

COMPOSITIONS AND METHODS OF MAKING THE SAME

[0001] The present disclosure claims priority from Australian Provisional Patent Application No. 2023903226, the entire content of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present disclosure relates to novel methods of assembling compositions comprising casein micelles; recombinant casein micelles, and their uses in the production of food products.

BACKGROUND

[0003] All references, including any patent or patent application cited in this specification are hereby incorporated by reference to enable full understanding of the invention. Nevertheless, such references are not to be read as constituting an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

[0004] Dairy food products, in particular cow's milk, is considered beneficial to a healthy diet, as it is a major source of calcium, as well as providing other essential nutrients, such as protein, vitamins (A, B12 and riboflavin) and minerals (P, Mg, K and Zn). However, despite the obvious health benefits to dairy foods, the production and consumption of dairy products in modern times face significant challenges. The shortage of arable land for food production, dwindling natural resources and effects of traditional land-based agricultural food production systems on the environment, coupled with consequences of climate change makes it difficult for conventional agriculture to cope with the food demand of a growing world population. This challenge; consumer interest in sustainable and ethical products and the increasing trend of food intolerance and allergy (such as to lactose or casein in dairy products) have attributed to the increased demand for sustainable, animal-free dairy product alternatives. However, many of the current alternatives, such as plant-based milks such as soy, almond or oat, do not contain the same nutrients as cow's milk, and the reliance on such products can lead to nutritional deficiency and health issues such as reduced bone mineral density.

[0005] Alternative food and nutrient production using microbes (bacteria, yeasts, fungi, microalgae), and macro algae is being explored. The goal of cellular agriculture is for sustainable, animal free food production capable of providing similar nutritional benefit.

[0006] However, milk is a deceptively simple food product, and cannot be easily replicated. Previous milk / dairy alternatives, including plant milks and recombinant protein-based

products, usually lack the physicochemical properties of milk and do not meet consumer expectations for flavour, aroma and/or other sensory properties. This is because milk is a complex emulsion of milk fat particles and milk protein complexes (called micelles) which are enriched with minerals, suspended within an aqueous fluid of sugars and protein. The conformation of the milk protein complex contributes to milk's characteristic appearance, mouth feel / texture and unique ability to deliver high protein and mineral nutritional value.

[0007] Accordingly, there remains an urgent need for improved methods of producing environmentally sustainable and less resource-intensive, animal-free dairy products that more closely replicate the taste, appearance, physicochemical and nutritional profile of animal milk, in particular, cow milk.

SUMMARY OF THE INVENTION

[0008] In one aspect disclosed herein, there is provided a method of assembling a casein micelle, the method comprising providing a composition comprising at least one casein protein in a yeast ferment; and agitating the composition of step (a) in the presence of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a micelle.

[0009] In an embodiment the at least one casein protein is selected from the group consisting of α S1, α S2, β and κ casein proteins or functional variants thereof. In one embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S2 casein or a functional variant thereof. In another embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S1 casein or a functional variant thereof. In another embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and κ casein or a functional variant thereof. In one embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof and κ casein or a functional variant thereof. In one embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof, κ casein or a functional variant thereof and α S2 casein or a functional variant thereof.

[0010] In an embodiment, the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:1 to 2:1. In another embodiment, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 10:13 to 5:1. In one embodiment, the mass ratio of the β casein or a functional

variant thereof to α S1 casein or a functional variant thereof to κ casein or a functional variant thereof is from about 1:1:1 to 5:5:1.

[0011] In one embodiment, the final concentration of the calcium salt is from about 7-8mM. In another embodiment, the final concentration of the phosphate salt is from about 5-6mM. In one embodiment, the composition comprising at least one casein protein in a yeast ferment further comprises a citrate buffer. In another embodiment, the final concentration of the citrate buffer is from about 2-3mM.

[0012] In one embodiment, the micelle has an average particle diameter of from about 50nm to 600nm. In another embodiment, the micelle has an average particle diameter of about 125nm to 490nm. In one embodiment, the micelle has an average particle diameter of about 150nm to 230nm.

[0013] In one embodiment, the micelle is colloidally stable.

[0014] In an embodiment, the at least one casein protein is a native casein protein. In one embodiment, the at least one casein protein is a recombinant casein protein produced in yeast cells or bacterial cells or plant cells. In an embodiment, the at least one casein protein is produced in yeast cells. In a preferred embodiment, the least one casein protein is produced by the yeast cells from which the yeast ferment is derived.

[0015] In an embodiment, the at least one casein protein is a mammalian casein protein. In another embodiment, the mammalian casein protein is a bovine casein protein, a sheep casein protein, a goat casein protein, a human casein protein or a combination thereof. In a preferred embodiment, the mammalian recombinant casein protein is a bovine casein protein.

[0016] In an embodiment, the yeast ferment is partially purified. In another embodiment, the partial purification is performed using by tangential flow filtration, ultrafiltration, dialysis and/or diafiltration. In another embodiment, the yeast ferment is cell-free.

[0017] In one aspect disclosed herein, there is provided a micelle comprising at least three recombinant casein proteins, wherein the at least three recombinant casein proteins are selected from the group consisting of: a recombinant α S1 casein or a functional variant thereof; a recombinant α S2 casein or a functional variant thereof; a recombinant κ casein or a functional

variant thereof; and a recombinant β casein or a functional variant thereof. In another embodiment, the micelle is the micelle produced using any one of the methods disclosed herein.

[0018] In one embodiment, the at least three recombinant casein proteins comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof. In another embodiment, the at least three recombinant casein proteins comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S2 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof. In another embodiment, the micelle comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof, recombinant κ casein or a functional variant thereof and recombinant α S2 casein or a functional variant thereof.

[0019] In one embodiment, the micelle has an average particle diameter of from about 50nm to 600nm. In another embodiment, the micelle has an average particle diameter of about 125nm to 490nm. In one embodiment, the micelle has an average particle diameter of about 150nm to 230nm.

[0020] In one embodiment, the micelle is colloiddally stable.

[0021] In one aspect disclosed herein, there is provided a composition comprising the micelles disclosed herein. In an embodiment, the composition further comprises whey proteins. In another embodiment, the whey proteins comprise β -lactoglobulin and α -lactalbumin. In another embodiment, the β -lactoglobulin and α -lactalbumin are recombinant β -lactoglobulin and recombinant α -lactalbumin. In another embodiment, the composition further comprises a fat, a sugar, calcium, phosphate and food flavouring and/or colouring.

[0022] In one embodiment, the composition does not comprise a constituent that is derived from an animal.

[0023] In one aspect disclosed herein, there is provided a composition comprising a micelle in a yeast ferment, wherein the micelle comprises at least one recombinant casein protein, wherein the recombinant casein protein is selected from the group consisting of: a recombinant α S1 casein or a functional variant thereof; a recombinant α S2 casein or a functional variant thereof; a recombinant κ casein or a functional variant thereof; and a recombinant β casein or a functional variant thereof.

[0024] In one embodiment, the micelle comprises or consists of recombinant β casein or a functional variant thereof and recombinant α S1 casein or a functional variant thereof; or comprises or consists of recombinant β casein or a functional variant thereof and recombinant κ casein or a functional variant thereof; or comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof; or comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof, recombinant κ casein or a functional variant thereof and recombinant α S2 casein or a functional variant thereof.

[0025] In one embodiment, the recombinant casein protein is produced in yeast cells or bacterial cells. In an embodiment, the at least one casein protein is produced in yeast cells. In yet another embodiment, the least one casein protein is produced by the yeast cells from which the yeast ferment is derived. In one embodiment, the yeast ferment has been partially purified. In another embodiment, the purification is performed by tangential flow filtration, ultrafiltration, dialysis and/or diafiltration. In another embodiment, the yeast ferment is cell-free.

[0026] In one aspect disclosed herein, there is provided a dairy substitute comprising the any one of the compositions or the micelles disclosed herein. In one embodiment, the dairy substitute is selected from a group consisting of milk, yogurt, cream, butter, cheese, ice-cream and custard.

BRIEF DESCRIPTION OF THE FIGURES

[0027] Figure 1 shows successful expression of recombinant milk proteins in *P. pastoris* ferment. (A) Example of HPLC chromatogram analysis detecting the presence of β -casein. (B) SDS-PAGE analysis of chromatographic fractionation of β -casein. Samples of the fermentation supernatant were chromatographed using an anion exchange stationary phase, whereby a salt gradient of 0-100% 1 M NaCl was passed over the stationary phase in order to elute proteins between 10-14 minutes retention time. (C) SDS analyses of partially purified protein fractions of recombinant β -casein, κ -casein and α -casein proteins.

[0028] Figure 2 shows micellarisation of sample M74. (A) Shows sample M74 before any additions (M74A), after calcium addition (M74B), after phosphate addition (M74C), and after overnight storage at 4°C (M74D). (B) shows the intensity based particle size distributions.

[0029] Figure 3 shows micellarisation of sample M75. (A) Shows sample M75 before any additions (M75A), after calcium addition (M75B), after phosphate addition (M75C), and after overnight storage at 4°C (M75D). (B) shows the intensity based particle size distributions.

[0030] Figure 4 shows micellarisation of sample M77. (A) Shows sample M77 before any additions (M77A), after calcium addition (M77B), after phosphate addition (M77C), and after overnight storage at 4°C (M77D). (B) shows the intensity based particle size distributions.

[0031] Figure 5 shows micellarisation of sample M78. (A) Shows sample M78 after phosphate addition (M78C). (B) shows the intensity based particle size distributions.

[0032] Figure 6 shows micellarisation of sample M79. (A) Shows sample M79 before any additions (M79A), after calcium addition (M79B), after phosphate addition (M79C). (B) shows the intensity based particle size distributions.

[0033] Figure 7 shows micellarisation of sample M80. (A) Shows sample M80 before any additions (M80A), after calcium addition (M80B), after phosphate addition (M80C), and after overnight storage at 4°C (M80D). (B) shows the intensity based particle size distributions.

[0034] Figure 8 shows micellarisation of sample M81. (A) Shows sample M81 before any additions (M81A), after calcium addition (M81B) and after phosphate addition (M81C). (B) shows the intensity based particle size distributions.

[0035] Figure 9 shows micellarisation of sample M82. (A) Shows sample M82 before any additions (M82A), after calcium addition (M82B), after phosphate addition (M82C), and after overnight storage at 4°C (M82D). (B) shows the intensity based particle size distributions.

[0036] Figure 10 shows micellarisation of sample M83. (A) Shows sample M83 before any additions (M83A), after calcium addition (M83B), after phosphate addition (M83C), and after overnight storage at 4°C (M83D).

[0037] Figure 11 shows micellarisation of sample M84. (A) Shows sample M84 before any additions (M84A), after calcium addition (M84B), after phosphate addition (M84C), and after overnight storage at 4°C (M84D). (B) shows the intensity based particle size distributions.

[0038] Figure 12 shows micellarisation of sample M85. (A) Shows sample M85 before any additions (M85A), after calcium addition (M85B), after phosphate addition (M85C), and after overnight storage at 4°C (M85D). (B) shows the intensity based particle size distributions.

[0039] Figure 13 shows micellarisation of sample M87. (A) Shows sample M87 before any additions (M87A), after calcium addition (M87B), after phosphate addition (M87C), and after overnight storage at 4°C (M87D). (B) shows the intensity based particle size distributions.

[0040] Figure 14 shows micellarisation of sample M88. (A) Shows sample M88 before any additions (M88A), after calcium addition (M88B), after phosphate addition (M88C), and after overnight storage at 4°C (M88D). (B) shows the intensity based particle size distributions.

[0041] Figure 15 shows micellarisation of sample M89. (A) Shows sample M89 before any additions (M89A), after calcium addition (M89B), after phosphate addition (M89C), and after overnight storage at 4°C (M89D). (B) shows the intensity based particle size distributions.

[0042] Figure 16 shows micellarisation of sample M90. (A) Shows sample M90 before any additions (M90A), after calcium addition (M90B), after phosphate addition (M90C), and after overnight storage at 4°C (M90D). (B) shows the intensity based particle size distributions.

[0043] Figure 17 shows micellarisation of sample M91. (A) Shows sample M91 before any additions (M91A), after calcium addition (M91B), after phosphate addition (M91C), and after overnight storage at 4°C (M91D). (B) shows the intensity based particle size distributions.

[0044] Figure 18 shows micellarisation of sample M92. (A) Shows sample M92 before any additions (M92A), after calcium addition (M92B), after phosphate addition (M92C), and after overnight storage at 4°C (M92D). (B) shows the intensity based particle size distributions.

[0045] Figure 19 shows micellarisation of sample M93. (A) Shows sample M93 before any additions (M93A), after calcium addition (M93B), after phosphate addition (M93C), and after overnight storage at 4°C (M93D). (B) shows the intensity based particle size distributions.

[0046] Figure 20 shows micellarisation of sample M94. (A) Shows sample M94 before any additions (M94A), after calcium addition (M94B), after phosphate addition (M94C), and after overnight storage at 4°C (M94D). (B) shows the intensity based particle size distributions.

[0047] Figure 21 shows micellarisation of sample M109. (A) Shows sample M109 before any additions (M109A), after calcium addition (M109B), after phosphate addition (M109C), and after overnight storage at 4°C (M109D). (B) shows the intensity based particle size distributions.

[0048] Figure 22 shows micellarisation of sample M111. (A) Shows sample M111 before any additions (M111A), after calcium addition (M111B), after phosphate addition (M111C), and after overnight storage at 4°C (M111D). (B) shows the intensity based particle size distributions.

[0049] Figure 23 shows micellarisation of sample M112. (A) Shows sample M112 before any additions (M112A), after calcium addition (M112B), after phosphate addition (M112C), and after overnight storage at 4°C (M112D). (B) shows the intensity based particle size distributions.

[0050] Figure 24 shows micellarisation of sample M118. (A) Shows sample M118 before any additions (M118A), after calcium addition (M118B), after phosphate addition (M118C), and after overnight storage at 4°C (M118D). (B) shows the intensity based particle size distributions.

[0051] Figure 25 shows micellarisation of sample M113, M114, M115 and M117. (Ai) Shows the samples before any additions (M113A, M114A, M115A and M117A) and (Aii) the intensity based particle size distributions; (Bi) after calcium addition (M113B, M114B, M115B and M117B) and (Bii) the intensity based particle size distributions; (Ci) after phosphate addition (M113C, M114C, M115C and M117C) and (Cii) the intensity based particle size distributions; (Di) after overnight storage at 4°C (M113D, M114D, M115D and M117D) and (Dii) the intensity based particle size distributions..

[0052] Figure 26 shows micellarisation of sample M121. (A) Shows sample M121 before any additions (M121A), after calcium addition (M121B), after phosphate addition (M121C), and after overnight storage at 4°C (M121D). (B) shows the intensity based particle size distributions.

[0053] Figure 27 shows micellarisation of sample M122. (A) Shows sample M122 before any additions (M122A), after calcium addition (M122B), after phosphate addition (M122C), and

after overnight storage at 4°C (M122D). (B) shows the intensity based particle size distributions.

[0054] Figure 28 shows stability of micelle compositions after a 10 minute heating to 75-80°C over several days. The samples from left to right, in (A) and (B) correspond to micellar composition after overnight incubation, day 1 control (no heat), day 1 (after heat treatment), day 2 (after heat treatment), day 3 (after heat treatment), day 6 (after heat treatment), day 7 (after heat treatment). (A) M176 comprising only recombinant β -casein. (B) M177 comprising recombinant β -casein and recombinant κ -casein. (C) shows the intensity based particle size distributions of M176 and M177.

[0055] Figure 29 shows stability of micelle compositions after a 10 minute heating to 75-80°C over several days. The samples from left to right, in (A) and (B) correspond to micellar composition after overnight incubation, day 1 control (no heat), day 1 (after heat treatment), day 2 (after heat treatment), day 3 (after heat treatment), day 6 (after heat treatment), day 7 (after heat treatment). (A) M178 comprising recombinant β -casein and α S1-casein. (B) M179 comprising recombinant β -casein, α S1-casein and recombinant κ -casein. (C) shows the intensity based particle size distributions of M176 and M177. (D) is a graph of the storage stability trials of the recombinant micelle compositions.

[0056] Figure 30 shows the particle size distributions of samples M70 – M74 comprising bovine β casein particles generated in the presence of Tris and NaCl.

[0057] Figure 31 shows micellarisation of sample M166; before any additions (A), after calcium addition (B), after phosphate addition (C), after overnight storage at 4°C (D), after 4 days of storage (G), after 5 days of storage (H), after 6 days of storage (I), after 7 days of storage (J) and after 8 days of storage (K). The graph shows the intensity based particle size distributions.

[0058] Figure 32 shows micellarisation of sample M167; before any additions (A), after calcium addition (B), after phosphate addition (C), after overnight storage at 4°C (D), after 4 days of storage (G), after 5 days of storage (H), after 6 days of storage (I), after 7 days of storage (J) and after 8 days of storage (K). The graph shows the intensity based particle size distributions.

[0059] Figure 33 shows micellarisation of sample M166; before any additions (A), after calcium addition (B), after phosphate addition (C), after overnight storage at 4°C (D), after 4 days of storage (G), after 5 days of storage (H), after 6 days of storage (I), after 7 days of storage (J) and after 10 days of storage (M). The graph shows the intensity based particle size distributions.

[0060] Figure 34 shows micellarisation of sample M166; before any additions (A), after calcium addition (B), after phosphate addition (C), after overnight storage at 4°C (D), after 4 days of storage (G), after 5 days of storage (H), after 6 days of storage (I), after 7 days of storage (J). The graph shows the intensity based particle size distributions.

[0061] Figure 35 shows the stability of the recombinant micelle compositions over 8-10 days in storage, at 4°C.

DETAILED DESCRIPTION OF THE INVENTION

[0062] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0063] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article, unless explicitly stated otherwise. By way of example, "an element" means one element or more than one element.

[0064] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0065] As used herein, the term "about" refers to a quantity, level, value, dimension, size, or amount that varies by as much as 10% (e.g., by 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%) to a reference quantity, level, value, dimension, size, or amount. The present description uses numerical ranges to quantify certain parameters relating to this disclosure. It should be understood that when numerical ranges are provided, such ranges are to be construed as providing support for claim limitations that recite the lower value of the range as well as claim limitations that recite the upper value of the range. For example, a disclosed numerical range

of 10 to 100 provides support for a claim reciting “greater than 10” (with no upper bounds) and a claim reciting “less than 100” (with no lower bounds) and provided support for and includes the end points of 10 and 100.

[0066] Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

[0067] The present disclosure relates generally to novel methods of assembling casein micelles, recombinant production of casein proteins, recombinant casein micelles, and compositions thereof. The present disclosure is predicated, at least in part, on the inventors' surprising finding that a composition of casein proteins in a yeast ferment can assemble into micelles. Previous methods of producing casein micelles invariably rely on purified fractions of casein proteins. The methods disclosed herein is advantageous as it does not require time-consuming and resource-intensive downstream processing and purification techniques to generate purified fractions of the casein proteins, prior to micellarisation. Further, the methods disclosed herein, advantageously allow assembly of colloiddally stable micelles, which approach the properties of cow milk micelles (i.e. size and/or protein and mineral content).

[0068] Herein, the terms “peptide”, “polypeptide”, “protein”, “enzyme” are to be understood as referring to a chain of amino acids linked by peptide bonds, irrespective of the number of amino acids forming said chain. The amino acids are typically represented by their one-letter or three-letters code, according to the following nomenclature: A: alanine (Ala); C: cysteine (Cys); D: aspartic acid (Asp); E: glutamic acid (Glu); F: phenylalanine (Phe); G: glycine (Gly); H: histidine (His); I: isoleucine (Ile); K: lysine (Lys); L: leucine (Leu); M: methionine (Met); N: asparagine (Asn); P: proline (Pro); Q: glutamine (Gln); R: arginine (Arg); S: serine (Ser); T: threonine (Thr); V: valine (Val); W: tryptophan (Trp) and Y: tyrosine (Tyr).

[0069] The term “native” as used herein refers to protein or nucleic acid that is derived from, or is naturally present and/or expressed in an organism or cell thereof. For example, a native bovine casein protein refers to a casein protein that is derived from a bovine, i.e. purified from bovine milk. In one example, a native ovine casein protein refers to a casein protein that is derived from an ovine, i.e. purified from sheep milk. In another example, a native caprine

casein protein refers to a casein protein that is derived from a caprine, i.e. purified from goat milk.

