

DAIRY-LIKE COMPOSITIONS AND RELATED METHODS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/234,193, filed on August 17, 2021, and U.S. Utility Application, 17/829,951 file don June 01, 2022, both of which applications are incorporated herein by reference in its entirety.

BACKGROUND

[0002] The clean food space is comprised of both plant-based and cell-based foods. Cell-based food is a large umbrella term that includes culturing muscle and fat cells to replace slaughtered meat and culturing bioengineered organisms to express recombinant animal proteins to replace other animal products such as dairy and eggs. The need to find an alternate source of animal protein comes from the inefficiencies and unsustainability of current animal food production.

[0003] Cheese is the third most unsustainable animal product globally (when measuring greenhouse gas emissions per kg of product), and the consumption of dairy cheese hasn't been slowed down by plant-based alternatives introduced into the market in the last 10 years. On the contrary, mozzarella cheese consumption is growing year on year in the US and in developing markets. Current cheese alternatives do not match the functionality, nutrition and taste of dairy cheese due to their lack of casein proteins.

SUMMARY

[0004] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

[0005] In some aspects, provided herein are consumable compositions. In some embodiments, the consumable composition may comprise a recombinant single variant of an alpha casein protein. In some embodiments, the single variant provides at least one dairy-like property to the consumable composition selected from the group consisting of adhesiveness, stretch, texture, mouthfeel, melt, browning, hardness, creaminess, taste, smell, and flexibility.

In some embodiments, the recombinant single variant may be not an animal-derived casein and has not been physically dissociated from a casein micelle. In some embodiments, the dairy-like property may be provided substantially from the recombinant single variant casein protein; and wherein the consumable composition may be comparable to a dairy-derived consumable composition in at least one of the dairy-like properties.

[0006] In some embodiments, the consumable composition may comprise one or more dairy-like properties selected from the group consisting of adhesiveness, stretch, firmness, viscosity, tackiness, chewiness, resilience, springiness, mouthfeel, melt, hardness, creaminess, and flexibility.

[0007] In some embodiments, the casein content of the consumable composition may comprise substantially the recombinant single variant of the alpha casein protein.

[0008] In some embodiments, the casein content of the consumable composition may comprise only the recombinant single variant of the alpha casein protein.

[0009] In some embodiments, the recombinant single variant of the alpha casein protein may comprise at least 95% or at least 97% of the casein content in the consumable composition.

[0010] In some embodiments, the recombinant single variant of the alpha casein protein may comprise at least 99% of the casein content in the consumable composition.

[0011] In some embodiments, the consumable composition lacks any additional caseins other than the recombinant single variant of the alpha casein protein.

[0012] In some embodiments, the single variant of the alpha casein protein may be not derived from caseinate.

[0013] In some embodiments, the composition may be free from any animal-produced proteins.

[0014] In some embodiments, the composition lacks any other animal-derived dairy proteins.

[0015] In some embodiments, the dairy-like property may be comparable to or improved as compared to a milk-derived consumable composition; wherein the milk-derived consumable composition may be identical to the consumable composition in all ingredients except the milk-derived consumable composition may comprise milk, one or more milk-derived proteins or a milk-derived ingredient instead of or in addition to the recombinant single variant of the alpha casein protein.

[0016] In some embodiments, the dairy-like property may be comparable to or improved as compared to a micellar casein composition wherein the micellar casein composition may be identical to the consumable composition in all ingredients except the micellar casein

composition may comprise micellar casein isolated from milk instead of or in addition to the recombinant single variant of the alpha casein protein.

[0017] In some embodiments, the recombinant single variant may be an alpha casein protein that may comprise at least one non-native post-translational modification.

[0018] In some embodiments, the recombinant single variant of the alpha casein protein further may comprise at least one native post-translational modification.

[0019] In some embodiments, the recombinant single variant may be an alpha casein protein lacking one or more post-translational modifications of a native alpha casein protein.

[0020] In some embodiments, the single variant of the alpha casein protein further may comprise at least one non-native post-translational modification.

[0021] In some embodiments, the single variant of the alpha casein protein may be not post-translational modified.

[0022] In some embodiments, the single variant of the alpha casein protein may be not phosphorylated.

[0023] In some embodiments, the single variant of the alpha casein protein may be an alpha-s1 casein protein. In some embodiments, the single variant of the alpha casein protein may be an alpha-s2 casein protein. In some embodiments, the composition may comprise a full-length alpha casein protein.

[0024] In some embodiments, the single variant of the alpha casein protein may comprise any one of SEQ ID Nos. 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.

[0025] In some embodiments, the single variant of the alpha casein protein may comprise an amino acid sequence of a bovine, caprine or ovine alpha casein protein or any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56 or a sequence with at least 70%, 80%, 85% or 90% identity to any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.

[0026] In some embodiments, the single variant of the alpha casein protein may comprise one or more non-native amino acids at the N-terminus.

[0027] In some embodiments, the single variant of the alpha casein protein may comprise a non-native methionine at the N-terminal position.

[0028] In some embodiments, the single variant of the alpha casein protein may be not derived from casein micelles.

[0029] In some aspects, described herein may be a dairy product analogue. The dairy product analogue may comprise any of the consumable compositions provided herein, wherein the analogue may be selected from the group consisting of a cheese analogue, a yogurt analogue, a cream analogue, and an ice cream analogue.

[0030] In some embodiments, the dairy product analogue may comprise the recombinant single variant alpha casein.

[0031] In some embodiments, the dairy product analogue further may comprise a fat or oil from a non-animal source. In some embodiments, the dairy product analogue lacks any animal-derived dairy proteins. In some embodiments, the dairy product analogue lacks any other casein proteins. In some embodiments, the recombinant single variant of the alpha casein protein may be not comprised in a micellar form within the dairy product analogue.

[0032] In some embodiments, the dairy product analogue may comprise one or more of (a) a plant-derived oil; (b) a plant-derived starch; (c) a sugar; and (d) a salt.

[0033] In some embodiments, the dairy product analogue may be a cheese analogue. In some embodiments, the cheese analogue may be a mozzarella analogue, a cheddar analogue or a parmesan analogue. In some embodiments, the cheese analogue may be a low-moisture cheese analogue. In some embodiments, the cheese analogue may be a soft cheese analogue. In some embodiments, the cheese analogue may be a hard cheese analogue.

[0034] In some embodiments, the cheese analogue may be a mozzarella analogue and wherein the single variant of the casein protein may be an alpha casein. In some embodiments, the alpha casein may be an alpha S1 casein. In some embodiments, the alpha S1 casein may be a bovine alpha S1 casein. In some embodiments, the alpha S1 casein may be a full-length casein. In some embodiments, the alpha casein may be an alpha S2 casein. In some embodiments, the alpha S2 casein may be a bovine alpha S2 casein. In some embodiments, the alpha S2 casein may be a full-length casein.

[0035] In some embodiments, a stretch of the cheese analogue may be comparable to or improved relative to a stretch of a dairy-derived cheese or dairy-derived cheese analogue.

[0036] In some embodiments, the cheese analogue has a comparable or greater melt area/time as compared to the melt area/time of a dairy-derived cheese or dairy-derived cheese analogue.

[0037] In some embodiments, an extensibility of the cheese analogue may be comparable to or improved relative to an extensibility of a dairy-derived cheese or dairy-derived cheese analogue. In some embodiments, a texture of the cheese analogue may be comparable to or improved relative to a texture of a dairy-derived cheese or dairy-derived cheese analogue. In

some embodiments, an adhesiveness of the cheese analogue may be reduced relative to an adhesiveness of a dairy-derived cheese or dairy-derived cheese analogue.

[0038] In some embodiments, the dairy-derived cheese or dairy-derived cheese analogue may comprise micellar casein. In some embodiments, the stretch of the cheese analogue may be improved relative to a stretch of a plant-derived cheese analogue. In some embodiments, a melt of the cheese analogue may be improved relative to a melt of a plant-derived cheese analogue. In some embodiments, a texture of the cheese analogue may be improved relative to a texture of a plant-derived cheese analogue. In some embodiments, an adhesiveness of the cheese analogue may be reduced relative to an adhesiveness of a plant-derived cheese analogue.

[0039] In some embodiments, the cheese analogue may comprise from at least 5% single variant of the casein protein w/w. In some embodiments, the cheese analogue may comprise from about 5% to about 30% single variant of the casein protein w/w. In some embodiments, the cheese analogue may comprise from about 10% to about 25% single variant of the casein protein w/w. In some embodiments, the cheese analogue may comprise from about 15% to about 25% single variant of the casein protein w/w.

[0040] In some embodiments, the cheese analogue may comprise at most 25mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise from 0mg to 25mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise from 0mg to 20mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise from 0mg to 10mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise from 5mg to 15mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise about 0mg of calcium per gram of casein. In some embodiments, the cheese analogue may comprise about 10mg of calcium per gram of casein.

[0041] In some embodiments, the cheese analogue may comprise from about 15% to about 30% fats w/w. In some embodiments, the cheese analogue may comprise from about 18% to about 28% fats w/w. In some embodiments, the cheese analogue may comprise from about 20% to about 25% fats w/w.

[0042] In some embodiments, the cheese analogue may comprise from about 0.5% to about 4% starch w/w. In some embodiments, the cheese analogue may comprise from about 1% to about 3% starch w/w. In some embodiments, the cheese analogue may comprise from about 2% to about 3% starch w/w. In some embodiments, the cheese analogue may comprise at most 10% starch w/w. In some embodiments, the cheese analogue may comprise at most 5% starch w/w.

[0043] In some embodiments, a ratio of the recombinant single variant alpha casein to emulsifying salts may be from 12:1 to 6:1. In some embodiments, the cheese analogue does not comprise emulsifiers other than the emulsifying salts.

[0044] In some embodiments, the dairy product analogue may be a yogurt analogue. In some embodiments, the single variant of the casein protein may be an alpha casein. In some embodiments, the alpha casein may be an alpha S1 casein. In some embodiments, the alpha S1 casein may be a bovine alpha S1 casein. In some embodiments, the alpha S1 casein may be a full-length casein. In some embodiments, the alpha casein may be an alpha S2 casein. In some embodiments, the alpha S2 casein may be a bovine alpha S2 casein. In some embodiments, the alpha S2 casein may be a full-length casein.

[0045] In some embodiments, the emulsification of the yogurt analogue may be comparable to or improved relative to an emulsification of a dairy-derived yogurt or dairy-derived yogurt analogue. In some embodiments, the firmness, tackiness, or viscosity of the yogurt analogue may be comparable to or improved relative to a dairy-derived yogurt or dairy-derived yogurt analogue. In some embodiments, the adhesiveness of the yogurt analogue may be reduced relative to a dairy-derived yogurt or dairy-derived yogurt analogue. In some embodiments, the dairy-derived yogurt or dairy-derived yogurt analogue may comprise micellar casein.

[0046] In some embodiments, the dairy-derived yogurt or dairy-derived yogurt analogue may be identical to the dairy product analogue in all ingredients except the dairy-derived yogurt or dairy-derived yogurt analogue may comprise micellar casein instead of the recombinant single variant of the alpha casein protein. In some embodiments, the emulsification of the yogurt analogue may be comparable to or improved relative to an emulsification of a plant-derived yogurt analogue. In some embodiments, the firmness, tackiness, or viscosity of the yogurt analogue may be comparable to or improved relative to a plant-derived yogurt analogue. In some embodiments, the adhesiveness of the yogurt analogue may be reduced relative to a plant-derived yogurt analogue.

[0047] In some embodiments, the plant-derived yogurt analogue lacks any dairy-proteins.

[0048] In some embodiments, the yogurt analogue may comprise from about 1% to about 4% single variant of the casein protein w/w. In some embodiments, the yogurt analogue may comprise from about 2% to about 4% single variant of the casein protein w/w. In some embodiments, the yogurt analogue may comprise from about 2% to about 6% fats w/w. In some embodiments, the yogurt analogue may comprise from about 4% to about 8% carbohydrates w/w.

