

PROTEIN PARTICLES COMPRISING BETA-LACTOGLOBULIN AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to protein particles comprising beta-lactoglobulin (BLG) protein, methods for preparation and use thereof.

BACKGROUND OF THE INVENTION

Various alternatives to dairy-based products have recently been introduced on the market as there is an increasing demand for such dairy-alternative or dairy-replacement products. There is a constant rush to develop dairy-alternative compositions having properties resembling traditional dairy products. It includes varying formulations as well as modulating their components.

Transglutaminase ("TG") is an enzyme that catalyzes a chemical reaction called transamidation or transglutamination. It facilitates the formation of covalent bonds between proteins or peptides by crosslinking specific amino acid residues. The reaction involves the transfer of an amine group (NH_2) from a donor molecule to an acyl group (C=O) of a glutamine residue in the acceptor molecule. This results in the formation of a covalent bond between the two molecules.

Transglutaminase can catalyze the crosslinking of various amino acids, but the primary target for transglutaminase-mediated crosslinking is the amino acid glutamine (Gln). Glutamine residues in proteins can serve as the acceptor sites for transglutaminase. However, the specific amino acid that gets crosslinked with glutamine can vary. The most common amino acid involved in the crosslinking reaction with glutamine is lysine (Lys). Lysine residues in proteins can act as the donor sites, providing the amine group (NH_2) necessary for the transamidation reaction catalyzed by transglutaminase.

Protein glutaminase ("PG") is an enzyme that catalyses the deamidation of glutamine amino acids in proteins. This modification can influence protein function, folding, and interactions. J. Wu et al. (Food chemistry 249, 136831, 2023) tested the effect of protein-glutaminase on the texture, rheology, microstructure and sensory properties of skimmed set-type yoghurt made from bovine skim milk.

There is a continuous need for the development of commercially viable methods for the preparation of alternative, non-animal dairy food products.

SUMMARY OF THE INVENTION

It has surprisingly been found that protein glutaminase (PG) can influence
5 transglutaminase (TG) activity, hypothetically through PGs' action on protein substrates. This finding contrasts with empirical data and reason, according to which two enzymes (PG and TG) utilizing the same substrate (glutamine residues in BLG) would compete for the substrate, and therefore be relatively less active when combined.

According to one aspect, the present invention provides a method for preparing a
10 population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins, and
- (ii) Enzymatically modifying BLG proteins in the composition of step (i), thus obtaining the population of protein particles comprising a BLG protein.

15 In some examples, step (ii) comprises contacting the composition of step (i) with an enzyme selected from a transglutaminase (TG), protein glutaminase (PG), a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. In some examples, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme. In some examples, step (ii) comprises contacting the composition of step (i)
20 with a protein glutaminase (PG) enzyme or a transglutaminase (TG) enzyme. In certain examples, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a corresponding population of protein particles in which BLG proteins were not enzymatically modified, e.g., at least partially polymerized or deamidated, preferably in which BLG proteins were not contacted with a PG enzyme and a
25 TG enzyme. In some examples, the characteristic is selected from the group consisting of (i) average particle size, such as increased average particle size, (ii) zeta potential, such as increased zeta potential, (iii) astringency, such as increased astringency, and (iv) any combination thereof. According to some examples, the resulting protein particles comprise covalently bound BLG proteins.

30 According to one aspect, the present invention provides a protein particle, obtainable or obtained by a method of any one of the examples of the described herein.

According to yet another aspect, the present invention provides a population of protein particles comprising a protein particle, the protein particle having at least one of the following characteristics:

- (i) comprising a plurality of non-animal BLG proteins,
- 5 (ii) having a diameter of 70 nm or above,
- (iii) having a Zeta potential of -17 mV or above, and
- (iv) optionally, comprising a protein glutaminase (PG) enzyme, and
- (v) optionally, comprising a transglutaminase (TG) enzyme.

According to some examples, the population of protein particles has a modulated
10 characteristic in comparison to a corresponding characteristic in a corresponding population of protein particles in which BLG proteins were not contacted with a PG enzyme and a TG enzyme. According to some examples, the modulated characteristic is selected (i) Average particle size, (ii) Zeta potential, (iii) Astringency, and (iv) Any combination of (i), (ii) and (iii). According to some examples, the modulated characteristic is selected from (i) Increased
15 average particle size, (ii) Increased Zeta potential, (iii) Decreased astringency, and (iv) Any combination of (i), (ii) and (iii).

According to another aspect, the present invention provides a method for preparing a gel comprising a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- 20 (i) Obtaining a composition comprising a population of protein particles by the method of any one of the examples described herein, and
- (ii) Lowering the pH of the composition of step (i), thus obtaining the gel comprising the population of the protein particle comprising BLG protein.

According to another aspect, the present invention provides a method for preparing a
25 gel comprising a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Enzymatically modifying BLG proteins in the composition of step (i), and
- (iii) Lowering the pH of the composition of step (ii), thus obtaining the gel
30 comprising the population of the protein particle comprising BLG protein.

In some examples, step (ii) comprises contacting the composition of step (i) with an enzyme selected from a transglutaminase, a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. In some examples, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme and/or a transglutaminase (TG) enzyme. In some examples the gel has a modulated characteristic in comparison to a corresponding characteristic in a corresponding gel substantially devoid of protein particles comprising covalently-bound BLG. In some examples, the modulated characteristic is selected from the group consisting of: (i) Gel strength, such as a Decreased gel strength, (ii) Gel astringency, such as a Decreased gel astringency, and (iii) any combination thereof.

According to some examples, step (iii) comprises at least one of (a) adding CaCl_2 to the composition obtained in step (ii), (b) adding an acidifier to the composition obtained in step (ii), and (c) adding lactic acid bacteria (LAB) to the composition obtained in step (ii).

According to one aspect, the present invention provides a gel, obtainable or obtained by a method of any one of the examples described herein.

According to a further aspect, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Obtaining a composition comprising a population of protein particles prepared by a method of any one of the examples described herein, and
- (ii) Formulating the composition obtained in step (i) into a dairy product.

According to another aspect, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Obtaining a gel comprising a population of protein particles prepared by a method of any one of the examples described herein, and
- (ii) Formulating the composition obtained in step (i) into a dairy product.

According to one aspect, the present invention provides a method for preparing an alternative dairy product comprising a protein particle comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Enzymatically modifying proteins in the composition of step (i),

- (iii) Optionally, lowering the pH of the composition of step (ii), and
- (iv) Formulating the composition obtained in step (ii) or in step (iii), if present, into a dairy product.

In some examples, step (ii) comprises contacting the composition of step (i) with an enzyme selected from a transglutaminase, a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. In some examples, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme or a transglutaminase (TG) enzyme. In some examples, enzymatically modified comprises enzymatically at least partially deamidated, enzymatically at least partially polymerized, or enzymatically at least partially deamidated and polymerized proteins. In some examples, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme. In some examples, the alternative dairy product has a modulated characteristic in comparison to a corresponding characteristic in a corresponding dairy product substantially devoid of modified BLG and/or protein particles comprising covalently-bound BLG. In some examples, the modulated characteristic of the alternative dairy product is selected from the group consisting of: (i) Dairy product gel strength, such as Decreased dairy product gel, (ii) Dairy product astringency, such as Decreased dairy product astringency, (iii) Average particle size, e.g. increased, (iv) Zeta potential, e.g. increased Zeta potential, (v) Viscosity, e.g. decreased Viscosity and (vi) a combination thereof. According to some examples, step (iii) comprises at least one of (a) adding CaCl_2 to the composition obtained in step (ii), (b) adding an acidifier to the composition obtained in step (ii), and (c) adding lactic acid bacteria (LAB) to the composition obtained in step (ii) and adding a stabilizer, thickener and/or texturizer to the composition obtained in step (ii). In some examples, step (iv) comprises mixing the composition obtained in step (ii) or in step (iii), if present, with a lipid, a mineral, a salt, a sugar, lactic acid bacteria (LAB), or any combination thereof. In some examples, step (iv) comprises mixing the composition obtained in step (ii) or in step (iii), if present, with a lipid, a mineral, a salt, a sugar, and LAB. In some examples, step (iv) further comprises homogenization and/or pasteurization of the alternative dairy product. In some examples, step (iv) further comprises homogenization and pasteurization of the alternative dairy product. In some examples, the alternative dairy product is selected from the group consisting of a milk composition, an alternative yogurt composition, an alternative soft cheese composition, an alternative ice cream composition, and an alternative hard cheese composition. In some examples, the alternative dairy product

is an alternative milk composition. In some examples, the alternative dairy product is an alternative cream cheese composition. In some examples, the alternative dairy product is an alternative yogurt composition. In some examples, the alternative dairy product is a non-animal alternative dairy product. In some examples, BLG protein is a recombinant BLG protein.

According to one aspect, the present invention provides a dairy product, obtainable or obtained by a method according to any one of the examples described herein

According to a further aspect, the present invention provides an alternative dairy product comprising (i) a population of protein particles, obtainable or obtained by the method of any one of examples described herein, (ii) a gel, obtainable or obtained by the method of any one of examples described herein or (iii) both (i) and (ii).

According to a further aspect, the present invention provides a population of protein particles comprising a beta-lactoglobulin (BLG) protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more, and/or (iii) comprise covalently bound BLG proteins.

According to one aspect, the present invention provides a gel, a population of protein particles comprising a beta-lactoglobulin (BLG) protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more, and/or (iii) comprise covalently bound BLG proteins.

According to a further aspect, the present invention provides a dairy product, comprising (a) a population of protein particles comprising a beta-lactoglobulin (BLG) protein or a gel comprising the population of protein particles, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more, and/or (iii) comprise covalently bound BLG proteins, (b) modified BLG comprising deamidated, polymerized or both deamidated and polymerized proteins, (c) a non-animal beta-lactoglobulin (BLG) protein, a protein glutaminase (PG) enzyme or residuals thereof, and a transglutaminase (TG) enzyme or residuals thereof, or (d) any combination of (a) – (c).

In some examples of the present invention, the dairy product comprises a protein glutaminase (PG) and transglutaminase (TG) enzymes. In some examples of the present invention, the dairy product comprises a lipid, a mineral, a salt, a sweetener, lactic acid

bacteria (LAB), stabilizer, texturizer or any combination thereof. In some examples of the present invention, the dairy product comprises a lipid, a mineral, a salt, a sugar, and LAB. In some examples, the dairy product is a homogenized and/or pasteurized dairy product. In some examples, the alternative dairy product is selected from the group consisting of a milk composition, a yogurt composition, a soft cheese composition, an ice cream composition, and a hard cheese composition. In some examples, the alternative dairy product is an alternative milk composition, an alternative yogurt composition, an alternative soft cheese composition, an alternative ice cream composition, or an alternative hard cheese composition. In some examples, the alternative dairy product is a non-animal dairy product.

10 In some examples, the alternative dairy product has a modulated characteristic in comparison to a corresponding characteristic in a corresponding dairy product substantially devoid of protein particles comprising modified BLG, e.g. covalently-bound BLG and/or devoid of deamidated BLG. In some examples, the modulated characteristic of the alternative dairy product is selected from the group consisting of (i) gel strength, such as a
15 decreased gel strength, (ii) astringency, such as a decreased dairy product astringency, (iii) average particle size such as increase in an average particle size, (iv) Zeta potential, e.g. increase in Zeta potential, (v) presence of modified BLG, e.g. polymerized and/or deamidated BLG and (vi) any combination of (i) to (v).

In some aspects and examples described herein, BLG protein is a recombinant BLG
20 protein.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows the results of gel strength measurements of gels comprising untreated BLG (“Control”), BLG treated with only TG (“FV”), or BLG treated with TG+PG (“SYG”).

Fig. 2 shows the effect of the treatment of BLG with SYG on gel strength (gram) and average
25 particle diameter (nm).

Fig. 3 shows the effect of the treatment of BLG with SYG on zeta-potential (mV) and average particle diameter (nm).

Fig. 4 shows the effect of shear stress on viscosity in an alternative yogurt without the use of SYG (dotted black line), in an alternative yogurt provided herein with the use of SYG
30 (solid black line), and in a commercial animal-based yogurt (dotted white line).

Fig. 5 shows the content of certain embodiments of alternative dairy products.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. In case of conflict, the patent specification, including definitions, will control.

Methods for obtaining protein particles

According to one aspect, the present invention provides a method for preparing a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins, and
- (ii) Modifying proteins in the composition of step (i), thus obtaining the population of protein particles comprising BLG protein.

According to some embodiments, modifying proteins comprises deamidating proteins, i.e. causing deamidation of glutamine amino acid(s) in proteins. According to some embodiments, modifying proteins comprises covalently linking different proteins, i.e. forming covalent bonds between proteins. According to some embodiments, modifying proteins comprises deamidating, e.g. glutamines and covalently linking proteins. According to some embodiments, modifying proteins comprises polymerizing proteins. Thus, according to some embodiments, the present invention provides a method for preparing a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins, and
- (ii) Polymerizing proteins in the composition of step (i), thus obtaining the population of protein particles comprising BLG protein.

According to some embodiments, modifying proteins, e.g. polymerizing proteins comprises crosslinking proteins. According to some embodiments, modifying proteins, e.g. polymerizing proteins comprises covalent polymerization of proteins. According to some embodiments, modifying proteins, e.g. polymerization, comprises enzymatic modification, e.g. enzymatic polymerization of proteins, i.e. mediated by enzymes. According to some

embodiments, modifying proteins, e.g. polymerization, comprises partial polymerization, e.g. partial enzymatic polymerization of proteins. According to some embodiments, the enzymatic polymerization of proteins comprises enzymatic crosslinking proteins. According to some embodiments, modifying proteins, e.g. polymerizing proteins, does not include protein aggregation. According to some embodiments, modifying proteins, e.g. polymerizing proteins, does not include complete protein denaturation. According to some embodiments, step (ii) further comprises deamidating proteins.

According to some embodiments, the modification of proteins is an enzymatic modification. According to some embodiments, the method comprises enzymatic polymerization of BLG proteins. Therefore, according to some embodiments, the present invention provides a method for preparing a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins, and
- (ii) Enzymatically modifying proteins in the composition of step (i), thus obtaining a population of protein particles comprising BLG protein.

In some embodiments, the modification is polymerization, thus step (ii) comprises enzymatically polymerizing proteins in the composition of step (i). In some embodiments, modification comprise deamidation of proteins. According to some embodiments, enzymatically modifying proteins comprises polymerization and deamidation of proteins.

The term "protein particles" as used herein refers to particles comprising proteins, said particles may be dispersed or suspended in the continuous phase formed by the vehicle, e.g. in aqueous solution. The particle as defined herein comprises protein(s) and optionally other components, which are combined together in accordance with the process of preparing the particle. In some embodiments, protein particles consist of proteins, e.g. consists of BLG proteins. In some embodiments, protein particles refer to particles comprising covalently bound BLG protein.

According to some embodiments, enzymatically modifying, e.g. polymerizing, proteins comprises the use of an enzyme selected from a transglutaminase (TG), protein glutaminase (PG), a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. According to some embodiments, enzymatic polymerizing proteins comprises contacting the composition of step (i) with the enzyme(s). According to some embodiments, enzymatic

polymerizing proteins comprises contacting the proteins in the composition of step (i) with the enzyme(s).

Without being bound to any theory or mechanism, according to some embodiments, the method further comprises increasing the susceptibility of BLG for polymerization.

- 5 According to some embodiments, the method comprises contacting the composition of step (i) with an enzyme capable of increasing the susceptibility of BLG for polymerization.

According to some embodiments, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme. According to some embodiments, step (ii) comprises contacting the composition of step (i) with a transglutaminase (TG) enzyme.

- 10 According to some embodiment, step (ii) comprises contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme. Thus, according to some embodiments, the present invention provides a method for preparing a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins, and
15 (ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme, thus obtaining the population of protein particles comprising a BLG proteins.

According to some embodiments, the present invention provides a method for preparing a population of protein particles comprising BLG protein, the method comprising
20 the steps of:

- (i) Providing a composition comprising BLG proteins, and
(ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme, thus obtaining the population of modified BLG proteins.

- 25 The term “beta-lactoglobulin” (BLG) refers to a beta-lactoglobulin protein that is typically present in cow's milk. As used in the present invention, the term “BLG” further refers to isoform B of BLG, i.e., beta-Lactoglobulin B (β -LG B), which is a small protein of 162 amino acids with a molecular mass of 18.2 kDa and optimum pH of 5.2 (UniProt D6QX31). Nevertheless, in some specific embodiments, the term “BLG” may refer to BLG-
30 A isoform or to a combination of BLG-A and BLG-B. According to some embodiments, BLG is a recombinant BLG. According to some embodiments, the BLG and/or the rBLG

have the amino acid sequence SEQ ID NO: 1. The term “BLG” encompasses known BLG variants, for example, known bovine BLG variants, and also analogs and chimera of the BLG. The term “analog”, “analog” and “sequence analog” are used herein interchangeably and refer to an analog of a peptide, polypeptide or protein having at least 70% sequence identity with the original peptide, wherein the analog retains the activity of the original peptide or protein. Thus, the terms “analog” and “active analog” may be used interchangeably. In some examples, the analog has at least 99%, 98%, 97%, 96% or 95% sequence identity with the original sequence. The term “analog” refers to a peptide, polypeptide or protein which contains substitutions, rearrangements, deletions, additions and/or chemical modifications in the amino acid sequence of the parent peptide. The substitutions of the amino acids may be conservative or non-conservative substitution. The non-conservative substitution encompasses substitution of one amino acid by any other amino acid. In one embodiment, the amino acid is substituted by a non-natural amino acid. The term “conservative substitution” as used herein denotes the replacement of an amino acid residue by another, without altering the overall conformation and biological activity of the peptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, shape, hydrophobic, aromatic, and the like). Amino acids with similar properties are well known in the art. For example, according to one table known in the art, the following six groups each contain amino acids that are conservative substitutions for one another: (1) Alanine (A), Serine (S), Threonine (T); (2) Aspartic acid (D), Glutamic acid (E); (3) Asparagine (N), Glutamine (Q); (4) Arginine (R), Lysine (K); (5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and (6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

According to some embodiments, the BLG protein comprises an amino acid sequence selected from SEQ ID NOs: 1-10. According to some embodiments, the term BLG also encompasses analogs thereof. According to some embodiments, the BLG analog comprises an amino acid sequence selected from SEQ ID NOs: 11-20.