[0070] The term “recombinant”, as used herein, refer to a biomolecule, *e.g.*, a gene or protein, or to a cell or microorganism. The term “recombinant” may be used in reference to cloned DNA isolates, chemically synthesized polynucleotides, or polynucleotides that are biologically synthesized by heterologous systems, as well as proteins or polypeptides encoded by such nucleic acids, *e.g.* enzymes. A “recombinant” nucleic acid is a nucleic acid linked to a nucleotide or polynucleotide to which it is not linked in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. As use herein, a “recombinant protein” refers to a protein that is expressed from an exogenously introduced nucleic acid. The amino acid sequence of the recombinant protein may be identical to its corresponding native protein, or it may contain amino acid sequence modifications / substitutions / mutations. As use herein, a “recombinant cell” refers to a cell that has introduced into it exogenous nucleic acid, typically exogenous DNA, such as a vector or other polynucleotides. The term includes the progeny of the original cell into which the exogenous DNA has been introduced. Thus, a “recombinant cell” as used herein generally refers to a cell that has been transformed, transfected or transduced with exogenous DNA. The host cell may be transformed, transfected or transduced in a transient or stable manner. The exogenous nucleic acid is typically introduced into a host cell so that it is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector. The term “recombinant cell” encompasses any progeny of a parent host cell that is not identical to the parent host cell due to the alterations introduced.

Milk proteins

[0071] The compositions and food products provided herein comprise one or more recombinant milk proteins. The term milk proteins refer generally to proteins found in milk. The major proteins in milk are broadly categorised as casein proteins and whey proteins. Casein is a family of phosphoproteins, comprising α S1 casein, α S2 casein, β casein, and κ casein. Whey proteins include α -Lactalbumin and β -lactoglobulin (the predominant whey proteins); as well as the low abundance immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and protease-peptones.

[0072] The casein and whey milk proteins referred to in the present disclosure can be casein and whey proteins from any mammalian species, including but not limited to cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque,

gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, and echidna. In one embodiment, the casein and whey proteins are derived from humans. In one embodiment, the casein and whey proteins are cow casein and whey proteins. In one embodiment, the casein and whey proteins are sheep casein and whey proteins. In one embodiment, the casein and whey proteins are goat casein and whey proteins. In another embodiment, the casein and whey proteins are buffalo casein and whey proteins. In one embodiment, the casein and whey proteins are human casein and whey proteins.

Casein Proteins

[0073] Casein is a family of phosphoproteins, comprising α S1 casein, α S2 casein, β casein, and κ casein. Mammalian milk comprise a range of casein content. For example, casein comprises 80% of the protein in cow's milk, while human milk is 20-60% casein. A component that gives dairy products such as cheese or yogurt its unique characteristics is the casein proteins that form micelles in milk.

[0074] One example of cow α S1 casein include NCBI Reference Sequence No: NP_851372.1. Examples of cow α S2 casein include NCBI Reference Sequence: Accession Nos: NP_776953.1 and NP_851372.1. Examples of cow β -casein include NCBI Reference Sequence Nos: XP_015327157.2 and XP_010804480.2. Examples of human β -casein include NCBI Reference Sequence Nos: NP_001289699.1 and NP_001882.1. Two well-known β -casein genetic variants are A1 β -casein and A2 β -casein, which differ by one amino acid at position 67, which is histidine in A1 β -casein and proline in A2 β -casein. Examples of cow κ casein include NCBI Reference Sequence Nos: Accession: NP_776719.1 and Accession: XP_024848756.1. Examples of human κ casein include NCBI Reference Sequence Nos: NP_005203.2, XP_016863250.1. Other examples of casein proteins and variant casein proteins can be found in Table 1. For the practice of the present disclosure, identification and selection of α S1 casein, α S2 casein, β -casein and κ casein proteins would be readily known to persons skilled in the art.

Casein Micelles

[0075] The term "micelle" typically refers to spherical colloidal particles formed from the aggregation of amphiphilic molecules. The term "micelle" generally describes a system that

exists in aqueous solvent, in which the amphiphile's hydrophilic zone is oriented outwards while the hydrophobic zone is oriented towards the particle's core.

[0076] In relation to dairy products and casein micelles, micelles are protein colloids comprised of casein proteins, wherein the casein micelles are microscopically dispersed insoluble particles suspended throughout another in a liquid, and can encapsulate components such as water, minerals, salts and/or vitamins. As used herein, the terms "micelle" and "casein micelle" are used interchangeably to refer to a micelle comprising casein proteins. The term "colloidally stable" as used herein refer to particles which remain suspended in equilibrium in the solution and do not coalesce / precipitate etc. over a period of time, for example, over an hour, over 2-5 hours, over 5-10 hours, over 10-20 hours, overnight, over 24 hours, over 2 days, over 3 days, over 4 days over 5 days, over 5-7 days, over 7-10 days, over 10-15 days.

[0077] In some embodiments, micelles described herein include micelles formed in a liquid solution. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not settle out (e.g. aggregate or precipitate) of the liquid solution. In some cases, the composition includes casein containing micelles and other forms of the caseins such as aggregates and/or monomeric forms of the proteins. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not substantially settle out (e.g. aggregate or precipitate) of the liquid solution over a range of temperatures. In this context, the casein containing micelles and the compositions thereof may be referred to as heat stable or temperature stable. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not substantially settle out (e.g. aggregate or precipitate) of the liquid solution in refrigerated conditions, from about 1°C to about 20°C, preferably from about 2°C to about 20°C, preferably from about 3°C to about 20°C, preferably from about 4°C to about 20°C, preferably from about 5°C to about 20°C, preferably from about 6°C to about 20°C, or more preferably from about 10°C to about 20°C. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not substantially settle out (e.g. aggregate or precipitate) of the liquid solution at room temperature. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not substantially settle out (e.g. aggregate or precipitate) of the liquid solution at temperatures exceeding room temperature. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not

substantially settle out (e.g. aggregate or precipitate) of the liquid solution even when heated to just below about 95°C. In some embodiments, casein containing micelles are present in the composition, where the micelles remain dispersed and do not substantially settle out (e.g. aggregate or precipitate) of the liquid solution even when heated to just below about 80°C. By "do not substantially settle out of the liquid solution", as used herein, is meant that at least about 50%, preferably at least about 55%, preferably at least about 60%, preferably at least about 65%, preferably at least about 70%, preferably at least about 75%, preferably at least about 80%, preferably at least about 85%, preferably at least about 90%, or more preferably at least about 95% of the casein containing micelles remain dispersed in the liquid solution or composition.

[0078] Casein micelles also provide numerous techno-functional properties to milk and milk products. They provide heat stability to milk, allowing for pasteurisation and consequently delivering a long shelf life; preventing curdling when milk is exposed to prolonged heat; providing desirable frothing/foaming in the presence of steam; support curd and gel formation leading to products such as cheese and yoghurt; etcetera. Micelles also contribute to visual properties, *i.e.*, the white colour resulting from light scattering by the micelle particles. In some embodiments, the casein micelles and compositions thereof remain in solution after heat treatment or storage. Unless otherwise indicated, the reference to micelles remaining in solution is also used interchangeably herein to denote micelle stability. It is to be understood that the reference herein to micelle stability (in solution), whether following exposure to heat, duration of storage, or both, does not mean the absence of, for example, micelle precipitation or aggregation, but rather that a significant number of micelles in the composition remain in solution. That number may depend on, for example, the intended use of the micelle composition, as it may be acceptable for certain uses or application that a proportion of micelles in the composition have aggregated or precipitated out of solution. As an illustrative example, less than about 70% of the micelles in the composition (e.g., about 70%, less than about 60%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, etc) have aggregated or precipitated out of solution. Thus, in an embodiment, less than about 70% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 60% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 50% of the

micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 40% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 35% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 30% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 25% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 20% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 15% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 10% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 5% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 4% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 3% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 2% of the micelles in the composition have aggregated or precipitated out of solution. In an embodiment, less than about 1% of the micelles in the composition have aggregated or precipitated out of solution. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C or more preferably after heating to about 40°C, for about 1 minute, preferably for about 2 minutes, preferably for about 3 minutes, preferably for about 5 minutes, preferably for about 7 minutes, preferably for about 10 minutes, preferably for about 15 minutes, or more preferably for about 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 80°C for about 2 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 80°C for about 3 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 80°C for about 5 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 80°C for about 7 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating to about 80°C for about 10 minutes. In some embodiments, the casein

micelles and compositions thereof remain in solution after heating to about 80°C for about 15 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after heating 75-80°C for 10 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to conditions suitable for pasteurisation. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to conditions suitable for ultra-high temperature (UHT) processing. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 40°C to 140°C for between 2 seconds to 30 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 50°C to 120°C for between 2 seconds to 30 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 50°C to 120°C for between 5 seconds to 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 120°C for between 2 seconds to 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 120°C for between 5 seconds to 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 100°C for between 5 seconds to 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 80°C for between 5 seconds to 20 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 80°C for between 5 seconds to 15 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to a temperature between 60°C to 80°C for between 5 seconds to 10 minutes. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to at least about 140°C for at least about 2-10 seconds or for at least 2-5 seconds. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to at least 80°C for at least about 15 to 30 seconds. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to at least 72°C for at least about 15 to 30 seconds. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for at least 24 hours. In some embodiments, the casein micelles and compositions thereof remain in solution after

being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for at least 48 hours. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for at least 72 hours. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least 4 days. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least about 5 days. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least about 6 days. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least about 7 days. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least about 8 days. In some embodiments, the casein micelles and compositions thereof remain in solution after being heated to about 90°C, preferably after heating to about 80°C, preferably after heating to about 70°C, preferably after heating to about 60°C, preferably after heating to about 50°C, or more preferably after heating to about 40°C, for up to at least about 10 days.

[0079] As described herein, casein micelles may be formed with isolated casein proteins, such as recombinant casein proteins or isolated native casein proteins purified / derived from milk. Micelles can be formed from recombinant or native α casein, such as α S1 casein or a functional

variant thereof, α S2 casein or a functional variant thereof, β casein or a functional variant thereof, κ casein or a functional variant thereof, and/or combinations thereof.

[0080] In some embodiments, the micelles and compositions described herein may comprise α S1 casein or a functional variant thereof. In some embodiments, the micelles and compositions described herein may not comprise α S1 casein or a functional variant thereof. In some embodiments, α S1 casein or a functional variant thereof comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90% or more preferably at least 95% of the casein in the micelle or the composition. In some embodiments, the casein micelle and compositions comprise only of α S1-casein.

[0081] In some embodiments, the micelles and compositions described herein may comprise α S2 casein or a functional variant thereof. In some embodiments, the micelles and compositions described herein may not comprise α S2 casein or a functional variant thereof. In some embodiments, α S2 casein or a functional variant thereof, comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90% or more preferably at least 95% of the casein in the micelle or the composition. In some embodiments, the casein micelle and compositions comprise only of α S2-casein.

[0082] In some embodiments, the micelles and compositions described herein may comprise β -casein proteins or a functional variant thereof. In some embodiments, the micelles and compositions described herein may not comprise β casein or a functional variant thereof. In some embodiments, the β -casein or a functional variant thereof, comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95% or preferably 100% of the casein in the micelle or the composition. In some embodiments, the casein micelle and compositions comprise only of β -casein. In some embodiments, the β -casein is A1 β -casein or a functional variant thereof, and comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%,

preferably at least 90% preferably at least 95% or preferably 100% of the β -casein in the micelle or the composition. In some embodiments, the β -casein is A2 β -casein or a functional variant thereof, and comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95% or preferably 100% of the β -casein in the micelle or the composition. In some embodiments, the β -casein of the micelles and compositions described herein comprises A1 β -casein or a functional variant thereof, and A2 β -casein or a functional variant thereof.

[0083] In some embodiments, the micelles and compositions described herein may comprise κ casein or a functional variant thereof. In some embodiments, the micelles and compositions described herein may not comprise κ casein or a functional variant thereof. In some embodiments, the κ casein or a functional variant thereof, comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95% or preferably 100% of the casein in the micelle or the composition. The casein in the composition may comprise from 0% to 100% κ casein or a functional variant thereof. In some embodiments, the κ casein or a functional variant thereof, comprises at least 1%, preferably at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95% or preferably 100% of the casein in the micelle or the composition. In some embodiments, the casein micelle and compositions comprise only of κ casein.

[0084] In some embodiments, the micelles and compositions described herein comprise recombinant β casein and/or a functional variant thereof, wherein the recombinant β casein and/or a functional variant thereof is the only casein in the micelle or the composition. In some embodiments, the micelles and compositions described herein comprises recombinant α S1 casein, recombinant κ casein and/or functional variants thereof, wherein the recombinant α S1 casein, recombinant κ casein and/or functional variants thereof are the only caseins in the micelle or the composition. In some embodiments, the micelles and compositions described herein comprise recombinant β casein, wherein the recombinant κ casein and/or functional

variants thereof, the recombinant β casein, recombinant κ casein and/or functional variants thereof, are the only caseins in the micelle or the composition. In some embodiments, the micelles and compositions described herein comprise recombinant β casein, recombinant α S1 casein and/or functional variants thereof, wherein the recombinant β casein recombinant α S1 casein and/or functional variants thereof are the only caseins in the micelle or the composition. In some embodiments, the micelles and compositions described herein comprise recombinant β casein, recombinant α S1 casein, recombinant κ casein and/or functional variants thereof, wherein the recombinant β casein, recombinant α S1 casein, recombinant κ casein and/or functional variants thereof are the only caseins in the micelle or the composition.

[0085] In some embodiments, where the micelles and compositions described herein comprise recombinant β casein, the recombinant β casein is the only casein in the micelle or the composition. In some embodiments, where the micelles and compositions described herein comprise recombinant α S1 casein and recombinant κ casein, the recombinant α S1 casein and recombinant κ casein are the only caseins in the micelle or the composition. In some embodiments, where the micelles and compositions described herein comprise recombinant β casein and recombinant κ casein, the recombinant β casein and recombinant κ casein are the only caseins in the micelle or the composition. In some embodiments, where the micelles and compositions described herein comprise recombinant β casein and recombinant α S1 casein, the recombinant β casein and recombinant α S1 casein are the only caseins in the micelle or the composition. In some embodiments, where the micelles and compositions described herein may comprise recombinant β casein, recombinant α S1 casein and recombinant κ casein, wherein the recombinant β casein, recombinant α S1 casein and recombinant κ casein are the only caseins in the micelle or the composition.

[0086] In some embodiments, the micelles and compositions described herein may comprise β casein or a functional variant thereof, and α S1 casein or a functional variant thereof. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:50 to 50:1, preferably from about 1:50 to 45:1, preferably from about 1:50 to 40:1, preferably from about 1:50 to 35:1, preferably from about 1:50 to 30:1, preferably from about 1:50 to 25:1, preferably from about 1:50 to 20:1, preferably from about 1:50 to 19:1, preferably from about 1:50 to 18:1, preferably from about 1:50 to 17:1, preferably from about 1:50 to 16:1, preferably from about 1:50 to 15:1, preferably from about 1:50 to 14:1, preferably from about 1:50 to 13:1, preferably from about 1:50 to 12:1,

preferably from about 1:50 to 11:1, preferably from about 1:50 to 10:1, preferably from about 1:50 to 9:1, preferably from about 1:50 to 9:1, preferably from about 1:50 to 8:1, preferably from about 1:50 to 7:1, preferably from about 1:50 to 6:1, preferably from about 1:50 to 5:1, preferably from about 1:50 to 4:1, preferably from about 1:50 to 3:1, preferably from about 1:50 to 2:1, or more preferably from about 1:50 to 1:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:48 to 50:1, preferably from about 1:45 to 50:1, preferably from about 1:43 to 50:1, preferably from about 1:40 to 50:1, preferably from about 1:38 to 50:1, preferably from about 1:35 to 50:1, preferably from about 1:33 to 50:1, preferably from about 1:30 to 50:1, preferably from about 1:28 to 50:1, preferably from about 1:25 to 50:1, preferably from about 1:23 to 50:1, preferably from about 1:20 to 50:1, preferably from about 1:18 to 50:1, preferably from about 1:15 to 50:1, preferably from about 1:14 to 50:1, preferably from about 1:13 to 50:1, preferably from about 1:12 to 50:1, preferably from about 1:11 to 50:1, preferably from about 1:10 to 50:1, preferably from about 1:9 to 50:1, preferably from about 1:8 to 50:1, preferably from about 1:7 to 50:1, preferably from about 1:6 to 50:1, preferably from about 1:5 to 50:1, preferably from about 1:4 to 50:1, preferably from about 1:3 to 50:1, preferably from about 1:2 to 50:1, or more preferably from about 1:1 to 50:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:9 to 9:1, preferably from about 1:8 to 8:1; preferably from about 1:7 to 7:1; preferably from about 1:6 to 6:1, preferably from about 1:5 to 5:1, preferably from about 1:4 to 4:1, preferably from about 1:3 to about 3:1, preferably from about 1:2 to 2:1 or more preferably from about 1:1 to 2:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:3 to 2:1.

[0087] In some embodiments, the micelles and compositions described herein may comprise β casein or a functional variant thereof, and κ casein or a functional variant thereof. In some embodiments, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 1:50 to 120:1, preferably from about 1:50 to 110:1, preferably from about 1:50 to 100:1, preferably from about 1:50 to 99:1, preferably from about 1:50 to 45:1, preferably from about 1:50 to 40:1, preferably from about 1:50 to 35:1, preferably from about 1:50 to 30:1, preferably from about 1:50 to 25:1, preferably from about 1:50 to 20:1, preferably from about 1:50 to 19:1, preferably from about 1:50 to 18:1, preferably from about 1:50 to 17:1, preferably from about 1:50 to 16:1, preferably from about 1:50 to 15:1, preferably from about 1:50 to 14:1, preferably from about 1:50 to 13:1, preferably from about

1:50 to 12:1, preferably from about 1:50 to 11:1, preferably from about 1:50 to 10:1, preferably from about 1:50 to 9:1, preferably from about 1:50 to 9:1, preferably from about 1:50 to 8:1, preferably from about 1:50 to 7:1, preferably from about 1:50 to 6:1, preferably from about 1:50 to 5:1, preferably from about 1:50 to 4:1, preferably from about 1:50 to 3:1, preferably from about 1:50 to 2:1, from about 1:50 to 1:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 1:48 to 50:1, preferably from about 1:45 to 50:1, preferably from about 1:43 to 50:1, preferably from about 1:40 to 50:1, preferably from about 1:38 to 50:1, preferably from about 1:35 to 50:1, preferably from about 1:33 to 50:1, preferably from about 1:30 to 50:1, preferably from about 1:28 to 50:1, preferably from about 1:25 to 50:1, preferably from about 1:23 to 50:1, preferably from about 1:20 to 50:1, preferably from about 1:18 to 50:1, preferably from about 1:15 to 50:1, preferably from about 1:14 to 50:1, preferably from about 1:13 to 50:1, preferably from about 1:12 to 50:1, preferably from about 1:11 to 50:1, preferably from about 1:10 to 50:1, preferably from about 1:9 to 50:1, preferably from about 1:8 to 50:1, preferably from about 1:7 to 50:1, preferably from about 1:6 to 50:1, preferably from about 1:5 to 50:1, preferably from about 1:4 to 50:1, preferably from about 1:3 to 50:1, preferably from about 1:2 to 50:1, or more preferably from about 1:1 to 50:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is 1:5 to 10:1, preferably from about 1:4 to 9:1, preferably from about 1:3 to 8:1, preferably from about 1:2 to 7:1, preferably from about 1:1 to 6:1, preferably from about 10: to 5:1, or more preferably from about 3:1 to 5:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 99:1 to 3:22. In some embodiments, the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 10:13 to 5:1.

[0088] In some embodiments, the micelles and compositions described herein may comprise β casein or a functional variant thereof, and α S2 casein or a functional variant thereof. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S2 casein or a functional variant thereof is from about 1:5 to 10:1, preferably from about 1:4 to 9:1, preferably from about 1:3 to 8:1, preferably from about 1:2 to 7:1, preferably from about 1:1 to 6:1, preferably from about 10: to 5:1, or more preferably from about 3:1 to 5:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof to α S2 casein or a functional variant thereof is from about 1:1 to 3:5.