[0049] In some embodiments, the dairy product analogue may be a beverage. In some embodiments, the single variant of the casein protein may be an alpha casein. In some embodiments, a smoothness of the beverage may be comparable to or improved relative to a smoothness of a dairy-derived beverage. In some embodiments, a texture of the beverage may be comparable to or improved relative to a texture of a dairy-derived beverage. In some embodiments, the emulsification of the beverage may be comparable to or improved relative to a dairy-derived beverage. In some embodiments, the beverage may comprise from about 0.5% to about 10% single variant of the casein protein w/w. In some embodiments, the beverage may comprise from about 0.1% to about 6% fats w/w.

INCORPORATION BY REFERENCE

[0050] 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

[0051] 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.

[0052] **FIG. 1** illustrates recombinantly produced full-length alpha S1 casein protein and two occurring truncated forms of alpha S1 casein.

[0053] **FIG. 2** illustrates a comparison of stretching ability (extensibility) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0054] **FIG. 3** illustrates a comparison of texture (on logarithmic scale) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0055] **FIG. 4** illustrates a comparison of melt profile between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0056] FIG. 5 illustrates a comparison of stretching ability (extensibility) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0057] FIG. 6 illustrates a comparison of melt between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0058] FIG. 7 illustrates a comparison of stretching ability (extensibility) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0059] FIG. 8 illustrates a comparison of texture (on logarithmic scale) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0060] FIG. 9 illustrates a comparison of melt profile between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0061] FIG. 10 illustrates a comparison of stretching ability (extensibility) between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0062] FIG. 11 illustrates a comparison of melt profile between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0063] FIG. 12 illustrates a comparison of an extensibility profile between the analogue cheese made using the methods described herein and a few commercially available cheeses and cheese analogues.

[0064] FIG. 13 illustrates a yogurt made using the methods described herein and a milk derived yogurt.

[0065] FIG. 14 illustrates a yogurt beverage made using the methods described herein and a milk derived yogurt beverage.

DETAILED DESCRIPTION OF THE INVENTION

[0066] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0067] Although the dairy industry is worth \$330 billion, research needs to be performed for a clean dairy solution using recombinant dairy proteins. As dairy cheese is an inefficient dairy product, in terms of resources needed per gram as well as being the hardest dairy product to accurately reproduce from just plant-based ingredients, presented herein are methods and compositions of dairy-like products made using recombinant proteins.

[0068] A component that gives dairy cheese its unique characteristics is the casein proteins. When milk or milk-derived ingredients are used in dairy products, the caseins are found in micelles. Micelles are protein colloids and typically in cow milk, the micelles are comprised of four casein proteins (alpha S1 casein, alpha S2 casein, beta casein, and kappa casein) that interact with insoluble calcium phosphate at the colloid centre. It is the micelles in milk that attract each other once chymosin is added to milk. This forms the curd, which is then used to make 99% of all cheeses. In case of yogurt, acidification of the micelle comprising liquid colloid may be performed using a starter culture of bacteria known for yogurt production. The current disclosure is based on the discovery that a recombinant non-naturally occurring single variant of casein can be used to generate dairy or dairy-like products without the presence of other caseins and without the formation of a micelle. People skilled in the art have attempted to isolate different casein proteins from milk or milk micelles for producing dairy like products but the inventors of this application have discovered for the first time that a single variant casein, in recombinant form, particularly in some embodiments lacking or having different post translational modifications (PTMs) from native casein, can provide dairy-like features without being present in a micellar form and without the association with or presence of other caseins or other dairy proteins.

[0069] One skilled in the art would know that micelles are complex structures with multiple proteins and would therefore not expect to be able to form dairy-like products such as cheese, cheese analogues, yogurt, and other dairy products using a single casein protein. The current disclosure is based on the surprising discovery made by the inventors that a single recombinantly produced casein protein, a single variant of casein, such as alpha casein, is able to form consumables without forming or being incorporated into micelles. The current disclosure also describes recombinantly made dairy products such as cheese analogues and other dairy analogue products, as well as powders using the compositions formed by the methods described herein.

[0070] One skilled in the art would know that micelles are complex structures with multiple proteins bearing multiple post-translational modifications and would therefore not expect to be able to form dairy-like products such as cheese, cheese analogues, yogurt, and other dairy

products with comparable or improved dairy-like properties using a single recombinant casein protein. The current disclosure is based on the surprising discovery made by the inventors that a single recombinant casein protein, a single variant of casein, such as alpha casein, is able to form consumables with comparable or improved dairy-like properties despite differences in post-translational modification and despite not being incorporated into a micelle.

[0071] The current disclosure also describes consumable compositions that incorporate truncated forms of a recombinantly made single variant of an alpha casein protein. Compositions described herein may comprise different truncated forms of casein proteins.

[0072] The consumable compositions described herein are formed from a recombinant single casein variant, such as the alpha casein variant. Recombinant casein protein may be expressed in a microbial organism, for example, bacteria such as gram-positive bacteria *Lactococcus lactis* and *Bacillus subtilis*, as well as a gram-negative model organism *E. coli*, as well as other host organisms such as yeasts, fungi, and plants. These recombinant casein proteins may be combined with other components (e.g., minerals, fats, sugars, and vitamins) to make dairy-like products, for example, cheese that behaves, smells, tastes, looks and feels like animal-derived dairy cheese. Such dairy-like products may have no: i) lactose, ii) cholesterol, iii) animal-derived saturated fats, iv) milk-derived whey proteins; and/or v) milk-derived casein proteins.

[0073] In some embodiments, the methods include producing a single variant of a casein protein in a bacterial host cell, such that such proteins are secreted from the cell into the surrounding media. In some examples, the methods include producing a single variant of an alpha casein protein in a bacterial host 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 bacterial host cell, such that such proteins are intracellular. Recombinant protein can then be isolated, purified or partially purified and used in methods for making compositions which can be used as a dairy ingredient, or emulsified with plant-based fats and other nutrients to form milk, cheese, yogurt or other dairy-like analogue products.

[0074] In some embodiments, the methods include producing compositions using a single casein from a variety of different species. In some examples, the methods include producing compositions using a single alpha casein variant from a variety of different species. The casein may be from human, Bovinae (cattle, bison, buffalo), Caprinae (sheep and goat), Equine (horses, zebra) and Camelus (camels). The single casein variant may be modified as compared

to a native alpha casein, for instance, truncated forms of native caseins. Compositions described herein may be produced without beta or kappa casein in some examples.

[0075] In some embodiments, recombinant caseins can be isolated, purified or partially purified from genetically modified microorganism or their cultivation broth.

[0076] The term “about” as used herein can mean within 1 or 2 standard deviations. Alternatively, “about” can mean a range of up to 10%, up to 5%, or up to 1% of a given value. For example, about can mean up to $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ of a given value.

[0077] The term “dairy protein” as used herein means a protein that has an amino acid sequence derived from a protein found in milk (including variants thereof).

[0078] The term “animal-derived dairy protein” as used herein means a protein derived from milk, such as a protein obtained and/or isolated from milk of a milk-producing organism, including but not limited to cow, sheep, goat, human, bison, buffalo, camel and horse. “Animal-derived casein protein” means casein protein obtained and/or isolated from milk of a milk-producing organism.

[0079] The term “recombinant dairy protein” as used herein means a protein that is expressed in a heterologous or recombinant organism that has an amino acid sequence derived from a protein found in milk (including variants thereof). “Recombinant casein protein” means a casein produced by a recombinant organism or in a heterologous host cell.

[0080] The term “single variant of casein” (also referred to as “single variant of a casein protein”) as used herein may describe a composition comprising or created from one variant of a casein protein amino acid sequence. For instance, a composition comprising a single variant of casein comprises only alpha casein. In some cases, the term “single variant of a casein protein” may describe a composition wherein a single casein protein provides one or more dairy-like properties to the composition irrespective of the presence of other caseins.

[0081] A composition comprising a single variant of casein may be created from only one casein protein amino acid sequence but may comprise truncated forms of the protein sequence in place of or in addition to the full-length version of the protein.

[0082] The term “single variant of alpha casein” (also referred to as “single variant alpha casein protein”) as used herein may describe a composition comprising or created from one variant of an alpha casein protein amino acid sequence. For instance, a composition comprising a single variant of alpha casein comprises only one of alpha S1 or alpha S2 casein. A composition comprising a single variant of alpha casein may be created from only one alpha casein protein amino acid sequence but may comprise truncated forms of the protein sequence

in place of or in addition to the full-length version of the protein. For instance, a composition comprising a single variant of alpha casein protein may comprise a mixture of full length alpha S1 casein protein and truncated forms thereof. A composition comprising a single variant of alpha casein may comprise only the full-length alpha S1 casein protein or only a truncated form of alpha S1 casein protein, or only a mixture of truncated forms of the alpha S1 protein.

[0083] A percentage of “sequence identity” as used herein in the context of polynucleotide or polypeptide (amino acid) sequences refers to the percentage of residues in two sequences that are the same when the sequences are aligned for maximum correspondence. There are a number of different algorithms known in the art that can be used to measure polynucleotide or polypeptide sequence identity. For instance, sequences can be compared using FASTA (e.g., using its default parameters as provided in the Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI), Gap (e.g., using its default parameters as provided in the Wisconsin Package Version 10.0, GCG, Madison, WI), Bestfit, ClustalW (e.g., using default parameters of Version 1.83), or BLAST (e.g., using reciprocal BLAST, PSI-BLAST, BLASTP, BLASTN) (see, for example, Pearson. 1990. *Methods Enzymol.* 183:63; Altschul et al. 1990. *J. Mol. Biol.* 215:403).

Compositions comprising Caseins

A. Casein proteins

[0084] Traditionally, cheese begins with animal-derived milk. The process of animal-derived cheese production includes precipitating micellar forms from milk wherein the micellar forms are in most cases complex protein mixtures (comprising multiple types of casein proteins such as alpha, beta and kappa proteins). Milk is acidified, the micelles shrink and dissociate slightly, then milk is renneted and made into curd, and the curd made into cheese. Cheese analogues may be created from animal milk by first precipitating casein micelles from milk using one of the following methods: 1) a sodium salt to make sodium caseinate, 2) an acid to make acid casein, 3) enzymatic coagulation with rennet to make rennet casein. Casein precipitated in this way from milk is then further processed with fats to create the cheese analogue.

[0085] Provided herein are consumable compositions with a single variant of casein, produced recombinantly, that is not in micellar form and not derived from milk or derived from milk casein. In some aspects, the consumable compositions of the disclosure are not only made of a single variant of casein but are able to provide dairy-like properties to food and beverage products without being in a micellar structure or being derived from a starting material having

a micellar structure. In some aspects, the consumable compositions of the disclosure are not only made using a single variant of casein but the single variant of casein is able to provide desired or improved dairy-like properties without the presence of other caseins and without the complexity of casein structures derived from milk. In some aspects, the consumable compositions of the disclosure comprise a single variant of alpha casein. In some alternative examples, the consumable compositions of the disclosure comprise a single variant of beta or kappa casein.

[0086] In some embodiments, compositions herein (and products made therefrom) do not include any dairy proteins other than a single variant of a casein protein. In some cases, the single variant of a casein protein is a single variant of the alpha casein protein. In some cases, compositions herein (and products made therefrom) do not include any whey proteins or any milk-derived whey proteins. In some embodiments, compositions herein (and products made therefrom) do not include any animal-derived dairy proteins. The compositions herein do not comprise any casein proteins isolated from any animal-derived products or micelles.

[0087] The compositions described herein comprise single variants of casein that are made through recombinant production. In some cases, the single variant casein in a consumable composition may be a modified casein protein relative to a native casein protein. The modifications in the single variant of a casein protein may comprise one or more amino acid insertions, deletions, or substitutions relative to a wild-type or native casein protein. In some cases, the single variant casein protein is the single variant of an alpha casein protein. In some examples, casein proteins may be beta or kappa or gamma casein proteins.