According to some embodiments, the composition of step (i) comprises at least 1 wt%, at least 1.5 wt%, at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt% of BLG protein. According to some embodiments, the composition of step (i) comprises from 1 to 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or

from 3 to 4 wt% of BLG. According to some embodiments, the BLG is a recombinant BLG (rBLG). According to some embodiments, the composition of step (i) comprises from 2 to 8 wt% of BLG. According to some embodiments, the composition of step (i) comprises from 2 to 6 wt% of BLG. According to some embodiments, the composition of step (i) comprises
5 from 3 to 8 wt% of BLG. According to some embodiments, the BLG is a non-animal BLG.

The term “transglutaminase” (TG, EC 2.3.2.13) refers to enzymes capable of catalyzing an acyl transfer reaction in which a γ -carboxy-amide group of a peptide-bound glutamine residue is the acyl donor. Primary amino groups in a variety of compounds may function as acyl acceptors with the subsequent formation of monosubstituted γ -amides of
10 peptide bound glutamine. When the ϵ -amino group of a lysine residue in a peptide chain serves as the acyl acceptor, the TG form intramolecular or intermolecular γ -glutamyl- ϵ -lysyl crosslinks.

The term “protein-glutaminase” (PG; EC 3.5.1.44) refers to an enzyme that specifically catalyzes the deamidation of glutamine residues within proteins.

15 In certain embodiments, the TG and/or PG enzymes have the following characteristics: (i) their activity can be stopped by simple actions such as temperature or pH shifts, (ii) the termination of their activity is irreversible, (iii) their activity does not affect the taste and/or smell of the final product and/or does not release undesirable compounds (such as ammonia), (iv) their activity can be monitored using simple laboratory methods and equipment, (v) their
20 activity is highly specific to the substrate and does not change other components in the product, (vi) the enzymes are highly active in low concentrations, and/or (vii) the enzymes are generally known as safe (GRAS) and can be used in the food industry.

According to some embodiments, the concentration of each one of the PG and the TG enzymes in step (ii) is at least 500 ppm. According to some embodiments, the concentration
25 of each one of the PG and the TG enzyme is at least 750 ppm. According to some embodiments, the concentration of each one of the PG and the TG enzymes in step (ii) is from 500 to 3000 ppm. According to some embodiments, the unit ratio of TG to PG is from 1:10 to 10:1. According to some embodiments, the unit ratio of TG to PG is from 1:5 to 5:1. According to some embodiments, the unit ratio of TG to PG is from 1:3 to 3:1. According
30 to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:3. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:2. According to some embodiments,

the unit ratio of TG to PG is from 1:1.2 to 1:1.7. According to some embodiments, the unit ratio of TG to PG is about 1:1.5.

According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.005 to 0.7 wt%. According to some
5 embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%,
10 about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%. According to some embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments, the initial concentration of the enzyme in the TG+PG combination is TG -100 U/g and PG - 150 U/g. The unit concentration of the enzymes may be therefore calculated from these parameters based on the concentration of the TG+PG combination in the final
15 product.

According to some embodiments, contacting the composition of step (i) with the PG and the TG enzymes is performed at pH 7 or above. According to some embodiments, contacting the composition of step (i) with the PG and the TG enzyme is performed at pH 7.5 or above. According to some embodiments, contacting the composition of step (i) with
20 the PG and the TG enzymes is performed at pH 8 or above. According to some embodiments, contacting the composition of step (i) with the PG and the TG enzymes is performed at pH of from 7.5 to 9.5 or at pH of from 8 to 9 or at pH 8. It is well known that enzymes have optimal activity conditions such as temperature and pH, and that the efficacy of the enzymatic process is further correlated with time. Subsequently, by varying conditions and
25 time, it is possible to arrive at similar results by reasonably varying all these conditions, and a person skilled in the art would contemplate what are the alternative/equivalent conditions.

According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins were not enzymatically modified, e.g. polymerized.
30 According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins were not contacted with a PG enzyme and a TG enzyme

during preparation of the particles. According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins are not covalently bound. According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins are not covalently crosslinked. According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins are aggregated. According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins are not deamidated. According to some embodiments, the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a population of protein particles in which BLG proteins are not covalently crosslinked and deamidated.

According to some embodiments, the modulated characteristic comprises average particle size. According to some embodiments, the modulated characteristic comprises zeta potential. According to some embodiments, the modulated characteristic comprises astringency. According to some embodiments, the modulated characteristic is selected from the group consisting of average particle size, zeta potential, astringency, and any combination thereof. According to some embodiments, the modulated characteristic is selected from the group consisting of increased average particle size, increased average zeta potential, decreased astringency and any combination thereof. According to some embodiments, the protein particles of the present invention have at least one of the followings: an increased average particle size, an increased average zeta potential, and a decreased astringency, in comparison to corresponding protein particles.

According to some embodiments, step (ii) is performed until at least one of the following is reached: (i) the average particle size of protein particles is increased by at least 10%, (ii) the average zeta potential of the protein particles is increased by at least 5%, or (iii) the astringency of protein particles is decreased by at least 10%.

As used herein, the term "particle size" refers to the longest dimension of the particles. For spherical particles, the term "particle size" refers to the diameter of the particle. The

particle size may be determined by any known method, for example, by a laser scattering particle size distribution analyzer.

According to some embodiments, the average particle size of the protein particles obtained in step (ii) is at least 50 nm. According to some embodiments, the average particle size of the protein particles obtained in step (ii) is at least 60 nm. According to some
5 embodiments, the average particle size of the protein particles obtained in step (ii) is at least 70 nm. According to some embodiments, the average particle size of the protein particles obtained in step (ii) is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230
10 nm.

According to some embodiments, the zeta potential of the protein particles obtained in step (ii) is -17 mV or more. The term “zeta-potential” and “average zeta-potential” are used herein interchangeably and refer to the electrical potential at the interface which separates the mobile fluid from the fluid that remains attached to the surface of a particle. According
15 to some embodiments, the zeta potential of the protein particles obtained in step (ii) is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles obtained in step (ii) is from -16.5 to -7 mV.

According to some embodiments, the protein particles obtained in step (ii) have an average particle size of at least 50 nm and a zeta potential of -17 mV and more. According
20 to some embodiments, the protein particles obtained in step (ii) have an average particle size of from 50 nm to 250 nm and zeta potential for -17 mV to -5 mV.

As described above, the protein particles of the present invention comprise covalently bound proteins, specifically covalently bound BLG proteins. According to some
25 embodiments, BLG is the sole whey protein in the composition of step (i). According to some embodiments, BLG is the sole whey protein in the protein particles. According to some embodiments, BLG is the sole milk protein in the composition of step (i). According to some embodiments, BLG is the sole milk protein in the protein particles. According to some
30 embodiments, BLG is the sole protein in the composition of step (i). According to some embodiments, BLG is the sole protein in the protein particles. According to some embodiments, the protein particles essentially consist of BLG protein. According to some
embodiments, the protein particles essentially consist of BLG protein, TG and PG.

According to some embodiments, the particles comprise TG and PG proteins. According to some embodiments, the protein particles are substantially devoid of casein proteins.

According to another aspect, the present invention provides protein particles obtained or obtainable by the method according to any one of the above embodiments. All terms,
5 embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

Methods for preparation of a gel comprising protein particles

According to yet another aspect, the present invention provides a method for preparing a gel comprising a population of protein particles comprising a BLG protein. Thus, in some
10 embodiments, the present invention provides a method for preparing a gel comprising a population of protein particles comprising a BLG protein, the method comprising the steps of:

- (i) Obtaining a composition comprising a population of protein particles prepared by the method of any one of the above embodiments and aspects, and
- 15 (ii) Lowering the pH of the composition of step (i), thus obtaining the gel comprising the protein particle comprising a BLG protein. (The method is referred to herein as Method A1.)

According to some embodiments, the present invention provides a method for preparing a gel comprising a population of protein particles comprising a BLG protein, the
20 method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Modifying proteins in the composition of step (i) by forming covalent bonds between proteins and/or deamidating proteins, and
- 25 (iii) Lowering the pH of the composition of step (ii), thus obtaining the gel comprising the protein particle comprising BLG protein. (The method is referred to herein as Method A2.)

All terms, embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

According to some embodiments, modifying comprises enzymatic modification of
30 proteins, i.e. mediated by enzymes. Therefore, according to some embodiments, the present

invention provides a method for preparing a gel comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Enzymatically modifying proteins in the composition of step (i), and
- 5 (iii) Lowering the pH of the composition of step (ii), thus obtaining the gel comprising the protein particle comprising BLG protein.

According to some embodiments, enzymatically modifying proteins comprises the use of an enzyme selected from a TG, a PG, a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. According to some embodiments, enzymatic modifying
10 proteins comprises contacting the composition of step (i) with the enzyme. According to some embodiments, enzymatic modifying proteins comprises contacting the proteins in the composition of step (i) with the enzyme.

According to some embodiments, enzymatically modifying proteins comprising contacting the composition of step (i) with a TG enzyme, PG enzyme or a combination of
15 TG and PG enzymes, as described in any one of the above aspects and embodiments. According to some embodiments, modifying proteins comprises polymerizing proteins.

According to any one of the above embodiments, enzymatic modification of proteins results in the formation of a population of protein particles comprising BLG protein. According to some embodiments, the protein particles comprise covalently bound proteins.
20 According to some embodiments, the protein particles comprise covalently bound BLG proteins. In some embodiments, the term “polymerized proteins” is considered equivalent to the terms “covalently bound proteins”, “covalently-bound proteins” and “inter-bound proteins” and may be replaced by them.

According to some embodiments, the composition comprising BLG protein (e.g. the
25 composition of step (i) in Method A2) comprises at least 1 wt%, at least 1.5 wt%, at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt%, from 1 to 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or from 3 to 4 wt% BLG. According to some embodiments, BLG protein is a recombinant BLG protein.

30 According to some embodiments, the step of enzymatically modifying proteins (e.g. step (ii) in Method A2) comprises contacting the composition comprising BLG protein (step

(i) in Method A2) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme.

Thus, according to some embodiments, the present invention provides a method for preparing a gel comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme, thus obtaining the population of protein particles comprising BLG protein, and
- (iii) Lowering the pH of the composition of step (ii), thus obtaining the gel comprising the protein particle comprising BLG protein.

According to some embodiments, the concentration of each one of the PG and the TG enzymes (e.g. in step (ii) in Method A2) is at least 100 ppm. According to some embodiments, the concentration of each one of the PG and the TG enzyme is at least 750 ppm. According to some embodiments, the concentration of each one of the PG and the TG enzyme is from 500 to 3000 ppm. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.005 to 0.7 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%, about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%. According to some embodiments, the unit ratio of TG to PG is from 1:5 to 5:1, from 1:3 to 3:1, from 1:1 to 1:3, from 1:1 to 1:2, or from 1:1.2 to 1:1.7. According to some embodiments, the unit ratio of TG to PG is about 1:1.5.

According to some embodiments, contacting the composition comprising BLG protein (e.g. composition of step (ii) in Method A2) with the PG and the TG enzymes is performed at pH above 7, 7.5 or above, at pH 8 or above, at pH of from 7.5 to 9.5, at pH of from 8 to 9 or at pH 8.

According to some embodiments, the average particle size of the protein particles in the resulting gel is at least 50 nm. According to some embodiments, the average particle size of the protein particles in the gel is at least 60 nm. According to some embodiments, the average particle size of the protein particles in the gel is at least 70 nm. According to some
5 embodiments, the average particle size of the protein particles in the gel is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm.

According to some embodiments, the zeta potential of the protein particles in the gel is -17 mV or more. According to some embodiments, the zeta potential of the protein
10 particles in the gel is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles in the gel is from -16.5 to -7 mV.

According to some embodiments, the gel strength of the gel is below 170g or below 160g. According to some embodiments, the gel strength of the gel is above 70 g. According to some embodiments, the gel strength of the gel is from 70 to 160g, from 70 to 120g, or
15 from 75 to 110 g. According to some embodiments, the gel strength of the gel is from 20 to 120g.

According to some embodiments, the gel comprises protein particles having an average particle size of at least 50 nm and zeta potential of -17 mV and more and has gel strength from 20 to 160g. According to some embodiments, the gel comprises protein
20 particles having an average particle size of from 50 nm to 250 nm and zeta potential for -17 mV to -5 mV, and has a gel strength from 70 to 160g.

According to some embodiments, the gel has a modulated characteristic in comparison to a corresponding characteristic in a corresponding gel substantially devoid of protein particles comprising covalently-bound BLG. According to some embodiments, the gel has a
25 modulated characteristic in comparison to a corresponding characteristic in a corresponding gel in which BLG proteins were not contacted with a PG enzyme and a TG enzyme during the preparation of protein particles.

According to some embodiments, the modulated characteristic comprises gel strength, gel astringency, or both gel strength and gel astringency. According to some embodiments,
30 the gel has (i) a decreased gel strength in comparison to the corresponding gel, (ii) a decreased gel astringency in comparison to the corresponding gel or (iii) both (i) and (ii).

The term "corresponding" as used herein generally refers to any product which is similar to a product provided by the present invention in any way except where specified otherwise. Such corresponding product(s) may (i) be made by a method or process which are different in a specified way or step from methods or processes provided by the present invention, (ii) comprise a specified additional ingredient compared to a product provided by the present invention, (iii) be devoid of a specified ingredient compared to a product provided by the present invention, and/or (iv) have a specified different property compared to a product provided by the present invention.

According to some embodiments, the step of enzymatically modifying (e.g. polymerizing and/or deamidating) proteins (e.g. step (ii) in Method A2) is performed until at least one of the following is reached: (i) the average particle size of protein particles is increased by at least 10%, (ii) the average zeta potential of the protein particles is increased by at least 5%, or (iii) the astringency of protein particles is decreased by at least 10%.

According to some embodiments, the content of Gln residues in the BLG proteins in the gel is lower than the content of Gln residues in native BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is less than 95% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, or less than 40% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is from 0% to 60%, from 5% to 55%, from 10% to 50%, from 15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

According to any one of the above embodiments, lowering the pH comprises (i) adding an acidifier, (ii) adding lactic acid bacteria (LAB) and subsequent fermentation, or both (i) and (ii). According to some embodiments, the step of lowering the pH comprises lowering

the pH to or below the isoelectric point of BLG. According to some embodiments, lowering the pH comprises LAB fermentation. According to some embodiments, the step of lowering the pH comprises lowering the pH to or below about pH 5.2.

5 According to some embodiments, BLG is the sole whey protein in the composition of step (i) of Method A2. According to some embodiments, BLG is the sole milk protein in the composition of step (i) of Method A2. According to some embodiments, BLG is the sole protein in the composition of step (i) of Method A2.

10 According to some embodiments, BLG is the sole milk protein in the protein particles. According to some embodiments, BLG is the sole whey protein in the protein particles. According to some embodiments, BLG is the sole protein in the protein particles. According to some embodiments, the protein particles essentially consist of BLG protein. According to some embodiments, the protein particles essentially consist of BLG protein, TG and PG. According to some embodiments, the particles comprise TG and PG proteins. According to some embodiments, the protein particles are substantially devoid of casein proteins.

15 According to some embodiments, BLG is the sole milk protein in the gel. According to some embodiments, BLG is the sole whey protein in the gel. According to some embodiments, BLG is the sole protein in the gel. According to some embodiments, the gel is substantially devoid of casein proteins.

20 According to some embodiments, the step of lowering pH (step (iii) in Method A2) comprises adding CaCl_2 to the composition obtained in previous step. Thus, the step of lowering pH comprises at least one of the followings:

- (i) Adding CaCl_2 to the composition obtained in the previous step,
 - (ii) Adding an acidifier to the composition obtained in the previous step, or
 - (iii) Adding lactic acid bacteria (LAB) to the composition obtained the in previous
- 25 step.

According to a further aspect, the present invention provides a gel obtained or obtainable by the methods of the present invention as described hereinabove. All terms, embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

30

Methods for preparing alternative dairy product

According to yet another aspect, the present invention provides a method for preparing an alternative dairy product comprising modified BLG protein and/or protein particles comprising BLG protein of the present invention. Thus, according to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Obtaining a composition comprising a population of protein particles prepared by a method of any one of the above aspects and embodiments, and
- 10 (ii) Formulating the composition obtained in step (i) into a dairy product. (The method is referred to herein as Method B1.)

According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- 15 (i) Obtaining a gel comprising a population of protein particles said gel is prepared by a method of any one of the above aspects and embodiments, and
- (ii) Formulating the composition obtained in step (i) into a dairy product. (The method is referred to herein as Method B2.)

According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Modifying proteins in the composition of step (i) as described in any one of the above aspects and embodiments, and
- 25 (iii) Formulating the composition obtained in step (ii) into a dairy product. (The method is referred to herein as Method B3.)

According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- 30 (i) Providing a composition comprising BLG proteins,
- (ii) Modifying proteins in the composition of step (i),

- (iii) Lowering the pH of the composition obtained in step (ii), and
- (iv) Formulating the composition obtained in step (iii) into a dairy product. (The method is referred to herein as Method B4.)

5 All terms, embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

According to some embodiments, the modifying comprises enzymatic modification of proteins, i.e. mediated by enzymes. Therefore, according to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a population of protein particles comprising BLG protein, the method comprising the steps of:

- 10 (i) Providing a composition comprising BLG proteins,
- (ii) Enzymatically modifying proteins in the composition of step (i),
- (iii) Optionally, lowering the pH of the composition of step (ii), and
- (iv) Formulating the composition obtained in step (ii) or in step (iii), if present, into a dairy product.

15 According to some embodiments, the modification of proteins comprises polymerization of the proteins. According to some embodiments, the modification of proteins comprises deamidation of the proteins. According to some embodiments, the modification of proteins comprises polymerization and deamidation of the proteins. According to some embodiments, the modification of proteins comprises at least partial
20 polymerization and/or deamidation of the proteins.

According to any one of the above embodiments, the modification is an enzymatic modification. According to any one of the above embodiments, enzymatic modification of proteins results in the formation of a population of protein particles comprising BLG protein. According to some embodiments, the protein particles comprise covalently bound proteins.
25 According to some embodiments, the protein particles comprise covalently bound BLG proteins. According to some embodiments, enzymatic modification of proteins results in the formation of a population of deamidated BLG proteins. According to any one of the above embodiments, enzymatic modification of proteins results in the formation of a population of protein particles comprising covalently bound deamidated BLG proteins. The term
30 "deamidated" when referring to proteins, specifically to BLG protein contemplates that at least one glutamine amino acid of the protein is deamidated. Therefore, the term "deamidated

protein" contemplates also partially deamidated proteins and the term has the meaning of at least partially deamidated protein. According to some embodiments from 1% to 99%, from 5 to 95%, from 10 to 90%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60%,
 5 from 1% to 30%, 2% to 30%, from 4% to 28%, from 6% to 26%, from 8% to 24%, from 10% to 22%, from 12% to 20%, from 14% to 18%, from 20% to 50%, from 22% to 48%, from 24% to 46%, from 26% to 44%, from 28% to 42%, from 30% to 40%, from 32% to 38%, or from 34% to 36% of the glutamine amino acid of the protein are deamidated.

