1 into a 10mL volumetric flask and dilute to volume with mobile phase.
This is standard solution WS2.
 4. Pipette 500 µL of S1 into a 5mL volumetric flask and dilute to volume with mobile phase.
20 This is standard solution WS3.
 5. Pipette 750 µL of S1 into a 5mL volumetric flask and dilute to volume with mobile phase.
This is standard solution WS4.
 6. Pipette 1.0 mL of S1 into a 5mL volumetric flask and dilute to volume with mobile phase.
This is the high working standard solution WS5.
 - 25 7. Using graduated disposable pipettes transfer 1.5mL of WS1-5 into separate vials.
Add 10 µL of 2-mercaptoethanol to each vial and cap. Vortex the solutions for 10 sec.
Let the standards stay at ambient temperature for about 1 hr.
 8. Filter the standards using 0.22 µm Cellulose Acetate syringe filters.
- 30 The purity of protein is measured using Kjeldahl ($N \times 6.38$) and the area % from standard solution WS5 using the HPLC.

$$\text{protein (mg)} = \text{"protein standard weight" (mg)} \times P1 \times P2$$

- 35 $P1 = P\% \text{ (Kjeldahl)}$
 $P2 = \text{protein area}\% \text{ (HPLC)}$

Sample preparation

1. Weigh the equivalent of 25mg of protein of the original sample into a 25mL volumetric flask.
2. Add approximately 20mL of mobile phase and let the sample dissolve for about 30min.
3. Add mobile phase to volume and add 167 μ L of 2-mercaptoethanol to the 25ml sample solution.
4. Sonicate for about 30min and afterwards let the sample stay at ambient temperature for about 1½ hours.
5. Mix the solution and filter using 0.22 μ L Cellulose Acetate syringe filters.

10 **HPLC system/columns**

Column Equilibration

1. Connect the GPC guard column and the two GPC analytical columns in series.
- 15 New columns are generally shipped in a phosphate-salt buffer.
2. Run water through a new column gradually from 0.1 to 0.5mL/min in 30 to 60mins. Continue flushing for about 1 hour.
3. Gradually decrease flow rate from 0.5mL/min to 0.1mL/min and replace with mobile phase in the reservoir.
- 20 4. Increase pump flow rate gradually from 0.1 to 0.5mL/min in 30 to 60mins to avoid pressure shock and leave at 0.5mL/min.
5. Inject ten samples to allow the column to be saturated and wait for the peaks to elute. This will aid in the conditioning of the column.
- This step is done without the need of waiting for each injection to be complete before injecting the next.
- 25 6. Equilibrate with the mobile phase at least 1 hour.

Calculation of the results

- Quantitative determination of the contents of the proteins to be quantified, e.g. alpha-lactalbumin, beta-lactoglobulin, and caseinomacropeptide, is performed by comparing the peak areas obtained for the corresponding standard proteins with those of the samples. The results are reported as g specific protein/100 g of the original sample or weight percentage of the specific protein relative to the weight of the original sample.

35 **EXAMPLE 2: CRYSTALLIZATION OF BETA-LACTOGLOBULIN FROM A CRUDE WHEY PROTEIN CONCENTRATE**

Protocol:

Lactose depleted UF retentate derived from sweet whey from a standard cheese production process and filtered through a 1.2 micron filter was used as feed for the BLG crystallization pro-

cess. The sweet whey feed was conditioned on an ultrafiltration setup using a Koch HFK-328 type membrane with a 46 mil spacer feed pressure of 1.5-3.0 bar, using a feed concentration of 21% TS (total solids) ± 5 and polished water (water filtered by reverse osmosis to obtain a conductivity of at most 0.05 mS/cm) as diafiltration medium. The temperature of the feed and retentate during ultrafiltration was approx. 12 degrees C. The pH was then adjusted by adding HCl to obtain a pH of approx. 5.40. Diafiltration continued until the drop in conductivity of the retentate was below 0.03 mS/cm over a 20 min period. The retentate was then concentrated to approx. 30% TS (approx. 23.1% total protein relative to the total weight of the concentrated retentate). A sample of the concentrated retentate was centrifuged at 3000 g for 5 minutes but no visible pellet was formed. The supernatant was subjected to HPLC analysis. The composition of the feed is shown in Table 1.

The concentrated retentate was seeded with 0.5 g/L pure BLG crystal material obtained from a spontaneous BLG crystallization (as described in Example 3 in the context of feed 2). The seeding material was prepared by washing a BLG crystal slurry 5 times in milliQ water, collecting the BLG crystals after each wash. After washing, the BLG crystals were freeze dried, grounded up using a pestle and mortar, and then passed through a 200 micron sieve. The crystallization seeds therefore had a particle size of less than 200 micron.

The concentrated retentate was transferred to a 300L crystallization tank where it was cooled to about 4 degrees C and kept at this temperature overnight with gentle stirring. Next morning, a sample of the cooled concentrated retentate was transferred to a test tube and inspected both visually and microscopically. Rapidly sedimenting crystals had clearly formed overnight. A lab sample of the mixture comprising both crystals and mother liquor was further cooled down to 0 degrees C in an ice water bath. The mother liquor and the crystals were separated by centrifugation at 3000 g for 5 minutes, and samples of the supernatant and pellet were taken for HPLC analysis. The crystals were washed once in cold polished water and then centrifuged again before freeze-drying the pellet.

Table 1 Concentration of selected components of the feed standardized to 95% w/w total solids.

Feed standardized to 95% TS		
Protein composition (% w/w relative to total protein)		
ALA		17.7
BLG		51.6
CMP		19.5
Other components (% w/w relative to total weight of the standardized feed)		

Ca		0.357
K		0.200
Mg		0.058
Na		0.045
P		0.280
fat		5.6
protein		79

BLG relative yield quantification by HPLC:

All samples were subjected to the same degree of dilution by adding polished water. The samples were filtered through a 0.22 micron filter. For each sample, the same volume was loaded on an HPLC system with a Phenomenex Jupiter® 5 µm C4 300 Å, LC Column 250 x 4.6 mm (Part Number:00G-4167-E0) and detected at 214 nm.

The samples were run using the following conditions:

Buffer A: MilliQ water, 0.1%w/w TFA

Buffer B: HPLC grade acetonitrile, 0.085%w/w TFA

Flow: 1ml/min

Gradient: 0-30 minutes 82-55%A and 18-45%B; 30-32 minutes 55-10%A and 45-90%B; 32.5-37.5 minutes 10%A and 90%B; 38-48 minutes 10-82%A and 90-18%B.

