INCORPORATION BY REFERENCE

[0089] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference

BRIEF DESCRIPTION OF THE DRAWINGS

[0090] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings.

[0091] **FIG. 1** is a basic technical diagram of the production process.

[0092] **FIG. 2** illustrates an exemplary protocol for cheese production.

[0093] **FIGS. 3A** and **3B** illustrate expression systems.

[0094] **FIG. 4A** shows yogurt-like gel and curd.

[0095] **FIG. 4B** illustrates curds made from micellar casein liquid colloid (supplemented with lactose) (left) and fat-free milk (right) via microbial acidification using bacterial starter culture. Firm, cuttable and cohesive curd was made in both cases. Bottom left: An example of curd made from micellar liquid colloid (supplemented with lactose) emulsified with deflavoured coconut oil via microbial acidification using bacterial starter culture.

[0096] **FIG. 4C** illustrates cheese and curd firmness (in g force used in stress-relaxation test) and relaxation for mozzarella-like cheese made from microbial casein liquid colloid vs fat-free milk using bacterial starter culture vs citric acid.

[0097] **FIG. 5A** illustrates texture profile and hardness / firmness (in g force) of mozzarella cheese.

[0098] **FIG. 5B** illustrates samples of mozzarella made from micellar casein melted and served on a ‘pizza’ for triangle test tasting.

[0099] **FIG. 6** illustrates average micelle diameter (in nm) of casein micelles (black) and submicelles (gray) induced using alpha casein, beta-casein and kappa casein in various salt conditions. Relative intensity proportions of micelle (black) and submicelle (gray) peaks detected are represented as arch sizes/angles.

[00100] **FIG. 7A** illustrates liquid milk-like colloids comprised of casein micelles induced using alpha casein, beta-casein and kappa casein in various salt conditions.

[00101] **FIG. 7B** shows liquid milk-like colloids subjected to acidification via citric acid (top row) and renneting coagulation via recombinant chymosin (middle row), which gave rise to dairy curds (bottom row, X where data was not collected). Samples B and F precipitated during acidification and renneting and formed curds on the bottom of the well. All other samples stayed in suspension during acidification and formed curds throughout. Curds were then dipped in hot water and stretched into pasta-filata cheese balls.

[00102] **FIG. 8** shows average micelle diameter (in nm) of casein micelles (black) and submicelles (gray) induced using alpha casein and kappa casein in various salt conditions. Relative intensity proportions of micelle (black) and submicelle (gray) peaks detected are represented as arch sizes/angles.

[00103] **FIG. 9A** shows hardness (in g force applied) of cheese made from liquid milk-like colloid of casein micelles assembled from alpha casein and kappa casein. Sodium caseinate (a source of mixed milk caseins) was used as a control. Error bars represent standard deviation on triplicate sample.

[00104] **FIG. 9B** shows cheese made from liquid milk-like colloid of casein micelles assembled from alpha casein and kappa casein (in triplicate).

[00105] **FIG. 10** shows liquid milk-like colloids of casein micelles induced using hypophosphorylated alpha casein (enzymatically dephosphorylated) and kappa casein in various salt conditions were subjected to acidification via citric acid (top row) and renneting coagulation via recombinant chymosin, which gave rise to dairy curds (bottom row, X where the curd was already used for cheese making). Samples F precipitated during acidification and renneting and formed curd on the bottom of the well. All other samples stayed in suspension during acidification and formed curds throughout. Curds were then dipped in hot water and stretched into pasta-filata cheese balls.

[00106] **FIG. 11** shows cheese made from liquid milk-like colloid of casein micelles assembled from hypophosphorylated alpha casein (enzymatically dephosphorylated) and kappa casein in various salt conditions.

[00107] **FIG. 12** shows average micelle diameter (in nm) of casein micelles (black) and submicelles (gray) induced using hypophosphorylated (enzymatically dephosphorylated) alpha casein and kappa casein in salt conditions at milk-like protein concentrations (2.8% total casein, 3.2% total casein). Relative intensity proportions of micelle (black) and submicelle (gray) peaks detected are represented as arch sizes/angles.

some embodiments the expression vector may contain any subset of the elements (b), (e), (f), and (g), including none of elements (b), (e), (f), and (g). Other expression elements and vector element known to one of skill in the art can be used in combination or substituted for the elements described herein.

[00194] Gram positive bacteria (such as *Lactococcus lactis* and *Bacillus subtilis*) may be used to secrete target proteins into the media, and gram-negative bacteria (such as *Escherichia coli*) may be used to secrete target proteins into periplasm or into the media. In some embodiments, the bacterially-expressed proteins expressed may not have any post-translational modifications (PTMs), which means they are not glycosylated and/or may not be phosphorylated.

[00195] Target casein proteins may be expressed and produced in *L. lactis* both in a nisin-inducible expression system (regulated by PnisA promoter), lactate-inducible expression system (regulated by P170 promoter) or other similar inducible systems, as well as a constitutively expressed system (regulated by P secA promoter), wherein both are in a food-grade selection strain, such as NZ3900 using vector pNZ8149 (lacF gene supplementation/rescue principle). The secretion of functional proteins may be enabled by the signal peptide of Usp45 (SP(usp45)), the major Sec-dependent protein secreted by *L. lactis*. For example, alpha-S1-casein and kappa casein may be co-expressed or individually expressed in *L. lactis* using a synthetic operon, where the gene order is kappa casein - alpha S1 casein, as shown in **FIGS. 3A** and **3B**.