[0089] In another embodiment, the micelles and compositions described herein comprise β casein or a functional variant thereof, α S1 casein or a functional variant thereof, and κ casein or a functional variant thereof. In some embodiments, the mass ratio of the β casein or a functional variant thereof, to α S1 casein or a functional variant thereof, to κ casein or a functional variant thereof is from about 60:50:1 to 1:1:1, preferably from about 55:50:1 to 1:1:1, preferably from about 53:46:1 to 1:1:1, preferably from about 50:40:1 to 1:1:1, preferably from about 45:35:1 to 1:1:1, preferably from about 40:30:1 to 1:1:1, preferably from about 30:20:1 to 1:1:1, preferably from about 20:10:1 to 1:1:1, preferably from about 1:1:10 to 1:1:1, preferably from about 1:10:1 to 1:1:1; preferably from about 10:1:1 to 1:1, preferably from about 1:1:5 to 1:1:1, preferably from about 1:1:4 to 1:1:1, preferably from about 1:1:3 to about 1:1:1, or more preferably from about 1:1:2 to 1:1:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof, to α S1 casein or a functional variant thereof, to κ casein or a functional variant thereof is from about 10:10:1 to about 5:5:1, preferably from about 9:9:1 to about 5:5:1, preferably from about 8:8:1 to about 5:5:1, preferably from about 7:7:1 to about 5:5:1, or more preferably from about 6:6:1 to about 5:5:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof, to α S1 casein or a functional variant thereof, to κ casein or a functional variant thereof is from about 10:20:1 to about 10:1:1, preferably from about 10:18:1 to about 10:1:1, preferably from about 10:15:1 to about 10:1:1, preferably from about 10:10:1 to about 10:1:1, or more preferably from about 10:5:1 to about 10:1:1. In some embodiments, the mass ratio of the β casein or a functional variant thereof, to α S1 casein or a functional variant thereof, to κ casein or a functional variant thereof, is from about 1:1:1 to 5:5:1.

[0090] The casein and other proteins described herein, including whey proteins, may be produced recombinantly. In some embodiments, the micelles and compositions described herein may comprise only recombinantly produced proteins. In some embodiments, the micelles and compositions described herein may comprise substantially only of recombinantly produced casein proteins. In another embodiment the micelles and compositions described herein may comprise a mixture of recombinantly produced and animal-derived native casein proteins.

[0091] The recombinantly expressed casein proteins may have post-translational modifications (such as glycosylation or phosphorylation patterns) different from animal- or plant-derived casein proteins. In some cases, the α S1, α S2, β and κ casein proteins comprises no post

translational modifications (PTMs). In some cases, the α S1, α S2, β and κ casein proteins comprises substantially reduced PTMs. As used herein, substantially reduced PTMs means at least 50% reduction of one or more types of PTMs as compared to the amount of PTMs in an animal-derived α casein protein. For instance, the α S1, α S2, β and κ casein proteins may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, or 99% less post-translationally modified as compared to animal-derived α casein. Alternatively, the α S1, α S2, β and κ casein proteins may comprise PTMs comparable to animal-derived α casein PTMs.

[0092] The PTMs in the α S1, α S2, β and κ casein proteins may be modified chemically or enzymatically. In some cases, the α S1, α S2, β and κ casein proteins comprise substantially reduced or no PTMs without chemical or enzymatic treatment. The compositions described herein may be generated using the α S1, α S2, β and κ casein proteins with reduced or no PTMs, wherein the lack of PTMs is not due to chemical or enzymatic treatments of the protein, such as producing the α S1, α S2, β and κ casein proteins through recombinant production where the recombinant protein lacks PTMs. The phosphorylation in the α S1 and α S2 casein proteins may be modified chemically or enzymatically. In some cases, the α S1 and α S2 casein proteins comprises substantially reduced or no phosphorylation without chemical or enzymatic treatment. For instance, α S1 and α S2 casein proteins may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, or 99% less phosphorylated as compared to animal-derived α S1 and α S2 casein. The compositions described herein may be generated using α S1 and α S2 casein protein with reduced or no phosphorylation, wherein the lack of phosphorylation is not due to chemical or enzymatic treatments, such as where recombinant production provides the α S1 and α S2 casein proteins with reduced or no phosphorylation.

[0093] κ casein: In some embodiments, the composition comprises κ casein proteins. In some embodiments, the protein content of the composition comprises from 0% to 100% κ casein protein. In some embodiments, the protein content of the composition comprises at least 1% κ casein protein. The protein content of the composition may comprise 100% or at most 50% or at most 30% κ casein protein. In an embodiment, the composition may comprise from 1% to 5%, preferably from 1% to 7%, preferably from 1% to 10%, preferably from 1% to 12%, preferably from 1% to 15%, preferably from 1% to 18%, preferably from 1% to 20%, preferably from 1% to 25%, preferably from 1% to 30%, preferably from 5% to 7%, preferably from 5% to 10%, preferably from 5% to 12%, preferably from 5% to 15%, preferably from 5% to 18%, preferably from 5% to 20%, preferably from 5% to 25%, preferably from 5% to 30%,

preferably from 7% to 10%, preferably from 7% to 12%, preferably from 7% to 15%, preferably from 7% to 18%, preferably from 7% to 20%, preferably from 7% to 25%, preferably from 7% to 30%, preferably from 10% to 12%, preferably from 10% to 15%, preferably from 10% to 18%, preferably from 10% to 20%, preferably from 10% to 25%, preferably from 10% to 30%, preferably from 12% to 15%, preferably from 12% to 18%, preferably from 12% to 20%, preferably from 12% to 25%, preferably from 12% to 30%, preferably from 15% to 18%, preferably from 15% to 20%, preferably from 15% to 25%, preferably from 15% to 30%, preferably from 18% to 20%, preferably from 18% to 25%, preferably from 18% to 30%, preferably from 20% to 25%, preferably from 20% to 30%, preferably from 25% to 30%, preferably from 30% to 35%, preferably from 35% to 40%, preferably from 40 to 45% or preferably from 45% to 50% κ casein protein. The protein content of the composition may comprise 1%, 5%, 7%, 10%, 12%, 15%, 18%, 20%, 25%, 30%, 35%, 40%, 45% or 50%, 60%, 70%, 80%, 90%, or 100% κ casein protein. The protein content of the composition may comprise at least 1%, 5%, 7%, 10%, 12%, 15%, 18%, 20%, 25%, 30%, 35%, 40% or 45% κ casein protein. The protein content of the composition may comprise at most 5%, 7%, 10%, 12%, 15%, 18%, 20%, 25%, 30%, 35%, 40%, 45% or 50% κ casein protein. In some instances, the composition may be produced using only κ casein. Alternatively, in some cases, the composition may be produced without any κ casein.

[0094] The κ casein protein may be produced recombinantly. In some cases, the composition may comprise only recombinantly produced κ casein protein. In certain cases, the composition may comprise substantially only recombinantly produced κ casein protein. In some cases, κ casein proteins may be 90%, 92%, 95%, 97%, 99% recombinant κ casein. Alternatively, the composition may comprise a mixture of recombinantly produced and animal- or plant-derived κ casein proteins.

[0095] Depending on the host organism used to express the κ casein, the κ casein proteins may have a posttranslational modification, such as glycosylation or phosphorylation pattern different from animal- or plant-derived κ casein protein. In some cases, the κ casein protein in the composition herein comprises no post-translational modifications (PTMs). In some cases, the κ casein protein comprises substantially reduced PTMs. As used herein, substantially reduced PTMs means at least 50% reduction of one or more types of PTMs as compared to the amount of PTMs in an animal- or plant-derived κ casein protein. For instance, κ casein proteins may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, or 99% less post-

translationally modified as compared to animal- or plant-derived κ casein. Alternatively, the κ casein protein may comprise PTMs comparable to animal- or plant-derived κ casein PTMs.

[0096] The PTMs in the κ casein protein may be modified chemically or enzymatically. In some cases, the κ casein protein comprises substantially reduced or no PTMs without chemical or enzymatic treatment. The composition may be generated using κ casein protein with reduced or no PTMs, wherein the lack of or reduction of PTMs is not due to chemical or enzymatic treatments, such as by producing recombinant κ protein in a host where the κ casein protein is not post-translationally modified or the level of PTMs is substantially reduced.

[0097] The glycosylation in the κ casein protein may be modified chemically or enzymatically. In some cases, the κ casein protein comprises substantially reduced or no glycosylation without chemical or enzymatic treatment. For instance, κ casein proteins may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, or 99% less glycosylated as compared to animal-derived κ casein. The composition described herein may be generated using κ casein protein with reduced or no glycosylation, wherein the lack of glycosylation is not due to chemical or enzymatic treatments post recombinant production.

[0098] The phosphorylation in the κ casein protein may be modified chemically or enzymatically. In some cases, the κ casein protein comprises substantially reduced or no phosphorylation without chemical or enzymatic treatment. For instance, κ casein proteins may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, or 99% less phosphorylated as compared to animal- or plant-derived κ casein. The composition may be generated using κ casein protein with reduced or no phosphorylation, wherein the lack of phosphorylation is not due to chemical or enzymatic treatments, such as by producing recombinant κ protein in a host where the κ casein protein is not post-translationally modified or the level of PTMs is substantially reduced.

[0099] The terms micelle "average particle diameter" or micelle diameters are used interchangeably herein and refer to the size of the micelles. The average particle diameter of the micelles as disclosed herein may be from about 10 nm to about 1500 nm, preferably from about 10 nm to about 1400 nm, preferably from about 10 nm to about 1300 nm, preferably from about 10 nm to about 1200 nm, preferably from about 10 nm to about 1100 nm, preferably from 10 nm to about 1000 nm, preferably from about 10 nm to about 900 nm, preferably from about 10 nm to about 800 nm, or preferably from about 10 nm to about 700 nm. In an

embodiment, the average particle diameter of the micelles is at least 10 nm. In an embodiment, the average particle diameter of the micelles is up to 700 nm. In an embodiment, the average particle diameter of the micelles is from 10 nm to 20 nm, preferably from 10 nm to 50 nm, preferably from 10 nm to 100 nm, preferably from 10 nm to 150 nm, preferably from 10 nm to 200 nm, preferably from 10 nm to 250 nm, preferably from 10 nm to 300 nm, preferably from 10 nm to 350 nm, preferably from 10 nm to 400 nm, preferably from 10 nm to 450 nm, preferably from 10 nm to 500 nm, preferably from 10 nm to 550 nm, preferably from 10 nm to 600 nm, preferably from 10 nm to 650 nm, preferably from 20 nm to 50 nm, preferably from 20 nm to 100 nm, preferably from 20 nm to 150 nm, preferably from 20 nm to 200 nm, preferably from 20 nm to 250 nm, preferably from 20 nm to 300 nm, preferably from 20 nm to 350 nm, preferably from 20 nm to 400 nm, preferably from 20 nm to 450 nm, preferably from 20 nm to 500 nm, preferably from 20 nm to 550 nm, preferably from 20 nm to 600 nm, preferably from 20 nm to 650 nm, preferably from 20 nm to 700 nm, preferably from 50 nm to 100 nm, preferably from 50 nm to 150 nm, preferably from 50 nm to 200 nm, preferably from 50 nm to 250 nm, preferably from 50 nm to 300 nm, preferably from 50 nm to 350 nm, preferably from 50 nm to 400 nm, preferably from 50 nm to 450 nm, preferably from 50 nm to 500 nm, preferably from 50 nm to 550 nm, preferably from 50 nm to 600 nm, preferably from 50 nm to 650 nm, preferably from 50 nm to 700 nm, preferably from 100 nm to 150 nm, preferably from 100 nm to 200 nm, preferably from 100 nm to 250 nm, preferably from 100 nm to 300 nm, preferably from 100 nm to 350 nm, preferably from 100 nm to 400 nm, preferably from 100 nm to 450 nm, preferably from 100 nm to 500 nm, preferably from 100 nm to 550 nm, preferably from 100 nm to 600 nm, preferably from 100 nm to 650 nm, preferably from 100 nm to 700 nm, preferably from 150 nm to 200 nm, preferably from 150 nm to 250 nm, preferably from 150 nm to 300 nm, preferably from 150 nm to 350 nm, preferably from 150 nm to 400 nm, preferably from 150 nm to 450 nm, preferably from 150 nm to 500 nm, preferably from 150 nm to 550 nm, preferably from 150 nm to 600 nm, preferably from 150 nm to 650 nm, preferably from 150 nm to 700 nm, preferably from 200 nm to 250 nm, preferably from 200 nm to 300 nm, preferably from 200 nm to 350 nm, preferably from 200 nm to 400 nm, preferably from 200 nm to 450 nm, preferably from 200 nm to 500 nm, preferably from 200 nm to 550 nm, preferably from 200 nm to 600 nm, preferably from 200 nm to 650 nm, preferably from 200 nm to 700 nm, preferably from 250 nm to 300 nm, preferably from 250 nm to 350 nm, preferably from 250 nm to 400 nm, preferably from 250 nm to 450 nm, preferably from 250 nm to 500 nm, preferably from 250 nm to 550 nm, preferably from 250 nm to 600 nm, preferably from 250 nm to 250 nm,

preferably from 250 nm to 700 nm, preferably from 300 nm to 350 nm, preferably from 300 nm to 400 nm, preferably from 300 nm to 450 nm, preferably from 300 nm to 500 nm, preferably from 300 nm to 550 nm, preferably from 300 nm to 600 nm, preferably from 300 nm to 650 nm, preferably from 300 nm to 700 nm, preferably from 350 nm to 400 nm, preferably from 350 nm to 450 nm, preferably from 350 nm to 500 nm, preferably from 350 nm to 550 nm, preferably from 350 nm to 600 nm, preferably from 350 nm to 650 nm, preferably from 350 nm to 700 nm, preferably from 400 nm to 450 nm, preferably from 400 nm to 500 nm, preferably from 450 nm to 500 nm, preferably from 450 nm to 550 nm, preferably from 450 nm to 600 nm, preferably from 450 nm to 650 nm, or preferably from 450 nm to 700 nm. In an embodiment, the average particle diameter of the micelles is from about 10nm to 1500nm. In an embodiment, the average particle diameter of the micelles is from about 50nm to 1000nm. In an embodiment, the average particle diameter of the micelles is from about 50nm to 600nm. In an embodiment, the average particle diameter of the micelles is from about 125nm to 490nm. In an embodiment, the average particle diameter of the micelles is from about 150nm to 230nm. In an embodiment, the average particle diameter of the micelles is about 10 nm, preferably about 20 nm, preferably about 50 nm, preferably about 100 nm, preferably about 150 nm, preferably about 200 nm, preferably about 250 nm, preferably about 300 nm, preferably about 350 nm, preferably about 400 nm, preferably about 450 nm, preferably about 500 nm, preferably about 600nm, or preferably about 700nm. In an embodiment, the average particle diameter of the micelles is at least 10 nm, preferably at least 20 nm, preferably at least 50 nm, preferably at least 100 nm, preferably at least 150 nm, preferably at least 200 nm, preferably at least 250 nm, preferably at least 300 nm, preferably at least 350 nm, preferably at least 400 nm or preferably at least 450 nm. In an embodiment, the average particle diameter of the micelles is up to 20 nm, preferably up to 50 nm, preferably up to 100 nm, preferably up to 150 nm, preferably up to 200 nm, preferably up to 250 nm, preferably up to 300 nm, preferably up to 350 nm, preferably up to 400 nm, preferably up to 450 nm, preferably up to 500 nm, preferably up to 600nm, preferably up to 700nm, preferably up to 700nm, preferably up to 800nm, preferably up to 900nm or preferably up to 1000nm.

Methods of micellarisation

[0100] As disclosed herein, there is provided a method of assembling a casein micelle, the method comprising (a) providing a composition comprising at least one casein protein in a yeast ferment or a bacterial ferment; and agitating the composition of step (a) in the presence

of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a casein micelle.

[0101] In one aspect, there is provided herein a method of assembling a casein micelle, the method comprising (a) providing a composition comprising at least one casein protein in a yeast ferment; and agitating the composition of step (a) in the presence of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a casein micelle.

[0102] In some embodiments, the at least one casein protein is selected from the group consisting of α S1, α S2, β and κ casein proteins or functional variants thereof. In one embodiment, the at least one casein protein is α S1 casein or a functional variant thereof. In one embodiment, the at least one casein protein is α S2 casein or a functional variant thereof. In one embodiment, the at least one casein protein is κ casein or a functional variant thereof. In one embodiment, the at least one casein protein is β casein or a functional variant thereof. In an embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S2 casein or a functional variant thereof. In an embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S1 casein or a functional variant thereof. In an embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof and κ casein or a functional variant thereof. In an embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof and κ casein or a functional variant thereof. In another embodiment, the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof, κ casein or a functional variant thereof and α S2 casein or a functional variant thereof. In one embodiment, the at least one casein protein is produced in yeast cells. In another embodiment, the at least one casein protein is produced by the yeast cells from which the yeast ferment is derived.

[0103] As disclosed herein, the casein micelles are formed using a composition comprising at least one casein protein in a yeast ferment. The term yeast ferment as used herein refers to a composition that comprises culture medium or growth medium used for the culture and growth of yeast. Yeast culture or growth medium can comprise of protein hydrolysates such as peptone; glycerol; amino acids; sugars (e.g. glucose / dextrose); vitamins; trace elements, buffers; and /or salts. Examples of yeast culture medium includes Yeast Extract Peptone Dextrose (YPD) Medium (1% yeast extract, 2% peptone, 2% dextrose (glucose)); Yeast Extract

Peptone Glycerol (YP glycerol) Medium (1% yeast extract, 2% peptone, 2% glycerol); Buffered Glycerol-complex Medium BMGY (1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.0, 1.34% yeast nitrogen base (YNB), 4×10^{-5} % biotin, 1% glycerol, buffered with 0.1 M potassium phosphate buffer, pH 6.0); and Buffered Minimal Methanol (BMM): 300 mM potassium phosphate pH 6.0, 1.34% YNB (yeast nitrogen base), 4×10^{-5} % biotin, 1% methanol. These medium can also be supplemented with antibiotics and/or selection supplements / nutrients (i.e. to support growth of auxotrophic yeast strains). Other suitable yeast culture or growth medium will be known and familiar to persons skilled in the art.

[0104] The yeast ferment may comprise yeast or yeast fragments. The yeast ferment may comprise of live yeast or yeast that has been inactivated. The yeast ferment may comprise proteins and other substances produced and secreted by the yeast. In some embodiments, the yeast ferment comprises the at least one casein protein is produced by the yeast cells from which the yeast ferment is derived.

[0105] In some embodiments, the yeast ferment is not purified (i.e. unprocessed yeast ferment). In one embodiment, the yeast ferment is substantially purified. In an embodiment, the yeast ferment is partially purified. The partially purified yeast ferment may comprise of yeast proteins and fragments thereof, and other substances produced and secreted by the yeast. Other components such as amino acids, salts and sugars, and / or fragments of yeast cells may persist in the partially purified yeast ferment. Partial purification does not seek to isolate a substantially purified fraction of a protein/of interest using time-consuming and potentially denaturing chromatography techniques, such as affinity chromatography, immune-affinity chromatography, HPLC and/or immunoprecipitation. The partial purification may function to concentrate the yeast ferment, remove undesirable salts, and/or to exclude large fragments of cellular debris or protein aggregates that may interfere with micellarisation efficiency. The partially purified yeast ferment can also be lyophilised or freeze dried, whether before or after partial purification (using TFF, ultrafiltration, dialysis and/or diafiltration) and can be reconstituted into a partially purified yeast ferment to any desired concentration.

[0106] In some embodiments, the yeast ferment is subjected to one or more steps to remove most or all of the yeast cells. In some embodiments, the yeast ferment is centrifuged to remove most or all yeast cells. In some embodiments, the yeast ferment is subjected to one or more filtration steps to remove most or all of the yeast cells. In some embodiments, the yeast ferment

is subjected to one or more filtration steps to remove most or all of the yeast cell debris. In one embodiment, the yeast ferment is filtered using a 0.2µm membrane. Other suitable filtration membranes for filtering the yeast ferment will be known to the skilled person. In some embodiments, ion exchange chromatography can be used if necessary to increase the purity of the ferment.

[0107] In some embodiments, the substantially cell-free yeast ferment is subject to dialysis to partially purify the yeast ferment, to remove low molecular weight proteins and degradation products. In another embodiment, the yeast ferment is subject to Tangential Flow Filtration (TFF) using a 10kDaMWCO membrane, Diafiltration against 10mM Tris-Hcl and/or sterilisation by 0.22µm filtration to generate a partially purified yeast ferment that is suitable for use in the methods disclosed herein. In some embodiments, Tangential Flow Filtration (TFF) using a 10kDaMWCO membrane, Diafiltration against 10mM Tris-Hcl and/or sterilisation by 0.22µm filtration can be used to generate a partially purified yeast ferment. In some embodiments, the partially purified yeast ferment is the TFF retentate. In some embodiments, the partially purified yeast ferment is the diafiltration retentate. In some embodiments, the partially purified yeast ferment is the ultrafiltration retentate. In some embodiments, the partially purified yeast ferment is the dialysis retentate.