Data treatment:

As all samples were treated in the same way, and we can directly compare the area of the BLG peaks to gain a relative yield. As the crystals only contain BLG and the samples all have been treated in the same way, the concentration of alpha-lactalbumine (ALA) and hence the area of ALA should be the same in all of the samples. Therefore the area of ALA before and after crystallization is used as a correction factor (cf) when calculating the relative yield.

$$cf_{\alpha} = \frac{\text{area of ALA}_{\text{before crystallization}}}{\text{area of ALA}_{\text{after crystallization}}}$$

The relative yield is calculated by the following equation:

$$Yield_{BLG} = \left(1 - \frac{cf_{\alpha} \times \text{area of BLG}_{\text{after crystallization}}}{\text{area of BLG}_{\text{before crystallization}}} \right) \times 100$$

Results:

From the obtained chromatograms from before and after crystallization of BLG from a sweet whey it is apparent that a large decrease in the concentration of BLG has occurred, and using the yield calculation as previously described the yield of removed BLG was determined to 64.5% w/w.

The crystal slurry was investigated by microscopy. The sample contained hexagonal crystals, many having a size considerably larger than 200 micron indicating that the observed crystals are not only the seeding crystals. The crystals easily shattered when pressed with a needle which confirmed that they were protein crystals.

A chromatogram of a washed crystal product show that BLG makes up 98.9% of the total area of the chromatogram. The purity of the BLG product can be increased even further by additional washing.

Conclusion:

This example demonstrates that surprisingly, it is possible to crystallize BLG selectively from a crude whey protein concentrate which contains more than 48% non-BLG protein relative to total protein and that the obtained BLG crystal isolate has an extremely high purity. This discovery opens up for a new approach for industrial milk protein separation, in which BLG is separated from the other protein components in a gentle way that preferably avoids extended exposure to high temperatures and problematic chemicals.

EXAMPLE 3: CRYSTALLISATION OF BLG IN THREE TYPES OF WHEY PROTEIN SOLUTIONS

Protocol:

Using the same experimental and analytical setup as in Example 2, three different types of whey protein-containing raw material were tested as feeds for crystallization. However, no seeding was used in the experiment performed with feed 2. Feed 1 and 2 were based on sweet whey and had been fat-reduced via a Synder FR membrane prior to treatment, as described in Example 2. Feed 3 was derived from an acid whey.

The composition of the three feeds can be seen below in Table 2, Table 3, and Table 4. Feed 3 was crystallized at 21% TS (total protein of 13.3% w/w relative to the total weight of the feed), a significantly lower concentration than the other two (total protein of 26.3% w/w in feed 1 and 25.0% w/w in feed 2).

The slurry of the crystallized feed 1 was centrifuged on a Maxi-Spin filter with a 0.45 micron CA membrane at 1500 g for 5 minutes. Then 2 volumes of MilliQ water were added to the filter cake before it was centrifuged again. The resulting filter cake was analyzed by HPLC. The pellet

from feed 2 was washed with 2 volumes of MilliQ water and centrifuged again under standard conditions before the pellet was analyzed by HPLC. The pellet from feed 3 was analyzed without washing.

- 5 Crystals made from feed 2 were diluted to 10%TS and pH adjusted to pH 7 using 1M NaOH to reverse the crystallization. NaCl was added to a crystal slurry from feed 2, 36%TS to reverse the crystallization.

10 *Table 2 The concentration of selected components of feed 1 (whey protein concentrate based on sweet whey). BDL= below detection limit in wet sample*

Feed 1 (standardized to 95% TS)	
Protein composition (% w/w relative to total protein)	
ALA	23.0
BLG	55.1
CMP	20.5
Other components (% w/w relative to the total weight of the standardized feed)	
Ca	0.387
K	0.290
Mg	0.066
Na	0.068
P	0.207
Fat	BDL
protein concentration	90

Table 3 The concentration of selected components of feed 2 (ALA-reduced whey protein concentrate based on sweet whey). BDL= below detection limit in wet, non-standardized sample.

Feed 2 standardized to 95% TS	
Protein composition (% w/w relative to total protein)	
ALA	12.2
BLG	70.0
CMP	17.1
Other components (% w/w relative to the total weight of the standardized feed)	
Ca	0.387
K	0.204
Mg	0.066
Na	0.051

P	0.174
fat	BDL
protein concentration	89

Table 4 The concentration of selected components of feed 3 (whey protein concentrate based on acid whey).

Feed 3 standardized to 95% TS	
Protein composition (% w/w relative to total protein)	
ALA	24.0
BLG	63.6
Other whey proteins	12.4
Other components (% w/w relative to the total weight of the standardized feed)	
Ca	0.205
K	0.051
Mg	0.013
Na	0.108
P	0.240
fat	5.1
protein concentration	79

5

Results:

Feed 1:

From chromatograms of the protein composition of the feed and the mother liquor it is evident that a large portion of BLG was recovered as crystals by the process. The yield (calculated as described in example 2) of isolated BLG is approx. 65% relative to the total amount of BLG in the feed.

10

From a microscope photo of a sample taken during the early stages of the crystallization period and a microscope photo of a sample which was taken when the crystallization had ended, it is clear that the BLG crystals are relatively fragile. Some of the crystals appear to break during stirring and are converted from hexagonal or rhombic shape to crystals fragment which still appear very compact and well-defined but have more irregular shapes.

15

From a chromatogram of the BLG crystals which was separated and washed on a spin filter it is seen that the purity is very high and the removal of other whey proteins is extremely efficient.

20

Feed 2:

From chromatograms of the protein composition of feed 2 and the obtained mother liquor, it is evident that a large portion of BLG has been removed, and the calculated yield was 82% relative to the total amount of BLG in the feed 2.

- 5 By studying feed 2 before and after crystallization, it is seen that during crystallization the feed transformed from a transparent liquid (in which the stirring magnet was visible) to a milky white, opaque liquid.

- 10 A microscope photo of the BLG crystals shows hexagonal shapes though the majority of the crystals are fractured.

A chromatogram of the isolated pellet of BLG crystals after being washed with 2 volumes of MilliQ water clearly shows that the crystals contain BLG in a very high purity.

- 15 Raising the conductivity (by adding NaCl) or altering the pH (by adjusting the pH to 7 by addition of NaOH) so that the environment no longer favours the crystalline structure gives, in both cases show that the milky white suspension turns in to a transparent liquid as the BLG crystals are dissolved.
- 20 The mineral composition of the crystal preparation obtained from feed 2 is provided in Table 5. We note that the phosphorus to protein ratio was very low, which makes the crystal preparation suitable as a protein source for patients having kidney diseases.

Table 5 The concentration of selected components in the crystal preparation obtained from feed 2.

Composition of the crystal preparation obtained from feed 2	% w/w relative to the composition standardized to 95% TS
Ca	0.119
K	0.047
Mg	0.019
Na	BDL
P	BDL (less than 0.026)
Total protein	93.4

- 25 Feed 3:

From chromatograms of the protein composition of feed 3 and the resulting mother liquor, it is evident that a large portion of BLG was isolated (a calculated yield of 70.3% relative to the total amount of BLG in the feed). If the protein content had been higher before crystallization, the obtained yield would have been even higher.