According to some embodiments, enzymatically polymerizing proteins comprises use
 10 of an enzyme selected from a transglutaminase, PG, a laccase, a peroxidase, a protein farnesyltransferase, and a sortase. According to some embodiments, enzymatic modifying proteins comprises contacting the composition of step (i) with the enzyme. According to some embodiments, enzymatic modifying proteins comprises contacting the proteins in the composition of step (i) with the enzyme. According to some embodiments, enzymatic
 15 modifying proteins comprises contacting proteins in the composition of step (i) with a protein glutaminase (PG) enzyme. According to some embodiments, enzymatic modifying proteins comprises contacting proteins in the composition of step (i) with a transglutaminase (TG) enzyme. According to some embodiments, enzymatic modifying proteins comprises contacting proteins in the composition of step (i) with a protein glutaminase (PG) enzyme
 20 and a transglutaminase (TG) enzyme.

According to some embodiments, the step of enzymatically modifying proteins (e.g. step (ii) in Methods B3 and B4) comprises contacting the composition comprising BLG protein (step (i) in these Methods) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme.

25 According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a protein particle comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme
 30 and a transglutaminase (TG) enzyme, thus obtaining the population of protein particles comprising BLG protein, and
- (iii) Formulating the composition obtained in step (ii) into a dairy product.

According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a protein particle comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- 5 (ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme, thus obtaining the population of modified BLG proteins, e.g. polymerized and/or deamidated BLG proteins,
- (iii) Lowering the pH of the composition obtained in step (ii), and
- (iv) Formulating the composition obtained in step (iii) into a dairy product.

10 According to some embodiments, the present invention provides a method for preparing an alternative dairy product comprising a protein particle comprising BLG protein, the method comprising the steps of:

- (i) Providing a composition comprising BLG proteins,
- (ii) Contacting the composition of step (i) with a protein glutaminase (PG) enzyme
- 15 and a transglutaminase (TG) enzyme, thus obtaining the population of protein particles comprising BLG protein,
- (iii) Lowering the pH of the composition obtained in step (ii), and
- (iv) Formulating the composition obtained in step (iii) into a dairy product.

According to some embodiments, the concentration of each one of the PG and the TG
20 enzymes in step (ii) of Methods B3 and B4 is at least 500 ppm. According to some embodiments, the concentration of each one of the PG and the TG enzyme is at least 750 ppm. According to some embodiments, the concentration of each one of the PG and the TG enzyme is from 500 to 3000 ppm. According to some embodiments, the unit ratio of TG to PG is from 1:10 to 10:1. According to some embodiments, the unit ratio of TG to PG is from
25 1:5 to 5:1. According to some embodiments, the unit ratio of TG to PG is from 1:3 to 3:1. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:3. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:2. According to some embodiments, the unit ratio of TG to PG is from 1:1.2 to 1:1.7. According to some embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments,
30 the combination of TG and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.005 to 0.7 wt%. According to some embodiments, the combination

of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%, about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%. According to some
5 embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments, the initial concentration of the enzyme in the TG+PG combination is TG 100 U/g and PG 150 U/g.

According to some embodiments, the alternative dairy product comprises at least 1
10 wt%, at least 1.5 wt%, at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt% of BLG. According to some embodiments, the alternative dairy protein comprises from 1 to 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or from 3 to 4 wt% BLG. According to some embodiments, the composition comprising BLG
15 protein (e.g. the composition of step (i) in Methods B3 and B4) comprises at least 1 wt%, at least 1.5 wt%, at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt%, from 1 to 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or from 3 to 4 wt% BLG. According to some embodiments, BLG protein is a recombinant BLG
20 protein. According to some embodiments, BLG is a recombinant BLG. According to some embodiments, the BLG and/or the rBLG have the amino acid sequence SEQ ID NO: 1. According to some embodiments, the BLG protein comprises an amino acid sequence selected from SEQ ID NOs: 1-10. According to some embodiments, the term BLG also encompasses analogs thereof. According to some embodiments, the BLG analog comprises
25 an amino acid sequence selected from SEQ ID NOs: 11-20. In some embodiments, the BLG is modified BLG protein. According to some embodiments, the BLG is polymerized and/or deamidated BLG protein.

According to some embodiments, contacting the composition comprising BLG protein (e.g. composition of step (i) in Methods B3 and B4) with the PG and the TG enzyme is
30 performed at pH above 7, 7.5 or above, at pH 8 or above, at pH of from 7.5 to 9.5, at pH of from 8 to 9 or at pH 8.

According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 50 nm. According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 60 nm. According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 70 nm. According to some embodiments, the average particle size of the protein particles in the alternative dairy product is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm.

According to some embodiments, the zeta potential of the protein particles in the alternative dairy product is -17 mV or more. According to some embodiments, the zeta potential of the protein particles in the alternative dairy product is from -17 to 20. According to some embodiments, the zeta potential of the protein particles in the alternative dairy product is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles in the alternative dairy product is from -16.5 to -7 mV. According to some embodiments, the zeta potential of the protein particles in the alternative dairy product is from 0 to 15 mV.

According to some embodiments, the gel strength of the gel in Methods B2 and B4 is below 170g or below 160g. According to some embodiments, the gel strength of the gel in Methods B2 and B4 is above 70 g. According to some embodiments, the gel strength of the gel in Methods B2 and B4 is from 70 to 160g, from 70 to 120g, or from 75 to 110 g. According to some embodiments, the gel strength of the gel in Methods B2 and B4 is from 20 to 150.

According to some embodiments, the alternative dairy product comprises protein particles having an average particle size of at least 50 nm and zeta potential of -17 mV and more and has a gel strength from 70 to 160g. According to some embodiments, the alternative dairy product comprises protein particles having an average particle size of from 50 nm to 250 nm and zeta potential for -17 mV to -5 mV, and has gel strength from 70 to 160g.

The terms "dairy product", "alternative dairy product", "dairy substitute" and "dairy alternative", are used herein interchangeably and refer to any consumable/edible product or foodstuff, which is not made from or derived from animals' milk. Such products may replace

animal-based products in one's diet by having the nutritional and/or rheologic and/or organoleptic and/or physicochemical properties of the corresponding traditional animal-milk-based products.

According to some embodiments, the alternative dairy product has a modulated
5 characteristic in comparison to a corresponding characteristic in a corresponding dairy product substantially devoid of modified BLG e.g. devoid of protein particles comprising covalently-bound BLG and/or deamidated BLG. According to some embodiments, the alternative dairy product has a modulated characteristic in comparison to a corresponding characteristic in a corresponding alternative dairy product in which BLG proteins were not
10 modified by e.g. contacting with a PG enzyme and a TG enzyme during the process of preparation of the protein particles present in the alternative dairy product. According to some embodiments, the modulated characteristic of the alternative dairy product is selected from the group consisting of (i) dairy product gel strength, (ii) dairy product astringency, (iii) viscosity, (iv) zeta potential, (v) amount of protein particles comprising BLG proteins,
15 (vi) average size of particles, and (vii) any combination of (i)-(vi).

According to some embodiments, the alternative dairy product has a decreased gel strength in comparison to the corresponding alternative dairy product. According to some embodiments, the alternative dairy product has a decreased astringency in comparison to the corresponding alternative dairy product. According to some embodiments, the alternative
20 dairy product has a decreased gel strength and a decreased astringency strength in comparison to the corresponding alternative dairy product. According to some embodiments, the alternative dairy product has a decreased viscosity in comparison to the corresponding alternative dairy product. According to some embodiments, the alternative dairy product has an increased viscosity in comparison to the corresponding alternative dairy product.
25 According to some embodiments, the alternative dairy product has an increase zeta potential in comparison to the corresponding alternative dairy product. According to some embodiments, the alternative dairy product has an increased amount of protein particles in comparison to the corresponding alternative dairy product. According to some embodiments, the average size of protein particles in the alternative dairy product is larger in comparison
30 to particles in the corresponding alternative dairy product. According to some embodiments, the average size of protein particles in the alternative dairy product is lower in comparison to particles in the corresponding alternative dairy product.

According to some embodiments, the step of enzymatically modifying proteins (e.g. step (ii) in Methods B3 and B4) is performed until at least one of the following is reached: (i) the average particle size of protein particles is increased by at least 10%, (i) the average particle size of protein particles is decreased by at least 10%, (ii) the average zeta potential of the protein particles is increased by at least 5%, or (iii) the astringency of protein particles is decreased by at least 10%, or (iv) the viscosity of the composition is reduced by 10%, or (v) the viscosity of the composition is increased by 10%.

According to any one of the above embodiments, lowering the pH, e.g. in step (iii) in Method B4, comprises: (i) adding an acidifier, (ii) adding lactic acid bacteria (LAB) and subsequent fermentation, or both (i) and (ii). According to some embodiments, the step of lowering the pH comprises lowering the pH to or below the isoelectric point of BLG. According to some embodiments, the step of lowering the pH comprises lowering the pH to or below pH 5.2. According to some embodiments, the step of lowering the pH comprises lowering the pH to or below pH 5.18. According to some embodiments, the step of lowering the pH comprises lowering the pH to or below pH 5.1.

According to some embodiments, the step of lowering pH (step (iii) in Method B4) comprises adding CaCl_2 to the composition obtained in the previous step. Thus, the step of lowering pH comprises at least one of the following:

- (i) Adding CaCl_2 to the composition obtained in the previous step,
- (ii) Adding an acidifier to the composition obtained in the previous step, or
- (iii) Adding lactic acid bacteria (LAB) to the composition obtained in the previous step.

According to some embodiments, BLG is the sole whey protein in the composition of step (i) of Methods B3 and B4. According to some embodiments, BLG is the sole milk protein in the composition of step (i) of Methods B3 and B4. According to some embodiments, BLG is the sole protein in the composition of step (i) of Methods B3 and B4. According to some embodiments, no additional milk protein is added in any steps of the method of preparation of the alternative dairy product. According to some embodiments, no additional whey protein is added in any steps of the method of preparation of the alternative dairy product. According to some embodiments, no additional protein is added in any steps of the method of preparation of the alternative dairy product.

According to some embodiments, BLG is the sole milk protein in the protein particles.
According to some embodiments, BLG is the sole whey protein in the protein particles.
According to some embodiments, BLG is the sole protein in the protein particles. According
to some embodiments, BLG is the sole milk protein in the alternative dairy product.
5 According to some embodiments, BLG is the sole whey protein in the alternative dairy
product. According to some embodiments, BLG is the sole protein in the alternative dairy
product. According to some embodiments, the alternative dairy product is substantially
devoid of casein proteins.

According to some embodiments, the step of formulating an alternative dairy product
10 (step (ii) in Methods B1 and B2, step (iii) in Method B3 and step (iv) in Method B4)
comprises mixing the composition obtained in the previous step with a lipid, a mineral, a
salt, a sugar, lactic acid bacteria (LAB), or any combination thereof. According to some
embodiments, the step of formulating the composition into an alternative dairy product (step
(ii) in Methods B1 and B2, step (iii) in Method B3 and step (iv) in Method B4) comprises
15 mixing the composition obtained in previous step with a lipid, a mineral, a salt, a sugar, and
lactic acid bacteria (LAB).

According to any one of the above embodiments, the step of formulating an
alternative dairy product comprises adding a mineral. According to some embodiments, the
mineral is a coagulation mineral salt. According to some embodiments, the coagulation
20 mineral salt is added before adding the LAB. The terms "coagulation salt", "coagulation
mineral" and "coagulation mineral salt" may be used herein interchangeably and refer to a
mineral, e.g., in the form of soluble salt, or ions thereof that initiate protein coagulation, as
known in the art. It is known that soluble salt upon dissolution disintegrates into ions forming
it. Thus, according to some embodiments, the term "salt" refers also to a dissolved
25 coagulation salt. Upon dissolution, coagulation mineral salt provides cations that initiate
coagulation. According to some embodiments, the coagulation mineral comprises one or
more salts of a mineral selected from calcium, magnesium, phosphorus, potassium,
selenium, and zinc. In some examples, the coagulation mineral is calcium or magnesium. In
some examples, the coagulation mineral salt is a calcium salt or a magnesium salt. In some
30 examples, the coagulation mineral salt is selected from calcium chloride, magnesium
chloride, and calcium lactate. According to some embodiments, the coagulation mineral salt
is calcium chloride. According to some embodiments, the method comprises adding from

about 0.0015 to about 0.35 wt% of the coagulation mineral salt. According to some embodiments, the method comprises adding from about 0.005 to about 0.25 wt%, from about 0.01 to about 0.20 wt% or from about 0.05 to about 0.2 wt% of the coagulation mineral salt, such as calcium chloride.

5 According to some embodiments, the salt is a flavoring salt. According to some embodiments, the salt is sodium chloride.

 According to some embodiments, the step of formulating an alternative dairy product comprises adding a stabilizer. According to some embodiments, the stabilizer is added in step (i). The term "stabilizer" as used herein refers to an additive to food which helps to
10 preserve its structure. Non-limiting examples of stabilizers are functional enzymatically treated potato starch such as Etenia 457, starch, Lcoust bean gum, pectin, Carrageenan, and any combination thereof. According to some embodiments, the method further comprises adding from about 0.01 to about 3 wt% of the stabilizer.

 According to any one of the above embodiments, the step of formulating an
15 alternative dairy product comprises adding a chelating agent at step (i). The terms "chelating agent", "chelating salt", "chelator", "coagulation mineral chelator" and "chelating mineral" are used herein interchangeably and refer to agents capable of chelating cations, such as divalent ions. In some embodiments, the chelating agent chelates divalent ions. In some
20 embodiments, the chelating agent is added to chelate divalent cation(s) to prevent early or spontaneous coagulation. In some examples, the chelating agent is a sodium salt. In some examples, the chelating agent is selected from sodium citrate, trisodium citrate, sodium phosphate, and sodium orthophosphate. In some examples, the salt is in its soluble form. In some other examples, the salt is in dry form. According to some embodiments, the method further comprises adding from about 0.01 to about 1 wt% of the stabilizer.

25 According to some embodiments, the step of formulating an alternative dairy product comprises adding a lipid. The term "lipid" refers to any lipid, fat and oil. According to some embodiments, the lipid is a non-animal lipid. The term "non-animal lipid" refers to any lipid, fat and oil that does not originate from an animal and/or milk. According to some
30 embodiment, the lipid is plant-derived lipid. According to some embodiments, the lipids comprise an oil. According to some embodiment, the oil is selected from shea oil, sunflower oil, coconut oil, rapeseed oil, nut oil, palm oil, kernel oil, olive oil, soya oil, cotton oil, and

cocoa butter (Theobroma oil). According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the method comprises adding from 1 to 40 wt% of the non-animal fat. According to some embodiments, the method comprises adding from 1 to 10 wt% of the non-animal fat. According to some embodiments, the method comprises adding from 10 to 40 wt%, from 15 to 35 wt% or from 20 to 30 wt% of the non-animal fat such as plant oil. According to some embodiments, the TG+PG combination is added before the addition of a lipid.

According to some embodiments, the step of formulating an alternative dairy product comprises adding a sweetener. According to some embodiments, the sweetener is sugar. According to some embodiments, sugar may be added in one step or in a plurality of steps. According to some embodiments, sugar is added in step (i). According to some embodiments, sugar is added after completion of the fermentation. The term "sugar" refers to any edible sugar, carbohydrate, or sugar substitute. According to some embodiments, the sugar is a non-animal sugar. According to some embodiment, the sugar is plant-derived sugar. According to some embodiments, the sugar is selected from a monosaccharide, disaccharide, and polysaccharide. According to some embodiments, the sugar is selected from glucose, fructose, mannose, xylose, arabinose, sucrose, dextrose, maltose, and galactose. According to some embodiments, the sugar is dextrose. According to some embodiments, the method comprises adding from 1 to 20 wt% of sugar. According to some embodiments, the method comprises adding from 1 to 5 wt% of sugar. According to some embodiments, the method comprises adding from 1 to 15 wt% of sugar. According to some embodiments, the method comprises adding from 1 to 5 wt%, or from 2 to 4 wt% 3.2% of dextrose.

According to some embodiments, the step of formulating an alternative dairy product comprises adding yeast extract. According to some embodiments, the method comprises adding from 0.005 to 0.5 wt% of yeast extract. According to some embodiments, the method comprises adding from 0.01 to 0.45 wt%, from 0.015 to 0.4 wt%, from 0.02 to 0.35 wt%,

from 0.025 to 0.3 wt%, from 0.01 to 0.08 wt%, from 0.01 to 0.07 wt%, from 0.01 to 0.06 wt%, from 0.015 to 0.05 wt%, from 0.02 to 0.04 wt%, or about 0.03 wt% of yeast extract.

According to any one of the above embodiments, the LAB is a non-pathogenic LAB capable of acidifying milk, generating flavor, texture, and any combination of the above.

- 5 According to any one of the above embodiments, the LAB is a non-pathogenic LAB capable of acidifying a dairy product, generating flavor, generating texture, and any combinations of the above. The LAB can be a mixed-strain or defined-strain cultures. In some examples, the bacteria culture is mesophilic. In some other examples, the bacteria culture is thermophilic. According to some embodiments, the LAB is selected from *Lactobacillus Bulgaricus*,
10 *Streptococcus Thermophilus*, *Streptococcus Group N1*, *Leuconostoc*, *Lactobacillus Acidophilus*, *Lactobacillus Casei*, *Lactobacillus Paracasei*, *Bifidobacterium Lactis*, *Lactococcus lactis* subsp. *Lactis*, *Leuconostoc Mesenteroides* subsp., *Lactobacillus Acidophilus*, and any combinations thereof. According to some embodiments, the LAB is *Lactobacillus Bulgaricus*. According to some embodiments, the LAB is *Streptococcus*
15 *Thermophilus*. According to some embodiments, the LAB is a combination of *Lactobacillus Bulgaricus* and *Streptococcus Thermophilus*.