[0108] In some embodiments, the partially purified yeast ferment is substantially free of insoluble yeast cell fragments and/or yeast-derived proteins. By "substantially free of insoluble yeast cell fragments and/or yeast-derived proteins" is meant that at least some of the insoluble yeast cell fragments and/or yeast-derived proteins has been removed from the harvested yeast ferment and that the level of any residual yeast cell fragments and/or yeast-derived proteins in the partially purified yeast ferment does not adversely impact the formation of recombinant micelles by the methods described herein. For example, the partially purified yeast ferment may suitably comprise 0.5%-95% less insoluble yeast cell fragments and/or yeast-derived proteins when compared to the amount of insoluble yeast cell fragments and/or yeast-derived proteins present in the harvested yeast ferment (i.e., prior to partial purification). Thus, in some embodiments, the partially purified yeast ferment comprises 0.5%-95% less insoluble yeast cell fragments and/or yeast-derived proteins than the harvested yeast ferment. In some embodiments, the partially purified yeast ferment comprises at least 1% less insoluble yeast cell fragments and/or yeast-derived proteins than the harvested yeast ferment. In some embodiments, the partially purified yeast ferment comprises at least 5% less insoluble yeast

[illegible]

cell fragments and/or yeast-derived proteins than the harvested yeast ferment. In some embodiments, the partially purified yeast ferment comprises at least 95% less insoluble yeast cell fragments and/or yeast-derived proteins than the harvested yeast ferment. In some embodiments, the partially purified yeast ferment comprises at least about 0.05% w/v recombinant casein (e.g., about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.11%, about 0.12%, about 0.13%, about 0.14%, about 0.15%, about 0.16%, about 0.17%, about 0.18%, about 0.19%, about 0.20%, about 0.25%, about 0.30%, about 0.40%, about 0.50%, about 0.60%, about 0.70%, about 0.80%, about 0.90%, and so on). In some embodiments, the insoluble yeast cell fragments and/or yeast-derived proteins are of at least about 10kDa in size. In some embodiments, the partially purified yeast ferment comprises at least about 0.01%-90% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.01% -50% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.01% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.01% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.02% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.03% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.04% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.05% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.1% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.2% w/v recombinant casein. In some embodiments, the partially purified yeast ferment comprises at least about 0.3% w/v recombinant casein (e.g., about 0.30%, about 0.31%, about 0.32%, about 0.33%, about 0.34%, about 0.35%, about 0.36%, about 0.37%, about 0.38%, about 0.39%, about 0.40%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 0.4% w/v recombinant casein (e.g., about 0.40%, about 0.41%, about 0.42%, about 0.43%, about 0.44%, about 0.45%, about 0.46%, about 0.47%, about 0.48%, about 0.49%, about 0.50%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 0.5% w/v recombinant casein (e.g., about 0.50%, about 0.51%, about 0.52%, about 0.53%, about 0.54%, about 0.55%, about 0.56%, about 0.57%, about 0.58%, about 0.59%, about 0.60%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 0.6% w/v recombinant casein (e.g., about 0.60%, about 0.61%, about 0.62%, about 0.63%, about 0.64%, about 0.65%, about 0.66%, about 0.67%, about 0.68%, about 0.69%, about 0.70%, and so on).

In some embodiments, the partially purified yeast ferment comprises at least about 0.7% w/v recombinant casein (e.g., about 0.70%, about 0.71%, about 0.72%, about 0.73%, about 0.74%, about 0.75%, about 0.76%, about 0.77%, about 0.78%, about 0.79%, about 0.80%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 0.8% w/v recombinant casein (e.g., about 0.80%, about 0.81%, about 0.82%, about 0.83%, about 0.84%, about 0.85%, about 0.86%, about 0.87%, about 0.88%, about 0.89%, about 0.90%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 0.9% w/v recombinant casein (e.g., about 0.90%, about 0.91%, about 0.92%, about 0.93%, about 0.94%, about 0.95%, about 0.96%, about 0.97%, about 0.98%, about 0.99%, about 1.00%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 1% w/v recombinant casein (e.g., about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 2% w/v recombinant casein (e.g., about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 3% w/v recombinant casein (e.g., about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 4% w/v recombinant casein (e.g., about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 5% w/v recombinant casein (e.g., about 5.0%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, about 5.6%, about 5.7%, about 5.8%, about 5.9%, about 6.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 6% w/v recombinant casein (e.g., about 6.0%, about 6.1%, about 6.2%, about 6.3%, about 6.4%, about 6.5%, about 6.6%, about 6.7%, about 6.8%, about 6.9%, about 7.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 7% w/v recombinant casein (e.g., about 7.0%, about 7.1%, about 7.2%, about 7.3%, about 7.4%, about 7.5%, about 7.6%, about 7.7%, about 7.8%, about 7.9%, about 8.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 8% w/v recombinant casein (e.g., about 8.0%, about 8.1%, about 8.2%, about 8.3%, about 8.4%, about 8.5%, about 8.6%, about 8.7%, about 8.8%, about 8.9%, about 9.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 9% w/v recombinant casein (e.g., about 9.0%, about 9.1%, about 9.2%, about 9.3%, about 9.4%,

about 9.5%, about 9.6%, about 9.7%, about 9.8%, about 9.9%, about 10.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 10% w/v recombinant casein (e.g., about 10.0%, about 10.1%, about 10.2%, about 10.3%, about 10.4%, about 10.5%, about 10.6%, about 10.7%, about 10.8%, about 10.9%, about 11.0%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 15% w/v recombinant casein (e.g., about 12.0%, about 12.5%, about 13%, about 13.5%, about 14%, about 14.5%, and so on). In some embodiments, the partially purified yeast ferment comprises at least about 20% w/v recombinant casein (e.g., about 16.0%, about 16.5%, about 17%, about 17.5%, about 18%, about 18.5%, about 19.5% and so on). In some embodiments, the partially purified yeast ferment comprises at least about 25% w/v recombinant casein (e.g., about 21.0%, about 21.5%, about 22%, about 22.5%, about 23%, about 23.5%, about 24%, about 24.5% and so on). In some embodiments, the partially purified yeast ferment comprises at least about 29% w/v recombinant casein (e.g., about 26.0%, about 26.5%, about 27%, about 27.5%, about 28%, about 28.5%, about 29%, about 29.5% and so on). In some embodiments, the partially purified yeast ferment comprises about 30% w/v recombinant casein. (e.g., about 31%, about 32%, about 33%, about 34%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39% and so on). In some embodiments, the partially purified yeast ferment comprises about 40% w/v recombinant casein (e.g., about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49% and so on). In some embodiments, the partially purified yeast ferment comprises about 50% w/v recombinant casein (e.g., about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59% and so on). In some embodiments, the partially purified yeast ferment comprises about 60% w/v recombinant casein (e.g., about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69% and so on). In some embodiments, the partially purified yeast ferment comprises about 70% w/v recombinant casein (e.g., about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79% and so on). In some embodiments, the partially purified yeast ferment comprises about 80% w/v recombinant casein (e.g., about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89% and so on).

[0109] To assemble the casein micelle, the composition comprising at least one casein protein in a yeast ferment is agitated in the presence of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a micelle. In one embodiment, the agitation can be performed by stirring, vibrating, or the composition, to bring the molecules in the composition

in contact with each other and have the casein proteins form micelles. In some embodiments, the agitation can be performed at speeds corresponding to 50rpm to 20000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 15000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 10000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 9000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 8000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 7000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 6000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 100rpm to 5000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 150 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 150 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 200rpm to 10000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 250rpm to 9000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 300rpm to 8000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 350rpm to 7000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 400rpm to 6000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 450rpm to 5000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 500 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 550 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 600 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 650 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 700 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 750 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 800 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 850 rpm to 12000rpm. In some embodiments, the agitation can be performed preferably at speeds corresponding to 900 rpm to 12000rpm.

[0110] To assemble the casein micelle, the period of time sufficient to allow assembly of a casein micelle may be from 1 second to about 20 hours. In another embodiment the period of time sufficient to allow assembly of a casein micelle may be from about 5 seconds to about 16 hours, from about 10 seconds to about 12 hours, from about 15 seconds to about 10 hours, from about 30 seconds to about 10 hours, from about 1 minute to about 10 hours, from about 5 minutes to about 8 hours, from about 10 minutes to about 6 hours, from about 15 minutes to about 5 hours. In another embodiment, the micelle may assemble instantaneously upon agitation in the presence of a calcium salt and a phosphate salt.

[0111] In an embodiment, the compositions described herein may comprise citrate. In an embodiment, the citrate may be selected from calcium citrate, potassium citrate, sodium citrate, trisodium citrate, tripotassium citrate and equivalents of any of the foregoing. In an embodiment, the final concentration of citrate in the composition is at least about 0.5mM. In an embodiment, the final concentration of citrate in the composition is at least about 0.75mM. In an embodiment, the final concentration of citrate in the composition is at least about 1mM. In an embodiment, the final concentration of citrate in the composition is from about 1 mM to about 35 mM. In an embodiment, the final concentration of citrate in the composition is at least about 1 mM. In an embodiment, the final concentration of citrate in the composition is at least about 2 mM. In an embodiment, the final concentration of citrate in the composition is at most about 15 mM or at most about 20 mM. In an embodiment, the final concentration of citrate in the composition is from about 1 mM to 2 mM, preferably from about 1 mM to 3 mM, preferably from about 1 mM to 4 mM, preferably from about 2 mM to 3 mM, preferably from about 2 mM to 4 mM, preferably from about 2 mM to 6 mM, preferably from about 2 mM to 8 mM, preferably from about 2 mM to 10 mM, preferably from about 2 mM to 12 mM, preferably from about 2 mM to 14 mM, preferably from about 2 mM to 16 mM, preferably from about 2 mM to 18 mM, preferably from about 2 mM to 20 mM, preferably from about 2 mM to 25 mM, preferably from about 2 mM to 30 mM, preferably from about 2 mM to 35 mM, preferably from about 4 mM to 6 mM, preferably from about 4 mM to 8 mM, preferably from about 4 mM to 10 mM, preferably from about 4 mM to 12 mM, preferably from about 4 mM to 14 mM, preferably from about 4 mM to 16 mM, preferably from about 4 mM to 18 mM, preferably from about 4 mM to 20 mM, preferably from about 4 mM to 25 mM, preferably from about 4 mM to 30 mM, preferably from about 4 mM to 35 mM, preferably from about 6 mM to 8 mM, preferably from about 6 mM to 10 mM, preferably from about 6 mM to 12 mM, preferably from about 6 mM to 14 mM, preferably from about 6 mM to 16 mM, preferably from about 6

mM to 18 mM, preferably from about 6 mM to 20 mM, preferably from about 6 mM to 25 mM, preferably from about 6 mM to 30 mM, preferably from about 6 mM to 35 mM, preferably from about 8 mM to 10 mM, preferably from about 8 mM to 12 mM, preferably from about 8 mM to 14 mM, preferably from about 8 mM to 16 mM, preferably from about 8 mM to 18 mM, preferably from about 8 mM to 20 mM, preferably from about 8 mM to 25 mM, preferably from about 8 mM to 30 mM, preferably from about 8 mM to 35 mM, preferably from about 10 mM to 12 mM, preferably from about 10 mM to 14 mM, preferably from about 10 mM to 16 mM, preferably from about 10 mM to 18 mM, preferably from about 10 mM to 20 mM, preferably from about 10 mM to 25 mM, preferably from about 10 mM to 30 mM, preferably from about 10 mM to 35 mM, preferably from about 12 mM to 14 mM, preferably from about 12 mM to 16 mM, preferably from about 12 mM to 18 mM, preferably from about 12 mM to 20 mM, preferably from about 14 mM to 16 mM, preferably from about 14 mM to 18 mM, preferably from about 14 mM to 20 mM, preferably from about 14 mM to 25 mM, preferably from about 14 mM to 30 mM, preferably from about 14 mM to 35 mM, preferably from about 16 mM to 18 mM, preferably from about 16 mM to 20 mM, preferably from about 16 mM to 25 mM, preferably from about 16 mM to 30 mM, preferably from about 16 mM to 36 mM, preferably from about 18 mM to 20 mM, preferably from about 18 mM to 25 mM, preferably from about 18 mM to 30 mM, preferably from about 18 mM to 35 mM, preferably from about 20 mM to 25 mM, preferably from about 20 mM to 30 mM, preferably from about 20 mM to 35 mM, preferably from about 25 mM to 30 mM, preferably from about 25 mM to 35 mM, or preferably from about 30 mM to 35 mM. In an embodiment, the final concentration of citrate in the composition is about 1mM, preferably about 2 mM, preferably about 4 mM, preferably about 6 mM, preferably about 8 mM, preferably about 10 mM, preferably about 12 mM, preferably about 14 mM, preferably about 16 mM, preferably about 18 mM, or preferably about 20 mM. In an embodiment, the final concentration of citrate in the composition is at least about 1mM, preferably at least about 2 mM, preferably at least about 4 mM, preferably at least about 6 mM, preferably at least about 8 mM, preferably at least about 10 mM, preferably at least about 12 mM, preferably at least about 14 mM, preferably at least about 16 mM or preferably at least about 18 mM. In an embodiment, the final concentration of citrate in the composition is up to about 4 mM, preferably up to about 6 mM, preferably up to about 8 mM, preferably up to about 10 mM, preferably up to about 12 mM, preferably up to about 14 mM, preferably up to about 16 mM, preferably up to about 18 mM, or preferably up to about 20 mM. In some embodiments, the casein protein is dispersed in a composition comprising citrate. In some embodiments, the composition comprising at least one casein protein in a yeast ferment

comprises citrate. In an embodiment, the final concentration of citrate in the composition is about 2.5mM. In an embodiment, the final concentration of citrate in the composition is about 1.25mM.

[0111] In some embodiments, the calcium salt is selected from calcium chloride, calcium carbonate, calcium citrate, calcium gluconate, calcium lactate, calcium gluconate, calcium acetate, equivalents thereof and combinations of any of the foregoing. In one embodiment, the calcium salt is calcium chloride. In one embodiment, the calcium salt is present in the composition comprising at least one casein protein in a yeast ferment before the step of agitation. In another embodiment, the calcium is added to the composition comprising at least one casein protein in a yeast ferment while the composition is being agitated. The final concentration of calcium in the composition may be from about 1 mM to about 100 mM. In an embodiment, the final concentration of calcium in the composition is from about 3 mM to about 100 mM. In an embodiment, the final concentration of calcium in the composition is from about 5 mM to about 100 mM. In an embodiment, the final concentration of calcium in the composition may be from about 5 mM to about 75 mM. In an embodiment, the final concentration of calcium in the composition may be from about 5 mM to about 50 mM. In an embodiment, the final concentration of calcium in the composition may be from about 5 mM to about 40 mM. In an embodiment, the final concentration of calcium in the composition may be from about 5 mM to about 30 mM. In an embodiment, the final concentration of calcium in the composition may be at least about 5 mM. In an embodiment, the final concentration of calcium in the composition may be at least about 6 mM. In an embodiment, the final concentration of calcium in the composition is at least about 7 mM. In an embodiment, the final concentration of calcium in the composition is at least about 8 mM. In an embodiment, the final concentration of calcium is from about 7 mM to 8mM. In an embodiment, the final concentration of calcium in the composition is at least about 10 mM. In an embodiment, the final concentration of calcium in the composition is at least about 20 mM. In an embodiment, the final concentration of calcium in the composition is at least about 30mM. In some embodiments, the final concentration of calcium in the composition is about 28 mM, or no more than about 28 mM, or preferably about 55 mM or no more than about 55 mM, preferably at least about 7 mM, preferably from about 1mM to 100mM, preferably from about 1mM to 70mM, preferably from about 1mM to 50mM, preferably from about 1mM to 30mM, preferably from about 1mM to 10mM, preferably from about 5 mM to 10 mM, preferably from about 5 mM to 15 mM, preferably from about 10 mM to 15 mM, preferably from about 10

mM to 20 mM, preferably from about 10 mM to 25 mM, preferably from about 10 mM to 30 mM, preferably from about 10 mM to 35 mM, preferably from about 10 mM to 40 mM, preferably from about 10 mM to 45 mM, preferably from about 10 mM to 50 mM, preferably from about 10 mM to 55 mM, preferably from about 15 mM to 20 mM, preferably from about 15 mM to 25 mM, preferably from about 15 mM to 30 mM, preferably from about 15 mM to 35 mM, preferably from about 15 mM to 40 mM, preferably from about 15 mM to 45 mM, preferably from about 15 mM to 50 mM, preferably from about 15 mM to 55 mM, preferably from about 20 mM to 25 mM, preferably from about 20 mM to 30 mM, preferably from about 20 mM to 35 mM, preferably from about 20 mM to 40 mM, preferably from about 20 mM to 45 mM, preferably from about 20 mM to 50 mM, preferably from about 20 mM to 55 mM, preferably from about 25 mM to 30 mM, preferably from about 25 mM to 35 mM, preferably from about 25 mM to 40 mM, preferably from about 25 mM to 45 mM, preferably from about 25 mM to 50 mM, preferably from about 25 mM to 55 mM, preferably from about 30 mM to 35 mM, preferably from about 30 mM to 40 mM, preferably from about 30 mM to 45 mM, preferably from about 30 mM to 50 mM, preferably from about 30 mM to 55 mM, preferably from about 35 mM to 40 mM, preferably from about 35 mM to 45 mM, preferably from about 35 mM to 50 mM, preferably from about 35 mM to 55 mM, preferably from about 40 mM to 45 mM, preferably from about 40 mM to 50 mM, preferably from about 40 mM to 55 mM, preferably from about 45 mM to 50 mM, preferably from about 45 mM to 55 mM, preferably from about 50 mM to 65 mM, preferably from about 55 mM to 75 mM, preferably from about 65 mM to 85 mM, preferably from about 75 mM to 95 mM, or preferably from about 85 mM to 100 mM. In an embodiment, the final concentration of calcium in the composition is about 1 mM, preferably about 5mM, preferably about 10 mM, preferably about 15 mM, preferably about 20 mM, preferably about 25 mM, preferably about 30 mM, preferably about 35 mM, preferably about 40 mM, preferably about 45 mM, preferably about 50 mM, preferably about 55 mM, preferably about 75mM or preferably about 100mM. In an embodiment, the final concentration of calcium in the composition is at least about 2mM, preferably about 5mM, preferably about 10 mM, preferably about 15 mM, preferably about 20 mM, preferably about 25 mM, preferably about 30 mM, preferably about 35 mM, preferably about 40 mM, preferably about 45 mM, preferably about 50 mM, preferably about 55 mM, preferably about 75mM or preferably about 100mM. In an embodiment, the final concentration of calcium in the composition is up to about 5mM, preferably up to about 10 mM, preferably up to about 15 mM, preferably up to about 20 mM, preferably up to about 25 mM, preferably up to about 30 mM, preferably up to about 35 mM, preferably up to about 40 mM, preferably up to about 45 mM,

preferably up to about 50 mM, preferably up to about 55 mM, preferably up to about 75mM or preferably up to about 100mM. In an embodiment, the final concentration of calcium in the composition is between 2-10mM. In an embodiment, the final concentration of calcium in the composition is between 3-8mM. In an embodiment, the final concentration of calcium in the composition is about 3mM. In an embodiment, the final concentration of calcium in the composition is about 3.75mM. In an embodiment, the final concentration of calcium in the composition is about 4mM. In an embodiment, the final concentration of calcium in the composition is about 5mM. In an embodiment, the final concentration of calcium in the composition is about 6mM. In an embodiment, the final concentration of calcium in the composition is about 6.5mM. In an embodiment, the final concentration of calcium in the composition is about 7mM. In an embodiment, the final concentration of calcium in the composition is about 7.5mM.