[0088] A single variant of an alpha casein protein may be a recombinant protein that is a truncated alpha casein protein relative to a wild-type or native alpha casein protein. The truncation may resemble a truncation found in nature (e.g., having a common number of amino acids). The truncation may be a non-naturally occurring truncation of the alpha casein protein. A single variant of an alpha casein protein may have a N-terminal truncation relative to a wild-type or native alpha casein protein. A single variant of an alpha casein protein may have a C-terminal truncation relative to a wild-type or native alpha casein protein. A single variant of an alpha casein protein may have an N-terminal truncation and a C-terminal truncation relative to a wild-type or native alpha casein protein.

[0089] In some embodiments, the single variant of an alpha casein in a consumable composition is an alpha S1 casein. In such compositions, the alpha S1 casein may comprise a modified alpha S1 casein, such as modified in a post-translational modification type (phosphorylation, glycosylation, position of such modifications or quantity of such

modifications). In some cases, the alpha S1 casein may be a full-length alpha S1 casein. In some cases, the compositions comprising an alpha S1 casein protein lacks any animal-derived proteins.

[0090] In some embodiments, the single variant of an alpha casein in a consumable composition is an alpha S2 casein. In such compositions, the alpha S2 casein may comprise a modified alpha S2 casein such as modified in post-translational modification type (phosphorylation, glycosylation, position of such modifications or quantity of such modifications). In some cases, the alpha S2 casein may be a full-length alpha S2 casein. In some cases, the compositions comprising an alpha S2 casein protein lacks any animal-derived proteins.

[0091] The compositions herein comprising a single variant of an alpha casein protein, are recombinant proteins and do not comprise alpha casein proteins isolated from casein micelles or alpha casein proteins isolated from any naturally occurring micellar forms or products comprising micelles or micellar forms.

[0092] In some embodiments, consumable compositions described herein comprise a single variant of a casein protein such as an alpha casein protein. In some cases, the casein content of the consumable composition comprises substantially a single variant of the casein protein. In some cases, the casein content of the consumable composition comprises only the single variant of the casein protein. In some cases, the single variant of the casein protein comprises at least 95% or at least 97% of the casein content in the consumable composition. In some cases, the single variant of the casein protein comprises at least 99% of the casein content in the consumable composition. In some cases, the consumable composition lacks any additional caseins other than the single variant of the casein protein. In preferred embodiments, the single variant of a casein is an alpha casein protein.

[0093] A single variant of an alpha casein protein may be from a ruminant species. A single variant of an alpha casein protein may be a bovine alpha casein protein. A single variant of an alpha casein may be a caprine alpha casein protein. A single variant of an alpha casein protein may be an ovine alpha casein protein. A single variant of an alpha casein protein may be an equine alpha casein protein. A single variant of an alpha casein protein may be a camel or camelid alpha casein protein. A single variant of an alpha casein protein may be a human alpha casein protein

[0094] A single variant of an alpha casein protein may be a mature form of an alpha casein (lacking a signal sequence, such as exemplified in SEQ ID NOs: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56) or a truncated form thereof

(exemplified as SEQ ID NOs: 4-12, 16-24). A single variant of an alpha casein protein may be a bovine alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1-3, 28-30, 39-41 or 48-50 or a truncated form thereof. A single variant of an alpha casein may be an ovine alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 13-15 or 42-44 or a truncated form thereof. A single variant of an alpha casein protein may be a caprine alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 25-27 or 45-47 or a truncated form thereof. A single variant of an alpha casein protein may be an equine alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 31-33 or 51-53 or a truncated form thereof. A single variant of an alpha casein protein may be a camel alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 34-36 or 54-56 or a truncated form thereof. A single variant of an alpha casein protein may be a human alpha casein protein, for instance, casein protein with at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% sequence identity to SEQ ID NO: 37-38 or a truncated form thereof.

[0095] The single variant of an alpha casein protein in a consumable composition may be an alpha S1 casein protein. The alpha S1 casein protein may be a full-length alpha S1 casein protein. In some cases, the alpha S1 protein is a truncated alpha S1 protein relative to a wild-type or native alpha S1 casein protein. In some cases, the alpha S1 casein protein has a N-terminal truncation relative to a wild-type or native alpha S1 casein protein. In some cases, the alpha S1 casein protein has a C-terminal truncation relative to a wild-type or native alpha S1 casein protein. In some cases, the alpha S1 casein may have a N-terminal truncation and a C-terminal truncation relative to a wild-type or native alpha S1 casein. In some cases, the alpha S1 protein lacks between 1 and 59 N-terminal amino acids. In some cases, the alpha S1 protein lacks between 1 to 5, 1 to 10, 1 to 20, 1 to 30, 1 to 50, 1 to 59 N-terminal amino acids. In some cases, a bovine alpha S1 protein with SEQ ID NO: 2 lacks between 1 to 59 N-terminal amino acids. In some cases, a bovine alpha S1 protein with SEQ ID NO: 2 lacks 22, 23, 24 or 25 N-terminal amino acids (see examples such as SEQ ID NOs: 4-12). In some cases, an ovine alpha S1 protein with SEQ ID NO: 14 lacks between 1 to 59 N-terminal amino acids (see examples such as SEQ ID NOs: 16-24). In some cases, an ovine alpha S1 protein with SEQ ID NO: 14 lacks 22, 23, 24 or 25 N-terminal amino acids.

[0096] In some cases, the alpha S1 casein is a mixture of a full-length alpha S1 casein and one or more truncated forms of the alpha S1 casein protein, such as any one or more of the truncated forms described herein.

[0097] In some cases, a composition herein made from a single variant of an alpha casein comprises a mixture of a full-length form and one or more truncated forms of the alpha casein protein and in such compositions the total alpha casein may be comprised of up to 20% wt/wt of the one or more truncated forms of the alpha S1 casein. The truncated forms may be any truncated forms of the full-length single variant alpha S1 casein protein, examples of which are provided elsewhere herein. In some cases, a composition herein made from a single variant of an alpha casein comprises only a truncated form of the alpha S1 casein protein.

[0098] In some cases, the single variant of an alpha casein in compositions (such as cheese analogues) comprises greater than 0% truncated forms, such as 0.1%, 0.2%, 0.5%, 0.8% wt/wt of the single variant of an alpha casein is a truncated form(s) of the alpha S1 protein. In some embodiments, the single variant of an alpha S1 casein comprises at least 1% wt/wt of one or more truncated forms of the alpha S1 casein. In some cases, the single variant of an alpha S1 casein comprises at most 20% wt/wt of one or more truncated forms of the alpha S1 casein. In some cases, the single variant of an alpha S1 casein comprises 1% to 3%, 1% to 5%, 1% to 7%, 1% to 10%, 1% to 12%, 1% to 15%, 1% to 20%, 3% to 5%, 3% to 7%, 3% to 10%, 3% to 12%, 3% to 15%, 3% to 20%, 5% to 7%, 5% to 10%, 5% to 12%, 5% to 15%, 5% to 20%, 7% to 10%, 7% to 12%, 7% to 15%, 7% to 20%, 10% to 12%, 10% to 15%, 10% to 20%, 12% to 15%, 12% to 20%, or 15% to 20% wt/wt of one or more truncated forms of the alpha S1 casein. In some cases, the single variant of an alpha S1 casein comprises of about 1%, 3%, 5%, 7%, 10%, 12%, 15%, or 20% wt/wt of one or more truncated forms of the alpha S1 casein. In some cases, the single variant of an alpha S1 casein comprises of at least 1%, 3%, 5%, 7%, 10%, 12% or 15% wt/wt of one or more truncated forms of the alpha S1 casein. In some cases, the single variant of an alpha S1 casein comprises of at most 3%, 5%, 7%, 10%, 12%, 15% or 20% wt/wt of one or more truncated forms of the alpha S1 casein. In such compositions, the remaining percentage of alpha S1 casein in the single variant of an alpha casein is the full-length form of the alpha S1 casein.

B. Post Translational Modifications

[0099] Depending on the host organism used to express the casein, the single variant of casein proteins, such as alpha casein proteins may have a glycosylation or phosphorylation pattern (post-translational modifications) different from animal-derived casein proteins. In

some cases, the single variant of casein protein, such as the alpha casein protein, comprises no post translational modifications (PTMs). In some cases, the single variant of casein protein, such as the alpha 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-derived casein protein. For instance, the single variant of alpha casein protein may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 99% less post-translationally modified as compared to animal-derived alpha casein. In some cases, the post-translationally modified caseins may be lacking one or more sites of post translational modifications found in animal-derived casein proteins.

[00100] In some cases, the single variant of casein protein, such as the alpha casein protein comprises one or more PTMs that differ from an animal-derived casein protein, for example, a modification at an amino acid within the single variant of alpha casein protein that is not modified in the animal-derived alpha casein protein or a modification that differs in chemical structure as compared to the animal-derived alpha casein protein, such as a different phosphorylation structure.

[00101] Alternatively, the single variant of casein protein, such as the alpha casein protein may comprise PTMs comparable to animal-derived casein PTMs. In some cases, the single variant of casein protein, such as the alpha casein protein comprises substantially increased PTMs. As used herein, substantially increased PTMs means at least 5% increase in one or more types of PTMs as compared to the amount of PTMs in an animal-derived casein protein. For instance, the single variant of alpha casein proteins may be 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 99% more post-translationally modified as compared to animal-derived alpha casein.

[00102] The PTMs in the casein protein, such as the alpha casein protein may be modified chemically or enzymatically. In some cases, the single variant of casein protein, such as the alpha casein protein, comprises substantially reduced or no PTMs without chemical or enzymatic treatment. Compositions may be generated using single variant of casein protein, such as the alpha casein protein with reduced or no PTMs, wherein the lack of PTMs is not due to chemical or enzymatic treatments of the protein, such as producing a single variant of alpha casein protein through recombinant production where the recombinant protein lacks PTMs.

[00103] The phosphorylation in the single variant of casein protein, such as the alpha casein protein may be modified chemically or enzymatically. In some cases, the single variant of casein protein comprises substantially reduced or no phosphorylation without chemical or enzymatic treatment. For instance, single variant of alpha casein proteins may be 50%, 55%,

60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 99% less phosphorylated as compared to animal-derived alpha casein. Compositions may be generated using single variant of casein protein, such as the alpha 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 single variant of casein protein with reduced or no phosphorylation.

[00104] Compositions may be generated using a single variant of caseins, such as the alpha casein protein which is a mixture of caseins with different PTMs. For instance, compositions may comprise single variants of alpha casein may comprise a mix of alpha caseins with no PTMs, reduced PTMs, nature comparable (or native-like) PTMs and/or increased PTMs. In some cases, compositions may comprise single variants of casein proteins, such as the alpha casein protein with native (nature comparable or animal-derived) PTMs, casein proteins lacking one or more types of PTMs, and/or no PTMs. Alternatively, compositions may comprise a single variant of casein proteins, such as the alpha casein protein with uniform PTMs. The PTM structures in such cases may include reduced PTMs, PTMs lacking one or more types of PTM. For instance, the consumable composition may comprise only a single variant of alpha casein with reduced phosphorylation.

Consumable compositions

A. Cheese and Cheese-like Analogues

[00105] The compositions of single variant casein described in this application may be used to create cheese analogues. A cheese analogue may comprise ingredients in addition to a recombinantly produced single variant of a casein protein. A cheese analogue may comprise ingredients in addition to a recombinantly produced single variant of an alpha casein protein. In some cases, a cheese analogue may comprise solvents such as water, fats, salts, starch, sugars, flavors, acids, pH stabilizers, carbohydrates, etc. A cheese analogue may comprise proteins other than the single variant of casein. For instance, the other proteins may comprise proteins (other than caseins) found in animal-derived dairy products. Alternatively, a cheese analogue may comprise proteins that are not found in animal-derived dairy products, such examples may include but are not limited to plant and/or microbial proteins.