A microscope photo of the BLG crystals isolated from feed 3 (substantially free of CMP) shows that the crystals had a rectangular shape as opposed to hexagonal. The rectangular crystals seemed more robust than the hexagonal ones. For a chromatogram of the isolated crystal pellet without washing; the chromatogram clearly shows that the crystals were BLG crystals despite having a rectangular shape instead of a hexagonal shape

Table 6 The concentration of selected components of the crystal preparation obtained from feed 3.

Composition of the crystal preparation obtained from feed 3	% w/w relative to the crystal preparation standardized to 95% TS
Ca	0.103
K	BDL
Mg	0.006
Na	0.035
P	0.041
Total protein	90

The crystal preparation derived from feed 3 contained 45 mg P/100 g protein. We note that the phosphorus to protein ratio is very low, which makes the crystal preparation suitable as a protein source for patients having kidney diseases.

Conclusion:

All three feeds were suitable for the BLG crystallization process. The BLG crystals were easily dissolved by adding salt or raising the pH or the temperature. The new method makes it possible to prepare BLG preparations with very low contents of phosphorus, which makes the preparations suitable as a protein sources for patients having kidney diseases.

EXAMPLE 4: PREPARATION OF SPRAY-DRIED BLG CRYSTALS AND DETERMINATION OF BULK DENSITY

A portion of the BLG crystals produced in Example 3 (using feed 2) was separated on a decanter centrifuge at 1200 g, 5180 RPM, 110 RPM Diff. with a 64 mil spacer (mil means 1/1000 inch) and a flow of 25-30 L/h. The BLG crystal phase was then mixed 1:1 with polished water and then separated again on the decanter centrifuge using the same settings. The BLG crystal phase was then mixed with polished water in order to make it into a slurry containing approx. 25% dry-matter and having a crystallinity of BLG of approx. 80, and subsequently dried on a pilot plant spray drier with an inlet temperature of 180 degrees C and an exit temperature of 85 degrees C without any preheating. The temperature of the liquid streams until spray-drying was 10-12 degrees C. The resulting powder sampled at the exit had a water content of 4.37 % w/w.

The crystallinity of BLG in the slurry was approximately 90%.

The inventors have also successfully separated a slurry of BLG crystals and mother liquor on a decanter centrifuge at 350 g, 2750 RPM, 150 RPM Diff. with a 64 mil spacer and a flow rate of 75 L/h. The BLG crystal phase was subsequently mixed 1:2 with polished water. The BLG crystal phase was then mixed with polished water in order to make it into a thinner slurry, and subsequently dried on a pilot plant spray drier using the same parameters as described above.

The bulk density of the spray-dried powder was then measured according to Example 1.17 and compared to the bulk density of a standard WPI dried on the same equipment. The standard WPI was found to have a bulk density (based on 625 stampings) of 0.39 g/mL, which is in the high end of the normal range for a WPI powder. However, the spray-dried BLG crystal preparation had a bulk density of 0.68 g/mL, more than 75% higher than the bulk density of the standard WPI. This is truly surprising and provides a number of both logistic and application-related advantages.

Table 7 The concentration of selected components of the spray-dried BLG crystal preparation of Example 7. BDL= below detection limit

Spray dried BLG crystal powder	
Protein composition (% w/w relative to total protein)	
ALA	0.7
BLG	97.4
CMP	BDL
Other components (% w/w relative to total weight of the BLG crystal powder)	
Ca	0.118
K	0.026
Mg	0.017
Na	BDL
P	BDL
water	3.8
protein concentration	94

A sample of the spray-dried BLG crystal preparation was subsequently resuspended in cold demineralised water and BLG crystals were still clearly visible by microscopy. Addition of citric acid or NaCl caused the BLG crystals to dissolve and transformed the opaque crystal suspension into a clear liquid.

The inventors have seen indications that extended heating during the drying step reduces the amount of BLG that is in crystal form. It is therefore preferred that the heat exposure of the BLG crystal preparation is as low as possible.

5 Conclusion:

This example demonstrates that slurries comprising BLG crystals can be spray-dried and that BLG crystals are still present in the resuspended spray-dried powder if the heating during the drying step is controlled.

10 The inventors furthermore found that the bulk density of a whey protein powder that contains BLG crystals is considerably higher than that obtained by normal spray-drying of dissolved protein streams. High density powders allows for more cost-effective packaging and logistics of the powder as less packaging material is required per kg powder and more powder (mass) can be transported by a given container or truck.

15

The high density powder also appears to be easier to handle and less fluffy and dusty during manufacture and use.

EXAMPLE 5: LOW PHOSPHORUS PROTEIN BEVERAGE

20 Six low phosphorus instant beverage powders were prepared using the purified BLG product from Example 3 (the crystal preparation obtained from feed 3). All the dry ingredients were blended to obtain an instant beverage powder and then mixed with demineralized water to obtain 10 kg of each sample and allowed to hydrate for 1 hour at 10 degrees C.

Table 8 Composition of the six beverage samples.

Ingredient % w/w	Beverage sample					
	A	B	C	D	E	F
Dried, purified BLG from Ex. 3, feed 3	5.0	10.0	5.0	10.0	5.0	10.0
Citric acid	To pH 3.5	To pH 3.5	To pH 3.0	To pH 3.0	To pH 4.0	To pH 4.0
Sucrose	10.0	10.0	10.0	10.0	10	10
Demineralised water	To 100%	To 100%	To 100%	To 100%	To 100%	To 100%

25

The turbidity of the sub-samples of the six samples was measured on a Turbiquant® 3000 IR Turbidimeter and the viscosity on a vicoman by Gilson. The results are shown in the table below.

Table 9 Measured viscosity and turbidity of the six beverage samples.

Sample	viscosity (Cp)	NTU
A	1.42	36.2
B	2.37	46.3
C	2.69	4.9
D	2.70	5.0
E	1.45	63.1
F	2.25	82.1

A photo of test tubes containing sub-samples of the six low phosphorous beverage samples is shown in Figure 3. From left to right, the sub-samples were sample A, B, C, D, E, and F. The visual inspection of the test tubes verified the turbidity measurements and documented that all beverage samples were transparent and that particularly samples C and D (pH 3.0) were very clear. The low viscosities demonstrate that the beverage samples were easily drinkable.

All ingredients used for preparing the beverage were low in phosphorus and did not contain unnecessary minerals. The obtained beverages therefore had a phosphorus content of approx. 45 mg P/100 g protein and generally had a very low mineral content. The six liquid food products prepared from the instant beverage powder were therefore suitable for use as instant protein beverages for kidney disease patients.