[0112] In an embodiment, the phosphate salt is selected from orthophosphates such as monosodium (dihydrogen) phosphate, di sodium phosphate, tri sodium phosphate, monopotassium (dihydrogen) phosphate, dipotassium phosphate, tripotassium phosphate; pyrophosphates such as disodium or dipotassium pyrophosphate, trisodium or tripotassium pyrophosphate, tetrasodium or tetrapotassium pyrophosphate; polyphosphates such as pent sodium or potassium tripolyphosphate, sodium or potassium tetrapolyphosphate, sodium or potassium hexametaphosphate. In one embodiment, the phosphate is present in the composition comprising at least one casein protein in a yeast ferment before the step of agitation. In another embodiment, the phosphate is added to the composition comprising at least one casein protein in a yeast ferment while the composition is being agitated. In an embodiment, the final concentration of phosphate in the composition is preferably from about 1 mM to about 70 mM. In an embodiment, the final concentration of phosphate in the composition is preferably from about 1 mM to about 65 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 60 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 55 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 50 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 2 mM to about 40 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 3 mM to about 40 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 4 mM to about 40 mM. In an embodiment the final concentration of phosphate in the

composition is preferably from about 5 mM to about 70 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 65 mM. The final concentration of phosphate in the composition is preferably from about 5 mM to about 60 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 55 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 50 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 1 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 3 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 5 mM. In an embodiment the final concentration of phosphate in the composition may be at least 8 mM. In an embodiment the final concentration of phosphate in the composition is preferably at most 30 mM or at most 40 mM, at most 45 mM, at most 50mM, at most 55 mM, at most 60 mM or at most 70mM. In an embodiment the final concentration of phosphate in the composition is preferably about 1mM to about 10mM, about 5mM to about 10mM, about 8 mM to about 10 mM, about 8 mM to about 15 mM, about 8 mM to about 20 mM, about 8 mM to about 25 mM, about 8 mM to about 30 mM, about 8 mM to about 35 mM, about 8 mM to about 40 mM, about 8 mM to about 45 mM, about 10 mM to about 15 mM, about 10 mM to about 20 mM, about 10 mM to about 25 mM, about 10 mM to about 30 mM, about 10 mM to about 35 mM, about 10 mM to about 40 mM, about 10 mM to about 45 mM, about 15 mM to about 20 mM, about 15 mM to about 25 mM, about 15 mM to about 30 mM, about 15 mM to about 35 mM, about 15 mM to about 40 mM, about 15 mM to about 45 mM, about 20 mM to about 25 mM, about 20 mM to about 30 mM, about 20 mM to about 35 mM, about 20 mM to about 40 mM, about 20 mM to about 45 mM, about 25 mM to about 30 mM, about 25 mM to about 35 mM, about 25 mM to about 40 mM, about 25 mM to about 45 mM, about 30 mM to about 35 mM, about 30 mM to about 40 mM, about 30 mM to about 45 mM, about 35 mM to about 40 mM, about 35 mM to about 45 mM, about 40 mM to about 45 mM, to about, about 40 mM to about 50 mM, about 40 mM to about 55 mM, about 40 mM to about 60 mM, about 40 mM to about 65 mM, about 40 mM to about 70 mM; about 45 mM to about 55 mM, about 45 mM to about 60mM, about 45 mM to about about 65 mM, about 50 mM to about 55 mM, about 50 mM to about 60 mM, about 50 mM to about 65 mM , about 50 mM to about 70 mM, about 55 mM to about 60 mM, about 55 mM to about 65 mM , about 55 mM to about 70 mM, about 60 mM to about 70 mM, about 65 mM to about 70 mM, or about 70 mM to about 75mM. The final concentration of phosphate in the composition may be about 5mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50mM, 60mM or

70mM. The final concentration of phosphate salt in the composition may be at least 5mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 50mM, 60mM or 70mM. The final concentration of phosphate salt in the composition may be at most 5mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 50mM, 60mM or 70mM. In an embodiment, the final concentration of phosphate in the composition is between 2-10mM. In an embodiment, the final concentration of phosphate in the composition is between 3-8mM. In an embodiment, the final concentration of phosphate in the composition is about 3mM. In an embodiment, the final concentration of phosphate in the composition is about 2.75mM. In an embodiment, the final concentration of phosphate in the composition is about 4mM. In an embodiment, the final concentration of phosphate in the composition is about 5mM. In an embodiment, the final concentration of phosphate in the composition is about 5.5 mM. In an embodiment, the final concentration of phosphate in the composition is about 6 mM. In an embodiment, the final concentration of phosphate in the composition is about 7mM. In an embodiment, the final concentration of phosphate in the composition is about 7.5mM. The composition containing casein micelles may comprise a combination of salts. In some embodiments, the composition comprises calcium, phosphate and citrate salts. In an embodiment, the molar ratio of calcium, phosphate and citrate salt in the composition is from about 1:1:1 to about 100:1:1, preferably from about 1:1:1 to about 90:1:1, preferably from about 1:1:1 to about 80:1:1 preferably from about 1:1:1 to about 70:1:1 preferably from about 1:1:1 to about 60:1:1, preferably from about 1:1:1 to about 50:1:1, preferably from about 1:1:1 to about 40:1:1, preferably from about 1:1:1 to about 30:1:1, preferably from about 1:1:1 to about 20:1:1, preferably from about 1:1:1 to about 10:1:1, preferably from about 1:1:1 to about 5:1:1, preferably from about 1:1:1 to about 3:1:1, preferably from about 1:1:1 to about 1:70:1, preferably from about 1:1:1 to about 1:60:1, preferably from about 1:1:1 to about 1:50:1, preferably from about 1:1:1 to about 1:40:1, preferably from about 1:1:1 to about 1:30:1, preferably from about 1:1:1 to about 1:20:1, preferably from about 1:1:1 to about 1:10:1, preferably from about 1:1:1 to about 1:5:1, preferably from about 1:1:1 to about 1:2:1, preferably from about 1:1:1 to about 1:1:35, preferably from about 1:1:1 to about 1:1:30, preferably from about 1:1:1 to about 1:1:25, preferably from about 1:1:1 to about 1:1:20, preferably from about 1:1:1 to about 1:1:15, preferably from about 1:1:1 to about 1:1:10, preferably from about 1:1:1 to about 1:1:5, preferably from about 1:1:1 to about 1:1:3, preferably from about 1:1:1 to about 100:70:1, preferably from about 1:1:1 to about 90:70:1, preferably from about 1:1:1 to about 80:70:1 preferably from about 1:1:1 to about 70: 70:1 preferably from about 1:1:1 to about 60:70:1, preferably from about 1:1:1 to about 50: 70:1, preferably from about 1:1:1 to about 40: 70:1,

preferably from about 1:1:1 to about 30: 70:1, preferably from about 1:1:1 to about 20: 70:1, preferably from about 1:1:1 to about 10:70:1, preferably from about 1:1:1 to about 5:70:1, preferably from about 1:1:1 to about 3:70:1, preferably from about 1:1:1 to about 1:70:1, preferably from about 1:1:1 to about 100:1:35, preferably from about 1:1:1 to about 100:1:30, preferably from about 1:1:1 to about 100:1:25, preferably from about 1:1:1 to about 100:1:20, preferably from about 1:1:1 to about 100:1:20, preferably from about 1:1:1 to about 100:1:15, preferably from about 1:1:1 to about 100:1:10, preferably from about 1:1:1 to about 100:1:5 preferably from about 1:1:1 to about 100:1:1, preferably from about 1:1:1 to about 1:70:35, preferably from about 1:1:1 to about 10:7:4. In an embodiment, the molar ratio of calcium, phosphate and citrate salt in the composition is preferably from about 3:2: 1 to about 6:4:1. In an embodiment, the molar ratio of calcium, phosphate and citrate salt in the composition is preferably about 3:1:1, 3:2:1, 3:3:1, 4:2:1, 4:3:1, 4:4:1, 5:2:1, 5:2:2, 5:3:1, 5:4:1, 5:5:1, 5:3:2, 5:4:2, 6:1:1, 6:2:1, 6:3:1 or 6:4:1.

[0113] Micelle formation in the composition may require solubilization of casein proteins in a solvent such as water and/or buffers. Salts may be added after the solubilization of casein proteins in a solvent. Alternatively, salts and casein proteins may be added to the solution simultaneously. Salts may be added more than once during micelle formation. For instance, calcium salts, phosphate salts and citrate salts may be added at regular intervals or in a continuous titration process and mixed in a solution comprising casein proteins until a micellar composition of desired quality is generated. In one example, salts may be added at regular interval until the composition reaches a desired absorbance. Different salts may be added at different times during the micelle formation process. For instance, calcium salts may be added before the addition of phosphate and citrate salts, or citrate salts may be added before the addition of calcium and phosphate salts, or phosphate salts might be added before the addition of calcium and citrate salts. In some embodiments, the methods disclosed herein comprises (a) providing a composition comprising at least one casein protein in a yeast ferment comprising citrate; and agitating the composition of step (a) in the presence of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a casein micelle.

[0114] The method of assembling a casein micelle as described herein can be performed at a range of temperatures, from about 2°C to about 50°C. That is the composition comprising at least one casein protein in a yeast ferment can be at a temperature, the agitation and the additives can be or held at a temperature from about 2°C to about 50°C. In some embodiments,

the method is performed at about 2°C, preferably at about 2°C to about 4°C, preferably at about 4°C to about 10°C, preferably at about 6°C to about 12°C, preferably at about 10°C to about 20°C, preferably at about 15°C to about 30°C, preferably at about 20°C to about 35°C, preferably at about 25°C to about 35°C, preferably at about 30°C to about 40°C or preferably at about 35°C to about 50°C. In some embodiments, the method is performed at about 37°C.

Recombinant Expression

[0115] Recombinant casein proteins may be expressed in a host cell. The cell may be a prokaryote or a eukaryote or an archaean cell. The prokaryotic cell may be any Gram-positive or Gram-negative bacterium. In some embodiments the bacterial cell is selected from the group of *Escherichia coli*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Streptococcus* and *Streptomyces*. In one embodiment, the bacteria may be *Bacillus subtilis*. In another embodiment, the bacteria may be *Clostridium saccharoperbutylacetonicum*. In some embodiments the bacterial cell is a lactic acid bacteria. In some embodiments the bacterial cell is a *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* spp. In one embodiment, the cell is a cyanobacteria cell. In some embodiments the cyanobacteria is a *Synechocystis* spp., *Cyanothece* spp., *Nostoc* spp., *Scytonema* spp., *Arthrospira* spp. such as *Arthrospira platensis*, *Arthrospira fusiformis* and *Arthrospira maxima*, or *Microcystis aeruginosa*. The cell may also be a eukaryotic cell, such as a yeast, fungal, algal, microalgal, mammalian, insect or plant cell. In some embodiments, the cell is an algae or a microalgae. In some embodiments, the algae or microalgae is a kelp or seaweed or sea lettuce (*Ulva* spp.), such as brown algae or *Sargassum* spp. including *Sargassum fusiforme*. In some embodiments, the algae or microalgae is *Chlorella* spp., *Dunaliella* spp., *Gracilaria* spp., *Eucheuma* spp., *Saccharina japonica*, *Gracilaria* spp., *Pyropia* spp., *Chlamydomonas* spp., *Haematococcus* spp., *Kphycus alvarezii* or *Undaria pinnatifida*. In some embodiments the algae or microalgae is *Ankistrodesmus* spp., *Botryococcus braunii*, *Cryptocodinium cohnii*, *Cyclotella* spp., *Hantzschia* spp., *Nannochloris* spp., *Nannochloropsis* spp., *Neochloris oleoabundans*, *Nitzschia* spp., *Phaeodactylum tricornutum*, *Scenedesmus* spp., *Schizochytrium* spp., *Stichococcus* spp., *Tetraselmis suecica* or *Thalassiosira pseudonana*.

[0116] In a particular embodiment, the cell is a yeast cell. In a further particular embodiment, the yeast cell is selected from the group of *Trichoderma*, *Aspergillus*, *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Torulaspora*, *Pichia*, *Thermus*, *Hansenula*, *Torulopsis*,

Komagataella, *Candida*, *Karwinskia* or *Yarrowia*. In representative embodiments, the yeast is selected from *Saccharomyces* species (e.g., *Saccharomyces cerevisiae*), *Kluyveromyces* species (e.g., *Kluyveromyces lactis*), *Torulaspota* species, *Yarrowia* species (e.g., *Yarrowia lipolitica*), *Schizosaccharomyces* species (e.g., *Schizosaccharomyces pombe*), *Pichia* species (e.g., *Pichia pastoris* or *Pichia methanolica*), *Hansenula* species (e.g., *Hansenula polymorpha*), *Torulopsis* species, *Komagataella* species, *Candida* species (e.g., *Candida boidinii*), and *Karwinskia* species. In another embodiment, the cell is *S. cerevisiae* or *S. pombe* or a *Pichia* species. In a preferred embodiment, the cell is the yeast *Pichia pastoris*.

[0117] In some embodiments, the methods include producing recombinant protein in the periplasmic space. In some embodiments, the methods include producing recombinant protein in the periplasmic space in a bacterial cell. In some embodiments, the methods include producing recombinant protein in a cell, such that such proteins are secreted from the cell into the surrounding media. In some embodiments, the methods include producing recombinant protein in a cell, such that such proteins are intracellular. Recombinant protein can then be purified or partially purified, isolated for use in methods for making micelles. In one embodiment, the at least one casein protein is secreted from the cell into the surrounding media. In one embodiment, the at least one casein protein is derived from the intracellular contents of a cell. In another embodiment, the at least one casein protein derived from a homogenised cell.

[0118] The term “recombinant protein” as used herein means a protein that is expressed in a heterologous or recombinant organism that has an amino acid sequence derived from the protein of interest. For example, a “recombinant casein protein” means a casein produced by a recombinant organism or in a heterologous host cell.

[0119] The terms “recombinant protein”, “recombinant casein” and the like, as used herein, extend to functional variants thereof. Preferably, a functional casein variant will have an amino acids sequence or structure that differs from the corresponding native protein, but retains at least some of the characteristics of the corresponding native protein, including, for example, the ability to at least partially replicate the flavour profile when utilised as an ingredient of a dairy-free milk product or composition, as described herein. In some embodiments, the functional variants comprise at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or preferably at least 99% sequence identity to SEQ ID NOs: 1-7. Non-limiting examples of functional casein variants include

truncated forms of the corresponding of the native proteins. The term “sequence identity” as used herein in the context of amino acid sequences is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a selected sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

[0120] One or more proteins used in the formation of cheese compositions may be produced recombinantly. In some cases, α SI, α S2, β and κ casein are produced recombinantly. In some embodiments, the recombinant α SI, β , α S2 and κ casein comprises one or more amino acid insertions, deletions or substitutions, or truncations relative to their corresponding native sequences. In some embodiments, the recombinant α SI, β , α S2 and κ casein further comprises glutamic acid and phenylalanine (EF) at the N terminus of the casein molecule, including the α SI, β , α S2 and κ casein molecules.

[0121] An α s1 and/or α s2 casein can have an amino acid sequence from any species. For example, recombinant α casein may have an amino acid sequence of cow, human, sheep, goat, buffalo, bison, horse or camel α casein. A casein nucleotide sequence may be codon-optimized for increased efficiency of production. Recombinant α casein can be a non-naturally occurring variant of an α casein. Such variant can comprise one or more amino acid insertions, deletions or substitutions, or truncations relative to a native α s1 and/or α s2 casein sequence.

[0122] K casein can have an amino acid sequence from any species. For example, recombinant κ casein may have an amino acid sequence of cow, human, sheep, goat, buffalo, bison, horse, or camel κ casein. K casein nucleotide sequence may be codon-optimized for increased efficiency of production. Exemplary κ casein amino acid sequences are provided in Table 1 below. Recombinant κ casein can be a non-naturally occurring variant of a κ casein. Such variant can comprise one or more amino acid insertions, deletions or substitutions, or truncations relative to a native κ sequence.

[0123] β casein can have an amino acid sequence from any species. For example, recombinant β casein may have an amino acid sequence of cow, human, sheep, goat, buffalo, bison, horse, or camel β casein. β casein nucleotide sequence may be codon-optimized for increased efficiency of production. Exemplary β casein amino acid sequences are provided in Table 1 below. Recombinant β casein can be a non-naturally occurring variant of a β casein. Such variant can comprise one or more amino acid insertions, deletions or substitutions, or truncations relative to a native κ sequence.

[0124] The α S1, α L1, β and κ caseins may be produced in the same host cell. Alternatively, α S1, α L1, β and κ caseins may be produced in different host cells. Expression of a target protein can be provided by an expression vector, a plasmid, a nucleic acid introduced into the host cell or integrated into the host cell genome, or by other means. For example, a vector for expression can include: (a) a promoter element, (b) a signal peptide, (c) a heterologous casein sequence, and (d) a terminator element. Where the host cell is a yeast cell, a yeast alpha-mating factor secretion signal can also be included.

Compositions

[0125] In one embodiment, the only milk proteins in the compositions disclosed herein may only comprise casein proteins. In one embodiment, the compositions disclosed herein may only comprise casein proteins in casein micelles. In another embodiment, the compositions disclosed herein may only comprise casein proteins in casein micelles may comprise casein proteins in the serum phase (*i.e.*, in the solution outside of the micelles). In one embodiment, the compositions disclosed herein may comprise casein proteins in casein micelles and casein proteins in the serum phase (*i.e.*, in the solution outside of the micelles).

[0126] In some embodiments, the composition may further comprise whey proteins. In another embodiment, the composition may not include any whey proteins. In one embodiment, the whey proteins comprise β -lactoglobulin and α -lactalbumin, wherein the β -lactoglobulin and α -lactalbumin can be recombinant β -lactoglobulin and α -lactalbumin or native β -lactoglobulin and α -lactalbumin.

[0127] In some embodiments, the composition may further comprise immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and protease-peptones. In another embodiment, the composition may not include immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and protease-peptones. In one embodiment, the immunoglobulins, serum albumin, lactoferrin,

lactoperoxidase, and protease-peptones can be recombinant immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and protease-peptones or native immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and protease-peptones.

[0128] In an embodiment, the compositions described herein (and products made therefrom) do not include any animal-derived proteins. In some embodiments, the compositions described herein (and products made therefrom) can include animal-derived proteins and/or native milk proteins.

[0129] A casein mixture in the composition may comprise α , β and/or κ casein proteins as described elsewhere herein. In some embodiments, the composition includes α casein and κ casein, but does not include β casein. Micelle formation in the composition herein may comprise addition of various salts to a solution comprising a casein mixture. Salts that may be added to a casein mixture may include calcium, phosphorous, citrate, potassium, sodium and/or chloride salts. In some cases, salt is comprised within the micelles. In some cases, salt is comprised in the composition such that a proportion of salt is comprised in the micelles and another portion of salt is in solution (*e.g.*, “outside” the micelles).

[0130] The composition containing casein micelles may comprise a calcium salt. The calcium salt may be selected from calcium chloride, calcium carbonate, calcium citrate, calcium glubionate, calcium lactate, calcium gluconate, calcium acetate, equivalents thereof and/or combinations thereof. The concentration of a calcium salt in the composition may be from about 10 mM to about 55 mM. In an embodiment, the concentration of a calcium salt in the composition is at least 10 mM. In an embodiment, the concentration of a calcium salt in the composition is about 1mM to 100mM, preferably from about 1mM to about 70mM, preferably from about 1mM to about 50mM, preferably from about 1mM to about 30mM, preferably from about 1mM to about 10mM, preferably from about 5 mM to about 10 mM, preferably from about 5 mM to about 15 mM, preferably from about 10 mM to about 15 mM, preferably from about 10 mM to about 20 mM, preferably from about 10 mM to about 25 mM, preferably from about 10 mM to about 30 mM, preferably from about 10 mM to about 35 mM, preferably from about 10 mM to about 40 mM, preferably from about 10 mM to about 45 mM, preferably from about 10 mM to about 50 mM, preferably from about 10 mM to about 55 mM, preferably from about 15 mM to about 20 mM, preferably from about 15 mM to about 25 mM, preferably from about 15 mM to about 30 mM, preferably from about 15 mM to about 35 mM, preferably from about 15 mM to about 40 mM, preferably from about 15 mM to about 45 mM, preferably

from about 15 mM to about 50 mM, preferably from about 15 mM to about 55 mM, preferably from about 20 mM to about 25 mM, preferably from about 20 mM to about 30 mM, preferably from about 20 mM to about 35 mM, preferably from about 20 mM to about 40 mM, preferably from about 20 mM to about 45 mM, preferably from about 20 mM to about 50 mM, preferably from about 20 mM to about 55 mM, preferably from about 25 mM to about 30 mM, preferably from about 25 mM to about 35 mM, preferably from about 25 mM to about 40 mM, preferably from about 25 mM to about 45 mM, preferably from about 25 mM to about 50 mM, preferably from about 25 mM to about 55 mM, preferably from about 30 mM to about 35 mM, preferably from about 30 mM to about 40 mM, preferably from about 30 mM to about 45 mM, preferably from about 30 mM to about 50 mM, preferably from about 30 mM to about 55 mM, preferably from about 35 mM to about 40 mM, preferably from about 35 mM to about 45 mM, preferably from about 35 mM to about 50 mM, preferably from about 35 mM to about 55 mM, preferably from about 40 mM to about 45 mM, preferably from about 40 mM to about 50 mM, preferably from about 40 mM to about 55 mM, preferably from about 45 mM to about 50 mM, preferably from about 45 mM to about 55 mM, preferably from about 50 mM to about 65 mM, preferably from about 55 mM to about 75 mM, preferably from about 65 mM to about 85 mM, preferably from about 75 mM to about 95 mM, or preferably from about 85 mM to about 100 mM. In an embodiment the concentration of a calcium salt in the composition is about 1 mM, about 5mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 75mM or about 100mM. In an embodiment the concentration of a calcium salt in the composition is at least about 2mM, about 5mM, about 10 mM, about 15 mM, about 20 mM, 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 75mM or about 100mM. In an embodiment the concentration of a calcium salt in the composition is at most 5mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM or 55 mM.