[00106] A cheese analogue described herein may comprise from 5% w/w to about 30% w/w of a recombinant single variant of a casein. In some cases, a cheese analogue may comprise at least 5% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some cases, a cheese analogue may comprise at most 30% w/w of

a recombinant single variant of a casein. In some cases, a cheese analogue may comprise from 5% to 7%, 5% to 10%, 5% to 15%, 5% to 20%, 5% to 25%, 5% to 30%, 7% to 10%, 7% to 15%, 7% to 20%, 7% to 25%, 7% to 30%, 10% to 15%, 10% to 20%, 10% to 25%, 10% to 30%, 15% to 20%, 15% to 25%, 15% to 30%, 20% to 25%, 20% to 30%, or 25% to 30% w/w of a recombinant single variant of an casein. In some cases, a cheese analogue may comprise about 5%, 7%, 10%, 15%, 20%, 25%, or 30% w/w of a recombinant single variant of casein.

[00107] A cheese analogue described herein may comprise from 5% w/w to about 30% w/w of a recombinant single variant of an alpha casein. In some cases, a cheese analogue may comprise at least 5% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a cheese analogue may comprise at most 30% w/w of a recombinant single variant of an alpha casein. In some cases, a cheese analogue may comprise from 5% to 7%, 5% to 10%, 5% to 15%, 5% to 20%, 5% to 25%, 5% to 30%, 7% to 10%, 7% to 15%, 7% to 20%, 7% to 25%, 7% to 30%, 10% to 15%, 10% to 20%, 10% to 25%, 10% to 30%, 15% to 20%, 15% to 25%, 15% to 30%, 20% to 25%, 20% to 30%, or 25% to 30% w/w of a recombinant single variant of an alpha casein. In some cases, a cheese analogue may comprise about 5%, 7%, 10%, 15%, 20%, 25%, or 30% w/w of a recombinant single variant of alpha casein. In some preferred cases, a cheese analogue may comprise from 10% to 25%, 15% to 25%, 18% to 25%, 20 to 25%, 10% to 20%, 15% to 20%, 18% to 20% w/w of a recombinant single variant of alpha casein.

[00108] In some cases, a cheese analogue may comprise 5% w/w to 40% w/w fats. Examples of fats which can be added to a cheese analogue include coconut, canola, high-oleic sunflower, palm oils. Other examples are provided elsewhere herein. In some cases, a cheese analogue may comprise at least 5% w/w fats. In some cases, a cheese analogue may comprise at most 40% w/w fats. In some cases, a cheese analogue may comprise from 5% w/w to 10% w/w, 5% w/w to 15% w/w, 5% w/w to 20% w/w, 5% w/w to 25% w/w, 5% w/w to 30% w/w, 5% w/w to 40% w/w, 10% w/w to 15% w/w, 10% w/w to 20% w/w, 10% w/w to 25% w/w, 10% w/w to 30% w/w, 10% w/w to 40% w/w, 15% w/w to 20% w/w, 15% w/w to 25% w/w, 15% w/w to 30% w/w, 15% w/w to 40% w/w, 20% w/w to 25% w/w, 20% w/w to 30% w/w, 20% w/w to 40% w/w, 25% w/w to 30% w/w, 25% w/w to 40% w/w, or 30% w/w to 40% w/w fats. In some cases, a cheese analogue may comprise about 5% w/w, 10% w/w, 15% w/w, 20% w/w, 25% w/w, 30% w/w, or 40% w/w fats.

[00109] In some cases, a cheese analogue may comprise 0% w/w to 50% w/w starch. Examples of starches which can be added to a cheese analogue include modified potato, corn. Other examples are provided elsewhere herein. In some cases, a cheese analogue may comprise

at least 0% w/w starch. In some cases, a cheese analogue may comprise at most 50% w/w starch. In some cases, a cheese analogue may comprise from 0% w/w to 10% w/w, 0% w/w to 20% w/w, 0% w/w to 30% w/w, 0% w/w to 40% w/w, 0% w/w to 50% w/w, 10% w/w to 20% w/w, 10% w/w to 30% w/w, 10% w/w to 40% w/w, 10% w/w to 50% w/w, 20% w/w to 30% w/w, 20% w/w to 40% w/w, 20% w/w to 50% w/w, 30% w/w to 40% w/w, 30% w/w to 50% w/w, or 40% w/w to 50% w/w starch. In some cases, a cheese analogue may comprise about 0% w/w, 10% w/w, 20% w/w, 30% w/w, 40% w/w, or 50% w/w starch. In some preferred cases, a cheese analogue may comprise from 0.5% to 1%, 0.5% to 2%, 0.5% to 3%, 0.5% to 4%, 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3%, 2% to 4%, 3% to 4% w/w starch.

[00110] Preferentially, a cheese analogue may comprise at most 30% w/w starch. In some cases, a cheese analogue may comprise 0% w/w to 30% w/w starch. In some cases, a cheese analogue may comprise at least 0% w/w starch. In some cases, a cheese analogue may comprise 0% w/w to 5% w/w, 0% w/w to 10% w/w, 0% w/w to 15% w/w, 0% w/w to 20% w/w, 0% w/w to 25% w/w, 0% w/w to 30% w/w, 5% w/w to 10% w/w, 5% w/w to 15% w/w, 5% w/w to 20% w/w, 5% w/w to 25% w/w, 5% w/w to 30% w/w, 10% w/w to 15% w/w, 10% w/w to 20% w/w, 10% w/w to 25% w/w, 10% w/w to 30% w/w, 15% w/w to 20% w/w, 15% w/w to 25% w/w, 15% w/w to 30% w/w, 20% w/w to 25% w/w, 20% w/w to 30% w/w, or 25% w/w to 30% w/w starch. In some cases, a cheese analogue may comprise 0% w/w, 5% w/w, 10% w/w, 15% w/w, 20% w/w, 25% w/w, or 30% w/w starch. In some cases, a cheese analogue may comprise at most 1% w/w, 5% w/w, 10% w/w, 15% w/w, 20% w/w, 25% w/w, or 30% w/w starch.

[00111] In some cases, a cheese analogue may comprise 0% w/w to 16% w/w salts such as calcium salts, emulsifying salts, table salts, etc. Examples of such salts are also provided elsewhere herein.

[00112] In some cases, a cheese analogue may comprise calcium salts such as calcium chloride. In some cases, a cheese analogue may comprise 0.1% to 6% w/w calcium salts. In some cases, a cheese analogue may comprise at least 0.1% w/w calcium salts. In some cases, a cheese analogue may comprise at most 6% w/w calcium salts. In some cases, a cheese analogue may comprise 0.1% to 1%, 0.1% to 2%, 0.1% to 3%, 0.1% to 4%, 0.1% to 5%, 0.1% to 6%, 1% to 2%, 1% to 3%, 1% to 4%, 1% to 5%, 1% to 6%, 2% to 3%, 2% to 4%, 2% to 5%, 2% to 6%, 3% to 4%, 3% to 5%, 3% to 6%, 4% to 5%, 4% to 6%, or 5% to 6% w/w calcium salts. In some cases, a cheese analogue may comprise about 0.1%, 1%, 2%, 3%, 4%, 5%, or 6% w/w calcium salts. In some cases, a cheese analogue may comprise less than 0.1%, 1%, 2%, 3%, 4%, 5%, or 6% w/w calcium salts. In some cases, a cheese analogue may comprise

more than 0.1%, 1%, 2%, 3%, 4% or 5% w/w calcium salts. In some cases, a cheese analogue may comprise calcium ions. The calcium ions may be added to a cheese analogue in the form of a calcium-based salt, for instance, calcium chloride.

[00113] In some cases, a cheese analogue may comprise 0% to 0.6% w/w calcium ions. In some cases, a cheese analogue may comprise at least 0% w/w calcium ions. In some cases, a cheese analogue may comprise at most 0.6% w/w calcium ions. In some cases, a cheese analogue may comprise 0% to 0.1%, 0% to 0.2%, 0% to 0.3%, 0% to 0.4%, 0% to 0.5%, 0% to 0.6%, 0.1% to 0.2%, 0.1% to 0.3%, 0.1% to 0.4%, 0.1% to 0.5%, 0.1% to 0.6%, 0.2% to 0.3%, 0.2% to 0.4%, 0.2% to 0.5%, 0.2% to 0.6%, 0.3% to 0.4%, 0.3% to 0.5%, 0.3% to 0.6%, 0.4% to 0.5%, 0.4% to 0.6%, or 0.5% to 0.6% w/w calcium ions. In some cases, a cheese analogue may comprise about 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 0.6% w/w calcium ions. In some cases, a cheese analogue may comprise at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 0.6% w/w calcium ions. In some cases, a cheese analogue may comprise at most 0.1%, 0.2%, 0.3%, 0.4%, or 0.5% w/w calcium ions.

[00114] In some cases, a cheese analogue may comprise 0 mg to 30 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise at least 0 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise at most 30 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise 0 mg to 5 mg, 0 mg to 10 mg, 0 mg to 15 mg, 0 mg to 20 mg, 0 mg to 25 mg, 0 mg to 30 mg, 5 mg to 10 mg, 5 mg to 15 mg, 5 mg to 20 mg, 5 mg to 25 mg, 5 mg to 30 mg, 10 mg to 15 mg, 10 mg to 20 mg, 10 mg to 25 mg, 10 mg to 30 mg, 15 mg to 20 mg, 15 mg to 25 mg, 15 mg to 30 mg, 20 mg to 25 mg, 20 mg to 30 mg, or 25 mg to 30 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise 0 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, or 30 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise at least 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, or 30 mg calcium ions per gram of casein. In some cases, a cheese analogue may comprise at most 1mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, or 30 mg calcium ions per gram of casein.

[00115] In some cases, a cheese analogue may comprise emulsifying salts such as disodium phosphate, trisodium citrate or other emulsifying salts. In some cases, a cheese analogue may comprise 0.1% to 6% w/w emulsifying salts. In some cases, a cheese analogue may comprise at least 0.1% w/w emulsifying salts. In some cases, a cheese analogue may comprise at most 6% w/w emulsifying salts. In some cases, a cheese analogue may comprise 0.1% to 1%, 0.1% to 2%, 0.1% to 3%, 0.1% to 4%, 0.1% to 5%, 0.1% to 6%, 1% to 2%, 1% to 3%, 1% to 4%, 1% to 5%, 1% to 6%, 2% to 3%, 2% to 4%, 2% to 5%, 2% to 6%, 3% to 4%, 3% to 5%, 3% to

6%, 4% to 5%, 4% to 6%, or 5% to 6% w/w emulsifying salts. In some cases, a cheese analogue may comprise about 0.1%, 1%, 2%, 3%, 4%, 5%, or 6% w/w emulsifying salts. In some cases, a cheese analogue may comprise less than 0.1%, 1%, 2%, 3%, 4%, 5%, or 6% w/w emulsifying salts. In some cases, a cheese analogue may comprise more than 0.1%, 1%, 2%, 3%, 4% or 5% w/w emulsifying salts. Alternatively, in some cases, the cheese analogue does not comprise any emulsifying salts. In some cases, the cheese analogue does not comprise any emulsifiers other than emulsifying salts.

[00116] In some embodiments, a cheese analogue may comprise a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of about 6:1, 7:1, 8:1, 9:1, 10:1, 11:1 or 12:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of about 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 or 9:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of about 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1 or 20:1.

[00117] In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of between about 3:1 and 6:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of between about 6:1 and 9:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of between about 8:1 and 10:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of between about 8:1 and 12:1. In some cases, a cheese analogue may have a ratio of casein (e.g., the recombinant single variant casein) to emulsifying salts (weight/weight) of between about 8:1 and 15:1.