- According to any one of the above embodiments, the step of formulating an alternative dairy product comprises LAB fermentation thereby obtaining a fermented dairy product. The terms "acidification" and "fermentation" refer to the process of reducing the
20 pH of the composition carried out using LAB. According to some embodiments, the alternative dairy product is allowed to ferment for at least 4.5, at least 6, at least 8, at least 12 hours or at least 16 hours. According to some embodiments, the alternative dairy product is fermented for from about 4.5 to about 48 hours. According to some embodiments, the alternative dairy product is fermented for from about 4.5 to about 24 hours. According to
25 some embodiments, the alternative dairy product is fermented for from about 6 to about 16 hours, from about 6 to about 12 hours, from about 7 to about 14 hours, or from about 8 to about 12 hours. According to some embodiments, the alternative dairy product is fermented at a temperature of from 30 to 45°C, from 34 to 42°C, from 35 to 40°C or at about 37°C. According to some embodiments, the alternative dairy product is allowed to ferment for from
30 4.5 to 48 hours at a temperature of from 30 to 45°C. According to some embodiments, the alternative dairy product is allowed to ferment for from 6 to 16 hours at a temperature of from 35 to 40°C. According to some embodiments, the alternative dairy product is allowed

to ferment for from 6 to 12 hours at a temperature of from 35 to 40°C. According to some embodiments, the method comprises fermentation for about 6, about 7, about 8, about 9, about 10, about 12, about 14 or about 16 hours at a temperature of from 35 to 40°C. According to some embodiments, the alternative dairy product is allowed to ferment until
5 the pH reaches the desired pH, e.g. from 3.9 to 4.7.

According to some embodiments, the combination of TG and PG enzymes is added before the acidification phase, e.g. before the fermentation step. According to some embodiments, adding the TG and PG enzymes combination before the fermentation step prevents obtaining a dense grainy texture of the final product.

10 According to any one of the above embodiments, the method further comprises adjusting the pH of a composition to the range of from 6.4 to 7.2 at any step before adding the LAB. According to some embodiments, the method comprises adjusting the pH of the composition to from 6.6 to about 7. According to some embodiments, the method comprises adjusting the pH of the composition to about 6.8.

15 According to some embodiments, the step of formulating an alternative dairy product comprises homogenization or pasteurization of the alternative dairy product. According to some embodiments, the step of formulating an alternative dairy product comprises homogenization and pasteurization of the alternative dairy product. According to some embodiments, the combination of TG and PG enzymes is added before the pasteurization
20 step.

The terms "homogenized" and "homogenization" refer to the process or to the product that passed the process of homogenization. Homogenization may be performed by any known method and/or device. According to some embodiments, the homogenization is performed in 1, 2, 3 or 4 stages. According to some embodiments, the homogenization is
25 performed at from about 50 to about 400 bar. According to some embodiments, homogenization is performed for from 2 to 120 minutes. According to the principles of the present invention, any homogenization stage and any homogenization pressure found to homogenize the compositions and products of the present invention are included. According to some embodiments, homogenization may be performed in two steps. Non-limiting
30 examples are stage homogenizing at 50 or 60 bar and then at 200 bar. According to some

embodiments, the composition is heated prior to homogenization, e.g. heated up to 50°C or up to 60°C or up to 70°C.

According to some embodiments, the pasteurization is performed at a temperature of less than 100°C, at times, less than 90°C; at times, less than 80°C. In some examples, during
5 pasteurization, the composition is mildly heated, typically at a temperature between 50°C and 100°C; at times at a temperature of between 50°C and 90°C, or at a temperature of between 50°C and 80°C. According to some embodiments, the composition is heated at a temperature of between 85°C and 95°C. In some examples, pasteurization is carried out at a temperature of about 90°C for several minutes. In some examples, pasteurization is carried
10 out at a temperature range of 85°C and 95°C, at times, at a temperature range of between about 87°C and 93°C. According to some embodiments, the duration of pasteurization is typically between 1 and about 10 minutes, at times, between about 2 and 9 minutes, at times between about 2 and 8 minutes, at times between about 3 and 7 minutes or at times for about 5 minutes. According to some embodiments, the pasteurization is carried out at a temperature
15 range of 80°C to 90°C for from 1 to 50 minutes. According to some embodiments, the pasteurization is followed by cooling the pasteurized composition to a temperature below 50°C, or below 40°C, or below 35°C, e.g., about 30°C. According to some embodiments, the pasteurization is followed by cooling the pasteurized composition to a temperature optimal for lactic acid bacteria growth. Thus, according to some embodiments, the
20 pasteurization is followed by cooling the pasteurized composition to from 60 to 70°C.

According to any one of the above embodiments, the alternative dairy product is selected from the group consisting of a milk composition, a yogurt composition, a soft cheese composition, an ice cream composition, and a hard cheese composition. According to some
embodiments, the alternative dairy product is a cream cheese composition. According to
25 other embodiments, the alternative dairy product is a yogurt composition.

According to some embodiments, the alternative dairy product is a non-animal alternative dairy product. The terms "non-animal" and "animal-free" refers to a product that is entirely free of animal-derived, and specifically free of milk-derived, components, such as BLG or other milk proteins. In this context, the term "milk" refers to milk from mammal
30 animals such as cow's, goat's and sheep's milk. While all components of such products are non-animal, the present invention specifically relates to products comprising at least one recombinant component or ingredient. The term "recombinant dairy ingredient" refers to any

ingredient, found in mammal dairy, that is recombinantly produced. According to one embodiment, the recombinant dairy ingredient is selected from a recombinant dairy protein, a recombinant dairy fat, and a recombinant dairy carbohydrate. According to some embodiments, the recombinant dairy ingredient is a recombinant dairy protein. According to some embodiments, the recombinant dairy protein is a recombinant whey protein. According to some embodiments, the recombinant dairy protein is BLG. According to some embodiments, the recombinant dairy protein is isoform A of BLG. According to some embodiments, the recombinant dairy protein is isoform B of BLG.

According to some embodiments, the fermentation may be carried out in the final container. According to some embodiments, the filling of the alternative dairy product into a final container is performed in aseptic conditions.

In certain embodiments, the resulting alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, a sweetener, and/or water. The term “sweetener” as used herein refers to an organic compound that is generally sweet in taste. For example, sweeteners are generally used to impart a sweet taste in edible products. A sweetener can include artificial sweeteners and natural sweeteners such as plant-derived sweeteners. A sweetener can be generally safe for consumption. A sweetener suitable for use according to the present disclosure can have a sweetness intensity that is lower, similar to or greater than that of sucrose depending on the desired sweetness in the final product. In some instances, the sweeteners have a sweetness intensity that is greater than that of sucrose. Those sweeteners can be high intensity sweeteners. Sweeteners are often classified as either nutritive (caloric) or non-nutritive (non-caloric), natural or synthetic. Examples of sweeteners include but are not limited to sucrose, dextrose, lactose, glucose, advantame, sorbitol, mannitol, liquid glucose, honey molasses, saccharin, sucralose, rebaudioside A stevia, rebaudioside M stevia, stevioside, mogroside IV, mogroside V, alitame, saccharin, neohesperidin dihydrochalcone, cyclamate, neotame, N- [3-(3-hydroxy- 4-methoxybenzyl yl) propyl] -L-a- aspartyl] -L- phenylalanine 1 -methyl ester, N- [3- (3- hydroxy-4-methoxyphenyl) -3-methylbutan yl] -L- a - aspartyl] -L- phenylalanine 1 -methyl ester, N- [3- (3- methoxy-4-hydroxyphenyl) propyl] -L- a - aspartyl] -L- phenylalanine 1 -methyl ester, curculin, cyclamate, aspartame, acesulfame potassium and others or mixtures thereof. In some embodiments, the sweetener is a sugar. In certain embodiments, the resulting alternative dairy product comprises BLG protein, and water. In certain embodiments, the

resulting alternative dairy product comprises BLG protein, PG and TG enzymes, and water. In certain embodiments, the resulting alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, and water. In certain embodiments, the resulting alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, and water.

5 In certain embodiments, the use of the TG and PG enzymes combination modulates the viscosity of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination increases the viscosity of the alternative dairy product. In certain
10 embodiments, the use of the TG and PG enzymes combination decreases the viscosity of the alternative dairy product. In certain embodiments, the viscosity of the alternative dairy product is below 500 cP. In certain embodiments, the viscosity of the alternative dairy product is below 450 cP. In certain embodiments, the viscosity of the alternative dairy product is below 200 cP. In certain embodiments, the viscosity of the alternative dairy product is below 100 cP. In certain embodiments, the viscosity of the alternative dairy product is between 70 and 130 cP. In certain embodiments, the use of the TG and PG
15 enzymes combination modulates the average particle diameter of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the average particle diameter of the alternative dairy product. In certain embodiments, the average particle diameter of the alternative dairy product is below 800 nm. In certain embodiments, the average particle diameter of the alternative dairy product is below 600 nm.
20 In certain embodiments, the average particle diameter of the alternative dairy product is 300 to 550 nm.

 In certain embodiments, the use of the TG and PG enzymes combination modulates the softness of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination modulates the firmness of the alternative dairy product. In certain
25 embodiments, the use of the TG and PG enzymes combination modulates the cohesiveness of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination modulates the consistency of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination modulates the work of cohesion of the alternative dairy product. In certain embodiments, the use of the TG and PG
30 enzymes combination modulates the average particle diameter of the alternative dairy product.

In certain embodiments, the use of the TG and PG enzymes combination increases the softness of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the firmness of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the cohesiveness of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the consistency of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the work of cohesion of the alternative dairy product. In certain embodiments, the use of the TG and PG enzymes combination decreases the average particle diameter of the alternative dairy product.

According to some embodiments, the alternative dairy product is an alternative milk.

According to some embodiments, the present invention provides a method of preparing an alternative milk, the method comprises the steps of:

- (i) Hydrating phosphate salts;
- (ii) Adding a stabilizer, such as gellan gum, followed by heating the solution to above 80 Celsius degrees and colling the resulted solution to below 60 Celsius degrees;
- (iii) Adding recombinant BLG, sucrose, calcium carbonate and NaCl;
- (iv) Adding TG and PG enzymes combination and incubating for at least 30 min;
- (v) Adding a fat; and
- (vi) Homogenizing and pasteurizing.

According to some embodiments, the phosphate salts are selected from sodium hexametaphosphate, dipotassium phosphate and monopotassium phosphate. According to some embodiments, the phosphate salts are added to the final concentration of from 0.05 to 1 wt%. According to some embodiments, the method comprises adding sodium hexametaphosphate, dipotassium phosphate and monopotassium phosphate; 0.155%, 0.8% and 0.1% respectively.

According to some embodiments, the stabilizer, such as gellan gum, was added to the final concentration of from 0.005 to 0.06 wt%, from 0.01 to 0.055 wt%, from 0.015 to 0.05 wt%, from 0.02 to 0.045 wt%, from 0.015 to 0.04 wt%, or from 0.02 to 0.03 wt% or about 0.025 wt%.

According to some embodiments, the method comprises adding from 2 wt% to 10 wt% BLG, such as rBLG. According to some embodiments, the method comprises adding about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt% of rBLG. According to some embodiments, the method comprises adding about 3
5 wt% rBLG. According to some embodiments, the method comprises adding about 8 wt% rBLG.

According to some embodiments, the method comprises adding from 0.1 to 10 wt% sucrose. According to some embodiments, the method comprises adding about 1 wt%, about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt%
10 of sucrose.

According to some embodiments, the method comprises adding from 0.05 to about 0.5 wt% of Arabic gum. According to some embodiments, the method comprises adding from 0.1 to about 0.3 wt% or about 0.2 wt% of Arabic gum.

According to some embodiments, the method comprises adding from about 0.1 to
15 about 0.5 wt% calcium carbonate, e.g. from 0.2 wt% to about 0.3 wt% (0.25%) calcium carbonate.

According to some embodiments, the method comprises adding from about 0.01 to about 0.3 NaCl, e.g. 0.01 to 0.1 wt%.

According to some embodiments, the method comprises adding TG and PG enzymes
20 combination at the concentration at least 500 ppm. According to some embodiments, the concentration of the added TG and PG enzymes combination is at least 750 ppm. According to some embodiments, the concentration of the added TG and PG enzymes combination is from 500 to 3000 ppm. According to some embodiments, the unit ratio of TG to PG is from 1:10 to 10:1. According to some embodiments, the unit ratio of TG to PG is from 1:5 to 5:1.
25 According to some embodiments, the unit ratio of TG to PG is from 1:3 to 3:1. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:3. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:2. According to some embodiments, the unit ratio of TG to PG is from 1:1.2 to 1:1.7. According to some embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments, the combination of TG
30 and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from

0.005 to 0.7 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%, about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%.

According to some embodiments, the TG and PG enzymes are incubated at a temperature of from 40 to 60°C for from 0.5 to 3 hours, e.g. for from 0.5 to 1.5 hours, e.g. for 1 hr.

According to some embodiments, the method comprises adding from 1 to 10 wt% of lipid. According to some embodiments, the method comprises adding from 1 wt% to 5 wt% of lipid. According to some embodiments, the method comprises adding about 1 wt%, about 1.5 wt%, about 2 wt%, about 2.5 wt%, about 3 wt%, about 3.5 wt%, about 4 wt%, about 4.5 wt% or about 5 wt% of lipid. According to some embodiments, the method comprises adding about 3 wt% lipid. According to some embodiments, the method comprises adding about 3.5 wt%. According to some embodiments, the method comprises adding about 4 wt%. According to some embodiment, the lipid is plant-derived lipid. According to some embodiments, the lipids comprise an oil. According to some embodiment, the oil is selected from shea oil, sunflower oil, coconut oil, rapeseed oil, nut oil, palm oil, kernel oil, olive oil, soya oil, cotton oil, and cocoa butter (Theobroma oil). According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the formulation is heated to up to 70 Celsius degrees or up to 65°C or up to 60°C before adding lipid.

According to some embodiments, the resulting alternative milk has a viscosity of from 80 to 2000 cP. According to some embodiments, the resulting alternative milk comprises from 1 to 5 wt% of rBLG and has a viscosity of from 80 to 120 cP. According to some embodiments, the resulting alternative milk comprises from 5 or above 5 wt% to 10 wt% of rBLG and has a viscosity below 2000 cP. of from 80 to 120 cP. According to some

embodiments, the resulting alternative milk comprises from 5 or above 5 wt% to 10 wt% of rBLG and has a viscosity of from 200 to 1800 cP or from 300 to 1500 cP.

According to some embodiments, the resulting alternative milk comprises from 1 to 5 wt% of rBLG and particles having an average particle size below 400 nm. According to some embodiments, the resulting alternative milk comprises from 1 to 5 wt% of rBLG and particles having an average size for from 250 to 400 nm.

According to some embodiments, the resulting alternative milk comprises from 5 or above 5 wt% to 10 wt% of rBLG and particles having an average particle size below 800 nm. According to some embodiments, the resulting alternative milk from 5 or from above 5 wt% to 10 wt% of rBLG and particles having an average particle size of from 300 to 800 nm. According to some embodiments, the resulting alternative milk from 5 wt% or from above 5 wt% to 10 wt% of rBLG and particles having an average particle size of from 400 to 700 nm. According to some embodiments, the resulting alternative milk has a viscosity and comprises particle size and defined above.

According to some embodiments, the method provides a modulated characteristic of the alternative milk in comparison to a method in which the proteins are not modified as described hereinabove, e.g. not modified by TG and PG enzymes combination. According to some embodiments, the modulated characteristic is selected from a group consisting of presence of protein particles, presence of TG or residuals thereof, presence of PG or residuals thereof, viscosity, average particle size and zeta-potential of the alternative milk. According to some embodiments, the method provides an alternative milk with a reduced average particle size in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative milk with an increased amount of protein particles in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative milk with an increased amount of TG or residuals thereof, PG or residuals thereof, or both TG and PG or residuals thereof in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative milk with an increased viscosity in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the

method provides an alternative milk with a decreased viscosity in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative milk with an increased zeta potential in comparison to an alternative milk obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the average particle size of particles is increased by at least 10%. According to some embodiments, the average particle size of particles is decreased by at least 10%. According to some embodiments, the average zeta potential is increased by at least 5%. According to some embodiments, the viscosity of the composition is reduced by 10%. According to some embodiments, the viscosity of the composition is increased by 10%.

In certain embodiments, the use of the TG and PG enzymes combination causes formation of protein particles in the alternative milk. In certain embodiments, the use of the TG and PG enzymes combination modulates the viscosity of the alternative milk. In certain embodiments, the use of the TG and PG enzymes combination increases the viscosity of the alternative milk. In certain embodiments, the use of the TG and PG enzymes combination decreases the viscosity of the alternative milk. In certain embodiments, the viscosity of the alternative milk is below 500 cP. In certain embodiments, the viscosity of the alternative milk is below 450 cP. In certain embodiments, the viscosity of the alternative milk is below 200 cP. In certain embodiments, the viscosity of the alternative milk is below 100 cP. In certain embodiments, the viscosity of the alternative milk is between 70 and 130 cP. In certain embodiments, the use of the TG and PG enzymes combination modulates the average particle diameter of the alternative milk. In certain embodiments, the use of the TG and PG enzymes combination decreases the average particle diameter of the alternative milk. In certain embodiments, the average particle diameter of the alternative milk is below 800 nm. In certain embodiments, the average particle diameter of the alternative milk is below 600 nm. In certain embodiments, the average particle diameter of the alternative milk is 300 to 550 nm.

According to some embodiments, the present invention provides a method of preparing an alternative milk, the method comprises the steps of:

- (i) Hydrating phosphate salts such as 0.5 to 1 wt% of dipotassium phosphate and 0.1 to 0.2 wt% of monopotassium phosphate;

- 5
- (ii) Adding from 0.01 to 0.03 wt% gellan gum followed by heating the solution to above 80 Celsius degrees and colling the resulted solution to below 60 Celsius degrees;
 - (iii) Adding from 2 to 5 wt% of recombinant BLG, from 1 to 3 wt% sucrose, from 0.1 to 0.4 calcium carbonate and from 0.01 to 0.08 NaCl;
 - (iv) Adding from 0.1 to 0.2 wt% of TG and PG enzymes combination and incubating for at least 60 min;
 - (v) Adding from 3 to 4 wt% of a plant fat; and
 - (vi) Homogenizing and pasteurizing.

10 According to some embodiments, the method comprises adding from 0.05 to 2 wt% of phosphates or polyphosphates. Examples of such phosphates are sodium hexametaphosphate, di-potassium phosphate and mono-potassium phosphate. According to some embodiments, the method comprises adding from 0.01 to 1 wt% of volatile Sulphur compounds scavenger such as L-cystine. According to some embodiments, the method of
15 preparing an alternative milk is as described in Example 4. According to some embodiments, the content of the resulting alternative milk is as defined in Fig. 5.

According to some embodiments, the alternative dairy product is an alternative cream cheese.