[0131] The composition containing casein micelles may comprise a phosphate salt. The phosphate salt may be selected from orthophosphates such as monosodium (dihydrogen) phosphate, di sodium phosphate, tri sodium phosphate, monopotassium (dihydrogen) phosphate, dipotassium phosphate, tripotassium phosphate; pyrophosphates such as disodium or dipotassium pyrophosphate, trisodium or tripotassium pyrophosphate, tetrasodium or tetrapotassium pyrophosphate; polyphosphates such as pent sodium or potassium tripolyphosphate, sodium or potassium tetrapolyphosphate, sodium or potassium hexametaphosphate. The final concentration of phosphate in the composition is from about 1

mM to about 70 mM. In an embodiment the final concentration of phosphate in the composition is from about 1 mM to about 65 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 60 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 55 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 1 mM to about 50 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 70 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 65 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 60 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 55 mM. In an embodiment the final concentration of phosphate in the composition is preferably from about 5 mM to about 50 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 1 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 3 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 5 mM. In an embodiment the final concentration of phosphate in the composition is preferably at least 8 mM. In an embodiment the final concentration of phosphate in the composition is preferably at most 30 mM or at most 40 mM, at most 45 mM, at most 50mM, at most 55 mM, at most 60 mM or at most 70mM. In an embodiment the final concentration of phosphate in the composition is preferably about 1mM to about 10mM, about 5mM to about 10mM, about 8 mM to about 10 mM, about 8 mM to about 15 mM, about 8 mM to about 20 mM, about 8 mM to about 25 mM, about 8 mM to about 30 mM, about 8 mM to about 35 mM, about 8 mM to about 40 mM, about 8 mM to about 45 mM, about 10 mM to about 15 mM, about 10 mM to about 20 mM, about 10 mM to about 25 mM, about 10 mM to about 30 mM, about 10 mM to about 35 mM, about 10 mM to about 40 mM, about 10 mM to about 45 mM, about 15 mM to about 20 mM, about 15 mM to about 25 mM, about 15 mM to about 30 mM, about 15 mM to about 35 mM, about 15 mM to about 40 mM, about 15 mM to about 45 mM, about 20 mM to about 25 mM, about 20 mM to about 30 mM, about 20 mM to about 35 mM, about 20 mM to about 40 mM, about 20 mM to about 45 mM, about 25 mM to about 30 mM, about 25 mM to about 35 mM, about 25 mM to about 40 mM, about 25 mM to about 45 mM, about 30 mM to about 35 mM, about 30 mM to about 40 mM, about 30 mM to about 45 mM, about 35 mM to about 40 mM, about 35 mM to about 45 mM, about 40 mM to about 45 mM, about , about 40 mM to about 50 mM, about 40 mM to about 55 mM, about 40 mM to about 60 mM, about 40 mM to about 65 mM, about 40 mM to about 70 mM; 45 mM to

about 55 mM, about 45 mM to about 60mM, about 40 mM to about 65 mM, about 50 mM to about 55 mM, about 50 mM to about 60 mM, about 50 mM to about 65 mM , about 50 mM to about 70 mM, about 55 mM to about 60 mM, about 55 mM to about 65 mM , about 55 mM to about 70 mM, about 60 mM to about 70 mM, about 65 mM to about 70 mM, about or 70 mM to about 75mM. In an embodiment the final concentration of phosphate in the composition is preferably about 5mM, about 8 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50mM, about 60mM or 70mM. In an embodiment the final concentration of phosphate salt in the composition is preferably at least about 5mM, at least about 8 mM, at least about 10 mM, at least about 15 mM, at least about 20 mM, at least about 25 mM, at least about 30 mM, at least about 35 mM, at least about 40 mM, at least about 50mM, at least about 60mM or at least about 70mM. In an embodiment the final concentration of phosphate salt in the composition is preferably at most 5mM, about at most 8 mM, about at most 10 mM, about at most 15 mM, about at most 20 mM, about at most 25 mM, about at most 30 mM, about at most 35 mM, about at most 40 mM, about at most 50mM, about at most 60mM or about at most 70mM.

[0132] The composition containing casein micelles may comprise a citrate salt. The citrate salt may be selected from calcium citrate, potassium citrate, sodium citrate, trisodium citrate, tripotassium citrate or equivalents thereof. The final concentration of citrate in the composition is from about 1 mM to about 35 mM. In an embodiment, the final concentration of citrate in the composition is at least 1 mM. In an embodiment, the final concentration of citrate in the composition is at least 2 mM. In an embodiment, the final concentration of citrate in the composition is at most 15 mM or at most 20 mM. In an embodiment, the final concentration of citrate in the composition is preferably from about 1 mM to about 2 mM, is preferably from about 1 mM to about 3 mM, from about 1 mM to about 4 mM, from about 2 mM to about 3 mM, from about 2 mM to about 4 mM, from about 2 mM to about 6 mM, from about 2 mM to about 8 mM, from about 2 mM to about 10 mM, from about 2 mM to about 12 mM, from about 2 mM to about 14 mM, from about 2 mM to about 16 mM, from about 2 mM to about 18 mM, from about 2 mM to about 20 mM, from about 2 mM to about 25 mM, from about 2 mM to about 30 mM, from about 2 mM to about 35 mM, from about 4 mM to about 6 mM, from about 4 mM to about 8 mM, from about 4 mM to about 10 mM, from about 4 mM to about 12 mM, from about 4 mM to about 14 mM, from about 4 mM to about 16 mM, from about 4 mM to about 18 mM, from about 4 mM to about 20 mM, from about 4 mM to about 25 mM, from about 4 mM to about 30 mM, from about 4 mM to about 35 mM, from about 6 mM to about 8

mM, from about 6 mM to about 10 mM, from about 6 mM to about 12 mM, from about 6 mM to about 14 mM, from about 6 mM to about 16 mM, from about 6 mM to about 18 mM, from about 6 mM to about 20 mM, from about 6 mM to about 25 mM, from about 6 mM to about 30 mM, from about 6 mM to about 35 mM, from about 8 mM to about 10 mM, from about 8 mM to about 12 mM, from about 8 mM to about 14 mM, from about 8 mM to about 16 mM, from about 8 mM to about 18 mM, from about 8 mM to about 20 mM, from about 8 mM to about 25 mM, from about 8 mM to about 30 mM, from about 8 mM to about 35 mM, from about 10 mM to about 12 mM, from about 10 mM to about 14 mM, from about 10 mM to about 16 mM, from about 10 mM to about 18 mM, from about 10 mM to about 20 mM, from about 10 mM to about 25 mM, from about 10 mM to about 30 mM, from about 10 mM to about 35 mM, from about 12 mM to about 14 mM, from about 12 mM to about 16 mM, from about 12 mM to about 18 mM, from about 12 mM to about 20 mM, from about 14 mM to about 16 mM, from about 14 mM to about 18 mM, from about 14 mM to about 20 mM, from about 14 mM to about 25 mM, from about 14 mM to about 30 mM, from about 14 mM to about 35 mM, from about 16 mM to about 18 mM, from about 16 mM to about 20 mM, from about 16 mM to about 25 mM, from about 16 mM to about 30 mM, from about 16 mM to about 36 mM, from about 18 mM to about 20 mM, from about 18 mM to about 25 mM, from about 18 mM to about 30 mM, from about 18 mM to about 35 mM, from about 20 mM to about 25 mM, from about 20 mM to about 30 mM, from about 20 mM to about 35 mM, from about 25 mM to about 30 mM, from about 25 mM to about 35 mM, from about 30 mM to about 35 mM. In an embodiment, the final concentration of citrate in the composition is about 1mM, about 2 mM, about 4 mM, about 6 mM, about 8 mM, about 10 mM, about 12 mM, about 14 mM, about 16 mM, about 18 mM, or about 20 mM. In an embodiment, the final concentration of citrate in the composition is at least about 1mM, at least about 2 mM, at least about 4 mM, at least about 6 mM, at least about 8 mM, at least about 10 mM, at least about 12 mM, at least about 14 mM, at least about 16 mM or at least 18 mM. In an embodiment, the final concentration of citrate in the composition is at most about 4 mM, at most about 6 mM, at most about 8 mM, at most about 10 mM, at most about 12 mM, at most about 14 mM, at most about 16 mM, at most about 18 mM, or at most about 20 mM.

[0133] The composition containing casein micelles may comprise a combination of salts. In some embodiments, the composition comprises calcium, phosphate and citrate salts. In an embodiment, a ratio of calcium, phosphate and citrate salt in the composition is from about 1:1:1 to about 100:1:1, from about 1:1:1 to about 90:1:1, from about 1:1:1 to about 80:1:1 from

about 1:1:1 to about 70:1:1 from about 1:1:1 to about 60:1:1, from about 1:1:1 to about 50:1:1, from about 1:1:1 to about 40:1:1, from about 1:1:1 to about 30:1:1, from about 1:1:1 to about 20:1:1, from about 1:1:1 to about 10:1:1, from about 1:1:1 to about 5:1:1, from about 1:1:1 to about 3:1:1, from about 1:1:1 to about 1:70:1, from about 1:1:1 to about 1:60:1, from about 1:1:1 to about 1:50:1, from about 1:1:1 to about 1:40:1, from about 1:1:1 to about 1:30:1, from about 1:1:1 to about 1:20:1, from about 1:1:1 to about 1:10:1, from about 1:1:1 to about 1:5:1, from about 1:1:1 to about 1:2:1, from about 1:1:1 to about 1:1:35, from about 1:1:1 to about 1:1:30, from about 1:1:1 to about 1:1:25, from about 1:1:1 to about 1:1:20, from about 1:1:1 to about 1:1:15, from about 1:1:1 to about 1:1:10, from about 1:1:1 to about 1:1:5, from about 1:1:1 to about 1:1:3, from about 1:1:1 to about 100:70:1, from about 1:1:1 to about 90:70:1, from about 1:1:1 to about 80:70:1 from about 1:1:1 to about 70: 70:1 from about 1:1:1 to about 60:70:1, from about 1:1:1 to about 50: 70:1, from about 1:1:1 to about 40: 70:1, from about 1:1:1 to about 30: 70:1, from about 1:1:1 to about 20: 70:1, from about 1:1:1 to about 10:70:1, from about 1:1:1 to about 5:70:1, from about 1:1:1 to about 3:70:1, from about 1:1:1 to about 1:70:1, from about 1:1:1 to about 100:1:35, from about 1:1:1 to about 100:1:30, from about 1:1:1 to about 100:1:25, from about 1:1:1 to about 100:1:20, from about 1:1:1 to about 100:1:20, from about 1:1:1 to about 100:1:15, from about 1:1:1 to about 100:1:10, from about 1:1:1 to about 100:1:5 from about 1:1:1 to about 100:1:1, from about 1:1:1 to about 1:70:35, from about 1:1:1 to about 10:7:4. In an embodiment, the molar ratio of calcium, phosphate and citrate salt in the composition is from about 3:2: 1 to about 6:4:1. In an embodiment molar ratio of calcium, phosphate and citrate salt in the composition is about 3:1:1, about 3:2:1, about 3:3:1, about 4:2:1, about 4:3:1, about 4:4:1, about 5:2:1, about 5:2:2, about 5:3:1, about 5:4:1, about 5:5:1, about 5:3:2, about 5:4:2, about 6:1:1, about 6:2:1, about 6:3:1 or about 6:4:1.

[0134] Micelle formation in the composition may require solubilization of casein proteins in a solvent such as water. Salts may be added after the solubilization of casein proteins in a solvent. Alternatively, salts and casein proteins may be added to the solution simultaneously. Salts may be added more than once during micelle formation. For instance, calcium salts, phosphate salts and citrate salts may be added at regular intervals or in a continuous titration process and mixed in a solution comprising casein proteins until a micellar composition of desired quality is generated. In one example, salts may be added at regular interval till the colloid reaches a desired absorbance. Different salts may be added at different times during the micelle formation process. For instance, calcium salts may be added before the addition of phosphate

and citrate salts, or citrate salts may be added before the addition of calcium and phosphate salts, or phosphate salts might be added before the addition of calcium and citrate salts.

[0135] Additional components may be added to the composition such that the composition is then milk-like and used for production of other dairy products. In some embodiments, fat is added to the composition. In some cases, fats may be essentially free of animal-derived fats. Fats used herein may include plant-based fats such as canola oil, sunflower oil, coconut oil, palm oil, cocoa butter, soybean oil, palm kernel oil, cottonseed oil, teaseed oil, rice bran oil, tomatoseed oil, peanut oil, sesame oil, corn oil, safflower, olive, grapeseed, walnut, avocado, hemp oil, or hydrogenated oils of the above or combinations thereof. Fats used herein may include hydrogenated plant-based fats such as hydrogenated versions of canola oil, sunflower oil, coconut oil, palm oil, cocoa butter, soybean oil, palm kernel oil, cottonseed oil, teaseed oil, rice bran oil, tomatoseed oil, peanut oil, sesame oil, corn oil, safflower, olive, grapeseed, walnut, avocado, hemp oil, or hydrogenated oils of the above or combinations thereof. The concentration of fats in the composition will ultimately depend on the dairy product or dairy substitute composition in question. As a starting point, the concentration of fat in the composition may be the same as that in the animal-derived dairy product. The concentration of fat in the composition can be the lower than that in the animal-derived dairy product. The concentration of fat in the composition can be the higher than that in the animal-derived dairy product. The concentration of fat in an animal-derived dairy product would be known to the person skilled in the art. For example, animal-derived milk can have a range of fat concentration; cow, sheep and goat milk range between 3.5%-7.5% milk fat. Buffalo milk can have twice the amount of fat as cow's milk. Cow milk, being the most popular animal milk, contains about 3.5% fat. Reduced fat milk can range from having no fat, to 0.1% fat content and from about 1%-2% fat content. Animal-derived yogurt can have no fat to about 10% fat content, depending on the type of yogurt. In another example, animal-derived cream can have 10%-over 60% fat content.

[0136] In an embodiment, the concentration of fats in the milk-like compositions is about 0% to about 40% in the composition. In an embodiment, the concentration of fats in the milk-like compositions is about 0% to about 30% in the composition. In an embodiment, the concentration of fats in the milk-like compositions is about 0% to about 20% in the composition. In an embodiment, the concentration of fats is at least 0.5% or at least about 1%. In an embodiment, the concentration of fats is at most 5%. In an embodiment, the concentration

of fats is about 0%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4% or about 5%. In an embodiment, the concentration of fats is from about 0 to about 0.5%, about 0.5% to about 1%, about 1% to about 3%, about 1% to about 4%, or about 1% to about 5%. In an embodiment, the concentration of fats is at most about 2%, about 3%, about 4%, or about 5%.

[0137] When the milk-like compositions, dairy products or dairy substitutes are provided in a concentrate, the skilled person would understand that the concentration of the respective components of milk-like compositions, dairy products or dairy substitutes would be adjusted accordingly. For example, with a double concentrate of a milk-like composition may comprise twice the concentration of fat, proteins, micelles and salts as a single strength milk-like composition. For example, with a triple concentrate of a milk-like composition may comprise three times the concentration of fat, proteins, micelles and salts as a single strength milk-like composition.

[0138] These compositions disclosed herein may comprise recombinant proteins that can be combined with plant-based media (minerals, fats, sugars, and vitamins) to make compositions of recombinant dairy products that behaves, smells, tastes, looks and feels like animal-derived dairy products.

[0139] The term “animal-derived” as used herein means a substance or protein derived from an animal, such as a protein obtained and/or isolated from a whole organism (from meat or milk), including but not limited to cow, sheep, goat, human, bison, buffalo, camel and horse. An “animal-derived casein protein” is taken to mean a casein protein obtained and/or isolated from a milk-producing animal. In one embodiment, the micelles and compositions do not comprise a constituent that is derived from an animal. In one embodiment, the micelles and compositions do not comprise a milk protein that is derived from an animal. In one embodiment, the micelles and compositions do not comprise a casein protein that is derived from an animal.

[0140] In some embodiments, the compositions of recombinant dairy products comprising the casein micelle described herein is a dairy product substitute. The compositions of recombinant dairy products may have no: i) lactose, ii) cholesterol, iii) saturated fats (depending on how it affects the taste and mouthfeel), and / or iv) whey proteins. In another embodiment, the compositions of recombinant dairy products may contain i) lactose, ii) cholesterol, iii) saturated fats (depending on how it affects the taste and mouthfeel), and / or iv) whey proteins.

[0141] The composition as described herein may further comprise sugars. Sugars used herein may include plant-based disaccharides and / or oligosaccharides. Examples of sugars include sucrose, glucose, fructose, galactose, lactose, maltose, mannose, allulose, tagatose, xylose, and arabinose.

[0142] Compositions with additional components may be generated by mixing different components at a temperature from 2°C to 45°C. In an embodiment, the composition with one or more recombinant proteins (such as a combination of other caseins or whey proteins) may be mixed with additives, fats and/or sugars at a temperature at about 2°C, at about 2°C to about 4°C, at about 4°C to about 10°C, at about 6°C to about 12°C, at about 10°C to about 20°C, at about 15°C to about 30°C, at about 20°C to about 35°C, at about 25°C to about 35°C, at about 30°C to about 40°C or at about 35°C to about 50°C.

[0143] It will be appreciated that the above-described terms and associated definitions are used for the purpose of explanation only and are not intended to be limiting.

[0144] In order that the disclosure may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

Example 1: Recombinant expression of milk proteins

[0145] Expression plasmids for the expression of milk proteins (β , κ , α_{s1} and α_{s2} casein, β lactoglobulin, and α -lactalbumin) were constructed for transformation into *Pichia pastoris*, using a variety of yeast plasmid vectors, with a variety of promotor (e.g. TEF, ILV5, TEF), terminator sequences (e.g. CYC1, AOD, AOX), antibiotic selection marker genes (e.g. Zeocin and Geneticin), secretion signal (e.g. α MF) and amino acid sequence arrangements.

[0146] Linerised plasmids were transformed into either one of *Pichia pastoris* expression strains BG10 (wildtype), BG16 and BG11. This is followed by small scale fermentation and screening of the strains for protein yield and expression dynamics based on total protein production, using SDS-PAGE, ELISA and/or HPLC. All six milk proteins were confirmed to be successfully produced and secreted by *P. pastoris*.

[0147] HPLC and SDS-PAGE analyses confirmed successful expression of β , κ and α s2 casein (Figure 1). In particular, SDS-PAGE analysis of partially purified yeast ferments showed the presence of multiple casein protein bands and peptides, as well as *Pichia* protein bands and peptides.

Example 2: Preparations of yeast ferment

[0148] The following methodology is for *Pichia* strains for expressing κ casein and α -S2 casein and are demonstrative of the process. Growth rates are also characterised in this fed-batch process followed by protein induction with a continuous methanol feed. The process was completed in a fully defined medium.

[0149] Yeast Extract Peptone Dextrose (YPD) Medium: 1% yeast extract, 2% peptone, 2% dextrose (glucose), supplemented with 20 g/L for solid medium. Yeast Extract Peptone Glycerol (YP glycerol) Medium: 1% yeast extract, 2% peptone, 2% glycerol, supplemented with 20 g/L for solid medium. YPD + Zeocin or YP glycerol + Zeocin: supplemented with 100 μ g/mL Zeocin when required for selection.

[0150] Buffered Glycerol-complex Medium BMGY: 1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.0, 1.34% yeast nitrogen base (YNB), 4×10^{-5} % biotin, 1% glycerol, buffered with 0.1 M potassium phosphate buffer, pH 6.0; supplemented with 100 μ g/mL Zeocin when required for selection.

[0151] Buffered Minimal Methanol (BMM): 300 mM potassium phosphate pH 6.0, 1.34% YNB (yeast nitrogen base), 4×10^{-5} % biotin, 1% methanol. BMM + Zeocin: BMM supplemented with 100 μ g/mL Zeocin when required for selection.

[0152] After fermentation / induction of recombinant protein expression, the yeast ferment supernatant were subject to filtration, ultrafiltration and / or diafiltration before proceeding to micellarisation.

[0153] For example, the yeast ferment supernatant is subjected to one or more of:

- i. 0.22 μ m filtration to remove yeast cells,
- ii. Tangential Flow Filtration (TFF) using a 10kDaMWCO membrane,
- iii. Diafiltration against 10mM Tris-Hcl,
- iv. sterilisation by 0.22 μ m filtration,

to generate partially purified yeast ferments.

Example 3: An exemplary method for micellarisation and preparation of milk-like product

[0154] Examples of solutions that can be used for micellarisation are provided in Table 2. Volumes are given for 1L as an example; they can be increased as required as long as the concentrations and proportions of mixing are maintained.

[0155] The protein fractions, (i.e. partially purified yeast ferments or purified casein protein fractions) and the salt solutions were brought to at least room temperature, or optimally warmed to 37 °C. The yeast ferments (1600 µl) were contained in a cuvette holder that incorporated a stirrer and was linked to a temperature control system.