EXAMPLE 6: CRYSTAL SEPARATION BY DYNAMIC CROSS-FLOW FILTRATION

Lactose-depleted UF retentate derived from sweet whey from a standard cheese production process, filtered through a 1.2 micron filter, was used as feed for the crystallization process. The sweet whey feed was conditioned on an ultrafiltration setup using a Koch HFK-328 type membrane with a 46 mil spacer, a feed pressure of 1.5-3.0 bar, using a feed concentration of 10% TS (total solids) ± 5 , and polished water (water filtered by reverse osmosis to obtain a conductivity of at most 0.05 mS/cm) as diafiltration medium. The temperature of the feed and retentate during ultrafiltration was approx. 12 degrees C. The pH was then adjusted by adding HCl to obtain a pH of approx. 5.60. Diafiltration continued until the conductivity of the retentate was below 1.30 mS/cm. The feed was then heated to 25 degrees C before the retentate was concentrated to approx. 27% TS (approx. 21% total protein relative to the total weight of the concentrated retentate). The permeate conductivity was 0.33 mS/cm at the end of the concentration. A sample of the concentrated retentate was centrifuged at 3000 g for 5 minutes but no visible pellet was formed.

The concentrated retentate was transferred to a 300L crystallization tank where it was cooled to about 6 degrees C and kept at this temperature overnight with gentle stirring. The next morning, the retentate had crystallized. The mother liquor and the crystals were separated by centrifugation at 3000 g for 5 minutes, and samples of the supernatant and pellet were taken for HPLC analysis. The yield of BLG from this process was calculated to be 67%.

The crystal slurry from the 300 L tank was used for a feed in an Andritz DCF 152S system using one disk membrane with a pore size of 500nm. The filtration was run at 8 degrees C, rotational speed was 32Hz, and the transmembrane pressure was 0.4 bar. The system works as a dead end filtration where retentate is built up in the filtration chamber, unlike a larger unit where the retentate would be continuously removed. The filtration was run in a stable manner for just over 40 minutes, at which point the solids, which had built up in the filtration chamber, started to influence the filtration.

The amount of crystal mass increased significantly during the DFC operation.

Conclusion: The DCF provides a stable and efficient means for separating the crystals from the ML. If needed, washing liquid could be added to the DCF.

EXAMPLE 7: DEGREE OF PROTEIN DENATURATION OF DIFFERENT WHEY PROTEIN PRODUCTS

The degree of protein denaturation of a commercial product and four BLG isolates were compared. The BLG isolates are suitable for preparing the instant beverage powder of the invention. The samples are described below.

Samples
A: BiPro (Commercially available WPI; Davisco, USA)
B: BLG crystal slurry as is - no drying (invention)
C: BLG crystal slurry freeze dried (invention)
D: BLG crystals redissolved (pH 7) and freeze-dried
E: BLG crystal slurry spray dried (invention)

Samples B-E were prepared the following way:
Crystal slurry was prepared as described in Example 6 and separated as described in Example 4. Some of the separated BLG slurry was taken out and split into four portions.

Sample B: The first portion of the separated BLG crystal slurry was re-dissolved without any drying by adjusting the pH of the BLG crystal slurry to 7.01 using a 3% NaOH; and the sample was then diluted to Brix 6 in order to make an approximately 5% protein solution.

Sample C: The second portion of the separated BLG crystal slurry was freeze-dried. The powder was then re-suspended in polished water, the pH was adjusted to 7.09 using a 3% NaOH, and the sample was then diluted to Brix 6 in order to make an approximately 5% protein solution.

- 5 Sample D: The third portion of the separated BLG crystal slurry was re-dissolved by adjusting the pH to 7.0 using a 3% NaOH, and then freeze-dried. The freeze-dried powder was then re-suspended in polished water, and the pH was measured to be 7.07. The sample was then diluted to Brix 6 in order to make an approximately 5% protein solution.
- 10 Sample E: The fourth portion of the separated BLG crystal slurry was treated and spray dried as described in Example 4. The powder was then re-suspended in polished water, and the pH was adjusted to 7.04 using a 3% NaOH. The sample was then diluted to Brix 6 in order to make an approximately 5% protein solution.
- 15 The degree of protein denaturation of each sample was determined according to Example 1.3 and the results are presented in the table below.

Table: Comparing the degree of protein denaturation of a commercially available WPI product (BiPro) with 4 BLG products which can be used in the instant beverage powder of the invention.

Sample	Total protein concentration at pH 7	Total concentration of soluble protein at pH 4.6	Degree of protein denaturation (%)
A: BiPro (Commercially available WPI)	5.11	4.54	11.15
B: BLG crystal slurry as is (no drying)	4.62	4.56	1.30
C: BLG crystal slurry freeze-dried	4.74	4.69	1.05
D: BLG crystals re-dissolved (pH 7) and freeze-dried	4.74	4.69	1.05
E: BLG crystal slurry spray-dried	4.75	4.71	0.84

Conclusion:

Regardless of the drying method, the BLG isolate have a surprisingly low degree of denatured protein; only a tenth of what can be found in the commercially available WPI used for comparison. It is particularly surprising that the spray-dried BLG crystal slurry product still has the lowest degree of denaturation of all products.

EXAMPLE 8: PRODUCTION OF A SPRAY-DRIED, ACIDIC BLG ISOLATE POWDER

Whey protein feed

Lactose-depleted UF retentate derived from sweet whey from a standard cheese production process was filtered through a 1.2 micron filter and had been fat-reduced via a Synder FR

membrane prior to being used as feed for the BLG crystallisation process. The chemical composition of the feed can be seen in Table 10. We note that all weight percentages of specific proteins, such as BLG, ALA, mentioned in this Example pertain to the weight percentage of the non-aggregated proteins relative to total protein.

5

Conditioning

The sweet whey feed was conditioned on an ultrafiltration setup at 20 degrees C, using a Koch HFK-328 type membrane (70 m² membrane) with a 46 mill spacer feed pressure 1.5-3.0 bar, to a feed concentration of 21% total solids (TS) ± 5 , and using as diafiltration medium polished
 10 water (water filtered by reverse osmosis to obtain a conductivity of at most 0.05 mS/cm). The pH was then adjusted by adding HCl so that the pH was approx. 5.5. Diafiltration continued until the drop in conductivity of the retentate was below 0.1 mS/cm over a 20 min period. The retentate was then concentrated until the permeate flow was below 1.43 L/h/m². A first sample of concentrated retentate was taken and subjected to centrifugation at 3000 g for 5 minutes.
 15 The supernatant of the first sample was used for the determination of BLG yield.

Crystallisation

The concentrated retentate was transferred to a 300L crystallisation tank where it was seeded with pure BLG crystal material made from rehydrated, spray-dried BLG crystals. Subsequently,
 20 the seeded whey protein solution was cooled from 20 degrees C to approx. 6 degrees C over approx. 10 hours to allow the BLG crystals to form and grow.

After cooling, a sample of the crystal-containing whey protein solution (the second sample) was taken and the BLG crystals were separated by centrifugation at 3000 g for 5 minutes. The su-
 25 pernatant and crystal pellets from the second sample were subjected to HPLC analysis as described below. The yield of crystallization was calculated as outlined below and determined to 57%.