20 According to some embodiments, the present invention provides a method of preparing an alternative cream cheese, the method comprises the steps of:

- (i) Hydrating dry powders of recombinant rBLG, carbohydrates, an antioxidant, yeast extract and a stabilizer, such as locust bean gum, until full dissolution of solids;
- (ii) Adjusting pH was adjusted to 6.7-6.8;
- 25 (iii) adding a TG and PG enzymes combination for at least 30 min, followed by inactivation of the TG and PG enzymes by heating;
- (iv) Adding a lipid, optionally with elevating the temperature until the fat was fully dissolved;
- (v) Homogenizing and pasteurizing the resulted composition;
- 30 (vi) Reducing the temperature to from 20 to 37°C, adding LAB and fermenting for from 10 to 24 hours; and
- (vii) Optionally packaging and storing the resulting alternative cream cheese.

According to some embodiments, steps (iv) may be performed before step (iii).

According to some embodiments, the method comprises adding from 2 wt% to 10wt% BLG such as rBLG. According to some embodiments, the method comprises adding about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt% of rBLG. According to some embodiments, the method comprises adding about 3 wt% rBLG. According to some embodiments, the method comprises adding from 2 wt% to 4 wt% BLG such as rBLG. According to some embodiments, the method comprises adding from 5 wt% to 7 wt% BLG such as rBLG. According to some embodiments, the method comprises adding about 8 wt% rBLG.

10 According to some embodiments, the method comprises adding from 0.1 to 10 wt% dextrose. According to some embodiments, the method comprises adding from 1 to 5 wt% dextrose. According to some embodiments, the method comprises adding from 2 to 3 wt% dextrose. According to some embodiments, the method comprises adding about 1wt%, about 2wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt% of dextrose. According to some embodiments, the method comprises adding about 2.8 wt% dextrose.

According to some embodiments, the method comprises adding from 0.1 to 5 wt% of starch. According to some embodiments, the method comprises adding from 0.5 to 4 wt% of starch. According to some embodiments, the method comprises adding about 1wt%, about 2wt%, about 3 wt%, or about 4 wt% of starch.

According to some embodiments, the method comprises adding from 0.01 to about 1 wt% of an antioxidant. According to some embodiments, the method comprises adding from 0.05 to about 0.1 wt% or about 0.08 wt% of the antioxidant. According to one embodiment, the antioxidant is a trisodium citrate, e.g. 0.08% trisodium citrate

25 According to some embodiments, the method comprises adding from 0.005 to about 1 wt% of yeast extract. According to some embodiments, the method comprises adding from 0.01 to about 0.06 wt% or about 0.03 wt% of the yeast extract.

According to some embodiments, the method comprises adding from 0.05 to about 1 wt% of a stabilizer. According to some embodiments, the stabilizer is selected from locust bean gum, Etenia 457, starch, pectin, Carrageenan, and any combination thereof. According

to some embodiments, the method comprises adding from 0.1 to about 0.6 wt% or about 0.3 wt% of locust bean gum.

According to some embodiments, the pH is adjusted to 6.7-6.8 prior to adding TG PG combination. According to some embodiments, the method comprises adding TG and PG enzymes combination at the concentration at least 500 ppm. According to some
5 embodiments, the concentration of the added TG and PG enzymes combination is at least 750 ppm. According to some embodiments, the concentration of the added TG and PG enzymes combination is from 500 to 3000 ppm. According to some embodiments, the unit ratio of TG to PG is from 1:10 to 10:1. According to some embodiments, the unit ratio of
10 TG to PG is from 1:5 to 5:1. According to some embodiments, the unit ratio of TG to PG is from 1:3 to 3:1. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:3. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:2. According to some embodiments, the unit ratio of TG to PG is from 1:1.2 to 1:1.7. According to some
15 embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.005 to 0.7 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some
20 embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%, about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%. According to some embodiments, the TG and PG enzymes are incubated at a temperature of from 40 to 60 Celsius degrees for from 0.5 to 3 hours, e.g. for from 0.5 to 1.5 hours, e.g. for 1 hr.

25 According to some embodiments, the method comprises adding from 10 to 30 wt% of a lipid. According to some embodiments, the method comprises adding from 12 wt% to 28 wt% of a lipid. According to some embodiments, the method comprises adding from 15 wt% to 25 wt% of lipid. According to some embodiments, the method comprises adding from 17 wt% to 22 wt% of a lipid. According to some embodiments, the method comprises
30 adding about 20 wt% a lipid. According to some embodiment, the lipid is plant-derived lipid. According to some embodiments, the lipids comprise an oil. According to some embodiment, the oil is selected from shea oil, sunflower oil, coconut oil, rapeseed oil, nut

oil, palm oil, kernel oil, olive oil, soya oil, cotton oil, and cocoa butter (Theobroma oil). According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some
5 embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the formulation is heated to up to 70 Celsius degrees or up to 65°C or up to 60°C before, during or after adding the lipid.

10 According to some embodiments, the acidification (i.e. fermentation) process was carried out for from 12 hr to 20 hr, from 14 hr to 18 hr or about 16 hr at from 22°C to 34°C, to at from 24°C to 32°C or at from 26°C to 30°C or at about 28°C.

 According to some embodiments, the resulting alternative cream cheese is cooled to 4-6°C and stored in refrigerator for further examinations.

15 According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 1000 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 900 nm. According to some
20 embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 300 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 400 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 500 to 1100 nm. According to some
25 embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 600 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 300 to 800 nm. According to some embodiments, the average particle size may depend on the concentration of BLG in the composition.

30 According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the softness of the resulting alternative cream cheese is

below 50 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the softness of the resulting alternative cream cheese is below 40 g or below 30 g or below 20 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the softness of the resulting alternative cream cheese is from 1 to 40 g or from 1 to 30 g or from 1 to 20 g or from 5 to 15g.

According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the firmness of the resulting alternative cream cheese is below 1000 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the firmness of the resulting alternative cream cheese is below 800 g, below 500 g or below 400 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the firmness of the resulting alternative cream cheese is from 50 to 1000g, from 100 to 500 g or from 100 to 400g.

According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is above -800 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is above -600 g., above -400g or above -300g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is from -800 to -1g, from -600 to -50g, from -300 to -100 g or from -250 to -100g.

According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the consistency of the resulting alternative cream cheese is below 20,000 g*sec. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the consistency of the resulting alternative cream cheese is below 10,000 g*sec, below 8,000 g*sec or below 5000 g*sec. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the consistency of the resulting alternative cream cheese is from 100 to 20,000 g*sec, from 1000 to 15,000 g*sec, from 1000 to 10,000 g*sec, or from 2,000 to 5,000 g*sec.

According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is above -1500 g. According to some embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is above -1200 g, above -1000g or above -800g. According to some
5 embodiments, the resulting alternative cream cheese comprises from 5 wt% to 10 wt% of rBLG and the cohesiveness of the resulting alternative cream cheese is from --1500 to -1g sec, from -1000 to -100g, from -800 to -200 g or from -600 to -200g.

According to some embodiments, the method provides a modulated characteristic of
10 the alternative cream cheese in comparison to a method in which the proteins are not modified as described hereinabove, e.g. not modified by TG and PG enzymes combination. According to some embodiments, the modulated characteristic is selected from presence of protein particles, presence of TG or residuals thereof, presence of PG or residuals thereof, an average particle size, softness, firmness, cohesiveness, consistency, work of cohesion,
15 viscosity, and zeta-potential of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination modulates the softness of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination modulates the firmness of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination modulates the cohesiveness of the alternative cream cheese. In
20 certain embodiments, the use of the TG and PG enzymes combination modulates the consistency of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination modulates the work of cohesion of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination modulates the average particle diameter of the alternative cream cheese.

25 In certain embodiments, the use of the TG and PG enzymes combination increases the softness of the alternative cream cheese. According to some embodiments, the use of the TG and PG enzymes combination increases the amount of protein particles in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the use of the TG and
30 PG enzymes combination increases the amount of TG or residuals thereof, PG or residuals thereof, or both TG and PG or residuals thereof in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes

combination. In certain embodiments, the use of the TG and PG enzymes combination decreases the firmness of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination decreases the cohesiveness of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination decreases the consistency of the alternative cream cheese. In certain embodiments, the use of the TG and PG enzymes combination decreases the work of cohesion of the alternative cream cheese. With respect to terms "cohesiveness" and work of cohesion", the level, i.e. values, refer to the absolute numbers of these parameters; therefore, for example, the change from -100% to -50% is considered as a reduction (decrease) in the level of these parameters. In certain embodiments, the use of the TG and PG enzymes combination decreases the average particle diameter of the alternative cream cheese.

In certain embodiments, the softness of the alternative dairy product is below 50 g. In certain embodiments, the softness of the alternative dairy product is between 8 and 20 g. In certain embodiments, the firmness of the alternative dairy product is below 2000 g. In certain embodiments, the firmness of the alternative dairy product is between 200 and 600 g. In certain embodiments, the cohesiveness of the alternative dairy product is above -1000 g. In certain embodiments, the cohesiveness of the alternative dairy product is between -400 and 100 g. In certain embodiments, the consistency of the alternative dairy product is below 22000 g sec. In certain embodiments, the consistency of the alternative dairy product is between 2500 and 7000 g sec. In certain embodiments, the work of cohesion of the alternative dairy product is above -1900 g sec. In certain embodiments, the work of cohesion of the alternative dairy product is between -800 and -300 g sec.

According to some embodiments, the method provides an alternative cream cheese with an increased amount of protein particles in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

According to some embodiments, the method provides an alternative cream cheese with a reduced average particle size in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

According to some embodiments, the method provides an alternative cream cheese with a reduced softness in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

5 According to some embodiments, the method provides an alternative cream cheese with a reduced firmness in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

According to some embodiments, the method provides an alternative cream cheese with an increased cohesiveness in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

10 According to some embodiments, the method provides an alternative cream cheese with a reduced consistency in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

According to some embodiments, the method provides an alternative cream cheese with an increased work of cohesion in comparison to an alternative cream cheese obtained
15 by a method in which the protein is not modified by TG and PG enzymes combination.

According to some embodiments, the method provides an alternative cream cheese with an increased viscosity in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative cream cheese with a decreased
20 viscosity in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination. According to some embodiments, the method provides an alternative cream cheese with an increased zeta potential in comparison to an alternative cream cheese obtained by a method in which the protein is not modified by TG and PG enzymes combination.

25 According to some embodiments, the average particle size of particles is decreased by at least 10%. According to some embodiments, the firmness is decreased by at least 50%. According to some embodiments, the cohesiveness is increased by at least 50%. According to some embodiments, the consistency is decreased by at least 50%. According to some embodiments, work of cohesion is increased by at least 50%.

According to some embodiments, the average zeta potential is increased by at least 5%. According to some embodiments, the viscosity of the composition is reduced by 10%. According to some embodiments, the viscosity of the composition is increased by 10%.

According to some embodiments, the present invention provides a method of preparing an alternative cream cheese, the method comprises the steps of:

- (i) Hydrating dry powders of recombinant from 2 to 9 wt% rBLG, from 1 to 3 wt% starch, from 1 to 4 wt% dextrose, from 0.04 to 0.1 wt% of trisodium citrate, from 0.01 to 0.05 wt% of yeast extract and from 0.1 to 0.5 wt% of locust bean gum until full dissolution of solids;
- (ii) Adjusting pH was adjusted to 6.5-7.0;
- (iii) adding a from 0.1 to 0.2 wt% of TG and PG enzymes combination for at least 30 min;
- (iv) Adding from 15 to 25 wt% of a fat, optionally with elevating the temperature until the fat was fully dissolved;
- (v) Homogenizing and pasteurizing the resulted composition;
- (vi) Reducing the temperature to from 20 to 37°C, adding a LAB and fermenting for from 12 to 20 hours; and
- (vii) Optionally packaging and storing the resulting alternative cream cheese.

According to some embodiments, the method of preparing a cream cheese is as described in Example 4. According to some embodiments, the content of the resulting alternative cream cheese is as defined in Fig. 5.

According to some embodiments, the alternative dairy product is an alternative yogurt.

According to some embodiments, the present invention provides a method of preparing an alternative yogurt, the method comprises the steps of:

- (i) Hydrating phosphate salts followed by addition of recombinant BLG (optionally as a powder), a sweetener, tri-sodium citrate, a thickener such as starch, a texturizer such as inulin and threonine;
- (ii) Adding a lipid optionally with elevating the temperature until the lipid is fully dissolved;

- (iii) adding a TG and PG enzymes combination for at least 30 min, followed by inactivation of the TG and PG enzymes, e.g. by heating
- (iv) Homogenizing and pasteurizing the resulted composition;
- (v) Adjusting pH was adjusted to 6.7-6.8 and the temperature to from 20 to 37°C;
- 5 (vi) Adding LAB and fermenting for from 10 to 24 hours until the pH reaches the value of from 4.0 to 4.5; and
- (vii) Optionally packaging and storing the resulting alternative yogurt.

According to some embodiments, steps (iv) may be performed before step (iii).

According to some embodiments, the method comprises adding from 2 wt% to 10wt% BLG such as rBLG. According to some embodiments, the method comprises adding about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt% of rBLG. According to some embodiments, the method comprises adding about 3 wt% rBLG. According to some embodiments, the method comprises adding from 2 wt% to 4 wt% BLG such as rBLG. According to some embodiments, the method comprises adding from 5 wt% to 7 wt% BLG such as rBLG. According to some embodiments, the method comprises adding about 8 wt% rBLG.

According to some embodiments, the method comprises adding from 0.1 to 10 wt% dextrose. According to some embodiments, the method comprises adding from 1 to 5 wt% dextrose. According to some embodiments, the method comprises adding from 2 to 4 wt% dextrose. According to some embodiments, the method comprises adding about 1wt%, about 2wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, or about 8 wt% of dextrose. According to some embodiments, the method comprises adding about 2.8 wt% dextrose.

According to some embodiments, the method comprises adding from 0.01 to about 1 wt% of an antioxidant. According to some embodiments, the method comprises adding from 0.05 to about 0.1 wt% or about 0.08 wt% of the antioxidant. According to one embodiment, the antioxidant is a trisodium citrate, e.g. 0.08% trisodium citrate.

According to some embodiments, the method comprises adding from 0.1 to 5 wt% of starch. According to some embodiments, the method comprises adding from 0.5 to 4 wt% of starch. According to some embodiments, the method comprises adding about 1wt%, about 2wt%, about 3 wt%, or about 4 wt% of starch.

According to some embodiments, the method comprises adding from 0.01 to about 1 wt% of threonine. According to some embodiments, the method comprises adding from 0.05 to about 0.5 wt%, from 0.05 to about 0.2 wt% or about 0.12 wt% of the threonine.

According to some embodiments, the method comprises adding from 0.1 to about 6 wt% of an inulin. According to some embodiments, the method comprises adding from 0.5 to about 3 wt%, from 1 to about 2 wt% or about 1.5 wt% of the inulin.

According to some embodiments, the pH is adjusted to 6.7-6.8 prior to adding TG PG combination. According to some embodiments, the method comprises adding TG and PG enzymes combination at the concentration at least 500 ppm. According to some embodiments, the concentration of the added TG and PG enzymes combination is at least 750 ppm. According to some embodiments, the concentration of the added TG and PG enzymes combination is from 500 to 3000 ppm. According to some embodiments, the unit ratio of TG to PG is from 1:10 to 10:1. According to some embodiments, the unit ratio of TG to PG is from 1:5 to 5:1. According to some embodiments, the unit ratio of TG to PG is from 1:3 to 3:1. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:3. According to some embodiments, the unit ratio of TG to PG is from 1:1 to 1:2. According to some embodiments, the unit ratio of TG to PG is from 1:1.2 to 1:1.7. According to some embodiments, the unit ratio of TG to PG is about 1:1.5. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.001 to 1 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.005 to 0.7 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.5 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of from 0.01 to 0.3 wt%. According to some embodiments, the combination of TG and PG is added to the final concentration of about 0.01, about 0.05 wt%, about 0.1wt%, about 0.15wt%, about 0.2 wt%, about 0.25wt% or about 0.3 wt%. According to some embodiments, the TG and PG enzymes are incubated at a temperature of from 40 to 60 Celsius degrees for from 0.5 to 3 hours, e.g. for from 0.5 to 1.5 hours, e.g. for 1 hr.

According to some embodiments, the method comprises adding from 0.5 to 8 wt% of a lipid. According to some embodiments, the method comprises adding from 1 wt% to 6 wt% of a lipid. According to some embodiments, the method comprises adding from 2 wt% to 5 wt% of lipid. According to some embodiments, the method comprises adding from 2

wt% to 4 wt% of a lipid. According to some embodiments, the method comprises adding about 3 wt% a lipid. According to some embodiment, the lipid is plant-derived lipid. According to some embodiments, the lipids comprise an oil. According to some embodiment, the oil is selected from shea oil, sunflower oil, coconut oil, rapeseed oil, nut
 5 oil, palm oil, kernel oil, olive oil, soya oil, cotton oil, and cocoa butter (Theobroma oil). According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined
 10 vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the formulation is heated to up to 70°C or up to 65°C or up to 60°C before, during or after adding the lipid.

According to some embodiments, the acidification (i.e. fermentation) process was carried out for from 12 hr to 20 hr, from 14 hr to 18 hr or about 16 hr at from 22°C to 34°C,
 15 to at from 24°C to 32°C or at from 26°C to 30°C or at about 28°C. According to some embodiments, the fermentation is performed until the pH reaches the value of from 3.6 to 4.5

According to some embodiments, the resulting alternative yogurt is cooled to 4-6°C and stored in refrigerator for further examinations.

20 According to some embodiments, the present invention provides a method of preparing an alternative yogurt, the method comprises the steps of:

- (i) Hydrating phosphate salts followed by addition of recombinant BLG (optionally as a powder) to the final concentration of from 2 to 4.5 %, dextrose from 2 to 4, tri-sodium citrate from 0.03 to 0.1%, starch from 2 to
 25 5%), inulin from 1 to 2% and threonine from 0.05 to 0.2 %;
- (ii) Adding a lipid optionally with elevating the temperature until the fat was fully dissolved;
- (iii) adding a TG and PG enzymes combination for at least 30 min, followed by inactivation of the TG and PG enzymes by heating
- 30 (iv) Homogenizing and pasteurizing the resulted composition;
- (v) Adjusting pH was adjusted to 6.7-6.8;

- (vi) Reducing the temperature to from 20 to 37°C, adding LAB and fermenting for from 10 to 24 hours until the pH reaches the value of from 4.0 to 4.5; and
- (vii) Optionally packaging and storing the resulting alternative yogurt.