[00118] In some cases, a cheese analogue may comprise table salts such as sodium chloride salts. In some cases, a cheese analogue may comprise 0.1% to 4% w/w sodium chloride. In some cases, a cheese analogue may comprise at least 0.1% w/w sodium chloride. In some cases, a cheese analogue may comprise at most 4% w/w sodium chloride. In some cases, a cheese analogue may comprise 0.1% to 1%, 0.1% to 2%, 0.1% to 3%, 0.1% to 4%, 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3%, 2% to 4%, or 3% to 4% w/w sodium chloride. In some cases, a cheese analogue may comprise about 0.1%, 1%, 2%, 3%, or 4% w/w sodium chloride. In some cases, a cheese analogue may comprise less than 0.1%, 1%, 2%, 3%, or 4% w/w sodium

chloride. In some cases, a cheese analogue may comprise more than 0.1%, 1%, 2% or 3% w/w sodium chloride.

[00119] In some examples, a single variant of casein, such as alpha casein proteins recombinantly produced (5-30% w/w of the cheese analogue) (exemplary optimal range 10-20% w/w) may be combined with water (30-65% w/w) (exemplary optimal range 45-55% w/w), fats (5-40% w/w) (exemplary optimal range 20-25% w/w), sodium chloride (salt) (0-4% w/w) (exemplary optimal range 0-1.5% w/w), calcium chloride (0-6% w/w) (exemplary optimal range 0-1.5% w/w), emulsifying salts (disodium phosphate, trisodium citrate) (0-6% w/w) (exemplary optimal range 0-3% w/w), starch (0-50% w/w) (exemplary optimal range 0-8% w/w), natural vegan flavors (0-5% w/w) (exemplary optimal range 0.5-1% w/w), and acid (0-5% w/w) (exemplary optimal range 0-1% w/w). Optional ingredients such as plant-based or other animal-free protein (0-30%) (exemplary optimal range 0-8% w/w), hydrocolloids (0-5%) (exemplary optimal range 0-2%), sugars such as mono-, di- and oligosaccharides (0-5% w/w) (exemplary optimal range 0-2% w/w), emulsifying agents such as mono- and diglycerides (0-2% w/w) (exemplary optimal range 0-0.5% w/w), natural flavor maskers, color additives (0-5% w/w), preservatives (0-1% w/w), anti-caking agents (0-2% w/w) and micronutrients such as vitamins (0-1%) can be incorporated into a cheese analogue as well.

[00120] In some embodiments, the following ingredients are pre-mixed: a recombinantly produced single variant of an alpha casein, fat(s), water, starch, salt(s) such as sodium chloride. In some embodiments, pH adjustment is performed at this stage to bring the composition to neutral pH, 6.8 – 7.2, using a pH adjuster such as sodium hydroxide (lye). Optional ingredients such as plant-based or other animal-free protein, sugars, hydrocolloids and emulsifying agents can be added at this step or at a later stage. Pre-mixing can occur at ambient or elevated temperatures (15 – 50 °C). In some cases, fats are pre-melted [30-70 °C] (exemplary optimal range 40-50 °C) and held at their melting temperature prior to incorporation. The calcium chloride and emulsifying salts may be added at the pre-mixing stage or at a later stage. Alternatively, the calcium chloride and emulsifying salts may be added consecutively, in any order: calcium chloride may be added before or after emulsifying salts. For instance, calcium chloride and emulsifying salts may be added in 2 stages over the course of 4 minutes to 1 hour (exemplary optimal range 10 to 20 minutes), with 2 minute to 30 minute (exemplary optimal range 5 to 10 min) incubation intervals at ambient or elevated temperature. Alternatively, a cheese analogue may be produced without calcium chloride or emulsifying salts. Calcium chloride can also be added at the end of the cheese analogue making process, before or after the acid addition.

[00121] The mixtures may be heated over a temperature ramp from pre-mixing temperature (ambient or elevated) to 50-95 °C (exemplary optimal range 75-90 °C), over a ramp period of 1 to 30 minutes (exemplary optimal range 1 to 5 mins), while being mixed mechanically. Heated mixtures may then be held for 0 to 20 minutes (exemplary optimal range 2 – 5 mins) at the final ramp temperature as ingredients are mechanically incorporated to form an emulsion. Mechanical incorporation (mixing) can be achieved using a variety of mixers, such as a vertical cutter mixer or a twin-screw mixer.

[00122] The acidity of the mixtures may be regulated by incorporating an acid, such as lactic or citric acid and continuing to mix briefly, to a final pH of about 5 – 6.5 (exemplary optimal range 5.7 – 6.2). Acidity may also be regulated by using glucono-delta-lactone earlier in the mixing process. The resulting mixtures may then be set into moulds, other shaping containers, or vacuum seal packaging. The resulting product may be chilled to 4 °C immediately after portioning into moulds to create the cheese analogue. Such cheese analogues can then be used as food products, toppings and incorporated into other food products.

[00123] In some embodiments, the amount of salts or minerals in a cheese may be altered to generate favorable qualities. For instance, in one example, an amount of calcium may be altered to improve melt, texture, stretch, etc. In one example, the amount of calcium in a cheese analogue may be reduced to improve the melt of the cheese analogue. In another example, the amount of calcium may be increased in a cheese analogue to improve the texture or stretch of the cheese analogue.

[00124] In some embodiments, a cheese analogue comprises a single variant casein and the single variant casein, such as an alpha casein, provides one or more properties of the cheese or cheese analogue to the composition.

[00125] The texture of a cheese analogue made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the texture of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Texture of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the texture of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Texture of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the texture of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein, or when compared to a plant-derived cheese analogue lacking dairy

proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Texture of a cheese analogue may be tested using a trained panel of human subjects or using a machine such as a texture analyzer.

[00126] The taste of a cheese analogue made with a single variant of a casein, such as alpha casein, such as by methods described herein may be comparable to the taste of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Taste of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the taste of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Taste of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved when compared to the taste of a cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Taste of a cheese may be tested using a trained panel of human subjects.

[00127] Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a browning ability which is comparable to a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a browning ability which is comparable to a similar type of cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a browning ability which is improved when compared to a similar type of cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Browning ability of a cheese analogue may be tested using an oven and computer imaging.

[00128] Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a melting ability which is comparable to a similar type of cheese

made using animal-derived dairy proteins, such as cheese made from animal milk. Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a melting ability which is comparable to a similar type of cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Cheese analogue compositions described herein having a single variant of a casein, such as alpha casein may have a melting ability which is improved when compared to a similar type of cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Melting ability of a cheese analogue may be tested using a modified Schreiber melt test and computer imaging. Exemplary assays that measure this melt property are provided in the examples section.

[00129] In some embodiments, a cheese or cheese analogue is analyzed for melt characteristics by heating, such as by heating on a hot plate at 95°C for 15 minutes and melt is assessed by the ratio of melted area to unmelted area, where melting is defined as a ratio of greater than or equal to 1. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a melt value of 1 or greater than 1. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a melt value greater than 1, such as 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6 or greater than 2.6. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a melt value greater than 1, such as between 1.0-1.3, 1.3-1.5, 1.5-1.7, 1.7-1.9, 1.9-2.1, 2.1-2.3 or 2.3-2.5. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a melt value greater than 1 and the melted area retains an opaque appearance. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a melt value greater than the melt value of a plant-based cheese analogue, for example the melt value the cheese analogue comprising a recombinant single variant alpha casein is 1.5x, 2x, 2.5 x or greater than 2.5x the melt value of the plant-based cheese analogue.

[00130] The stretching ability of a cheese analogue made with a single variant of a casein, such as alpha casein, such as by methods described herein, may be comparable to the stretching ability of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Stretching ability of a cheese made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the

stretching ability of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Stretching ability of a cheese made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the stretching ability of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein, or when compared to a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Stretching ability of a cheese may be tested using a trained panel of human subjects or using a machine such as a texture analyzer. Stretch of a cheese analogue made using the methods described herein may be greater than 2.5 cm when measured on a texture analyzer post cooking. Exemplary assays that measure this stretch property are provided in the examples section.

[00131] In some embodiments, a cheese or cheese analogue is analyzed for stretch using a texture analyzer, such as by heating the cheese in an oven at 90°C for 10 min in an extensibility rig and measuring the extensibility on a texture analyzer as the distance to failure (i.e., the distance at which all of the strands of cheese break). In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a stretch value of about 200 mm (20 cm). In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a stretch of at least 40mm, at least 50mm, at least 60mm, at least 80mm, at least 100mm, at least 120 mm, at least 140mm, at least 160mm, at least 180 mm, at least 190 mm, at least 200 mm, at least 210 mm, at least 220 mm, at least 230 mm, at least 240 mm, at least 250 mm or greater than 250 mm. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a stretch of between 40-70mm, 70-100mm, 100-125mm, 125-150mm, 150-180 mm, 180-200 mm, 200-225mm, 225-250mm or 250-300 mm. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a stretch greater than the stretch of a plant-based cheese analogue, for example the stretch of the cheese analogue comprising a recombinant single variant alpha casein is at least 2x, at least 3x, at least 4x, at least 5x, at least 7x, at least 10x, the stretch of the plant-based cheese analogue. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has a stretch comparable to the stretch of a low-moisture milk-derived cheese (such as a low-moisture mozzarella) or a caseinate-containing cheese, such as an imitation mozzarella cheese. In some embodiments, the cheese analogue comprising a

recombinant single variant alpha casein has a stretch that is within 10-20%, 15-25%, 20-40% or 10-150% of the stretch of a low moisture milk-derived cheese (such as a low moisture mozzarella) or a caseinate-containing cheese, such as an imitation mozzarella cheese.

[00132] The hardness of a cheese analogue made with a single variant of a casein, such as alpha casein such as by methods described herein may be comparable to the hardness of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Hardness of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the hardness of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Hardness of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved when compared to the hardness of a cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Hardness of a cheese analogue may be tested using a trained panel of human subjects or using a machine such as a texture analyzer.

[00133] The adhesiveness of a cheese analogue made with a single variant of a casein, such as alpha casein such as by methods described herein may be comparable to the adhesiveness of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Adhesiveness of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the adhesiveness of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Adhesiveness of a cheese analogue made by methods described herein having a single variant of a casein, such as alpha casein may be reduced when compared to adhesiveness of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Adhesiveness of a cheese analogue made by methods described herein having a single variant of a casein, such as alpha casein may be reduced when compared to adhesiveness of a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Adhesiveness of a cheese analogue may be tested using a trained panel of human subjects or using a machine such as a texture analyzer. Adhesiveness

of a cheese analogue made using the methods described herein may be less than 2 (g*sec) on the texture analyzer test. Exemplary assays that measure this adhesiveness property are provided in the examples section.

[00134] Adhesiveness of a cheese analogue may be tested using a texture analyzer, for example a TA.XT Plus Texture Analyzer equipped with a TA-18 ½" ball probe, such as where the adhesiveness represents the force required to remove the cheese from the probe. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has an adhesiveness of between 0.1-3.0 g*sec. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has an adhesiveness of less than 3.0 g*sec, less than 2.5 g*sec, less than 2.0 g*sec, less than 1.5 g*sec, less than 1.0 g*sec, or less than 0.5 g*sec. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has an adhesiveness such as between 0.1-2.5 g*sec, 0.1-2.0 g*sec, 0.1-1.5 g*sec, 0.1-1.0 g*sec, 0.1-0.5 g*sec, 0.5-2.5 g*sec, 0.5-2.0 g*sec, 0.5-1.5 g*sec, 0.5-1.0 g*sec, or 0.5-1.5 g*sec. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has an adhesiveness that is reduced as compared to low moisture milk-derived cheese (such as a low moisture mozzarella) or a caseinate-containing cheese, such as an imitation mozzarella cheese. In some embodiments, the adhesiveness of the cheese analogue comprising a recombinant single variant alpha casein when compared to the adhesiveness of low moisture milk-derived cheese (such as a low moisture mozzarella) or a caseinate-containing cheese (such as an imitation mozzarella cheese) is reduced by at least 2x, at least 3x, at least 5x, at least 10x, at least 20x, or more than 20x reduced. In some embodiments, the cheese analogue comprising a recombinant single variant alpha casein has an adhesiveness that is reduced as compared to plant-based cheese analogue. In some embodiments, the adhesiveness of the cheese analogue comprising a recombinant single variant alpha casein when compared to the adhesiveness of plant-based cheese analogue is reduced by at least 2x, at least 3x, at least 5x, at least 10x, at least 15x, at least 20x, or more than 20x reduced.