Table 10 Chemical composition of the feed

Feed standardized to 95% total solids		
Protein composition % w/w of total protein		
ALA		10.2
BLG		59.6
Other proteins		30.2
Selected other components % w/w		
Ca		0.438
K		0.537

Mg		0.077
Na		0.131
P		0.200
Fat		0.220
protein concentration		87

BLG yield determination using HPLC:

- 5 The supernatants of the first and second samples were subjected to the same degree of dilution by adding polished water and the diluted supernatants were filtered through a 0.22µm filter. For each filtered and diluted supernatant the same volume was loaded on an HPLC system with a Phenomenex Jupiter® 5 µm C4 300 Å, LC Column 250 x 4.6 mm, Ea. and detected at 214nm.
- 10 The samples were run using the following conditions:
 Buffer A: MilliQ water, 0.1%w/w TFA
 Buffer B: HPLC grade acetonitrile, 0.085%w/w TFA
 Flow: 1mL/min
 Column temperature: 40 degrees C
- 15 Gradient: 0-30 minutes 82-55%A and 18-45%B; 30-32 minutes 55-10%A and 45-90%B; 32.5-37.5 minutes 10%A and 90%B; 38-48 minutes 10-82%A and 90-18%B.

Data treatment:

- As both supernatants were treated in the same way, one can directly compare the area of the
- 20 BLG peaks to calculate a relative yield. As the crystals only contain BLG and the samples all have been treated in the same way, the concentration of alpha-lactalbumin (ALA) and hence the area of ALA should be the same in all of the samples. Therefore, the area of ALA before and after crystallisation is used as a correction factor (cf) when calculating the relative yield.

$$cf_{\alpha} = \frac{\text{area of ALA}_{\text{before crystallization}}}{\text{area of ALA}_{\text{after crystallization}}}$$

25

The relative yield is calculated by the following equation:

$$Yield_{BLG} = \left(1 - \frac{cf_{\alpha} \times \text{area of BLG}_{\text{after crystallization}}}{\text{area of BLG}_{\text{before crystallization}}} \right) \times 100$$

Acid dissolution of BLC crystals

The remainder of the material from the crystallisation tank was separated using a decanter at 350 g, 2750 RPM, 150 RPM Diff. with a 64 spacer and a feed flow of 75 L/h before separation the feed was mixed 1:2 with polished water. The BLG crystal/solid phase from the decanter was then mixed with polished water in order to make it into a thinner slurry before a phosphoric acid was added to lower the pH to approx. 3.0 in order to quickly dissolve the crystals.

After dissolving the BLG crystals, the pure BLG protein liquid was concentrated to 15 Brix on the same UF setup as used to prepare the feed for crystallisation and the pH was adjusted to final pH of approx. 3.8. The liquid BLG isolate was then heated to 75 degrees for 5 minutes and subsequently cooled to 10 degrees C. The heat-treatment was found to reduce the microbial load from 137.000 CFU/g prior to the heat-treatment to <1000 CFU/g after the heat-treatment. The heat-treatment did not cause any protein denaturation and the intrinsic tryptophan fluorescence ratio (330nm/350nm) was determined to 1.20 indicating native confirmation of the BLG molecules.

The BLG was dried on a pilot plant spray drier with an inlet temperature of 180 degrees C and an exit temperature of 75 degrees C. The resulting powder sampled at the exit had a water content of approx. 4 % w/w, the chemical composition of the powder is shown in Table 11. A sample of the dried powder was dissolved and the degree of protein denaturation was determined to 1.5% and the intrinsic tryptophan fluorescence emission ratio (I330/I350) was measured to 1.20.

Table 11 The composition of the BLG isolate powder (BDL=below the detection limit)

BLG isolate powder standardized to 95% total solids		
Protein composition % w/w of total protein		
ALA		0.4
BLG		98.2
Other protein		1.4
Other selected components % w/w		
Ca		BDL
K		BDL
Mg		BDL
Na		BDL
P		0.781
fat		0.09
protein concentration		90

The bulk density (625 taps) of the spray-dried powder was estimated at 0.2-0.3 g/cm³.

Conclusion: By using the above described process, we were able to produce a high-purity BLG product that can be heat-treated with substantially no protein denaturation or protein unfolding during processing. The heat-treatment greatly lowered the bacteria levels without damaging the protein product.

The inventors have seen indications that even higher bulk density can be obtained by increasing the protein content prior to spray-drying. Also, the inventors have observed that even lower degrees of denaturation are obtained if the entry and/or exit temperature used for spray-drying are reduced.

EXAMPLE 9: PRODUCTION OF A SPRAY-DRIED, PH-NEUTRAL BLG ISOLATE POWDER

When using the same protocol and experimental setup as in Example 2, the lactose-reduced whey protein isolate shown in Table 12 was conditioned and used for feed for crystallization. The yield of crystallization was calculated to be 68%.

We note that all weight percentages of specific proteins, such as BLG and ALA, mentioned in this Example pertain to the weight percentage of the non-aggregated proteins relative to total protein.

Table 12 Composition of the feed

FEED standardized to 95% total solids		
Protein composition % w/w of total protein		
ALA		9.1
BLG		59.1
Other protein incl. CMP		31.6
Other selected components % w/w		
Ca		0.445
K		0.574
Mg		0.074
Na		0.128
P		0.211
fat		0.513
protein concentration		84

The remainder of the material from the crystallization tank was separated on a decanter at 350 g, 2750 RPM, 150 RPM Diff. with a 64 spacer and a feed flow of 75 L/h. before separation the feed was mixed 1:2 with polished water. The BLG crystal/solid phase from the decanter was then mixed with polished water in order to make it into a thinner slurry before 0.1 M potas-

sium hydroxide was added to adjust the pH to approx. 7 in order to quickly dissolve the crystals.

After dissolving the crystals, the pure BLG protein liquid was concentrated to brix 15 on a the same UF setup as used to prepare the whey protein solution for crystallization and the pH was adjusted to the final pH of 7.0. The BLG was dried on a pilot plant spray drier with an inlet temperature of 180 degrees C and an exit temperature of 75 degrees C. The resulting powder sampled at the exit had a water content of approx. 4 % w/w. The composition of the powder is shown in Table 13. After drying, some of the powder was dissolved in demineralized water and the degree of protein denaturation was determined to 9.0% and the intrinsic tryptophan fluorescence ratio (330nm/350nm) was 1.16.

Table 13 Chemical composition of the BLG isolate powder

BLG isolate powder standardized to 95% total solids		
Protein composition % w/w of total protein		
ALA		0.2
BLG		98.9
Other protein		0.9
Other selected components (% w/w)		
Ca		0.003
K		2.343
Mg		BDL
Na		BDL
P		0.629
fat		0.329
protein concentration		88

The bulk density (625 taps) of the spray-dried powder was estimated at 0.2-0.3 g/cm³.

Conclusion: By using the above described process, we are able to produce a pH-neutral, high-purity BLG product with minimum to no protein denaturation during processing. The inventors have seen indications that even higher bulk density can be obtained by increasing the protein content prior to spray-drying. Also, the inventors have observed that even lower degrees of denaturation are obtained if the entry and/or exit temperature used for spray-drying are reduced. The level of denaturation may furthermore be reduced by reducing the mineral content prior to spray-drying.