According to some embodiments, the homogenization is performed at 400-800 Bar at
5 60-70°C, e.g. using GEA, Lab homogenizer Panda Plus 2000, Italy, two stages homogenization.

According to some embodiments, the pasteurization is made using a tubular heat exchanger (e.g. 80°C, 5 min holding time; HTST/UHT Mini Pilot System, Armfield)

According to some embodiments, the LAB the starter cultures are added at the amount
10 of 0.005-0.05%.

According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 1mV in comparison to a corresponding alternative yogurt. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative
15 yogurt by at least 2mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 3mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 4mV. According to
20 some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 5mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles by at least 6mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 7mV. According to some embodiments, the methods provided herein increase the average zeta
25 potential of the protein particles in the alternative yogurt by at least 8mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles by at least 9mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by at least 10mV. According to any one of the above embodiments, the change is in comparison to a
30 corresponding alternative yogurt.

According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 1mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 2mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 3mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in about in the alternative yogurt 4mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 5mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles by about 6mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 7mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 8mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 9mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt by about 10mV. According to any one of the above embodiments, the change is in comparison to a corresponding alternative yogurt.

According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt from about 1mV to about 20mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt from about 1mV to about 15mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt from about 1mV to about 10mV. According to some embodiments, the methods provided herein increase the average zeta potential of the protein particles in the alternative yogurt from about 1mV to about 5mV. According to any one of the above embodiments, the change is in comparison to a corresponding alternative yogurt.

In certain embodiments, the alternative dairy product has a different yield stress from a corresponding alternative dairy product which was not produced according to the methods

of the present invention. In certain embodiments, the alternative dairy product has a similar yield profile as a corresponding animal-based dairy product. In certain embodiments, the alternative dairy product has a similar yield stress as a corresponding animal-based dairy product. In certain embodiments, the alternative dairy product has a yield stress of below 500 Pa. In certain embodiments, the alternative dairy product has a yield stress of below 100 Pa. In certain embodiments, the alternative dairy product has a yield stress of below 50 Pa. In certain embodiments, the alternative dairy product has a yield stress of between 10 and 100 Pa. In certain embodiments, the alternative yogurt has a yield stress of below 500 Pa. In certain embodiments, the alternative yogurt has a yield stress of below 100 Pa. In certain embodiments, the alternative yogurt has a yield stress of below 50 Pa. In certain embodiments, the alternative yogurt has a yield stress of between 10 and 100 Pa.

According to some embodiments, the method of preparing an alternative milk is as described in **Example 4**. According to some embodiments, the content of the resulting alternative milk is as defined in **Fig. 5**.

According to a further aspect, the present invention provides an alternative dairy product obtained or obtainable by the methods of the present invention as described hereinabove. All terms, embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

According to another aspect, the present invention provides a population of protein particles comprising BLG protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more or (iii) comprise covalently bound BLG proteins. According to some embodiments, the population of protein particles comprising BLG protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more and (iii) comprise covalently bound BLG proteins.

According to some embodiments, the average particle size of the protein particles is at least 60 nm. According to some embodiments, the average particle size of the protein particles is at least 70 nm. According to some embodiments, the average particle size of the protein particles is from 60 to 500 nm. According to some embodiments, the average particle size of the protein particles is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm. According to some embodiments, the zeta potential of the protein

particles is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles is from -16.5 to -7 mV. According to some embodiments, the protein particles have an average particle size of from 50 nm to 250 nm and zeta potential for -17 mV to -5 mV.

- 5 According to some embodiments, BLG proteins are crosslinked via amid bonds. According to some embodiments, BLG proteins are crosslinked via amid bond between Glu and Lys amino acids.

- According to some embodiments, the protein particles comprise at least 25 wt% BLG proteins. According to some embodiments, the protein particles comprise at least 30 wt%, at least 35wt%, at least 40wt%, at least 45%, at least 50wt%, at least 55wt%, at least 60wt%, at least 65wt%, at least 70wt%, at least 75wt% or at least 80wt% BLG proteins. According to some embodiments, the protein particles comprise at least 85 wt% BLG proteins. According to some embodiments, the protein particles comprise at least 90 wt% BLG proteins. According to some embodiments, the protein particles comprise at least 95 wt% BLG proteins. According to some embodiments, the protein particles comprise at least 95 wt% BLG proteins. According to some embodiments, the protein particles comprise at least 98 wt% BLG proteins. According to some embodiments, the protein particles comprise from 80 to 98wt%, from 85 to 95wt% or from 87 to 92 wt% of BLG proteins. According to some embodiments, the term "wt%" refers to dry weight. According to some embodiments, the protein particles consist essentially of water and proteins. According to some embodiments, the protein particles consist essentially of water and BLG proteins. According to some embodiments, the protein particles consist essentially of BLG proteins.

- According to some embodiments, BLG is the sole whey protein in the protein particles. According to some embodiments, BLG is the sole milk protein in the protein particles. According to some embodiments, BLG is the sole protein in the protein particles. According to some embodiments, the protein particles are devoid of any additional whey protein, milk protein or any other protein aside BLG protein. According to some embodiment, the protein particles consist of BLG proteins and water. According to some embodiment, the protein particles consist of BLG proteins.

According to some embodiments, the protein particles essentially consist of BLG protein, TG and PG. According to some embodiments, the particles comprise TG and PG proteins or residuals thereof.

According to another aspect, the present invention provides a composition comprising
5 protein particles of the present invention and a carrier. All terms, embodiments and definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

The term “carrier” as used herein refers to any compound or composition useful as a vehicle, for dispersing, for facilitating storage or stability of the particles. Non-limiting
10 examples of the carrier are liquids such as water, solid or semi solid carriers.

According to some embodiments, the protein particles obtained by the methods of the present invention have the above-defined characteristics.

According to another aspect, the present invention provides a gel comprising a population of protein particles of the present invention. All terms, embodiments and
15 definitions disclosed in any one of the above aspects apply and are encompassed herein as well.

According to some embodiments, the population of protein particles is obtained or obtainable by the methods of the present invention. According to some embodiments, the gel comprises a plurality of protein particles comprising BLG protein, wherein the protein
20 particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more, or (iii) comprise covalently bound BLG proteins. According to some embodiments, the population of protein particles comprising BLG protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more, and (iii) comprise covalently bound BLG proteins. According
25 to some embodiments, the average particle size of the protein particles is at least 60 nm. According to some embodiments, the average particle size of the protein particles is at least 70 nm. According to some embodiments, the average particle size of the protein particles is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm. According to some
30 embodiments, the zeta potential of the protein particles is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles is from -16.5 to -7 mV. According to some embodiments, the protein particles have an average particle size of from

50 nm to 250 nm and zeta potential of -17 mV to -5 mV. According to some embodiments, the gel strength of the gel is below 170g or below 160g. According to some embodiments, the gel strength of the gel is above 70 g. According to some embodiments, the gel strength of the gel is from 70 to 160g, from 70 to 120g, or from 75 to 110 g. According to some
5 embodiments, the gel strength of the gel is from 20 to 150g.

According to some embodiments, BLG proteins in the protein particles are crosslinked via amid bonds. According to some embodiments, BLG proteins are crosslinked via amid bond between Glu and Lys amino acids of the proteins.

According to some embodiments, the gel comprises at least 1 wt%, at least 1.5 wt%,
10 at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt%, from 1 to 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or from 3 to 4 wt% BLG. According to some embodiments, BLG protein is a recombinant BLG protein.

According to some embodiments, the gel comprises water as a carrier. According to
15 some embodiments, the pH of the gel equals to or below the isoelectric point of BLG. According to some embodiments, the pH of the gel equals to or below 5.2.

According to some embodiments, the gel comprises protein glutaminase (PG) and transglutaminase (TG) enzymes. According to some embodiments, the gel comprises traces of PG and/or TG.

20 According to some embodiments, the content of Gln residues in the BLG proteins in the gel is lower than the content of Gln in native BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is less than 95% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is less than 90%, less than 85%, less
25 than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, or less than 40% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%,
30 from 20% to 60% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the gel

is from 0% to 60%, from 5% to 55%, from 10% to 50%, from 15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiment, the native, non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

5 According to some embodiments, the gel has a modulated characteristic in comparison to a corresponding gel substantially devoid of protein particles comprising covalently-bound BLG. According to some embodiments, the modulated characteristic comprises an average particle size, zeta potential, gel strength, gel astringency, or both gel strength and gel astringency. According to some embodiments, the gel has (i) a decreased gel strength in
10 comparison to the corresponding gel, (ii) a decreased gel astringency in comparison to the corresponding gel, (iii) an increased amount of protein particles in comparison to the corresponding gel, or (iv) any combination of (i)-(iii).

 According to some embodiments, BLG is the sole whey protein in the protein particles. According to some embodiments, BLG is the sole milk protein in the protein particles.
15 According to some embodiments, BLG is the sole protein in the protein particles. According to some embodiments, the protein particles are devoid of any additional whey protein, milk protein or any other protein aside BLG protein. According to some embodiments, BLG is the sole whey protein in the gel. According to some embodiments, BLG is the sole milk protein in the gel. According to some embodiments, BLG is the sole protein in the gel.
20 According to some embodiments, the gel is devoid of any additional whey protein, milk protein or any other protein aside BLG protein.

 According to some embodiments, the gel prepared by the methods of the present invention has the characteristics as described hereinabove.

Alternative Dairy product

25 According to another aspect, the present invention provides an alternative dairy product, comprising a population of modified BLG proteins. According to some embodiments, the present invention provides a dairy product, comprising a population of at least partially deamidated BLG proteins. According to some embodiments, the present invention provides a dairy product, comprising a population of covalently bound
30 (polymerized) BLG proteins. According to some embodiments, the present invention

provides a dairy product, comprising a population of covalently bound (polymerized) and deamidated BLG proteins.

According to another aspect, the present invention provides an alternative dairy product, comprising a population of protein particles comprising covalently bound BLG
5 such as covalently bound rBLG. According to some embodiments, BLG is a recombinant BLG. According to some embodiments, the BLG and/or the rBLG have the amino acid sequence SEQ ID NO: 1. According to some embodiments, the BLG protein comprises an amino acid sequence selected from SEQ ID NOs: 1-10. According to some embodiments, the term BLG also encompasses analogs thereof. According to some embodiments, the BLG
10 analog comprises an amino acid sequence selected from SEQ ID NOs: 11-20.

According to some embodiments, the protein particles are obtainable or obtained by the method of any one of the above aspects and embodiments. According to some embodiments, the present invention provides a dairy product, comprising a gel comprising a population of protein particles obtainable or obtained by the method of any one of the
15 above aspects and embodiments. According to some embodiments, the dairy product comprises the protein particles or gel comprising the protein particles as described in any one of the above embodiments and aspects.

In certain embodiments, the alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, a sweetener, and/or water. In certain embodiments, the alternative
20 dairy product comprises BLG protein, and water. In certain embodiments, the alternative dairy product comprises BLG protein, PG and TG enzymes, and water. In certain embodiments, the alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, and water. In certain embodiments, the alternative dairy product comprises BLG protein, PG and TG enzymes, a fat, and water. The term "sweetener" refers to any natural and
25 artificial substance that provides a sweet taste in foods and beverages. According to some embodiments, the term sweetener excludes lactose. The term "sweetener" also comprises sugars and carbohydrates.

In certain embodiments, the alternative dairy product comprises BLG protein, PG and TG enzymes or residuals thereof, a fat, a sugar, and water. In certain embodiments, the
30 alternative dairy product comprises 1% to 15% BLG protein, 0.01% to 0.6% PG and TG enzymes combination, 1% to 50% fat, 1% to 10% sweetener such as sugar, and water. In

certain embodiments, the alternative dairy product comprises 1% to 10% BLG protein, 0.05% to 0.3% PG and TG enzymes, 1% to 20% fat, 1% to 5% sugar, and water. In certain embodiments, the alternative dairy products are selected from alternative Cream Cheese, alternative Yogurt and/or alternative Milk. In certain embodiments, the alternative Cream Cheese, Yogurt or Milk comprises BLG protein, PG and TG enzymes, a fat, a sugar, and water. In certain embodiments, the alternative Cream Cheese, Yogurt or Milk comprises 1% to 15% BLG protein, 0.01% to 0.6% PG and TG enzymes, 1% to 50% fat, 1% to 10% sugar, and water. In certain embodiments, the alternative Cream Cheese, Yogurt or Milk comprises 1% to 10% BLG protein, 0.05% to 0.3% PG and TG enzymes, 1% to 20% fat, 1% to 5% sugar, and water. According to any one of the above embodiments, the alternative dairy product comprises protein particles comprising covalently bound BLG proteins. According to some embodiments, from up to 80% of the BLG is modified, i.e. deamidated and/or polymerized. According to some embodiments, from 5% to 80% of the BLG is modified. According to some embodiments, from 5% to 80 %, from 10% to 75 %, from 15% to 70 %, from 20% to 65 %, from 25% to 60 %, from 30% to 55 %, or from 35% to 50 % of the BLG is modified. According to some embodiments, from 5% to 45 %, from 10% to 40 %, from 15% to 35 %, from 20% to 30 %, from 25% to 30 % is modified of the BLG is modified. According to some embodiments, from 30% to 80%, from 35% to 75%, from 40% to 70%, from 45% to 65%, from 50% to 60% of the BLG is modified. According to some embodiments, from 5% to 80 %, from 10% to 75 %, from 15% to 70 %, from 20% to 65 %, from 25% to 60 %, from 30% to 55 %, or from 35% to 50 % of the BLG proteins are covalently bound and form particles. According to some embodiments, from 5% to 95 %, from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the BLG proteins are at least partly deamidated.

According to some embodiments, the alternative dairy product comprises a population of protein particles comprising BLG protein, wherein the protein particles: (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more and/or (iii) comprise covalently bound BLG proteins. According to some embodiments, the population of protein particles comprising BLG protein, wherein the protein particles (i) have an average particle size of 50 nm or more, (ii) have an average zeta potential of -17 mV or more and (iii) comprise covalently bound BLG proteins. According to some embodiments, the average particle size of the protein particles is at least 60 nm. According

to some embodiments, the average particle size of the protein particles is at least 70 nm. According to some embodiments, the average particle size of the protein particles is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm. According to some
5 embodiments, the zeta potential of the protein particles is from -17 to -5 mV. According to some embodiments, the zeta potential of the protein particles is from -16.5 to -7 mV.

According to some embodiments, the alternative dairy product comprises a gel comprising a population of protein particles. According to some embodiments, the gel strength of the gel is below 170g or below 160g. According to some embodiments, the gel
10 strength of the gel is above 70 g. According to some embodiments, the gel strength of the gel is from 70 to 160g, from 70 to 120g, or from 75 to 110 g. According to some embodiments, the gel strength of the gel is from 20 to 150g.

According to some embodiments, the alternative dairy product comprises protein glutaminase (PG) and transglutaminase (TG) enzymes. According to some embodiments,
15 the alternative dairy product comprises traces/residuals of PG and/or TG.

According to some embodiments, BLG is the sole whey protein in the protein particles. According to some embodiments, BLG is the sole milk protein in the protein particles. According to some embodiments, BLG is the sole protein in the protein particles. According to some embodiments, the protein particles are devoid of any additional whey protein, milk
20 protein or any other protein aside BLG protein. According to some embodiments, the protein particles essentially consist of BLG protein. According to some embodiments, the protein particles essentially consist of BLG protein, TG and PG. According to some embodiments, the particles comprise TG and PG proteins.

According to some embodiments, BLG is the sole whey protein in the alternative dairy
25 product. According to some embodiments, BLG is the sole milk protein in the alternative dairy product. According to some embodiments, BLG is the sole protein in the alternative dairy product. According to some embodiments, the alternative dairy product is devoid of any additional whey protein, milk protein or any other protein aside BLG protein. According to some embodiments, the alternative dairy product is devoid of any casein proteins.

30 According to some embodiments, no casein protein is added during the preparation of the alternative dairy product. According to some embodiments, no casein protein is added at

the concentration in which casein is present in mammalian milk during the preparation of the alternative dairy product.

According to some embodiments, the alternative dairy product does not comprise, or is devoid of, alpha-S1, alpha-S2, β and/or κ casein proteins. According to some
5 embodiments, the alternative dairy product does not comprise, or is devoid of, alpha-S1, alpha-S2, β and/or κ casein proteins at the concentration in which it is present in mammalian milk.

According to some embodiments, the alternative dairy product further comprises a lipid, a mineral, a salt, a sugar, lactic acid bacteria (LAB), or any combination thereof.
10 According to some embodiments, the alternative dairy product further comprises a lipid, a mineral, a salt, a sugar, and lactic acid bacteria (LAB).

According to some embodiments, the alternative dairy product comprises at least 1 wt%, at least 1.5 wt%, at least 2 wt%, at least 2.5 wt%, at least 3 wt%, at least 3.5 wt%, at least 4 wt%, at least 4.5 wt%, at least 5 wt%, at least 6 wt%, or at least 6.5 wt%, from 1 to
15 10 wt%, from 2 to 8 wt%, from 3 to 6 wt%, from 4 to 5 wt%, from 4 to 8 wt%, from 3 to 8 wt%, or from 3 to 4 wt% of BLG. According to some embodiments, BLG protein is a recombinant BLG protein.

According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is lower than the content of Gln residues in native BLG.
20 According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is less than 95% of the Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less
25 than 45%, or less than 40% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the content of Gln residues in the native, non-modified BLG
30 protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is from 0% to 60%, from 5% to 55%, from 10% to 50%, from

15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the native, non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

According to some embodiments, the alternative dairy product comprises up to 0.35 wt% of a coagulation mineral salt. According to some embodiments, the alternative dairy product comprises from about 0.0015 to about 0.35 wt% of a coagulation mineral salt(s). According to some embodiments, the alternative dairy product comprises from about 0.002 to about 0.30 wt% of a coagulation mineral salt(s). According to some embodiments, the alternative dairy product comprises from about 0.005 to about 0.25 wt% of a coagulation mineral salt(s). According to some embodiments, the alternative dairy product comprises from about 0.01 to about 0.20 wt% of a coagulation mineral salt(s). According to some embodiments, the alternative dairy product comprises from about 0.05 to about 0.2 wt% of a coagulation mineral salt(s). According to some embodiments, the alternative dairy product comprises from about 0.1 to about 0.25 wt% of a coagulation mineral salt(s). According to some embodiments, the coagulation mineral salt is calcium chloride.