[00135] The creaminess of a cheese analogue made with a single variant of a casein, such as alpha casein such as by methods described herein may be comparable to the creaminess of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Creaminess of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the creaminess of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Creaminess of a cheese analogue made using the compositions described herein having a single variant of a casein,

such as alpha casein may be improved when compared to the creaminess of a cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)).

[00136] The mouthfeel of a cheese analogue made with a single variant of a casein, such as alpha casein such as by methods described herein may be comparable to the mouthfeel of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Mouthfeel of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the mouthfeel of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Mouthfeel of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved when compared to the mouthfeel of a cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)).

[00137] The opacity of a cheese analogue made with a single variant of a casein, such as alpha casein such as by methods described herein may be comparable when heated (e.g., melted) to the opacity of a similar type of cheese made using animal-derived dairy proteins, such as cheese made from animal milk. Opacity of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable when heated to the opacity of a cheese or cheese analogue made using micellar form of casein, such as cheese made from milk, or cheese analogue made from caseinate or rennet casein. Opacity of a cheese analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved when heated as compared to the opacity of a cheese or cheese analogue made using a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)).

[00138] The cheese analogue or similar compositions described herein may be low-moisture cheese analogues. For instance, the low moisture cheese analogue may comprise from 45-52% w/w moisture. The low moisture cheese analogue may comprise less than 52% w/w moisture.

B. Yogurt or yogurt analogues

[00139] The single variant casein compositions, such as compositions comprising alpha casein described herein may be used to generate consumable compositions such as yogurt analogues or yogurt-like compositions. For instance, single variant alpha casein compositions may be used to form a yogurt analogue product. Yogurt analogue products may be formed without the formation of a micelle or a micelle-like composition. Yogurt analogue products made using the compositions described herein may provide similar or equivalent features (such as texture, creaminess, firmness, tackiness, adhesiveness, viscosity, smell and taste) to an animal-derived dairy yogurt or a dairy yogurt analogue made using micellar form of casein, such as one made from caseinate, rennet casein or micellar casein. Yogurt analogue products made using the compositions described herein may provide an improvement in one or more features (such as texture, creaminess, firmness, tackiness, adhesiveness, viscosity, smell and taste) when compared to a plant-derived yogurt analogue lacking any dairy proteins (i.e., a yogurt-like product made either using no protein, or using plant-derived proteins such as pea, chickpea, nut and/or other vegetable protein as the sole source of protein (meaning they do not contain any casein)).

[00140] A yogurt analogue described herein may comprise from 1% w/w to about 20% w/w of a recombinant single variant of a casein. In some cases, a yogurt analogue may comprise at least 1% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some cases, a yogurt analogue may comprise at most 20% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some cases, a yogurt analogue may comprise 1% to 2%, 1% to 4%, 1% to 6%, 1% to 8%, 1% to 10%, 1% to 15%, 1% to 20%, 2% to 4%, 2% to 6%, 2% to 8%, 2% to 10%, 2% to 15%, 2% to 20%, 4% to 6%, 4% to 8%, 4% to 10%, 4% to 15%, 4% to 20%, 6% to 8%, 6% to 10%, 6% to 15%, 6% to 20%, 8% to 10%, 8% to 15%, 8% to 20%, 10% to 15%, 10% to 20%, or 15% to 20% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some cases, a yogurt analogue may comprise 1%, 2%, 4%, 6%, 8%, 10%, 15%, or 20% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some preferred embodiments, a yogurt analogue may comprise from 2% to 5% w/w of a recombinant single variant of a casein.

[00141] A yogurt analogue described herein may comprise from 1% w/w to about 20% w/w of a recombinant single variant of an alpha casein. In some cases, a yogurt analogue may comprise at least 1% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a yogurt analogue may comprise

at most 20% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a yogurt analogue may comprise 1% to 2%, 1% to 4%, 1% to 6%, 1% to 8%, 1% to 10%, 1% to 15%, 1% to 20%, 2% to 4%, 2% to 6%, 2% to 8%, 2% to 10%, 2% to 15%, 2% to 20%, 4% to 6%, 4% to 8%, 4% to 10%, 4% to 15%, 4% to 20%, 6% to 8%, 6% to 10%, 6% to 15%, 6% to 20%, 8% to 10%, 8% to 15%, 8% to 20%, 10% to 15%, 10% to 20%, or 15% to 20% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a yogurt analogue may comprise 1%, 2%, 4%, 6%, 8%, 10%, 15%, or 20% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some preferred embodiments, a yogurt analogue may comprise from 2% to 5% w/w of a recombinant single variant of an alpha casein.

[00142] A yogurt analogue described herein may comprise 0.5% to 20% w/w fats. A yogurt analogue described herein may comprise at least 0.5% w/w fats. A yogurt analogue described herein may comprise at most 20% w/w fats. A yogurt analogue described herein may comprise 0.5 % to 1 %, 0.5 % to 4 %, 0.5 % to 6 %, 0.5 % to 8 %, 0.5 % to 10 %, 0.5 % to 12 %, 0.5 % to 15 %, 0.5 % to 18 %, 0.5 % to 20 %, 1 % to 4 %, 1 % to 6 %, 1 % to 8 %, 1 % to 10 %, 1 % to 12 %, 1 % to 15 %, 1 % to 18 %, 1 % to 20 %, 4 % to 6 %, 4 % to 8 %, 4 % to 10 %, 4 % to 12 %, 4 % to 15 %, 4 % to 18 %, 4 % to 20 %, 6 % to 8 %, 6 % to 10 %, 6 % to 12 %, 6 % to 15 %, 6 % to 18 %, 6 % to 20 %, 8 % to 10 %, 8 % to 12 %, 8 % to 15 %, 8 % to 18 %, 8 % to 20 %, 10 % to 12 %, 10 % to 15 %, 10 % to 18 %, 10 % to 20 %, 12 % to 15 %, 12 % to 18 %, 12 % to 20 %, 15 % to 18 %, 15 % to 20 %, or 18 % to 20 % w/w fats. A yogurt analogue described herein may comprise 0.5 %, 1 %, 4 %, 6 %, 8 %, 10 %, 12 %, 15 %, 18 %, or 20 % w/w fats. A yogurt analogue described herein may comprise at most 0.5 %, 1 %, 4 %, 6 %, 8 %, 10 %, 12 %, 15 %, or 18% w/w fats. A yogurt analogue described herein may comprise at least 0.5 %, 1 %, 4 %, 6 %, 8 %, 10 %, 12 %, 15 %, or 18% w/w fats. Alternatively, a yogurt analogue may not comprise any fats.

[00143] A yogurt analogue described herein may comprise 0% to 10% w/w starch. A yogurt analogue described herein may comprise at least 0.5% w/w starch. A yogurt analogue described herein may comprise at most 10% w/w starch. A yogurt analogue described herein may comprise 0.5 % to 1 %, 0.5 % to 2 %, 0.5 % to 4 %, 0.5 % to 6 %, 0.5 % to 8 %, 0.5 % to 10 %, 1 % to 2 %, 1 % to 4 %, 1 % to 6 %, 1 % to 8 %, 1 % to 10 %, 2 % to 4 %, 2 % to 6 %, 2 % to 8 %, 2 % to 10 %, 4 % to 6 %, 4 % to 8 %, 4 % to 10 %, 6 % to 8 %, 6 % to 10 %, or 8 % to 10 % w/w starch. A yogurt analogue described herein may comprise 0.5 %, 1 %, 2 %, 4 %, 6 %, 8 %, or 10 % w/w starch. A yogurt analogue described herein may comprise at most

0.5 %, 1 %, 2 %, 4 %, 6 %, 8 %, or 10 % w/w starch. A yogurt analogue described herein may comprise at least 0.5 %, 1 %, 2 %, 4 %, 6 %, 8 %, or 10 % w/w starch.

[00144] A yogurt analogue described herein may comprise proteins other than the single variant of casein. For instance, the other proteins may comprise proteins (other than caseins) found in animal-derived dairy products. Alternatively, a yogurt analogue may comprise proteins that are not found in animal-derived dairy products, such examples may include but are not limited to plant and/or microbial proteins.

[00145] In some embodiments, a yogurt analogue comprises a single variant casein and the single variant casein, such as an alpha casein, provides one or more properties of the yogurt analogue to the composition.

[00146] The texture of a yogurt analogue made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the texture of a similar type of yogurt made using animal-derived dairy proteins, such as yogurt made from animal milk. Texture of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the texture of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein or rennet casein. Texture of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the texture of a yogurt or yogurt analogue made using micelles, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet casein, or when compared to a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Texture of a yogurt analogue may be tested using a trained panel of human subjects or using a machine such as a texture analyzer. Texture properties may include, for example, one or more of firmness, tackiness, adhesiveness, and viscosity of the yogurt or yogurt analogue.

[00147] The smoothness or creaminess of a yogurt analogue made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the smoothness of a similar type of yogurt made using animal-derived dairy proteins, such as yogurt made from animal milk. Smoothness of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the smoothness of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet

casein. Smoothness of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the smoothness of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinate, micellar casein, or rennet casein, or when compared to a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)).

[00148] The smell of a yogurt analogue made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the smell of a similar type of yogurt made using animal-derived dairy proteins, such as yogurt made from animal milk. Smell of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the smell of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet casein. Smell of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the smell of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet casein, or when compared to a plant-derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)).

[00149] The taste of a yogurt analogue made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the taste of a similar type of yogurt made using animal-derived dairy proteins, such as yogurt made from animal milk. Taste of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the taste of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet casein. Taste of a yogurt analogue made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the taste of a yogurt or yogurt analogue made using micellar form of casein, such as yogurt made from milk, or yogurt analogue made from caseinates, micellar casein, or rennet casein, or when compared to a plant-

derived cheese analogue lacking dairy proteins (i.e., a cheese-like product made either with plant-derived protein such as pea, chickpea, nut and/or other vegetable protein as the sole/primary protein source, or with no protein (such as cheese-like products made primarily with starch)). Taste of a yogurt analogue may be tested using a trained panel of human subjects.

C. Beverages

[00150] The single variant casein compositions, such as compositions comprising alpha casein described herein may be used to generate consumable compositions such as beverages. For instance, single variant alpha casein compositions may be used to form a milk-like or yogurt-like drink. Beverage products may be formed without the formation of a micelle or a micelle-like composition. Beverage products made using the compositions described herein may provide similar or equivalent features (such as texture, creaminess and taste) to an animal-derived dairy beverage or a dairy beverage made using micellar form of casein such as one made from caseinates or micellar casein. Beverage products made using the compositions described herein may provide an improvement in one or more features (such as texture, creaminess and taste) when compared to a beverage made with a plant-derived protein.

[00151] In some cases, the beverage may be selected from the group consisting of a juice product, a broth, a soup, a soda, a soft drink, a nutritional drink, an energy drink, a sports drink, a recovery drink, a heated drink, a coffee-based drink, a tea-based drink, a milk-based drink, a yogurt-like drink, a shake, a non-dairy, plant-based mild drink, infant formula drink, a meal replacement drink. In some embodiments, the beverage comprises carbonation.