EXAMPLE 10: PREPARATION OF COATED BLG ISOLATE POWDER

Using a spray-dried BLG isolate, produced as described in example 4 or example 9, a coated BLG isolate was produced in a fluid bed (DIOSNA, MINILAB XP no. 365-146I). The inlet temperature was 60 degrees C. The powder temperature was between 40 and 50 degrees C for the duration of the process and the air flow was 25-35m³ per hour. The coating material was dissolved in 50 g of demineralized water and slowly injected into the fluid bed, where it was nebulized. For each batch, 500 g of the spray dried BLG isolate was used. After the coating material had been added, the drying continued until the coated BLG isolate had a moisture content of 4-5%. Using this setup a BLG isolate coated with 25 g of citric acid and a BLG isolate coated with 30 g of trisodium citrate was produced.

Test samples were prepared as shown in the table and analyzed with regard to solubility as described below. In addition to the 10 % w/w solution described, a 30 % w/w solution of BLG isolate powder were prepared and tested. The test samples were further analyzed with regard to wettability as described below. In addition the test samples were evaluated sensorically by two trained test persons according to the parameters set out in example 1.11.

Using the BLG isolate powder from example 8, a powder coated with lecithin was produced in the same fluid bed as above. 500 g of powder was added to the fluid bed inlet temperature was 75 degrees C and the air flow was 25m³/h. When the powder temperature reached 38 degrees C, 50 mL of water was added slowly via the nebulizer. The powder temperature was allowed to rise up to 45 degrees C, and 5 ml lecithin was injected through the nebulizer. The powder was heated to 65 degrees C and dried until it contained less than 5 % moisture.

Solubility test: The solubility and readiness of an instant powder to dissolve can be measured by the present test. 10 gram of the powder is added to 90 grams of demineralized water (8 degrees C) in a sealable transparent test tube. The mixture is shaken vigorously by hand for 30 seconds. The mixture is evaluated immediately and left to stand for 1 minute, whereafter the mixture is evaluated again. The evaluation is carried out by visual inspection of the following parameters: transparency of liquid phase, foam formation, color and to which extend the powder has dissolved.

Results

Sample #	Powder %w/w
1	10% neutral uncoated BLG isolate, pH 7.02
2	10% WPI coated with lecithin
3	10% BLG isolate coated with lecithin
4	10% BLG isolate coated with Citric acid
5	10% BLG isolate coated with trisodium citrate

6	10% standard WPI, no coating
7	30% BLG coated w. trisodium citrate
8	30% neutral BLG isolate, no coating, pH 7.02
9	30% standard WPI, no coating

#	First evaluation	After 1 minutes evaluation	Taste
1	Easily dissolved, Foam formation, no visible particles in the liquid or in the foam	Stable foam, volume increase in liquid phase, liquid phase completely clear	Whey taste: 0-1,
2	Easily dissolved, Foam formation, no visible particles in the liquid or in the foam, some yellow color	Stable foam but less foam compared to the other test mixtures. liquid phase turbid and a bit yellow	Whey taste: 7 with some undertaste
3	Easily dissolved, Foam formation, no visible particles in the liquid or in the foam, slightly yellow color	Stable foam but less foam compared to sample 2. liquid phase turbid and a bit yellow	whey taste: 1-2, with some undertaste, as #2
4	Easily dissolved, Foam formation, no visible particles in the liquid or the foam	Stable foam, liquid phase turbid but no discoloration	pleasant Whey taste: 0-1 Citric acid: 6
5	Easily dissolved, Foam formation, no visible particles in the liquid or the foam	Stable foam, liquid phase slightly turbid but no discoloration	Slight taste of minerals Whey taste: 2-3
6	Easily dissolved, Foam formation, no visible particles in the liquid or the foam, slightly yellow color	Stable foam, slightly yellow color	whey taste: 8
7	Easily dissolved, Foam formation, no visible particles in the liquid or the foam	Stable foam, liquid phase turbid but no discoloration	Not tasted

8	Easily dissolved, Foam formation, no visible particles in the liquid or the foam	Stable foam, Volume increase in liquid phase, liquid phase completely clear	Neutral but with a milky hint and a bit protein taste Whey taste: 6
9	Foam formation, some small visible parti- cles in the foam, some yellow discoloration	Stable foam, some yellow discoloration	Not tasted

Conclusion: The BLG isolate powders procured had a good agglomerated structure and easily dissolved in water. All coated BLG isolate powders (samples 1, 3-5 and 7-8) performed equal to or better than a normally coated WPI (test sample 2) in this test. When testing the 30% version it was surprisingly easy to dissolve the BLG isolates, both coated and uncoated (test samples 1, 3-5 and 7-8) and therefore it is believed that there is a possibility to go even higher than 30% in protein concentration. Both the coated and uncoated BLG isolates in 30 % solution (samples 7-8) were easily dissolved as compared with the standard WPI (sample 9), which had visible particles in the foam.

Wettability: The method is used to describe the wettability of a powder. The wettability is defined as the time it takes before the entire sample is wet. 0.5 grams of the powder is measured out and placed on the surface of 100 g of demineralized water (5 degrees C) in a cylindrical container with a diameter of 5 cm. The time from placing the powder on the surface of the water to the powder was dissolved or has passed through the water surface is measured.

Results

Sample #	Powder	Time to dissolve (minutes)	comments
10	Neutral BLG isolate, uncoated	22	The powder was not as evenly distributed over the surface and this is believed to be the reason for the slightly slower wetting
11	WPI coated with. lecithin	18	
12	Neutral BLG isolate coated with lecithin	16	
13	BLG isolate coated with Citric acid	+80	Almost no wetting occurred.

14	BLG isolate coated with. trisodium citrate	17	
15	Standard WPI	+80	After 80 minutes the test was terminated and roughly 2/3 of the WPI had been dissolved at this point

Conclusion: The coated BLG isolate powders (sample 10, 12, 14) wetted in a similar way as at normal instantized WPI (samples 11, 15) with the exception of the BLG isolate powder coated with citric acid (sample 13). It was surprising that non coated BLG isolate powder wetted much better than a standard WPI (sample 6).

EXAMPLE 11: WETTABILITY OF UNCOATED BLG ISOLATES

In the present example, the wettability of an uncoated acidic BLG isolate and an uncoated neutral BLG isolate is compared with the wettability of an uncoated whey protein isolate (WPI),
The wettability is defined as the time it takes before the entire sample is wet. 0.5 grams of the powder is measured out and placed on the surface of 100 g demineralized water (10 degrees C) in a cylindrical container with a diameter of 5 cm. The time from placing the powder on the surface of the water to the powder was dissolved or has passed through the water surface is measured.

results

Sample #	Powder	Time to dissolve (minutes)	comments
1	WPI	+55	5-10% of the powder was still left on the surface after 55 minutes
2	acidic BLG isolate (pH3.7), uncoated	27	Completely wetted and dissolved
3	neutral BLG isolate (pH7), uncoated	15	Completely wetted and dissolved

Conclusion: It was surprising that uncoated BLG isolate powder (samples 2, 3) wetted much better than a standard WPI (sample 1).