According to some embodiments, the alternative dairy product comprises a lipid (fat). According to some embodiments, the lipid is a non-animal lipid. According to some embodiment, the lipid is plant-derived lipid. According to some embodiments, the lipids comprise an oil. According to some embodiment, the oil is selected from shea oil, sunflower oil, coconut oil, rapeseed oil, nut oil, palm oil, kernel oil, olive oil, soya oil, cotton oil, and cocoa butter (Theobroma oil). According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the alternative dairy product comprises from 1 to 40 wt% of the non-animal lipid. According to some embodiments, the alternative dairy product comprises from 1 to 10 wt% of the non-animal lipid. According to some embodiments, the alternative dairy product comprises from 10 to 40 wt%, from 15 to 35 wt% or from 20 to 30 wt% of the non-animal lipid such as plant oil.

According to some embodiments, the alternative dairy product comprises a sweetener. According to some embodiments, the sweetener is a sugar, therefore, according to some embodiments, the alternative dairy product comprises sugar. According to some embodiments, the sugar is a non-animal sugar. According to some embodiment, the sugar is plant-derived sugar. According to some embodiments, the sugar is selected from a monosaccharide, disaccharide, and polysaccharide. According to some embodiments, the sugar is selected from glucose, fructose, mannose, xylose, arabinose, sucrose, dextrose, maltose, and galactose. According to some embodiments, the sugar is dextrose. According to some embodiments, the alternative dairy product comprises from 1 to 20 wt% of sugar.

5 According to some embodiments, the alternative dairy product comprises from 1 to 5 wt% of sugar. According to some embodiments, the alternative dairy product comprises from 1 to 15 wt% of sugar. According to some embodiments, the alternative dairy product comprises from 1 to 5 %, from 2 to 4 or about 3.2% of dextrose.

According to any one of the above embodiments, the alternative dairy product comprises LAB or traces of LAB.

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According to some embodiments, the alternative dairy product comprises adding yeast extract. According to some embodiments, the alternative dairy product comprises from 0.005 to 0.5 wt% of yeast extract. According to some embodiments, the alternative dairy product comprises 0.01 to 0.45 wt%, from 0.015 to 0.4 wt%, from 0.02 to 0.35 wt%, from 0.025 to 0.3 wt%, from 0.01 to 0.06 wt%, from 0.015 to 0.05 wt%, from 0.02 to 0.04 wt%, or about 0.03 wt% of yeast extract.

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According to some embodiments, the alternative dairy product comprises a flavoring salt. According to some embodiments, the salt is sodium chloride.

According to some embodiments, the alternative dairy product comprises at least one of a stabilizer, a thickener, a texturizer and a preservative.

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According to some embodiments, the alternative dairy product is a homogenized or pasteurized dairy product. According to some embodiments, the alternative dairy product is a homogenized and pasteurized dairy product.

According to any one of the above embodiments, the alternative dairy product is selected from the group consisting of a milk composition, a yogurt composition, a soft cheese

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composition, an ice cream composition, and a hard cheese composition. According to one embodiment, the alternative dairy product is a cream cheese composition. According to another embodiment, the alternative dairy product is a yogurt composition. According to some embodiments, the alternative dairy product is a non-animal dairy product.

5 According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 50 nm. According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 60 nm. According to some embodiments, the average particle size of the protein particles in the alternative dairy product is at least 70 nm. According to some embodiments, the average
10 particle size of the protein particles in the alternative dairy product is from 60 to 500 nm. According to some embodiments, the average particle size is from 60 to 300 nm, from 60 to 250 nm, from 70 to 250 nm, or from 80 to 230 nm.

 According to any one of the above embodiments, the alternative dairy product has at least one modulated characteristic in comparison to a corresponding characteristic in a
15 corresponding alternative dairy product substantially devoid of modified BLG, such as deamidated BLG and/or protein particles comprising covalently-bound BLG. According to some embodiments, the modulated characteristic of the alternative dairy product is (i) particle size, (ii) viscosity; (iii) dairy product gel strength, (iv) dairy product astringency, (v) presence of protein particle, or any combination thereof. According to some embodiments,
20 the alternative dairy product has an increased population of protein particles, e.g. BLG comprising particles. According to some embodiments, the alternative dairy product has a decreased dairy product gel strength in comparison to a corresponding alternative dairy product substantially devoid of particles comprising covalently-bound BLG. According to some embodiments, the alternative dairy product has a decreased astringency in comparison
25 to a corresponding alternative dairy product substantially devoid of particles comprising covalently-bound BLG. According to some embodiments, the alternative dairy product has a decreased dairy product gel strength and a decrease astringency in comparison to a corresponding alternative dairy product substantially devoid of particles comprising covalently-bound BLG.

30 According to some embodiments, the present invention provides an alternative milk comprising: from 0.1 to 1 wt% phosphate salt, from 0.01 to 0.03 wt% of a stabilizer, from 2 to 5 wt% of rBLG, from 1 to 3 wt% of a sweetener, from 0.1 to 0.4 calcium carbonate, from

0.01 to 0.08 NaCl; from 0.1 to 0.2 wt% of TG and PG enzymes combination or residuals thereof; and from 3 to 4 wt% of a non-animal lipid. According to some embodiments, the present invention provides an alternative milk comprising: from 0.5 to 1 wt% of dipotassium phosphate and 0.1 to 0.2 wt% of monopotassium phosphate; from 0.01 to 0.03 wt% gellan
5 gum; from 2 to 5 wt% of rBLG, from 1 to 3 wt% sucrose, from 0.1 to 0.4 calcium carbonate and from 0.01 to 0.08 NaCl; from 0.1 to 0.2 wt% of TG and PG enzymes combination; and from 3 to 4 wt% of a plant fat. According to some embodiments, the alternative milk comprises from 0.05 to 2 wt% of polyphosphates. According to some embodiments, the alternative milk comprises from 0.01 to 1 wt% of L-cystine. According to some embodiment,
10 the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According
15 to some embodiments, the alternative milk has a viscosity of from 80 to 2000 cP. According to some embodiments, the resulting alternative milk comprises from 1 to 5 wt% of rBLG and has a viscosity of from 80 to 120 cP. According to some embodiments, the resulting alternative milk comprises from 5 or above 5 wt% to 10 wt% of rBLG and has a viscosity below 2000 cP. of from 80 to 120 cP. According to some embodiments, the resulting
20 alternative milk comprises from 5 or above 5 wt% to 10 wt% of rBLG and has a viscosity of from 200 to 1800 cP of from 300 to 1500 cP. According to some embodiments, the resulting alternative milk comprises particles having an average particle size below 400 nm. According to some embodiments, the resulting alternative milk comprises particles having an average size of from 250 to 400 nm. According to some embodiments, the method of
25 preparing an alternative milk is as described in Example 4. According to some embodiments, the content of the resulting alternative milk is as defined in Fig. 5. According to any one of the above embodiments, the alternative milk comprises a plurality of protein particles comprising covalently bound BLG. According to any one of the above embodiments, the alternative milk comprises deamidated BLG. According to some embodiments, the content
30 of Gln residues in the BLG proteins in the alternative dairy product is lower than the content of Gln residues in native BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is less than 95% of the Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative dairy product is less than 90%, less than 85%,

less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, or less than 40% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative milk is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative milk is from 0% to 60%, from 5% to 55%, from 10% to 50%, from 15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the native, non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

According to some embodiments, the present invention provides an alternative cream cheese comprising: from 2 to 9 wt% rBLG, from 1 to 3 wt% of a thickener such as starch, from 1 to 4 wt% of a sweetener, from 0.04 to 0.1 wt% of trisodium citrate, from 0.01 to 0.05 wt% of yeast extract and from 0.1 to 0.5 wt% of a stabilizer, from 0.1 to 0.2 wt% of TG and PG enzymes or residuals thereof and from 15 to 25 wt% of a non-animal lipid. According to some embodiments, the present invention provides an alternative cream cheese comprising: from 2 to 9 wt% rBLG, from 1 to 3 wt% starch, from 1 to 4 wt% dextrose, from 0.04 to 0.1 wt% of trisodium citrate, from 0.01 to 0.05 wt% of yeast extract and from 0.1 to 0.5 wt% of locust bean gum, from 0.1 to 0.2 wt% of TG and PG enzymes and from 15 to 25 wt% of a fat. According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, inter-esterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 1000 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size below 900 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 300 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average

particle size of from 400 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 500 to 1100 nm. According to some embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 600 to 1100 nm. According to some
5 embodiments, the resulting alternative cream cheese comprises particles having an average particle size of from 300 to 800 nm. According to some embodiments, the average particle size may depend on the concentration of BLG in the composition. In certain embodiments, the softness of the alternative dairy product is below 50 g. In certain embodiments, the softness of the alternative dairy product is between 8 and 20 g. In certain embodiments, the
10 firmness of the alternative dairy product is below 2000 g. In certain embodiments, the firmness of the alternative dairy product is between 200 and 600 g. In certain embodiments, the cohesiveness of the alternative dairy product is above -1000 g. In certain embodiments, the cohesiveness of the alternative dairy product is between -400 and 100 g. In certain embodiments, the consistency of the alternative dairy product is below 22000 g*sec. In
15 certain embodiments, the consistency of the alternative dairy product is between 2500 and 7000 g*sec. In certain embodiments, the work of cohesion of the alternative dairy product is above -1900 g*sec. In certain embodiments, the work of cohesion of the alternative dairy product is between -800 and -300 g*sec. According to some embodiments, the method of preparing a cream cheese is as described in Example 4. According to some embodiments,
20 the content of the resulting alternative cream cheese is as defined in Fig. 5. According to any one of the above embodiments, the alternative cream cheese comprises a plurality of protein particles comprising covalently bound BLG. According to any one of the above embodiments, the alternative cream cheese comprises deamidated BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative cream cheese
25 is lower than the content of Gln residues in native BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative cream cheese is less than 95% of the Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative cream cheese is less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%,
30 less than 60%, less than 55%, less than 50%, less than 45%, or less than 40% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative cream cheese is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the content of Gln

residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative cream cheese is from 0% to 60%, from 5% to 55%, from 10% to 50%, from 15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the native, non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

According to some embodiments, the present invention provides an alternative yogurt comprising from 1 to 5 wt% rBLG; from 0.1 to 0.2 wt% of TG and PG enzymes, from 1 to 5 wt% of a non-animal lipid, from 1 to 5 wt% of a thickener such as starch, from 0.1 to 1 wt% of phosphate salts, from 1 to 5 wt% of a sweetener, from 0.01 to 0.1 wt% of TSC, from 1 to 2 wt% of a texturizer, such as inulin. According to some embodiments, the present invention provides an alternative yogurt comprising from 1 to 5 wt% rBLG; from 0.1 to 0.2 wt% of TG and PG enzymes, from 1 to 5 wt% of a plant fat, from 1 to 5 wt% of starch, from 0.1 to 0.6 wt% of DPP, from 0.01 to 0.1 wt% MPP, from 1 to 5 wt% of dextrose, from 0.01 to 0.1 wt% of TSC, from 1 to 2 wt% of inulin. According to some embodiments, the alternative yogurt further comprises 0.05 to 0.3 threonine and/or from 0.3 to 1 wt% of a vegetable oil. According to some embodiments, the zeta potential of the alternative yogurt is above 5 or above 6 or from 6 to 20. According to some embodiment, the fat is a coconut fat, such as modified coconut fat. According to some embodiment, the fat is a shea fat. According to some embodiment, the fat is a coconut fat, such as modified coconut oil and a shea fat. According to some embodiment, the fat is fractionated, non-hydrogenated, interesterified, and/or refined vegetable fat. According to some embodiment, the fat is a mixture of an oil (i.e. liquid at the ambient temperature) and solid fat. According to any one of the above embodiments, the alternative yogurt comprises a plurality of protein particles comprising covalently bound BLG. According to any one of the above embodiments, the alternative yogurt comprises deamidated BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative yogurt is lower than the content of Gln residues in native BLG. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative yogurt is less than 95% of the Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative yogurt is less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, or less than 40% of the content of Gln residues in the native, non-modified

BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative yogurt is from 10% to 95%, from 15% to 90%, from 20% to 85%, from 25% to 80%, from 30% to 75%, from 35% to 70%, from 40% to 65%, from 35% to 60%, from 20% to 60% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the content of Gln residues in the BLG proteins in the alternative yogurt is from 0% to 60%, from 5% to 55%, from 10% to 50%, from 15% to 45%, from 20% to 40%, from 25% to 35% of the content of Gln residues in the native, non-modified BLG protein. According to some embodiments, the native, non-modified BLG protein comprises the amino acid sequence SEQ ID NO: 1.

10 In certain embodiments, the alternative yogurt has a yield stress of below 500 Pa. In certain embodiments, the alternative yogurt has a yield stress of below 100 Pa. In certain embodiments, the alternative yogurt has a yield stress of below 50 Pa. In certain embodiments, the alternative yogurt has a yield stress of between 10 and 100 Pa.

The terms “a,” “an,” and “the” are used herein interchangeably and mean one or more.

15 The term “and/or” is used to indicate one or both stated cases may occur, for example A and/or B includes, (A and B) and (A or B).

The term “or,” as used herein, denotes alternatives that may, where appropriate, be combined; that is, the term “or” includes each listed alternative separately as well as their combination if the combination is not mutually exclusive.

20 The terms “comprising”, “comprise(s)”, “include(s)”, “having”, “has” and “contain(s)”, are used herein interchangeably and have the meaning of “consisting at least in part of”. When interpreting each statement in this specification that includes the term “comprising”, features other than that or those prefaced by the term may also be present. Related terms such as “comprise” and “comprises” are to be interpreted in the same manner. The terms
25 “have”, “has”, “having” and “comprising” may also encompass the meaning of “consisting of” and “consisting essentially of”, and may be substituted by these terms. The term “consisting of” excludes any component, step or procedure not specifically delineated or listed. The term “consisting essentially of” means that the composition or component may include additional ingredients, but only if the additional ingredients do not materially alter
30 the basic and novel characteristics of the claimed compositions or methods.

As used herein, the term “about”, when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of +/-10%, or +/-5%, +/-1%, or even +/-0.1% from the specified value.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

Example 1. Enzymatic modification of beta-lactoglobulin by transglutaminase and protein glutaminase.

The enzymatic modification of beta lactoglobulin (BLG) with transglutaminase or with a combination of transglutaminase (TG) and protein glutaminase (PG) was performed according to the enzyme manufacturer recommendations. Briefly, BLG (5 w/w %) and tri-sodium citrate (TSC, 0.16 w/w %) were dissolved in deionized water and mixed for 40 min at 40 °C to achieve full hydration of the protein. Next, the solution was heated to 50 °C and pH was adjusted to pH=8.0 using 1M NaOH. Subsequently, transglutaminase (TG, marked as “FV”) or TG with protein glutaminase (TG+PG, marked as “SYG”, TG 100 U/g + PG 150 U/g) (ACTIVA® FV and ACTIVA® SYG, respectively) were added (0.05, 0.1 or 0.15 w/w %, corresponding to 500ppm, 1000ppm and 1500ppm) to samples. The beaker was covered with parafilm, to reduce enzyme oxidation, and gently stirred. After 60 min, the temperature was rapidly increased to 90 °C and held for 5 min to fully inactivate the enzyme(s) and the solution was immediately cooled on ice.

Example 2. Gel preparation, measurement of gel strength, particle size and Zeta-potential.

To evaluate the effect of TG and TG+PG enzymatic modification on BLG gel formation and its strength, the following procedure was executed: CaCl₂ solution (decreases coagulation time, creates firmer gels, and increases curd yield; 3 gr in 7 mL deionized water) was added (1 µL :1 mL ratio) to the modified BLG solution prepared as explained in **Example 1** and mixed for another 30 min. Next, glucono delta-lactone (GDL, an acidifier; 0.5 gr per 100 mL solution) was directly added, gently stirred for 10 min and poured (23 mL) into texture analyzer Teflon cups. The cups were incubated for 24 hours at 38 °C to

form BLG-based gels. The measurement of gel strength was carried out via a texture analyzer (Stable Micro Systems, TA.XTplusC, England) with a P/75 compression plate. Calibration settings were as follows: Return distance: 75 mm, Return speed 10 mm/sec, Contact force: 50 kg. Gel strength parameters measurements were repeated three times for
5 each sample in at least two individual repetitions.

To evaluate the instant effect of TG or TG+PG enzymatic modification on particle size, the different samples were entered to 4 mL plastic cuvettes and the average particle size was determined using photon cross-correlation spectroscopy technique (Nanophox, Sympatec GmbH, Germany). Laser intensity was adjusted to ~84%, desired temperature was
10 25 °C, measuring mode was cross correlation. Size measurements were repeated at least three times for each sample in two individual repetitions and results are shown as an average of 3 samples for 99% of the population $X_{99,3}$.

Zeta-potential is the electrical charge present on the surface of a protein and is a key parameter that influences their stability and behavior in solution. It is a measure of the
15 potential difference between the protein surface and the surrounding solution, determining the extent of electrostatic repulsion or attraction between proteins, which in turn affects their aggregation, solubility, and interactions with other molecules. To assess the impact of TG or TG+PG enzymatic modification on BLG zeta-potential, the solutions were diluted in phosphate buffer pH=7.5 and inserted to folded capillary zeta cells. The zeta-potential was
20 measured using Malvern Zetasizer instrument at 25 °C. Size measurements were repeated six times and results are presented as average zeta-potential (mV).

The results of the average particle size and zeta potential are presented in **Figs. 1-3**.

Fig. 1 shows that TG alone had an insignificant effect on the strength of BLG gel. In contrast, the addition of PG to TG (SYG, 500 ppm) decreases gel strength by about 75%
25 (from ~100 to ~25). This implies that a combination of TG and PG was necessary to modify a characteristic of BLG protein, and subsequently, modify a characteristic of gel formed from BLG solution. Further, it can be seen from **Fig. 2** that treatment of BLG with increasing concentration of TG+PG enzymes and subsequent appearance of protein particles with increasing size led to a reduced gel strength, reduction from about 250 g for untreated BLG
30 to about 70-80g after treatment with 1500 ppm.

Further, it can be seen from **Fig. 2** that there is an inverse correlation between particle size and gel strength. It further seems that there is a maximum of TG+PG enzymes concentrations above which the gel strength does not decrease below about 70-80g.

5 It can be seen from **Fig. 3** that increasing the concentration of TG+PG enzymes caused a change in zeta potential of particles comprising BLG proteins. As follows from the results, zeta potential changes from about -20 mV to about -12 mV upon increasing the concentration of TG+PG enzymes from 0 to 1500 ppm.