[0156] A solution of calcium chloride (75 mM, 200 µL or 10% of final volume) was injected into the yeast ferment solution over minutes, at a rate of 40 µl/min (or rate of 2% final volume per minute). The mixture was allowed to equilibrate at 37 °C for 20-30 minutes.

[0157] 200 µl of a solution of dipotassium hydrogen orthophosphate (55 mM or 10% of final volume / 100mL) was then injected over two hours at 1.667ul/min (or at a rate of 0.08% final volume per minute). At this point, the pH level can be monitored, and adjusted if required to about 6.7 using 1M NaOH.

[0158] The re-micellarised casein solution was mixed with whey / sucrose solution in the ratio 80:20. The resulting composition provides a 'skim milk' milk-like product.

[0159] To make a full fat milk-like composition, fat is included and dispersed in the skim product at 3.4%, and homogenised. The composition can be pasteurised then chilled rapidly to 4°C.

[0160] The particle size was measured using dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern, Worcestershire, UK). using either undiluted or diluted samples (if protein concentration was too high). Measurements were made at 25 °C using a scattering angle of 173° and laser with a wavelength 633 nm. A cumulate analysis correlation function was used to obtain the intensity mean diameter (z-average diameter) of each sample by using a viscosity of 1 mPa.s (water) and a refractive index of n=1.343 and absorptivity of 0.001 (Day et al. (2015) Journal of Dairy Science, 98:3633). At least three cumulant z average

measurements were obtained for each sample. Data was analysed using the general purpose model. For representation in graphs data from three distribution curves was averaged.

[0161] Photography was undertaken using a Colourview III microscope camera fitted with an Olympus OM system 50mm f3.5 macro lens. Samples were illuminated from two sides with LED ring lights and images captured using Cell D software at 400ms exposure at f11.

Example 4: An exemplary method for micellarisation and preparation of milk-like products using recombinant casein proteins in a yeast ferment

[0162] Recombinant protein preparations can be at relatively low concentration compared to purified cow caseins above, and the micellarisation method was modified to accommodate the changed concentrations and volumes.

[0163] The different mixtures of yeast ferments (partially purified by TFF ultrafiltration and diafiltration using a 10kDaMWCO membrane) comprising recombinant casein proteins with or without native bovine casein proteins (dissolved in a 3.125mM citrate buffer) (M74-M122) used to test micelle formation are shown in Table 3.

[0164] Images and particle sizing data of the samples were recorded for each stage of the micellarisation trials, as shown in Figures 3-30: (A) Before addition of any solutions (i.e. ferments / protein solutions); (B) after addition of calcium containing solution; (C) after addition of phosphate containing solution; and (D) after overnight storage at 4°C. Intensity based particle size distributions for each micellarisation trial are shown as (E) in Figures 3-30. Unless otherwise specified, the final concentration of salt components in micellarisation trials was 2.48mM Na Citrate, 7.43mM CaCl₂, and 5.45 mM K₂HPO₄. The concentration of the proteins provided are that of the final concentration of the corresponding protein in the composition.

[0165] The mass ratios of protein components in each micellarisation trial and the Z average particle size diameter in nm is shown in Table 4.

[0166] Samples M74-M81 are micellarisation experiments using recombinant β casein alone, or with bovine κ casein.

[0167] Sample M74: Recombinant β casein 1.36 g/L in 10mM Tris pH 6.7, 30mM CaCl₂, 22 mM K₂HPO₄ (Figure 2).

[0168] The first trial (M74) was carried out using a final calcium content of 30mM. 30mM of calcium is equivalent to the calcium content of normal (i.e. cow milk) skim milk. Upon addition of calcium and phosphate, precipitation occurred, mostly likely a result of an excess of calcium and phosphate the in mix. Particle sizing data is unreliable for samples that precipitate / sediment.

[0169] Sample M75: Recombinant β casein 1.36g/L in 10mM Tris pH 6.7, 7.5mM CaCl_2 , 5.5 mM K_2HPO_4 (Figure 3).

[0170] A pale milky suspension formed upon addition of calcium and phosphate. Comparison with M83 ('no protein' control) suggests that components in M75 were able to stabilise this level of calcium and phosphate against precipitation. Particle size data suggests the particles are around 398 nm.

[0171] Sample M77: Recombinant β casein 1.35g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 4).

[0172] This trial tested the inclusion of a concentrated citrate solution (250mM). Stable particles about 355nm were formed, with some increase in particle size on overnight storage to about 433 nm.

[0173] Sample M78: Recombinant β casein 1.35 g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (CaCl_2 and K_2HPO_4 addition made at 4°C) (Figure 5).

[0174] M78 was undertaken to observe whether cooling the solution during the calcium loading phase had any effect. This resulted in little to no difference when compared to M75 and M77.

[0175] Sample M79: Recombinant β casein 1.26 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.076 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 6).

[0176] The combination of recombinant β casein and bovine κ casein at about 38:62 mass ratio reduced the particle size from a z average of around 350nm (i.e., the average particle size of sample M77 after the addition of phosphate containing solution) to 178nm.

[0177] Sample M80: Recombinant β casein 1.3 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.38 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 7).

[0178] The combination of recombinant β casein and bovine κ casein at about 23:77 mass ratio resulted in a z average particle size of 196nm.

[0179] Sample *M81*: Recombinant β casein 1.18 g/L in 10mM Tris pH 6.7 and bovine κ casein 1.51 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 8).

[0180] The combination of recombinant β casein and bovine κ casein at about 44:46 mass ratio resulted in a z average particle size of 156nm.

[0181] Samples M82-M87 are micellarisation experiments using recombinant αS1 .

[0182] Sample *M82*: Recombinant αS1 casein 3.17 g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 9).

[0183] Addition of calcium and phosphate resulted in formation of precipitate (as can be seen by settling of the precipitate after overnight storage). The observed precipitation response was stronger than that of recombinant β casein preparation, consistent with the predicted number and distribution of calcium and protein interacting sites on αS1 -cn.

[0184] Sample *M83* ('no protein' control): Solution containing 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 . Calcium and orthophosphate solutions were injected into a citrate solution (Figure 10).

[0185] In the absence of proteins, an unstable precipitate forms, which settles out almost completely. This precipitate is most likely a form of calcium phosphate, which has a low solubility threshold.

[0186] Sample *M84*: Recombinant αS1 casein 3.07 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.38 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 11).

[0187] The combination of recombinant αS1 casein and bovine κ casein at about 89:11 mass ratio resulted in a precipitate, but the precipitate was more diffuse. The upper phase of this sample was more turbid, suggesting some stable particles may have formed

[0188] Sample *M85*: Recombinant αS1 casein 2.97 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.76 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 12).

[0189] Similarly to sample *M84*, the combination of recombinant α S1 casein and bovine κ casein at about 80:20 mass ratio resulted in a precipitate. However, the upper phase of this sample was more turbid than *M84*, suggesting *M85* generated a greater concentration of stable particles.

[0190] Sample *M87*: Recombinant α S1 casein 2.77 g/L in 10mM Tris pH 6.7 and bovine κ casein 1.516 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 13).

[0191] The combination of recombinant α S1 casein and bovine κ casein at about 65:35 mass ratio resulted in the formation of stable particles.

[0192] *M88-M94* are micellarisation experiments using recombinant α S1 and recombinant β casein and bovine κ casein.

[0193] Sample *M88*: Recombinant α S1 casein 1.58 g/L and recombinant β casein 0.67 g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 14).

[0194] The combination of recombinant α S1 casein and recombinant β casein at about 70:30 mass ratio resulted in the formation of both stable and unstable particles.

[0195] Sample *M89*: Recombinant α S1 casein 1.49 g/L and recombinant β casein 0.63 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.76 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 15).

[0196] The combination of recombinant α S1 casein, recombinant β casein, and bovine κ casein at about 52:22:26 mass ratio resulted in the formation of stable 3-casein particles which did not precipitate after overnight storage at 4°C.

[0197] Sample *M90*: Recombinant α S1 casein 0.94 g/L and recombinant β casein 0.95 g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 16), at about 50:50 mass ratio of recombinant α S1 and recombinant β casein resulted in the formation of stable particles.

[0198] Sample *M91*: Recombinant α S1 casein 0.5 g/L and recombinant β casein 1.14 g/L in 10mM Tris pH 6.7, 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 17), at

about 30:70 mass ratio of recombinant α S1 and recombinant β casein resulted in the formation of stable particles.

[0199] Sample *M92*: Recombinant α S1 casein 0.93 g/L, recombinant β casein 0.93 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.214 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 18).

[0200] The combination of recombinant α S1 casein, recombinant β casein, and bovine κ casein at about 45:45:10 mass ratio (consistent with ratios observed in cow milk micelles) resulted in the formation of stable particles.

[0201] Sample *M93*: Recombinant α S1 casein 0.93 g/L and recombinant β casein 0.93g/L in 10mM Tris pH 6.7 and bovine κ casein 0.457 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 19), at about 40:40:20 mass ratio of recombinant α S1, recombinant β casein and bovine κ casein resulted in the formation of stable particles.

[0202] Sample *M94*: Recombinant α S1 casein 0.89 g/L and recombinant β casein 0.88 g/L in 10mM Tris pH 6.7 and bovine κ casein 0.762 g/L, reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 (Figure 20), at about 35:35:30 mass ratio of recombinant α S1, recombinant β casein and bovine κ casein resulted in the formation of stable particles.

[0203] Sample *M109*: Bovine α S1 casein 3.02 g/L reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of precipitate (as can be seen by settling of the precipitate). (Figure 21).

[0204] The following samples (M111-M122) were conducted using a ferment comprising recombinant κ casein processed by ultrafiltration and diafiltration that have been suspended in buffer (as described earlier) or in water (see M113-M115)., to analyse the behaviour of the recombinant κ casein. The concentration of recombinant κ casein in these ferments could not be accurately determined, therefore the proportions of κ casein are estimates.

[0205] Sample *M111*: Bovine α S1 casein 2.98 g/L and recombinant κ casein (a ferment processed by ultrafiltration and diafiltration; concentration undetermined) reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of stable particles. (Figure 22).

[0206] Sample *M112*: Bovine α S1 casein 2.98 g/L and an independent ferment (fermented differently) comprising recombinant κ casein (processed by ultrafiltration and diafiltration, concentration undetermined) reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , also resulted in formation of stable particles (Figure 23). M111 and M112 demonstrated that recombinant κ casein could stabilise bovine α S1 casein.

[0207] Sample *M118*: Bovine α S1 casein 3 g/L and another independent ferment (different to M111 and M112) of recombinant κ casein that had been processed using dialysis and freeze drying (concentration undetermined) reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of stable particles (Figure 24).

[0208] Sample M113-M115 are similar experiments, using the same ferment of recombinant κ casein, but testing different amounts of recombinant κ casein. For these experiments, the proteins were suspended in water instead of buffer (while maintaining the same final citrate, calcium and phosphate concentrations).

[0209] Sample *M113*: Bovine β casein 1.49 g/L and recombinant κ casein reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in initial formation of stable particles, with some precipitation observed after overnight storage (Figure 25).

[0210] Sample *M114*: Bovine β casein 1.49g/L and recombinant κ casein reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of stable particles, with some precipitation observed after overnight storage (Figure 25). M114 contained twice the amount of recombinant κ casein of M113.

[0211] Sample *M115*: Bovine β casein 1.49g/L and recombinant κ casein reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of a precipitate (Figure 25). M115 contained half the amount of recombinant κ casein than M113.

[0212] Sample *M117*: Bovine β casein 1.49g/L and recombinant κ casein the same ferment of recombinant κ casein used in M113-M115, but dissolved in buffer reacted with 2.48mM Na Citrate, 7.43mM CaCl_2 , 5.45 mM K_2HPO_4 , resulted in formation of stable particles, with minimal precipitation forming after overnight storage (Figure 25). M117 contained 4 times the amount of recombinant κ casein than M113, and it appears that increasing the proportion of recombinant κ casein is conducive for the formation of stable particles.

[0213] Sample *M121*: Bovine α casein 1.5 g/L, 1.5g g/L bovine β casein and recombinant κ casein derived from an independent ferment (concentration undetermined) was reacted with 2.48mM Na Citrate, 7.43mM CaCl₂, 5.45 mM K₂HPO₄. This resulted in formation of stable particles (Figure 26).

[0214] Sample *M122*: Bovine α casein 1.5 g/L, and 1.5g/L bovine β casein reacted with 2.48mM Na Citrate, 7.43mM CaCl₂, 5.45 mM K₂HPO₄, resulted in formation of stable particles, with some precipitation forming after overnight storage (Figure 27).

Table 1: Exemplary amino acid and nucleotide sequences of milk proteins

SEQ ID NO	Sequence
1 (α S1 casein protein) Uniprot P02662; NCBI NP_851372.1	MKLLILTCLVAVALARPKHPIKHQGLPQEVLNENLLRFFVA PFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESSISSE EIVPNSVEQKHQKEDVPSERYLGYLEQLRLKKYKVPQLEI VPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQ FYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKT TMPLW
2 (α S2 casein protein) Uniprot P02663; NCBI NP_776953.1	MKFFIFTCLLAVALAKNTMEHVSSEESIISQETYKQEKDMA INPSKENLCSTFCKEVVRNANEEEEYSIGSSSEESAEVATEEVK ITVDDKHYYKALNEINQFYQKFPQYLQYLYQGPIVLNPWDQ VKRNAVPITPTLNREQLSTSEENSKKTVDMESTEVFTKKTKL TEEEKNRLNFLKKISQRYQKFALPQYLKTVYQHQAAMKPW IQPKTKVIPYVRYL
3 (β casein protein; A1-isoform) Uniprot P02666; NCBI AAB29137.1	MKVLILACLVALALARELEELNVPGEIVESLSSSEESITRINK KIEKFQSEEQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLP QNIPPLTQTPVVPPFLQPEVMGVSKVKEAMAPKHKEMPFP KYPVEPFTESQLTLTDVENLHLPLLLQSWMHQPHQPLPPT VMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQE PVLGPVRGPFPIIV

4 (κ casein protein) Uniprot P02668; NCBI NP_776719.1	MMKSFFLVVTILALTLPFLGAQEQNQEQPIRCEKDERFFSDK IAKYIPIQYVLSRYPYGLNYYQQKPVALINNQFLPYPPYAK PAAVRSPAQLQWQVLSNTVPAKSCQAQPTTMARHPHPLS FMAIPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVATLE DSPEVIESPPEINTVQVTSTAV
5 (β casein protein; A2-isoform) Uniprot P02666; NCBI AAB29137.1	MKVLILACLVALALARELEELNVPGEIVESLSSSEESITRINK KIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFGPIPNLSP QNIPPLTQTTPVVVPFLQPEVMGVSKVKEAMAPKHKEMPPF KYPVEPFTESQSLTLTDVENLHLPLLLQSWMHQPHQPLPPT VMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQE PVLGPVRGPFPIIV
6 (β lactoglobulin protein) Uniprot P02754; NCBI NP_776354.2	MKCLLLALALTCGAQALIVTQTMKGLDIQKVAGTWYSLA MAASDISLLDAQSAPLRVYVEELKPTPEGDLEILLQKWENG ECAQKKHAEKTKIPAVFKIDALNENKVLVLDTDYKKYLLFC MENSAEPEQSLACQCLVRTPEVDDEALEKFDKALKALPMHI RLSFNPTQLEEQCHI
7 (β lactoalbumin protein) Uniprot P00711; NCBI NP_776803.1	MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVS LPEWVCTTFHTSGYDTQAIVQNNSTEYGLFQINNKIWCKD DQNPSSNICNISCDKFLDDDLTDDIMCVKKILDKVGI NYWLAHKALCSEKLDQWLCEKL
8 (α S1 casein protein; with EF at the N terminus)	EFMKLLILTCLVAVALARPKHPIKHQGLPQEVLENENLLRFFV APFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESS SEEIVPNSVEQKHQKEDVPSEYLYGYLEQLLRLKKYKVPQL EIVPNSAEERLHSMKEGIHAQQKEPMIGVQNQELAYFYPELFR QFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEK TTMLPW

9 (β casein protein; with EF at the N terminus)	EFMKVLILACLVALALARELEELNVPGEIVESLSSEESITRIN KKIEKFQSEEQQTEDELQDKIHPFAQTQSLVYPFGPIHNSL PQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPF PKYPVEPFTESQSLTLTDVENLHPLPLLQSWMHQPHQPLPP TVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQ EPVLGPVRGPFPIIV
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Table 2: Examples of micellarisation buffers

Solution	Preparation
0.0125M Citrate buffer	Dissolve 3.67g sodium citrate (Mw 294.1) in water, make up to 1 litre.
0.3M calcium buffer	Dissolve 44.103g calcium chloride (Mw 147.01) in water, make up to 1 litre
0.22M Phosphate buffer	Dissolve 38.12g K ₂ HPO ₄ (MW 174.18) (dihydrate) in water, make up to 1 litre
1M NaOH	Dissolve 40g NaOH in water, make up to 1 litre
Whey/sucrose solution	Dissolve 2.941g sodium citrate, 2.961g K ₂ HPO ₄ , and 0.88g calcium chloride in water, adjust pH to 6.7 using 1M NaOH, make up to 1 litre
Caseinate/sucrose solution	Dissolve sodium caseinate (43.75g/kg) and sucrose (12.5g/kg) in citrate buffer and allow to hydrate overnight
WPI / Sucrose solution	Dissolve WPI (35g/kg) and sucrose (10g/kg) in whey buffer

Table 3: Final concentrations of protein and salt components in micellarisation trials

	Recomb α S1 casein (g/L)	Recomb β casein (g/L)	Recomb κ casein (g/L)	Bovine α casein (g/L)	Bovine β casein (g/L)	Bovine κ casein (g/L)	Total protein (g/L)
M74	0.00	1.36	0.00	0.00	0.00	0.00	1.36
M75	0.00	1.36	0.00	0.00	0.00	0.00	1.36
M77	0.00	1.35	0.00	0.00	0.00	0.00	1.35
M78	0.00	1.35	0.00	0.00	0.00	0.00	1.35
M79	0.00	1.26	0.00	0.00	0.00	0.76	2.02
M80	0.00	1.30	0.00	0.00	0.00	0.38	1.68
M81	0.00	1.18	0.00	0.00	0.00	1.51	2.69
M82	3.17	0.00	0.00	0.00	0.00	0.00	3.17
M83	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M84	3.07	0.00	0.00	0.00	0.00	0.38	3.45
M85	2.97	0.00	0.00	0.00	0.00	0.76	3.73
M87	2.77	0.00	0.00	0.00	0.00	1.516	4.29
M88	1.58	0.67	0.00	0.00	0.00	0.00	2.26
M89	1.49	0.63	0.00	0.00	0.00	0.76	2.87
M90	0.94	0.95	0.00	0.00	0.00	0.00	1.89
M91	0.5	1.14	0.00	0.00	0.00	0.00	1.63
M92	0.93	0.93	0.00	0.00	0.00	0.214	2.07
M93	0.91	0.91	0.00	0.00	0.00	0.457	2.28
M94	0.89	0.88	0.00	0.00	0.00	0.762	2.54
M109	0.00	0.00	0.00	3.02	0.00	0.00	3.02
M111	0.00	0.00	n/d	2.98	0.00	0.00	n/d
M112	0.00	0.00	n/d	2.98	0.00	0.00	n/d
M113	0.00	0.00	n/d	0.00	1.49	0.00	n/d
M114	0.00	0.00	n/d	0.00	1.49	0.00	n/d
M115	0.00	0.00	n/d	0.00	1.49	0.00	n/d
M117	0.00	0.00	n/d	0.00	1.49	0.00	n/d
M118	0.00	0.00	n/d	3	0.00	0.00	n/d
M121	0.00	0.00	n/d	1.5	1.50	0.00	n/d
M122	0.00	0.00	0.00	1.50	1.50	0.00	3.00
M164	2.50	0.00	0.00	0.00	0.00	0.00	2.5
M165	2.50	0.00	1.88	0.00	0.00	0.00	4.38
M166	0.00	2.50	0.00	0.00	0.00	0.00	2.5
M167	0.00	2.50	1.88	0.00	0.00	0.00	4.38
M168	1.25	1.25	0.00	0.00	0.00	0.00	2.5
M169	1.25	1.25	1.88	0.00	0.00	0.00	4.38

Table 4: Mass ratios of protein components in micellarisation trials and Z average particle diameter after micellarisation 'C' sample

	Recomb α S1 casein	Recomb β casein	Recomb κ casein	Bovine α casein	Bovine β casein	Bovine κ casein	Z average particle diameter (nm)
M74	0	100	0	0	0	0	N/A
M75	0	100	0	0	0	0	398
M77	0	100	0	0	0	0	355
M78	0	100	0	0	0	0	278
M79	0	38	0	0	0	62	178
M80	0	23	0	0	0	77	196
M81	0	44	0	0	0	46	156
M82	100	0	0	0	0	0	N/A
M83	0	0	0	0	0	0	N/A
M84	89	0	0	0	0	11	N/A
M85	80	0	0	0	0	20	N/A
M87	65	0	0	0	0	35	291
M88	70	30	0	0	0	0	N/A
M89	52	22	0	0	0	26	302
M90	50	50	0	0	0	0	297
M91	30	70	0	0	0	0	228
M92	45	45	0	0	0	10	275
M93	40	40	0	0	0	20	252
M94	35	35	0	0	0	30	342
M109	0	0	0	100	0	0	1728
M111	0	0	1	99	0	0	466
M112	0	0	1	99	0	0	587
M113	0	0	1	0	99	0	512
M114	0	0	1	0	99	0	599
M115	0	0	1	0	99	0	537
M117	0	0	1	0	99	0	579
M118	0	0	1	99	0	0	6811
M121	0	0	1	46	53	0	499
M122	0	0	0	50	50	0	969
M164	100	0	0	0	0	0	245
M165	57	0	43	0	0	0	200
M166	0	100	0	0	0	0	214
M167	0	57	43	0	0	0	242
M168	50	50	0	0	0	0	158
M169	28.5	28.5	43	0	0	0	175

Example 5: Stability of casein micelle-comprising compositions

[0215] The stability of casein-micelle compositions comprising

- 2.5 g/L recombinant β -casein, 25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4
- 2.5 g/L, recombinant β -casein and 1.88 g/L recombinant κ -casein, 1.25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4
- 1.25 g/L recombinant β -casein and 1.25 g/L recombinant αS1 -casein, 1.25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4
- 1.25 g/L, recombinant β -casein, 1.25 g/L, recombinant αS1 -casein, and 1.88 g/L recombinant κ -casein, 1.25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4

were assessed. The casein micelle compositions were generated using the methods disclosed and held overnight. The compositions were then heated to 75-80°C for 10 minutes, and the appearance of the compositions and the micelle particle size was monitored over several days (Table 5, Figures 28 and 29).