EXAMPLE 12: PREPARATION OF INSTANT BEVERAGE POWDER

Preparation of an instant powder used as a nutritional supplement.

100 g of the instant powder is prepared by blending the following ingredients: 91 grams of whey protein concentrate comprising at least 85% w/w of BLG as prepared in example 4, and 4 grams of soja lecithin.

- 5 100 grams of the instant powder contains 360 Kcal with an energy distribution as follows: 2 E% from lipid, 1 E% from carbohydrate and 97 E% from protein. Food products prepared from the instant powder can be used as a protein supplement for treatment of patients with or at risk of malnutrition e.g. by mixing the instant powder with water to obtain a beverage or by adding the powder to regular meals. 10-15 grams of the instant powder is stirred into water having a
10 temperature of 15-25 degrees C. The prepared instant powder drink has a pleasant taste, colour and viscosity.

EXAMPLE 13: PREPARATION OF INSTANT BEVERAGE POWDER

Preparation of a nutritionally complete instant powder comprising BLG.

- 100 g of the instant powder is prepared by blending 18.2 grams of vegetable oil (a mixture
15 consisting of palm kernel oil, coconut oil, rapeseed oil and sunflower oil), 56.4 grams of glucose syrup, and 20 grams of whey protein concentrate comprising at least 90% w/w of BLG as prepared in example 4 and about 3 gram soja lecithin.

- Further is added: potassium citrate, sodium citrate, sodium chloride, magnesium hydrogen phosphate, potassium hydrogen phosphate, magnesium chloride, cholin chloride, calcium carbonate, calcium phosphate, sodium L-ascorbate, aroma, iron sulphate, L-ascorbin acid, zinc sulphate, magnesium citrate, DL-alpha-tocopheryl acetate, manganese sulphate, nicotin amide, D-biotin, copper sulphate, calcium-D-pantothenate, pteroyl monoglutaminic acid, sodium fluoride, DL-alpha-tocopherol, thiamin hydrochloride, pyridoxine hydrochloride, carotenoids, retinyl palmitate, riboflavin, cyanocobalamin, cholecalciferole, chrom chloride, sodium molybdenum, potassium iodide, sodium selenite and phytomenadion to obtain an instant powder having the
20 following nutritional profile: 381 µg RE vitamin A, 0.91 mg carotenoids, 3.3 µg vitamin D, 5.9 mg α-TE vitamin E, 24 µg vitamin K, 0.69 mg vitamin B1, 0.74 mg vitamin B2, 8.3 mg-NE niacin, 2.5 mg pantothenic acid, 0.79 µg vitamin B6, 123 µg folic acid, 0.98 µg vitamin B12, 24 µg biotine, 60 mg vitamin C, 156 mg cholin, 1.17 gram salt, 469 mg sodium, 705 mg potassium,
25 578 mg chloride, 371 mg calcium, 337 mg phosphorous, 107 mg magnesium, 7.4 mg iron, 5.6 mg zinc, 0.83 mg copper, 1.5 mg manganese, 0.5 g fluoride, 48 µg molybdenum, 26 µg selenium, 25 µg chrome, 61 µg iodide.

- The instant powder contains 462 Kcal/100 gram with an energy distribution as follows: 35.6 E%
35 from lipid, 48.7 E% from carbohydrate and 15.7 E% from protein. Food products prepared from the instant powder can be used as nutritionally complete food products for treatment of patients with or at risk of malnutrition e.g. by mixing the instant powder with water to obtain a drink or to be used for tube feeding. 22 gram of the instant powder is stirred into 85 ml cold

water (which has been boiled) to obtain a drink containing 100 kcal. 33 grams of the instant powder is stirred into 78 ml cold water (which has been boiled) to obtain a drink containing 150 kcal. The prepared instant powder beverage has a pleasant taste, colour and viscosity.

5 In the claims which follow and in the preceding description of the invention,
except where the context requires otherwise due to express language or necessary implication,
the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive
sense, i.e. to specify the presence of the stated features but not to preclude the presence or
addition of further features in various embodiments of the invention.

0 It is to be understood that, if any prior art publication is referred to herein, such
reference does not constitute an admission that the publication forms a part of the common
general knowledge in the art, in Australia or any other country.

CLAIMS

1. An instant beverage powder comprising at least 5% w/w beta-lactoglobulin (BLG), wherein:
i. at least 91 % w/w of the total amount of protein is comprised by BLG,
and furthermore comprising at least one additional ingredient selected from the group
consisting of vitamins, flavouring agent, colouring agent, sweeteners, antioxidants, food
acid, lipids, carbohydrate, prebiotics, probiotics, anti-foaming agents and non-whey pro-
tein.

2. The powder according to any of claim 1, wherein said powder further comprises one or more
of:

- i. a sweetener, e.g. a sugar sweetener and/or a non-sugar sweetener,
- ii. a flavoring agent,
- iii. at least one food acid, e.g. citric acid or other suitable food acids,
- iv. the sum of the amounts of Na, K, Mg, and Ca of the instant beverage is at most
10 mmol/g protein and

wherein a 10% w/w solution of the powder in demineralized water has a pH in the range
of 2-8.

3. The powder according to any one of the preceding claims, wherein said powder comprises at
least 92% w/w BLG relative to total protein, at least 93% w/w BLG relative to total protein, at
least 94% w/w BLG relative to total protein, at least 95% w/w BLG relative to total protein, at
least 96% w/w BLG relative to total protein, at least 97% w/w BLG relative to total protein, at
least 98% w/w BLG relative to total protein or at least 99% w/w BLG relative to total protein.

4. The powder according to any one of the preceding claims wherein the powder comprises a
water content in an amount of at most 6% w/w, such as at most 5% w/w, preferably at most
4% w/w, more preferably at most 3% w/w, and even more preferably at most 2% w/w.

5. The powder according to any one of the preceding claims, wherein said powder comprises:
i. at most 6% w/w water
ii. at least 15% w/w total protein relative to total solids
iii. at least 91% w/w BLG relative to total protein,
wherein said powder is a dry powder.

6. The powder according to any one of the preceding claims, wherein said powder has a bulk
density of at least 0.30 g/mL, preferably at least 0.4 g/mL, more preferably 0.5 g/mL or even
more preferably 0.5 g/mL.

7. The powder according to any one of the preceding claims, wherein the sum of ALA and caseinomacropeptide (CMP) comprises at least 40% w/w of the non-BLG protein of the powder, preferably at least 60% w/w, even more preferably at least 70% w/w, and most preferably at least 85% w/w of the non-BLG protein of the powder.