It is known that zeta-potential is correlated with protein astringency. Thus, the change in values of zeta-potential can reduce astringency of BLG-based products such as yogurt.

10 To summarize, the data shown in **Figs. 2 and 3** indicates that increasing the concentration of TG+PG enzyme produced protein particles with increasing average particle diameter. It can be seen that there is a strong correlation between treating BLG with 500-1500 ppm of TG+PG enzymes and average particle diameter which increases from about 70 to about 230 nm.

15 Without being limited to any particular theory, it is hypothesized that minor aggregation of untreated BLG may occur due to thermal processing. However, such BLG aggregates are formed mostly due to non-covalent bonds. Apparently, most of the protein particles formed due to TG+PG activity comprise covalently bound BLG.

Example 3. Viscosity and color measurements.

20 The effect of TG or TG+PG enzymatic modification on BLG solution's viscosity was measured using an IKA ROTAVISC SBS me-vi HELI Complete using a SP1 spindle (100 rpm @ 4°C). Viscosity measurements were repeated at least three times for each sample in two individual repetitions.

25 To evaluate the effect of TG or TG+PG enzymatic modification on color, the different samples were transferred to a designated measurement cell and placed on a benchtop colorimeter (CR5; Konica Minolta Bench-top, Japan) utilizing the CIE L*a*b* color space. All measurements were repeated at least four times for each sample in two individual repetitions. The results are presented in **Table 1** below.

Table 1.

	Color (Hunter Lab)	Viscosity (cP)
Without TG+PG (Reference)	-	6.78
SYG (TG+PG: 0.05 w/w %)	$\Delta E < 1$	6.42
FV (TG: 0.05 w/w %)	$\Delta E < 1$	9.36

It can be seen that TG or TG+PG showed minimal effect on color and viscosity of a BLG solution. ΔE is a color difference metric that quantifies the perceptual distance between two colors. $\Delta E < 1$ is considered imperceptible to most observers.

5 **Example 4. Alternative dairy product preparation and analysis**

Materials.

 Recombinant beta lactoglobulin (rBLG) powder, transglutaminase and protein
glutaminase (Activa SYG, Ajinomoto), dextrose, table salt, plant fat (e.g. coconut and shea
or modified coconut fat, Crokvitrol 703, Bunge), trisodium citrate (CS-Chemicals, Israel),
10 starch (Etenia 457, Avebe), locust bean gum (LBG Sicily), yeast extract (NuCel® 581 PW,
Procelys), gum arabic (Instantgum AA, Nexira), calcium carbonate (Calcipur 90 KP, Omya),
gellan gum (Gellaneer, DSM), sodium hexametaphosphate (Joha B-50, ICL), dipotassium
phosphate and monopotassium phosphate (Sigma, Israel).

Methods.

15 Alternative milk preparation

 Alternative milk was prepared according to the following procedures: first, the
phosphate salts (sodium hexametaphosphate, dipotassium phosphate and monopotassium
phosphate; 0.155%, 0.8% and 0.1% respectively) were hydrated in distilled water for 20 min
at 25 Celsius degrees. Next, gellan gum was added, hydrated for 10 min, and activated by
20 heating the solution to 90 Celsius degrees for 30 sec holding time (via Thermomix TM5,
Germany; mixing speed was set to 500 RPM at reverse knife mode). Immediately after, the
solution was cooled to 50 Celsius degrees and all other ingredients (besides TG PG and fat)
were added as following: recombinant BLG powder (final BLG concentrations in the milk
product were 3% and 8%, respectively), sucrose (2%), Arabic gum (0.18%), calcium

carbonate (0.25%) and NaCl (0.05%). The ingredients were mixed at 50 Celsius degrees for 1 hr. The enzymatic reaction was initiated by adding TG+PG combination (0%, 0.05% or 0.15%), for 1 hr and reducing the RPM to 50. Subsequently, the temperature was raised to 65 Celsius degrees and fat was introduced to the solution (3.5%). The solution was dispersed
5 using Turrax (T 25 digital ULTRA-TURRAX, IKA, Germany) for 10 min at 1,000 RPM and then homogenized at 600 Bar at 65 Celsius degrees (GEA, Lab homogenizer Panda Plus 2000, Italy, two stages homogenization). Finally, the alternative milk was pasteurized using Thermomix (85 Celsius degrees, two sec holding time) and immediately stored at 4 Celsius degrees for further examination. All concentrations refer to % w/w in the final product.

10 Alternative cream cheese preparation

Alternative cream cheese was prepared according to the following procedures: First, dry powders of recombinant beta lactoglobulin (final BLG concentrations in the cream cheese product were 3% and 8%, respectively), starch (2%), dextrose (2.8%), trisodium citrate (0.08%), yeast extract (0.03%) and locust bean gum (0.3%) were mixed and hydrated
15 in distilled water for 20 min. The temperature gradually elevated from 25 to 40 Celsius degrees until the protein was fully dissolved. Next, the pH was adjusted to 6.7-6.8 using 1M NaOH. The enzymatic reaction was executed by elevating the temperature to 50°C and adding TG+PG combination (0%, 0.05% or 0.15%) for one hour. Next, fat (20%) was added while gradually elevating the temperature to 65°C until the fat was fully melted. The solution
20 was dispersed using Turrax (T 25 digital ULTRA-TURRAX, IKA, Germany) for 10 min at 1,000 RPM and then homogenized at 500 Bar at 65 Celsius degrees (GEA, Lab homogenizer Panda Plus 2000, Italy, two stages homogenization). Subsequently, the solution was pasteurized via Thermomix (85 Celsius degrees, 30 sec holding time) and immediately cooled down to 28°C. To initiate the fermentation process, a cream cheese bacterial culture
25 (Lyofast V M01 N, Sacco) was added under slow agitation for 2 min. The acidification process was carried out for 16 hr at 28 Celsius degrees. Finally, the cream cheese was cooled to 4-6°C and stored in refrigerator for further examinations. All concentrations refer to % w/w in the final product.

Alternative yogurt preparation

30 Alternative Yogurt was prepared according to the following procedures: First, the phosphate salts (dipotassium phosphate and monopotassium phosphate; 0.3825% and

0.051% respectively) were hydrated in distilled water for 20 min at 25 Celsius degrees. Subsequently, the temperature was raised to 55 Celsius degrees and the following ingredients were added and mixed for 1 hr: Recombinant BLG powder (3.59%, final BLG concentrations in the yogurt product was 3%), dextrose (3.2%), tri-sodium citrate (0.07%), starch (3.2%), inulin (1.5%) and threonine (0.12%). Then, fat (3.0%) was added to the solution and dispersed using a Turrax device (T 25 digital ULTRA-TURRAX, IKA, Germany). Once dispersed, the enzymatic reaction was initiated by adding TG-PG (0.15%) and reducing the RPM to 50 (after 1 hr, the enzyme was inactivated by elevating the temperature to 70 Celsius degrees for 15 min while increasing the RPM to 500). The emulsion was homogenized at 600 Bar at 65 Celsius degrees (GEA, Lab homogenizer Panda Plus 2000, Italy, two stages homogenization), pasteurized using a tubular heat exchanger (80 Celsius degrees, 5 min holding time; HTST/UHT Mini Pilot System, Armfield) and cooled down to 43 Celsius degrees. Finally, the starter cultures were added (0.01%) and the yogurt was incubated until reaching a pH of 4.0-4.3 (approximately 12-14 hr). The fermented curd was agitated and stored at 4°C until further examination. All concentrations refer to % w/w in the final product.

Viscosity measurements - The viscosity of the different alternative milk samples (3% and 8% BLG, at three different TG PG concentration: 0, 0.05 and 0.15%) was examined using viscometer (ROTAVISC lo-vi I HELI Complete, IKA, Germany) using a T-SP-1 spindle at 200 RPM. The measurement was carried out by filling a 50 mL falcon with the milk sample at 10 Celsius degrees. In addition, the viscosity of commercial bovine milk was measured according to the same procedure. Measurements were performed twice, and results are a calculated average of both measurements.

Particle size measurements - The average particle diameter of both alternative milk and cream cheese products were evaluated using a Photon Cross Correlation Spectroscopy (PCCS) NANOPHOX (Sympatec GmbH, Germany) at 25 Celsius degrees. The samples were diluted in a ratio of 1:150 (sample: buffer phosphate, pH =7.2) and results are a calculated average of two replicates.

Texture analyzer measurements - The penetration test for the different alternative cream cheese samples (3% and 8% BLG, at three different TG-PG concentration: 0, 0.05 and 0.15%) was performed using texture analyzer (Ta.xt Express C, Stable Micro Systems, UK) using a cylinder probe. The measurement was carried out in a 150 mL cup filled with

100 mL cream cheese at 10 Celsius degrees. Measurements were performed twice, and results are a calculated average of both measurements. The Back Extrusion Rig test for the different cream cheese samples (3% and 8% BLG, at three different TG PG concentration: 0, 0.05 and 0.15%) was performed using texture analyzer (Ta.xt Express C, Stable Micro
 5 Systems, UK) using a disc plunger. The measurement was carried out in a 150 mL cup filled with 100 mL cream cheese at 10 Celsius degrees. Measurements were performed twice, and results are a calculated average of both measurements.

Results.

Results herein show the effect of TG+PG addition on an alternative milk product comprised of two different BLG concentrations. As observed in **Table 2**, addition of TG+PG had a significant effect on the alternative milk's viscosity, especially in milk prepared with 8% BLG. Thus, the addition of 0.15% enzymes mix decreased the viscosity of the milk by 4-fold, creating a thick yet flowing liquid. Without the addition of TG-PF enzyme mixture, the milk showed solid-like texture attributes similar to that of a yogurt. The trend of changing
 15 the viscosity was seen also in alternative milk comprising 3% BLG, although to a lesser extent. Considering that *a priory* alternative milk comprising 3% BLG has a viscosity below 120 cP, the effect of the addition of TG+PG during the preparation of such alternative milk is less prominent. The average particle diameter showed a similar trend in 3% and 8% BLG-based milk products. It was concluded that once the concentration of enzyme was increased
 20 the overall average diameter decreased.

Table 2. Viscosity (cP) and average particle diameter (nm) of the alternative milk prepared with different BLG concentrations and different TG-PG concentrations.

<i>BLG (%)</i>	<i>TG-PG (%)</i>	<i>Viscosity (cP)</i>	<i>Particle Diameter (nm)</i>
3	0	75.0	466.5
3	0.05	89.1	377.5
3	0.15	98.4	350.8
8	0	2133.0	833.0
8	0.05	1303.0	862.0
8	0.15	412.5	522.0
Bovine Milk		70 - 130	150 - 250

Results herein (Table 3) show the effect of TG+PG addition on an alternative cream cheese product comprised of two different BLG concentrations. Like results obtained for milk, the addition of TG+PG had a significant effect on cream cheese texture, especially for alternative cream cheese comprising 8% BLG. The values of all texture parameters measured (softness, firmness, and cohesiveness) were 5-10-fold lower in treated alternative cream cheese comprising 8% BLG in comparison to untreated one. Surprisingly, for both 3% and 8% BLG-comprising alternative cream cheese, increasing the TG+PG concentration from 0.05% to 0.15% did not cause to further significant decrease in the values of texture attributes.

It was also observed that addition of TG-PG during the preparation of an alternative cream cheese brought to a significant reduction in an average particle size diameter: from 1.2-1.3 microns for untreated products to 500-929 nm to treated ones.

Table 3. Texture attributes (softness, firmness, & cohesiveness) & average particle diameter (nm) of alternative cream cheese prepared with different BLG concentrations and different TG PG concentrations.

BLG (%)	TG-PG (%)	Softness (g)	Firmness (g)	Cohesiveness (g)	Consistency (g sec)	Work of Cohesion (g sec)	Particle Diameter (nm)
3	0	12.05	512.96	-270.86	6309.76	-705.11	1260.00
3	0.05	11.40	439.23	-261.74	5197.22	-555.38	500.45
3	0.15	13.10	505.51	-308.28	6260.74	-677.68	615.62
8	0	59.11	2166.89	-1193.13	22626.86	-1925.19	1372.50
8	0.05	8.56	234.98	-152.22	3091.02	-405.25	816.12
8	0.15	10.48	312.18	-202.95	4005.5	-490.77	929.67
Cream Cheese made from Bovine Milk		8 - 20	200 - 600	(-400) - (-100)	2500 - 7000	(-800) - (-300)	100 – 850000

Another interesting, unexpected finding was the importance of the stage in which the TG PG solution is added. It has been found that in order to obtain the effects demonstrated herein, the TG+PG solution is preferably added before any pasteurization step. Specifically, it has been found that (i) the addition of the enzymes before the acidification phase does not decrease the astringency of the final product, and (ii) the addition of the enzymes during the acidification phase forms a highly dense grainy texture in the final product.

Another unexpected finding regarding the importance of the stage in which the TG+PG solution is added is that in order to obtain the effects demonstrated herein, the TG+PG solution is preferably added before the addition of any fats to the composition. Specifically, it has been found that the addition of fats before the TG+PG solution reduces the effect of TG+PG or the speed of its development. In cases where the fats are added before
5 the TG+PG solution, the TG and PG enzymes take longer to produce the same effects (e.g., two hours incubation instead of 1 hour at 50 °C).

The zeta potential of the BLG-comprising particles in alternative yogurt formulations was measured as described in Example 2. The results are presented in Table 4.

10 **Table 4.** Average Zeta potential of particles in yogurt formulations

Sample	Average zeta potential
Control (untreated compound)	4.25
Cow-milk-based yogurt	4.99
TG+PG	11.15

Figure 4 demonstrates the essential role of TG+PG in conferring characteristics of animal-based products to alternative dairy products. Specifically, it is evident from Figure 4 that alternative yogurts produced without TG+PG have different viscosities and react to shear stresses in a much different way than alternative yogurts produced with TG+PG. It can
15 be seen that the viscosity and shear stress of alternative yogurts comprising BLG modified by TG+PG is very similar to those of the commercial cow-milk-based dairy products. This behavior is very prominent not only in the rheologic sense, but also, to a degree, in the organoleptic sense.

Example 5. Exemplary TG+PG-based Cream Cheese, Yogurt and Milk Formulations.

20 Alternative milk, yogurt and cream cheese were prepared as described in Example 4. Alternative yogurt was prepared in a process similar to preparation of alternative cream cheese. **Fig. 5** summarizes components of certain non-limiting embodiments these Cream Cheese, Yogurt and Milk Formulations. Other embodiments are described herein.

Although the present invention has been described herein above by way of preferred
25 embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

CLAIMS

1. A method for preparing an alternative dairy product comprising a protein particle comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:
 - (i) Providing a composition comprising non-animal BLG proteins,
 - 5 (ii) Enzymatically modifying proteins in the composition of step (i) by contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme,
 - (iii) Optionally, lowering the pH of the composition of step (ii), and
 - (iv) Formulating the composition obtained in step (ii) or in step (iii), if present, into
10 a dairy product.
2. An alternative dairy product, obtainable or obtained by a method according to claim 1.
3. An alternative dairy product, comprising a modified non-animal beta-lactoglobulin (BLG) protein, wherein the modified BLG protein comprises at-least-partly deamidated and
15 covalently bound BLG protein.
4. The alternative dairy product of claim 3, comprising:
 - (i) a non-animal beta-lactoglobulin (BLG) protein,
 - (ii) a protein glutaminase (PG) enzyme or residuals thereof, and
 - (iii) a transglutaminase (TG) enzyme or residuals thereof.
- 20 5. The method or alternative dairy product of any one of claims 1 to 4, wherein the alternative dairy product is selected from the group consisting of an alternative milk composition, an alternative yogurt composition, an alternative soft cheese composition, an alternative ice cream composition, and an alternative hard cheese composition.
6. The method or alternative dairy product of claim 5, wherein the alternative dairy
25 product is selected from the group consisting of an alternative milk composition, an alternative yogurt composition, and an alternative cream cheese composition.
7. The method or alternative dairy product of any one of claims 1 to 6, wherein the alternative dairy product has a modulated characteristic in comparison to a corresponding characteristic in a corresponding alternative dairy product substantially devoid of a PG
30 enzyme and a TG enzyme or residuals thereof.

8. The method or alternative dairy product of claim 7, wherein the modulated characteristic is selected from the group consisting of:

- (i) Average particle size,
- (ii) Zeta potential,
- 5 (iii) Viscosity,
- (iv) Gel strength,
- (v) Astringency, and
- (vi) Any combination of (i), (ii), (iii), (iv) and (v).

9. The method or alternative dairy product of claim 8, wherein the modulated
10 characteristic is selected from the group consisting of:

- (i) Increase in an average particle size,
- (ii) Increase in Zeta potential,
- (iii) Decreased viscosity,
- (iv) Decreased gel strength,
- 15 (v) Decreased astringency, and
- (vi) Any combination of (i), (ii), (iii), (iv) and (v).

10. A method for preparing a population of protein particles comprising a beta-lactoglobulin (BLG) protein, the method comprising the steps of:

- (i) Providing a composition comprising non-animal BLG proteins, and
- 20 (ii) Enzymatically modifying BLG proteins in the composition of step (i), by contacting the composition of step (i) with a protein glutaminase (PG) enzyme and a transglutaminase (TG) enzyme, thus obtaining the population of protein particles comprising a BLG protein.

11. A population of protein particles, obtainable or obtained by a method of claim 10.

25 12. A population of protein particles comprising a protein particle, the protein particle having the following characteristics:

- (i) comprising a plurality of non-animal modified BLG proteins,
- (ii) having a diameter of 70 nm or above,
- (iii) having a Zeta potential of -17 mV or above, and
- 30 (iv) optionally, comprising a protein glutaminase (PG) enzyme, and
- (v) optionally, comprising a transglutaminase (TG) enzyme.

13. The method or population of protein particles of any one of claims 10 to 12, wherein the population of protein particles has a modulated characteristic in comparison to a corresponding characteristic in a corresponding population of protein particles in which BLG proteins were not contacted with a PG enzyme and a TG enzyme.

5 14. The method of claim 13, wherein the modulated characteristic is selected from the group consisting of:

- (i) Average particle size,
- (ii) Zeta potential,
- (iii) Astringency, and
- 10 (iv) Any combination of (i), (ii) and (iii).

15. The method of claim 14, wherein the modulated characteristic is selected from the group consisting of:

- (i) Increased average particle size,
- (ii) Increased Zeta potential,
- 15 (iii) Decreased astringency, and
- (iv) Any combination of (i), (ii) and (iii).

16. The method, alternative dairy product, or population of protein particles of any one of claims 1 to 15, wherein the alternative dairy product or population of protein particles is substantially devoid of a casein protein.

20