[00152] The compositions described herein may be used to generate beverage compositions such as milk or milk-like compositions. For instance, a single variant of an alpha casein may be used to form a milk-like analogue product without the formation of a micelle or a micelle-like composition. Milk-like analogue products made using the compositions described herein may provide similar or equivalent features (such as texture, creaminess and taste) as compared to an animal milk, or a milk-like analogue product made using micelles, such as one made from caseinate or rennet casein. Milk-like analogue products made using the compositions described herein may provide improvement in one or more features (such as texture, creaminess and taste) as compared to a plant-derived milk analogue.

[00153] A beverage described herein may comprise from 0.5% w/w to about 10 % w/w of a recombinant single variant of a casein, such as an alpha casein. In some cases, a beverage may comprise at least 0.5% w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some cases, a beverage may comprise at most 10% w/w of a recombinant single variant of a casein, such as any of the single variant of

caseins, such as an alpha casein, described herein. In some cases, A beverage described herein may comprise 0.5 % to 1 %, 0.5 % to 2 %, 0.5 % to 4 %, 0.5 % to 6 %, 0.5 % to 8 %, 0.5 % to 10 %, 1 % to 2 %, 1 % to 4 %, 1 % to 6 %, 1 % to 8 %, 1 % to 10 %, 2 % to 4 %, 2 % to 6 %, 2 % to 8 %, 2 % to 10 %, 4 % to 6 %, 4 % to 8 %, 4 % to 10 %, 6 % to 8 %, 6 % to 10 %, or 8 % to 10 % w/w of a recombinant single variant of a casein. A beverage described herein may comprise 0.5 %, 1 %, 2 %, 4 %, 6 %, 8 %, or 10 % w/w of a recombinant single variant of a casein, such as any of the single variant of caseins described herein. In some preferred embodiments, a beverage may comprise from 0.5% to 5% w/w of a recombinant single variant of a casein.

[00154] A beverage described herein may comprise from 0.5% w/w to about 10% w/w of a recombinant single variant of an alpha casein. In some cases, a beverage may comprise at least 0.5% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a beverage may comprise at most 10% w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a beverage may comprise 0.5 % to 1 %, 0.5 % to 2 %, 0.5 % to 4 %, 0.5 % to 6 %, 0.5 % to 8 %, 0.5 % to 10 %, 1 % to 2 %, 1 % to 4 %, 1 % to 6 %, 1 % to 8 %, 1 % to 10 %, 2 % to 4 %, 2 % to 6 %, 2 % to 8 %, 2 % to 10 %, 4 % to 6 %, 4 % to 8 %, 4 % to 10 %, 6 % to 8 %, 6 % to 10 %, or 8 % to 10 % w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some cases, a beverage may comprise 0.5 %, 1 %, 2 %, 4 %, 6 %, 8 %, or 10 % w/w of a recombinant single variant of an alpha casein, such as any of the single variant of alpha caseins described herein. In some preferred embodiments, a beverage may comprise from 0.5% to 5% w/w of a recombinant single variant of an alpha casein.

[00155] In some embodiments, a beverage comprises a single variant casein and the single variant casein, such as an alpha casein, provides one or more properties of the beverage to the composition.

[00156] The texture of a beverage made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the texture of a similar type of beverage made using animal-derived dairy proteins, such as yogurt drinks made from animal milk. Texture of a beverage made using the compositions described herein having a single variant of a casein, such as alpha casein may be comparable to the texture of a beverage made using micellar form of casein, such as milk, yogurt, or beverage made from caseinate or micellar casein. Texture of a beverage made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when

compared to the texture of a beverage made using micellar form of casein, such as milk, yogurt, or beverage made from caseinate, micellar casein, or when compared to a beverage made with a plant-derived protein. Texture of a beverage may be tested using a trained panel of human subjects and using a machine such as viscometer for viscosity measurements. Texture features may include for example, viscosity, smoothness, mouthfeel, graininess and creaminess.

[00157] The taste of a beverage made with a single variant of casein, alpha casein for instance, such as by methods described herein, may be comparable to the taste of a similar type of beverage made using animal-derived dairy proteins, such as yogurt drinks made from animal milk. Taste of a beverage made using the compositions described herein having a single variant of a casein, such as alpha casein, may be comparable to the taste of a beverage made using micellar form of casein, such as milk, yogurt, or beverage made from caseinate, micellar casein or rennet casein. Taste of a beverage made using the compositions described herein having a single variant of a casein, such as alpha casein may be improved/more desirable when compared to the taste of a beverage made using micellar form of casein, such as milk, yogurt, or beverage made from caseinate, micellar casein, rennet casein, or when compared to a beverage made with a plant-derived protein.. Taste of a beverage may be tested using a trained panel of human subjects.

D. Other Consumable Compositions

[00158] In some embodiments, analogues of food, dairy or dairy-like products may be produced using compositions comprising a single variant of an alpha casein protein described herein. Dairy or dairy-like analogue products which can be made using the compositions described herein may include analogues of milk, cream, milkshakes, creamers (liquid and powder forms), ice cream, condensed milk, yogurt or cheese. Cheese analogues or cheese-like products which do not come from real curd or were not made via coagulation of a liquid colloid may also be made using the compositions comprising a single variant of an alpha casein protein, comprising the full-length single variant of alpha casein and optionally truncated forms thereof described herein.

[00159] The single variant alpha casein compositions described herein may be used to generate consumable compositions such as dairy cream analogues or cream-like compositions. For instance, single variant alpha casein compositions may be used to form a dairy cream analogue product. Dairy cream analogue products may be formed without the formation of a micelle or a micelle-like composition. Cream analogue products made using the compositions described herein may provide similar or equivalent features (such as texture, creaminess and taste) to an animal-derived dairy cream, or a dairy cream analogue made using micelles such

as one made from caseinate or rennet casein. Cream analogue products made using the compositions described herein may provide an improvement in one or more features (such as texture, creaminess and taste) when compared to a plant-derived cream analogue.

[00160] The compositions described herein may be used to generate consumable compositions such as ice cream analogue compositions. For instance, single variant alpha casein compositions may be used to form an ice cream analogue product. Ice cream analogue products may be formed without the formation of a micelle or a micelle-like composition. Ice cream analogue products made using the compositions described herein may provide similar or equivalent to an animal-derived dairy ice cream analogue or an ice cream analogue made using micelles, such as one made from caseinate or rennet casein. Ice cream analogue products made using the compositions described herein may provide an improvement in one or more features (such as texture, creaminess and taste) when compared to a plant-derived ice cream analogue.

[00161] The compositions described herein may be used to generate various consumable compositions, including but not limited to beverages (such as nutritional drinks, dairy-related drinks, etc.), salad dressings, baking ingredients, cooking ingredients, etc. For instance, the single variant alpha casein compositions described herein may be used to generate a yogurt beverage, a ranch dressing, etc. As an additional example, the single variant alpha casein compositions described herein may be used to generate ingredients used for baking and cooking.

E. Other components

[00162] The compositions described herein may be used as ingredients in generating consumable compositions such as food products. The food products may include cheese-analogues, yogurt analogue products, and other food products described elsewhere herein. Such consumable compositions may comprise one or more ingredients in addition to the single variant casein protein. The ingredients may include but are not limited to solvents, salts, sugar, fats, flavorings, colorants, etc.

[00163] The consumable compositions comprising a single variant casein protein may comprise salts such as calcium, phosphorous, citrate, potassium, sodium and/or chloride salts. The calcium salt may be selected from calcium chloride, calcium carbonate, calcium citrate, calcium gluconate, calcium lactate, calcium gluconate, calcium acetate, equivalents thereof and/or combinations thereof. The phosphate salt may be selected from orthophosphates such as monosodium (dihydrogen) phosphate, disodium phosphate, trisodium 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 citrate salt may be selected from calcium citrate, potassium citrate, sodium citrate, trisodium citrate, tripotassium citrate or equivalents thereof. The consumable composition may comprise a combination of salts. In some embodiments, the consumable composition comprises calcium, phosphate and citrate salts. In some embodiments, the consumable composition comprises calcium and phosphate salts. In some embodiments, the consumable composition comprises calcium and citrate salts. In some embodiments, the consumable composition comprises phosphate and citrate salts.

[00164] In some embodiments, fat is added to the consumable 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, or combinations thereof. Fats used herein may include microbially-made recombinant animal or plant fats. Fats used herein may include mammalian cell-cultured recombinant animal or plant fats.

[00165] Consumable composition as described herein may further comprise sugars. Sugars used herein may include plant-based monosaccharides, disaccharides and/ or oligosaccharides. Examples of sugars include sucrose, glucose, fructose, galactose, lactose, maltose, mannose, allulose, tagatose, xylose, and arabinose.

[00166] The consumable food compositions made from a single variant casein protein described herein and the methods of making such compositions may including adding or mixing with one or more ingredients. For example, food additives may be added in or mixed with the compositions. Food additives can add volume and/or mass to a composition. A food additive may improve functional performance and/or physical characteristics. For example, a food additive may prevent gelation or increased viscosity due to the lipid portion of the lipoproteins in the freeze-thaw cycle. An anticaking agent (cellulose, potato starch, corn starch, starch blends) may be added to make a free-flowing composition. Carbohydrates can be added to increase resistance to heat damage, e.g., less protein denaturation during drying and improve stability and flowability of dried compositions. Food additives include, but are not limited to, starch (e.g., potato, modified potato, corn, rice), food coloring, pH adjuster (e.g. glucono-delta-lactone, sodium hydroxide), natural flavouring (e.g., mozzarella, parmesan, butter, cream, colby, provolone, asiago, etc.), artificial flavoring, flavor enhancer, flavour maskers, batch marker, food acid (e.g., lactic acid, citric acid), filler, anticaking agent (e.g., sodium

silicoaluminate), antigreening agent (e.g., citric acid), food stabilizer, foam stabilizer or binding agent, antioxidant, acidity regulatory, bulking agent, color retention agent, whipping agent (e.g., ester-type whipping agent, triethyl citrate, sodium lauryl sulfate), emulsifier (e.g., lecithin, monoglycerides, diglycerides), humectant, thickener, pharmaceutical excipient, solid diluent, nutrient, sweetener, glazing agent, preservative (e.g., sorbic acid, nisin), vitamins (e.g. vitamin B, vitamin D, vitamin A), dietary elements, carbohydrates, polyol, gums, starches, flour, oil, and bran. In some cases, flavoring may comprise a mozzarella flavoring, cheddar flavoring, parmesan flavoring or other similar cheese flavorings.

[00167] Food coloring includes, but is not limited to, FD&C Yellow #5, FD&C Yellow #6, FD&C Red #40, FD&C Red #3, FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, carotenoids (e.g., saffron, β -carotene), annatto, betanin, butterfly pea, caramel coloring, chlorophyllin, elderberry juice, lycopene, carmine, pandan, paprika, turmeric, curcuminoids, quinoline yellow, carmoisine, Ponceau 4R, Patent Blue V, and Green S.

[00168] Ingredients for pH adjustment include, but are not limited to, Tris buffer, potassium phosphate, sodium hydroxide, potassium hydroxide, citric acid, sodium citrate, sodium bicarbonate, and hydrochloric acid.

F. End-User Products

[00169] Consumable compositions of a single variant casein protein described herein may be used as ingredients to make a final product for an end-user. For instance, a cheese product or a cheese analogue described herein may be used by end-user to make a final product such as pizza, Italian food toppings, Mexican food toppings, frozen meals, toppings for savory baked goods, soups, macaroni cheese, cheese sticks, etc. In some examples, a yogurt analogue may be used to make yogurt like products or products that contain yogurt as an ingredient. In some examples, consumable compositions may be used to make milk like products or milk analogues or other beverages.