8. The powder according to any one of the preceding claims, wherein the sum of the amounts of Na, K, Mg, and Ca is at most 10 mmol/g protein

9. The powder according to any one of the preceding claims, wherein said powder has an energy content in the range of about 200-500 kcal/100 grams of powder.

10. The powder according to any one of the preceding claims, wherein the energy amount of the protein is at least 7 E%, preferably at least 25 E%, more preferably at least 30 E%, even more preferably at least 40 E%.

11. The powder according to any one of the preceding claims, wherein the contribution of energy from lipid is in the range of 0-60 E%.

12. The powder according to any one of the preceding claims, wherein the contribution of energy from carbohydrate is in the range of 0-90 E%.

13. The powder according to any one of the preceding claims, wherein said powder in a 10% w/w solution in demineralised water has a pH in the range of 2-8 at room temperature.

14. The powder according to any one of the preceding claims, wherein said powder has a degree of protein denaturation of at most 10%, preferably at most 4%, more preferably at most 1%, even more preferably at most 0.4% and even more preferably at most 0.1%.

15. The powder according to any one of the preceding claims, wherein said powder has a crystallinity of BLG of at least 20%, preferably at least 40%.

16. The powder according to any one of the preceding claims, for use in a method for the treatment of patients with or at risk of malnutrition.

17. The powder according to any one of the preceding claims, for use in a method for the treatment of kidney disease.

18. A kit comprising the powder according to any one of claims 1-17, said kit comprising

- i. a tool for measuring said powder, and
- ii. a container having a lid for opening and closing the container,

wherein said container is for mixing said powder with a liquid to form a food product, and said container is adapted for drinking the food product directly from the container.

19. A method for preparing a liquid food product according, comprising a liquid and the instant beverage powder according to any one of the claims 1-17, said method comprising

- i. Adding a powder according to any one of claims 1-17 to a liquid,
- ii. Optionally adding at least one further ingredient, and
- iii. Mixing the powder and liquid obtained to form a uniform mixture.

20. The method according to claim 19, wherein the further ingredient is selected from fruits or vegetables.

21. The method according to any one of claims 19-20, wherein the mixing is performed by shaking.

1/2

Figure 1

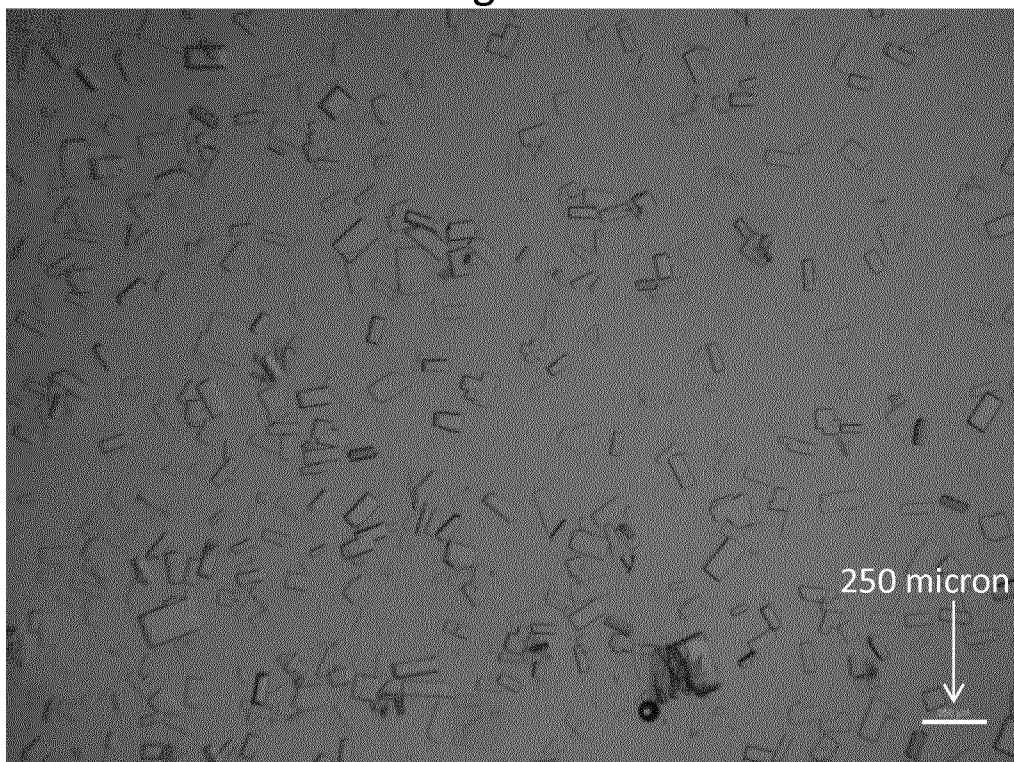
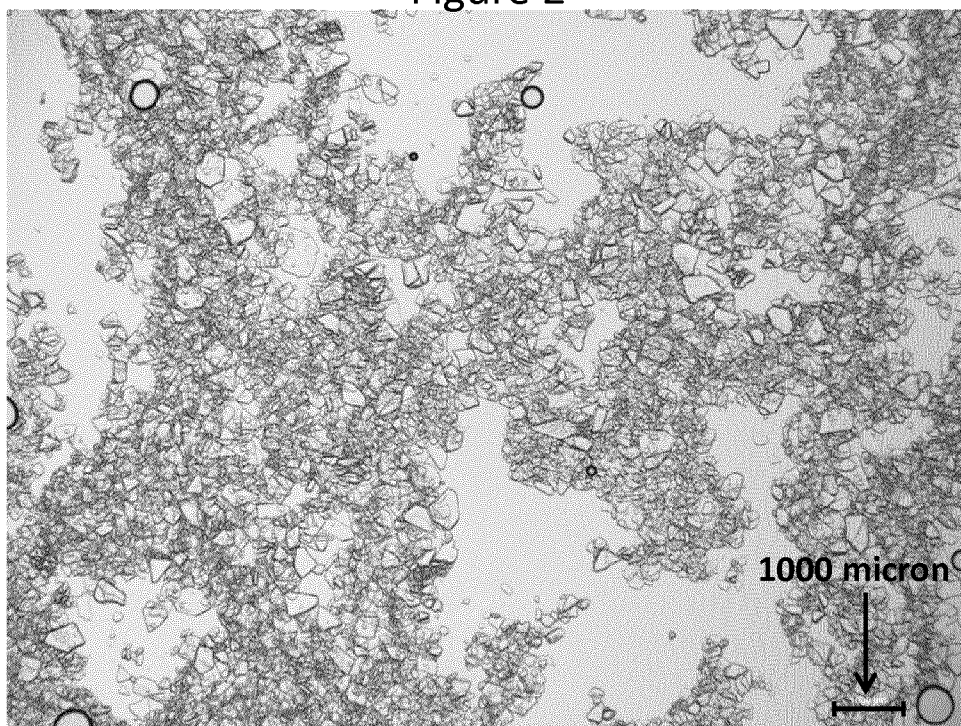


Figure 2



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Figure 3