Table 5: Particle size measurements (diameter in nm) of casein micelle-compositions at assembly (D0), after overnight incubation (D1) and after heating (Heat+), including at days 2, 3, 6 and 7.

Sample	D0	D1	Heat+ D1	Heat+ D2	Heat+ D3	Heat+ D6	Heat+ D7
β -casein only	240	238	249	278	267	264	261
β -casein and κ -casein	244	288	303	292	290	274	302
β -casein and αS1 -casein	159	168	215	221	225	225	217
β -casein, αS1 -casein and κ -casein	214	222	243	244	250	248	252

Example 6: Effects of background salt on micellarisation

[0216] Experiments were conducted to establish if micellarisation could be achieved in compositions comprising 20mM Tris, 20mM Tris 300mM NaCl and/or citrate (see Figure 30).

[0217] Sample *M70* comprised bovine β casein in 20mM Tris pH 6.7, reacted with 7.5mM CaCl_2 , and 5.5 mM K_2HPO_4 . A suspension formed upon addition of calcium and phosphate, and with large particle size of about 1000nM, that precipitated upon overnight storage at 4°C.

[0218] Sample *M71* comprised bovine β casein in 20mM Tris, 300mM NaCl, pH 6.7 reacted with 7.5mM CaCl_2 , and 5.5 mM K_2HPO_4 . No milky suspension formed upon addition of calcium. However, a milky suspension appeared to form upon addition of phosphate, with particle sizes around 100-200nM. However, these particles were not stable, as they precipitated upon overnight storage at 4°C.

[0219] Sample *M72* comprised bovine β casein in 20mM Tris, 3mM citrate pH6.7, pH 6.7 reacted with 7.5mM CaCl_2 , and 5.5 mM K_2HPO_4 . A pale milky suspension formed upon addition of calcium and phosphate. Stable particles were formed with particle sizes around 200-300nM, though some loss of stability after overnight storage as the solution appeared less milky.

[0220] Sample *M73* comprised bovine β casein in 20mM Tris, 300mM NaCl, 3.1mM citrate, pH 6.7. No milky suspension formed upon addition of calcium. No milky suspension formed upon addition of phosphate, though some precipitate can be seen. If particles were formed, they were much smaller, around 50nM and unstable.

Example 7: Micelle formation by recombinant caseins

[0221] The recombinant β casein and αS1 -casein casein proteins in the partially purified ferments were shown to be successfully in forming casein micelles individually, in combination with each other and with other bovine caseins. *M75* also demonstrated that casein micelle comprising only recombinant β casein (i.e. no additional bovine casein) can be formed. Further experiments (described below) also demonstrated that the recombinant caseins together could form a recombinant casein micelle (i.e. without bovine caseins). To our knowledge, this is the first time a casein micelle has been assembled using only recombinant yeast-expressed casein proteins.

[0222] Initial experiments (M164) using only recombinant α S1 casein (2.5 g/L), in 1.25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4 , showed that initial particle formation that precipitated out after 3 days. The particle size was estimated at 244nM after the addition of calcium and phosphate, and 312nM the next day.

[0223] In the following experiments, the recombinant κ -casein was partially purified using dialysis against reverse-osmosis purified water then concentrated to dryness (e.g. speed vac concentrator) and reconstituted in a citrate solution.

[0224] Sample M165 comprises 2.5 g/L recombinant α S1-casein and 1.88g/L recombinant κ -casein in 1.25mM Na Citrate, 3.75mM CaCl_2 , and 2.75 mM K_2HPO_4 ; and resulted in formation of stable particles. The particle size was estimated at 200nM after the addition of calcium and phosphate, and 205nM the next day. Further stability analysis indicated that M165 particles maintained stability up to at least 8 days.

[0225] For M166-M169, the recombinant β casein was derived from a partially purified ferment comprising recombinant β casein that was freeze dried, and then resuspended in citrate (to increase concentration of recombinant β casein).

[0226] Sample M166 comprises only 2.5 g/L recombinant β casein, in 1.25mM Na Citrate, 3.75mM CaCl_2 , 2.75 mM K_2HPO_4 . The calcium levels are lower in M166 than that used in previous M75, M77 and M78 experiments. M166 resulted in formation of stable particles. The particle size was estimated at 213nM after the addition of calcium and phosphate, and 234nM the next day. The particles were still stable after 8 days (See Figure 31).

[0227] Sample M167 comprises 2.5 g/L recombinant β casein and 1.88g/L recombinant κ -casein 1.25mM Na Citrate, 3.75mM CaCl_2 , and 2.75 mM K_2HPO_4 . Stable particles were formed. The particle size was estimated at 242nM after the addition of calcium and phosphate, and 271nM the next day. The particles were still stable after 8 days (See Figure 32).

[0228] Sample M168 comprises 1.25 g/L recombinant β casein, 1.25 g/L recombinant α S1 casein in 1.25mM Na Citrate, 3.75mM CaCl_2 , and 2.75 mM K_2HPO_4 . Stable particles were formed. The particle size was estimated at 158nM after the addition of calcium and phosphate, and 166nM the next day. The particles were still stable after 10 days (See Figure 33).

[0229] Sample M169 comprises 1.25 g/L recombinant β casein, 1.25 g/L recombinant α S1 casein, and 1.88g/L recombinant κ -casein in 1.25mM Na Citrate, 3.75mM CaCl_2 , and 2.75 mM K_2HPO_4 . Stable particles were formed. The particle size was estimated at 177nm after the addition of calcium and phosphate, and 185nm the next day. The particles were still stable after 10 days (See Figure 34).

[0230] A comparison of the stability of the recombinant casein micelles are shown in Figure 35.

Table 6: Particle size measurements (diameter in nm) of casein micelle-compositions before addition of any solutions (i.e. ferments) and after sequential addition of calcium containing solution and addition of phosphate containing solution; and after overnight storage at 4°C.

	Before addition of any salt	Addition of calcium	Addition of phosphate	After overnight storage
M165	154	154	200	206
M166	281	210	213	234
M167	316	239	242	271
M168	168	157	158	166
M169	226	177	175	185

Example 8: β -lactoglobulin particle formation in yeast ferment

[0231] Recombinant bovine β -lactoglobulin (yeast expressed) was used to assess if other milk proteins are capable of micelle-like particle formation seen in yeast ferment. When 50ul of 300mM CaCl_2 and 50ul of 220mM K_2HPO_4 was added to 400 μL of 40mg/mL solution of recombinant partially purified β -lactoglobulin in partially purified yeast ferment (i.e., also containing some yeast proteins) in a base buffer of 12.5mM Na citrate, 34mM NaCl pH6.7 (final concentrations: β -lactoglobulin: 32mg/ml, Na citrate: 10mM; NaCl: 27.2mM; CaCl_2 : 30mM; K_2HPO_4 : 22mM) calcium phosphate crystal did not immediately form, likely due to β -lactoglobulin's known calcium quenching effect. However, no colour change (i.e., no milky

suspension) is observed and precipitation happens quickly after the initial addition of calcium and phosphate (particles or aggregates of about 400nm are detected). The precipitate is obvious after 24 hours, and peaks at 48 hours.

CLAIMS

1. A method of assembling a casein micelle, the method comprising
 - a) providing a composition comprising at least one casein protein in a yeast ferment; and
 - b) agitating the composition of step (a) in the presence of a calcium salt and a phosphate salt for a period of time sufficient to allow assembly of a micelle.
2. The method of claim 1, wherein the at least one casein protein is selected from the group consisting of α S1, α S2, β and κ casein proteins or functional variants thereof.
3. The method of claim 1 or claim 2, wherein the at least one casein protein is β casein or a functional variant thereof.
4. The method of claim 1 or claim 2, wherein the at least one casein protein is α S1 casein or a functional variant thereof.
5. The method of claim 1 or claim 2, wherein the at least one casein protein is α S2 casein or a functional variant thereof.
6. The method of claim 1 or claim 2, wherein the at least one casein protein is κ casein or a functional variant thereof.
7. The method of any one of claims 1-3 and 5, wherein the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S2 casein or a functional variant thereof.
8. The method of any one of claims 1-4, wherein the at least one casein protein comprises or consists of β casein or a functional variant thereof and α S1 casein or a functional variant thereof.
9. The method of any one of claims 1-3 and 6, wherein the at least one casein protein comprises or consists of β casein or a functional variant thereof and κ casein or a functional variant thereof.
10. The method of any one of claims 1-4, and 8-9, wherein the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof and κ casein or a functional variant thereof.
11. The method of any one of claims 1-10, wherein the at least one casein protein comprises or consists of β casein or a functional variant thereof, α S1 casein or a functional variant thereof, κ casein or a functional variant thereof and α S2 casein or a functional variant thereof.

12. The method of any one of claims 8, and 10-11, wherein the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof is from about 1:3 to 2:1.
13. The method of any one of claims 9-11, wherein the mass ratio of the β casein or a functional variant thereof to κ casein or a functional variant thereof is from about 99:1 to 3:22.
14. The method of claims 11 or claim 12, wherein the mass ratio of the β casein or a functional variant thereof to α S1 casein or a functional variant thereof to κ casein or a functional variant thereof is from about 1:1:1 to 53:46:1.
15. The method of any one of the preceding claims, wherein the final concentration of the calcium salt is from about 3-35mM.
16. The method of any one of the preceding claims, wherein the final concentration of the phosphate salt is from about 2-30mM.
17. The method of any one of the preceding claims, wherein the composition comprising at least one casein protein in a yeast ferment further comprises a citrate buffer.
18. The method of claim 17, wherein the final concentration of the citrate buffer is from about 2-3mM.
19. The method of any one of the preceding claims, wherein the micelle has an average particle diameter of from about 50nm to 600nm.
20. The method of claim 19, wherein the micelle has an average particle diameter of about 125nm to 490nm.
21. The method of claim 19 or claim 20, wherein the micelle has an average particle diameter of about 150nm to 230nm.
22. The method of any one of the preceding claims, wherein the micelle is colloiddally stable.
23. The method of any one of the preceding claims, wherein the at least one casein protein is a recombinant casein protein produced in yeast cells or bacterial cells or plant cells.
24. The method of claim 23, wherein the at least one casein protein is produced in yeast cells.
25. The method of claim, wherein at the least one casein protein is produced by the yeast cells from which the yeast ferment is derived.
26. The method of any one of claims 1-22, wherein the at least one casein protein is a native casein protein.

27. The method any one of the preceding claims, wherein at least one casein protein is a mammalian casein protein.
28. The method of claim 27, wherein the mammalian casein protein is a bovine casein protein, a sheep casein protein, a goat casein protein, a human casein protein or a combination thereof.
29. The method of claim 28, wherein the mammalian recombinant casein protein is a bovine casein protein.
30. The method of any one of claims 1-3, and 26 wherein the at least one casein protein comprises or consists of recombinant β casein or a functional variant thereof and a native κ casein.
31. The method of any one of claims any one of claims 1-4, and 26, wherein the at least one casein protein comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof, and a native κ casein.
32. The method of any one of claims 1-2, 6 and 26, wherein the at least one casein protein comprises or consists of recombinant κ casein or a functional variant thereof, and native α S1 casein.
33. The method of any one of claims 1-2, 6 and 26, wherein the at least one casein protein comprises or consists of recombinant κ casein or a functional variant thereof, and native β casein.
34. The method of any one of claims 1-2, 6, and 26, wherein the at least one casein protein comprises or consists of recombinant κ casein or a functional variant thereof, native α S1 casein and native β casein.
35. The method of any one of the preceding claims, wherein the yeast ferment is partially purified to remove molecules larger than 5 kDa.
36. The method of claim 35, wherein the partial purification is performed using by tangential flow filtration, ultrafiltration, dialysis and/or diafiltration.
37. The method of any one of the preceding claims, wherein the yeast ferment is cell-free.
38. The method of any one of the preceding claims, further comprising isolating the assembled micelle.
39. A micelle when produced using the method of any one of claims 1-38.
40. A micelle comprising at least three recombinant casein proteins, wherein the at least three recombinant casein proteins are selected from the group consisting of:
 - a) a recombinant α S1 casein or a functional variant thereof;
 - b) a recombinant α S2 casein or a functional variant thereof;

- c) a recombinant κ casein or a functional variant thereof; and
 - d) a recombinant β casein or a functional variant thereof.
41. The micelle of claim 40, wherein the at least three recombinant casein proteins comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof.
 42. The micelle of claim 40 or claim 41, wherein the at least three recombinant casein proteins comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S2 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof.
 43. The micelle of any one of claims 40-42, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof, recombinant κ casein or a functional variant thereof and recombinant α S2 casein or a functional variant thereof.
 44. The micelle of any one of the claims 40-43, wherein the micelle has average particle diameter of about 50nm to 600nm.
 45. The micelle of claim 44, wherein the micelle has average particle diameter of about 125nm to 490nm.
 46. The micelle of claim 44 or claim 45, wherein the micelle has average particle diameter of about 150nm to 230nm.
 47. The micelle of any one of the claims 40-46, wherein the micelles are colloidally stable.
 48. The micelle of any one of the claims 40-47, wherein the recombinant casein protein is produced in yeast cells or bacterial cells.
 49. The micelle of any one of the claims 40-47, wherein the recombinant casein protein is produced in yeast cells.
 50. The micelle of any one of the claims 40-49, wherein the recombinant casein protein is a mammalian casein protein.
 51. The micelle of claim 50, wherein the mammalian casein protein is a bovine casein protein, a sheep casein protein, a goat casein protein, a human casein proteins or a combination thereof.
 52. The micelle of claim 51, wherein the mammalian casein protein is a bovine casein protein.
 53. The micelle of any one of claims 39-52, wherein the micelle is temperature stable from about 2°C to about 95°C.

54. The micelle of claim 52, wherein the micelle is temperature stable up to about 80°C.
55. A composition comprising the micelle of any one of claims 39-54.
56. The composition of claim 55, further comprising whey proteins.
57. The composition of claim 56, wherein the whey proteins comprise β -lactoglobulin and α -lactalbumin.
58. The composition of claim 57, wherein the β -lactoglobulin and α -lactalbumin are recombinant β -lactoglobulin and recombinant α -lactalbumin.
59. The composition of any one of claims 55-58, further comprising a fat, a sugar, calcium, phosphate and food flavouring and/or colouring.
60. The composition of any one of claims 55-59, wherein the composition does not comprise a constituent that is derived from an animal.
61. A dairy substitute comprising the composition of any one of claims 55-60, or the micelle of claims 39-54.
62. The dairy substitute of claim 61, wherein the dairy substitute is selected from a group consisting of milk, yogurt, cream, butter, cheese, ice-cream and custard.
63. A composition comprising a micelle in a yeast ferment, wherein the micelle comprises at least one recombinant casein protein, wherein the at least one recombinant casein protein is selected from the group consisting of:
 - a) a α S1 casein or a functional variant thereof;
 - b) a α S2 casein or a functional variant thereof;
 - c) a κ casein or a functional variant thereof; and
 - d) a β casein or a functional variant thereof.
64. The composition of claim 63, wherein the at least one recombinant casein protein is β casein or a functional variant thereof.
65. The composition of claim 63, wherein the at least one recombinant casein protein is α S1 casein or a functional variant thereof.
66. The composition of claim 63, wherein the at least one recombinant casein protein is α S2 casein or a functional variant thereof.
67. The composition of claim 63, wherein the at least one recombinant casein protein is κ casein or a functional variant thereof.
68. The composition of any one of claims 63-65 and 66, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof and recombinant α S2 casein or a functional variant thereof.

69. The composition of any one of claims 63-65, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof and recombinant α S1 casein or a functional variant thereof.
70. The composition of any one of claims 63-64 and 67, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof and recombinant κ casein or a functional variant thereof.
71. The composition of any one of claims 63-65, and 67, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof and recombinant κ casein or a functional variant thereof.
72. The composition of any one of claims 63-67, wherein the micelle comprises or consists of recombinant β casein or a functional variant thereof, recombinant α S1 casein or a functional variant thereof, recombinant κ casein or a functional variant thereof and recombinant α S2 casein or a functional variant thereof.
73. The composition of claim 63 or claim 69, wherein the mass ratio of the recombinant β casein or a functional variant thereof to recombinant α S1 casein or a functional variant thereof is from about 1:3 to 2:1.
74. The composition of claim 63 or 70, wherein the mass ratio of the recombinant β casein or a functional variant thereof to recombinant κ casein or a functional variant thereof is from about 99:1 to 3:22.
75. The composition of claim 63 or claim 71, wherein the mass ratio of the recombinant β casein or a functional variant thereof to recombinant α S1 casein or a functional variant thereof to recombinant κ casein or a functional variant thereof is from about 1:1:1 to 53:46:1.
76. The composition of any one of claims 63-75, further comprising a calcium salt.
77. The composition of claim 76, wherein the final concentration of the calcium salt is from about 7-8mM.
78. The composition of any one of claims 63-77, further comprising a phosphate salt.
79. The composition of claim 78, wherein the final concentration of the phosphate salt is from about 5-6mM.
80. The composition of any one of claims 63-79, further comprising a citrate buffer.
81. The composition of claim 80, wherein the final concentration of the citrate buffer is from about 2-3mM.

82. The composition of any one of claims 63-81, wherein the micelle has average particle diameter of from about 50nm to 600nm.
83. The composition of claim 82, wherein the micelle has average particle diameter of about from 125nm to 490nm.
84. The composition of claim 83, wherein the micelle has average particle diameter of about from 150nm to 230nm.
85. The composition of any one of claims 63-84, wherein the micelles are colloidally stable.
86. The composition of any one of claims 61-85, wherein the recombinant casein protein is produced in yeast cells or bacterial cells.
87. The composition of claim 86, wherein the at least one recombinant casein protein is produced in yeast cells.
88. The composition of claim 87, wherein at the least one recombinant casein protein is produced by the yeast cells from which the yeast ferment is derived.
89. The composition of any one of the claims 63-88, wherein the recombinant casein protein is a mammalian casein protein.
90. The composition of claim 89, wherein the recombinant mammalian casein protein is a recombinant bovine casein protein, a recombinant sheep casein protein, a recombinant goat casein protein, a recombinant human casein proteins or a combination thereof.
91. The composition of claim 90, wherein the recombinant mammalian casein protein is a bovine casein protein.
92. The composition of any one of claims 63-91, wherein the yeast ferment has been partially purified to remove molecules larger than 5 kDa.
93. The composition of claim 92, wherein the purification is performed by tangential flow filtration, ultrafiltration, dialysis and/or diafiltration.
94. The composition of claim 63-93, wherein the yeast ferment is cell-free.
95. The composition of any one of claims 56-61 and 64-94, wherein the composition is temperature stable from about 2°C to about 95°C.
96. The composition of claim 95, wherein the composition is temperature stable to about 80°C.