Bacillus subtilis Design

[00196] *B. subtilis*, unlike *L. lactis*, has multiple intracellular and extracellular proteases, which may interfere with protein expression. In some embodiments, *B. subtilis* strains are modified to reduce the type and amount of intracellular and/or extracellular proteases, for example strains which have deletions for 7 (KO7) and 8 (WB800N) proteases, respectively, may be used.

[00197] In order to drive the recombinant protein secretion, the signal peptide of amyQ, alpha-amylase of *Clostridium thermocellum* may be used. Additionally, native casein signal peptide sequences may be expressed heterologously in *B. subtilis*. Each casein protein has its own signal peptide sequence and may be used in the system. The signal proteins may be cross-combined with the casein proteins. The pHT01 vector may be used as a transformation and expression shuttle for inducible protein expression in *B. subtilis.* The vector is based on the strong σ^A-dependent promoter preceding the *groES-groEL* operon of *B. subtilis*, which has been converted into an efficiently controllable (IPTG-inducible) promoter by addition of the lac

CLAIMS

WHAT IS CLAIMED IS:

1. An edible composition comprising a coagulated colloid, wherein the coagulated colloid comprises α casein protein and κ casein protein associated in a micellar form, wherein at least one of the α (alpha) casein protein and the κ (kappa) casein protein is recombinantly produced; and wherein the edible composition does not contain β (beta) casein protein.

2. The edible composition of claim 1, wherein the recombinantly produced casein is produced from a bacterial host cell.

3. The edible composition of claim 2, wherein the bacterial host cell is selected from the group consisting of Lactococci sp., Lactococcus lactis, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis and Bacillus megaterium, Brevibacillus choshinensis, Mycobacterium smegmatis, Rhodococcus erythropolis and Corynebacterium glutamicum, Lactobacilli sp., Lactobacillus fermentum, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum, Synechocystis sp. 6803 and E.coli.

4. The edible composition of any one of claims 1-3, wherein the α casein protein completely lacks or is substantially reduced in post-translational modification as compared to animal-derived α casein.

5. The edible composition of claim 4, wherein the α casein protein completely lacks or is substantially reduced in phosphorylation as compared to animal-derived α casein.

6. The edible composition of any one of claims 1-5, wherein the κ casein protein completely lacks or is substantially reduced in post-translational modification as compared to animal-derived κ casein.

7. The edible composition of claim 6, wherein the κ casein protein completely lacks or is substantially reduced in glycosylation, phosphorylation or both glycosylation and phosphorylation as compared to animal-derived κ casein.

8. The edible composition of any one of claims 1-7, wherein the ratio of α casein protein to κ casein protein is from 1:1 to 15:1.

9. The edible composition of any one of claims 1-8, wherein the ratio of α casein protein to κ casein protein is from 1:1 to 5:1.

10. The edible composition of any one of claims 1-9, wherein the α casein protein is αS1 or αS2.

11. The edible composition of any one of claims 1-10, wherein the α casein protein has an amino acid sequence comprising one of SEQ ID NO. 1-26 or a variant thereof with at least 80% sequence homology.

12. The edible composition of any one of claims 1-11, wherein the κ casein protein has an amino acid sequence comprising one of SEQ ID NO. 27-40 or a variant thereof with at least 80% sequence homology.

13. The edible composition of any one of claims 1-12, further comprising at least one salt selected from the group consisting of a calcium salt, a citrate salt and a phosphate salt.

14. The edible composition of any one of claims 1-13, wherein the coagulated colloid is comprised in a dairy-like product, a cheese, a curd, a renneted curd, or a yogurt.

15. The edible composition of any one of claims 1-14, wherein the edible composition is a cheese and wherein the cheese has one or more of (a) a fat content more than 0% to 50%, (b) a fat derived from a plant-based source or (c) a sugar is derived from a plant-based source.

16. The edible composition of any one of claims 1-14, wherein:

a. the edible composition is a cheese and wherein the cheese is capable of melting and browning when heated;
b. the edible composition is a cheese selected from the group consisting of pasta-filata like cheese, paneer, cream cheese, cottage cheese, an aged cheese or a renneted curd; or
c. the edible composition is a cheese and wherein the texture or hardness of the cheese is comparable to an animal-derived dairy cheese.

17. A method for producing an edible composition, comprising: combining a recombinant α casein protein, a recombinant κ casein protein and at least one salt under conditions, wherein the α casein protein and the κ casein protein form a micellar form in a liquid colloid, wherein the micellar form does not include β casein protein; and subjecting the liquid colloid to a first condition to form coagulates.

18. A liquid colloid comprising a micellar form, wherein the micellar form comprises a recombinant α casein protein, a recombinant κ casein protein and at least one salt, wherein the micellar form does not include β casein protein.

19. The liquid colloid of claim 18, wherein the α casein protein, the κ casein protein or a combination thereof completely lack or are substantially reduced in post-translational modifications.

20. The liquid colloid of claim 18, wherein (a) the α casein protein completely lacks or is substantially reduced in phosphorylation as compared to an animal-derived α casein, (b) the κ casein protein completely lacks or is substantially reduced in glycosylation as compared to an animal-derived κ casein, (c) the κ casein protein completely lacks or is substantially reduced in phosphorylation as compared to an animal-derived κ casein or (d) any of (a), (b) and (c).

21. A yogurt composition formed from the liquid colloid of claim 18.