Recombinant Expression

[00170] One or more proteins used in the formation of cheese compositions may be produced recombinantly. In some cases, a single variant casein protein (e.g., a single variant of alpha S1 or a single variant of alpha S2) is produced recombinantly. The single variant casein protein, e.g., a single variant alpha S1 casein protein or a single variant alpha S2 casein protein can have an amino acid sequence from any species. For example, a recombinant alpha casein protein may have an amino acid sequence of cow, sheep, goat,

buffalo, horse, human, deer or camel alpha casein. The nucleotide sequence encoding the casein protein may be codon-optimized for increased efficiency of production. Exemplary alpha casein protein sequences are provided in Table 1 below for use in recombinant production of a single variant casein protein. A recombinant single variant casein protein can be a non-naturally occurring variant of an casein. Such variant can comprise one or more amino acid insertions, deletions, or substitutions relative to a native casein sequence.

[00171] Such a variant can have at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NOs: 1-56. In some cases, a variant may be a truncated form of the alpha S1 casein protein such as one with at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO.: 4-12, 16-24.

[00172] A recombinant single variant casein protein, such as a single variant alpha S1 casein protein, is recombinantly expressed in a host cell. As used herein, a “host” or “host cell” denotes any protein production host selected or genetically modified to produce a desired product. Exemplary hosts include bacteria, yeast, fungi, plants, insects and mammalian cells. In some cases, a bacterial host cell such as *Lactococcus lactis*, *Bacillus subtilis* or *Escherichia coli* may be used to produce alpha casein proteins and/or its truncated forms. Other host cells include bacterial host such as, but not limited to, *Lactococci* sp., *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* and *Synechocystis* sp. 6803.

[00173] In some embodiments, a full-length single variant alpha casein protein and/or truncated forms thereof are produced recombinantly in a host cell. For example, full-length and truncated single variant alpha S1 casein proteins may be produced in the same host cell and such production can originate from the same open reading frame (i.e., the same expression cassette) and truncated forms generated for example, by post-translational proteolytic cleavage or produced from separate open reading frames, such as with an expression cassette encoding the full-length alpha casein variant and one or more expression cassettes encoding the truncated open reading frames for the truncated forms of the alpha casein variant. Alternatively, full-length and truncated single variant alpha S1 casein proteins may be produced in different host cells. Expression of a target protein can be provided by an expression vector, a plasmid, a nucleic acid integrated into the host genome or 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.

[00174] Expression vectors that can be used for expression of casein include those containing an expression cassette with elements (a), (b), (c) and (d). In some embodiments, the signal peptide (b) and/or terminator element (d) need not be included in the vector. In some cases, a signal peptide may be part of the native signal sequence of the casein protein, for instance, the protein may comprise a native signal sequence as bolded in SEQ ID NOs: 1, 13, 25, 28, 31, 34, 37, 39, 42, 45, 48, 51 or 54. In some cases, the vector may comprise a mature protein sequence, as exemplified in SEQ ID NOs: 2, 3, 4-12, 14, 15, 16-24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56 with a heterologous signal sequence. In some cases, the protein may comprise no signal sequence but instead an initiator methionine, as exemplified in SEQ ID NOs: 3, 5, 7, 9, 11, 12, 15, 17, 19, 21, 23, 24, 27, 30, 33, 36, 41, 44, 47, 50, 53 or 56. In general, the expression cassette is designed to mediate the transcription of the transgene when integrated into the genome of a cognate host microorganism or when present on a plasmid or other replicating vector maintained in a host cell.

[00175] To aid in the amplification of the vector prior to transformation into the host microorganism, a replication origin (e) may be contained in the vector. To aid in the selection of microorganism stably transformed with the expression vector, the vector may also include a selection marker (f). The expression vector may also contain a restriction enzyme site (g) that allows for linearization of the expression vector prior to transformation into the host microorganism to facilitate the expression vectors stable integration into the host genome. In 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 elements known to one of skill in the art can be used in combination or substituted for the elements described herein.

[00176] 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. Both gram positive and gram-negative bacteria may be used to produce proteins intracellularly. In such examples, the cells may be lysed to recover the protein.

[00177] Single variant 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 truncates thereof may be co-expressed or individually expressed in *L. lactis* using a synthetic operon.

[00178] *B. subtilis* 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.

[00179] In order to drive the recombinant protein secretion, the signal peptide of amyQ, alpha-amylase of *Clostridium thermocellum* may be used or another bacterial signal peptide known in the art. 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 operator. pHT01 is an *E. coli/B. subtilis* shuttle vector that provides ampicillin resistance to *E. coli* and chloramphenicol resistance to *B. subtilis*.

[00180] Single variant casein proteins may be produced in *E. coli* using safe laboratory strains such as *E. coli* BL21 (exemplary strains BL21 (DE3) or BL21 AI) or their derivatives, or a wild-type like K12 strains (exemplary strains MG1655 or W3110) or their derivatives. Inducible (such as IPTG-inducible, lactose-inducible, arabinose-inducible, rhamnose-inducible), auto-inducible (such as phosphate depletion based) and constitutive promoters may be used to drive the casein expression. Single variant casein proteins may be produced intracellularly, or may be secreted into the periplasm and/or supernatant. In order to drive the recombinant protein secretion, bacterial signal peptides of Sec-dependent secretion pathway (such as OmpA, OmpC, OmpT, pelB, LamB), SRP secretion pathway (such as TolA, DsbA, DsbC, TorT) and TAT secretion pathway (such as TorA, SufI) can be used.

Table 1: Sequences

SEQ ID No.	Name	Sequence
1	Bovine A lpha S1 Casein	MKLLILTCLVAVALAR PKHPIKHQGLPQEVNLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSEKTTMPLW
2	Bovine A lpha S1 Casein Mature protein	RPKHPIKHQGLPQEVNLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSEKTTMPLW
3	Bovine A lpha S1 Casein Mature protein with Methionine	MRPKHPIKHQGLPQEVNLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSEKTTMPLW
4	Bovine alpha S1 casein (B. Taurus) aa 23-199	FFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
5	Bovine alpha S1 casein (B. Taurus) aa 23-199 with Methionine	MFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
6	Bovine alpha S1 casein (B. Taurus) aa 24-199	FVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
7	Bovine alpha S1 casein (B. Taurus) aa 24-199 with Methionine	MFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
8	Bovine alpha S1 casein (B. Taurus) aa 25-199	VAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
9	Bovine alpha S1 casein (B. Taurus) aa 25-199 with Methionine	MVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
10	Bovine alpha S1 casein (B. Taurus) aa 26-199	APFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW
11	Bovine alpha S1 casein (B. Taurus) aa 26-199 with Methionine	MAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDA PSFSDIPNPIGSENSGKTTMPLW

SEQ ID No.	Name	Sequence
12	Bovine alpha S1 casein (B. Taurus) aa 60-199	MEAESISSSEEIVPNSVEQKHQKEDVPSEYLGYLEQLRLKKYKVPQL EIVPNSAEERLHSMKEGIHAQQKEPMIGVNPQELAYFYPELFRQFYQL DAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
13	Ovine Alpha S1 casein	MKLLILTCLVAVALAR PKHPIKHQGLSSEVLNENLLRFVVPFPEVFRK ENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEIVPNSAEQKYIQ KEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPAH QKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWYYLPLGTQYTD APSFSDIPNPIGSENSGKITMPLW
14	Ovine Alpha S1 casein Mature protein	RPKHPIKHQGLSSEVLNENLLRFVVPFPEVFRKENINELSKDIGSESIE DQAMEDAKQMKAGSSSSSEEIVPNSAEQKYIQKEDVPSEYLGYLEQ LLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPAHQKQPMIAVNQELAY FYPQLFRQFYQLDAYPSGAWYYLPLGTQYTDAPSFSDIPNPIGSENSG KITMPLW
15	Ovine Alpha S1 casein Mature protein with Methionine	MRPKHPIKHQGLSSEVLNENLLRFVVPFPEVFRKENINELSKDIGSES IEDQAMEDAKQMKAGSSSSSEEIVPNSAEQKYIQKEDVPSEYLGYLE QLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPAHQKQPMIAVNQEL AYFYPQLFRQFYQLDAYPSGAWYYLPLGTQYTDAPSFSDIPNPIGSEN SGKITMPLW
16	Ovine alpha S1 casein aa 23-199	FVVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEI VPNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQL HSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWY YLPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
17	Ovine alpha S1 casein aa 23-199 with Methionine	MFVVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSS EEIVPNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEE QLHSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGA WYYLPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
18	Ovine alpha S1 casein aa 24-199	VVVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEI VPNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQL HSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWY YLPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
19	Ovine alpha S1 casein aa 24-199 with Methionine	MVVVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSE EIVPNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEE QLHSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGA WYYLPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
20	Ovine alpha S1 casein aa 25-199	VVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEI PNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQLH SMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWYY LPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
21	Ovine alpha S1 casein aa 25-199 with Methionine	MVVPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEI VPNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQL HSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWY YLPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
22	Ovine alpha S1 casein aa 26-199	APFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEIV NSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQLHS MKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWYYL PLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
23	Ovine alpha S1 casein aa 26-199 with Methionine	MAPFPEVFRKENINELSKDIGSESIEDQAMEDAKQMKAGSSSSSEEIV PNSAEQKYIQKEDVPSEYLGYLEQLRLKKYNVPQLEIVPKSAEEQLH

SEQ ID No.	Name	Sequence
		SMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWWY LPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
24	Ovine alpha S1 casein aa 60-199	MKAGSSSSSEIIVPNSAEQKYIQKEDVPSEYLYGYLEQLRLKKYNVPQ LEIVPKSAEEQLHSMKEGNPAHQKQPMIAVNQELAYFYPQLFRQFY QLDAYPSGAWWY LPLGTQYTDAPSFSDIPNPIGSENSGKITMPLW
25	Caprine Alpha S1 casein	MKLLILTCLVAVALAR PKHPINHRGLSPEVPNENLLRFVVPFPEVFR KENINELSKDIGSESTEDQAMEDAKQMKAGSSSSSEIIVPNSAEQKYI QKEDVPSEYLYGYLEQLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPA HQKQPMIAVNQELAYFYPQLFRQFYQLDAYPSGAWWY LPLGTQYTD APSFSDIPNPIGSENSGKITMPLW
26	Caprine Alpha S1 casein Mature Protein	RPKHPINHRGLSPEVPNENLLRFVVPFPEVFRKENINELSKDIGSEST EDQAMEDAKQMKAGSSSSSEIIVPNSAEQKYIQKEDVPSEYLYGYLE QLLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPAHQKQPMIAVNQEL AYFYPQLFRQFYQLDAYPSGAWWY LPLGTQYTDAPSFSDIPNPIGSEN SGKITMPLW
27	Caprine Alpha S1 casein Mature Protein with Methionine	MRPKHPINHRGLSPEVPNENLLRFVVPFPEVFRKENINELSKDIGSES TEDQAMEDAKQMKAGSSSSSEIIVPNSAEQKYIQKEDVPSEYLYG L EQLRLKKYNVPQLEIVPKSAEEQLHSMKEGNPAHQKQPMIAVNQEL AYFYPQLFRQFYQLDAYPSGAWWY LPLGTQYTDAPSFSDIPNPIGSE NSGKITMPLW
28	Buffalo Alpha S1 Casein	MKLLILTCLVAVALAR PKQPIKHQGLPQGVLENLLRFVVPFPEVFG KEKVNELSTDIGSESTEDQAMEDIKQMEASISSSEIIVPISVEQKHIQ KEDVPSEYLYGYLEQLRLKKYNVPQLEIVPNLAEELHSMKEGIHAQ QKEPMIGVNQELAYFYPQLFRQFYQLDAYPSGAWWY VPLGTQYPDAPS FSDIPNPIGSENSEKTTMPLW
29	Buffalo Alpha S1 Casein Mature Protein	RPKQPIKHQGLPQGVLENLLRFVVPFPEVFGKEKVNELSTDIGSEST EDQAMEDIKQMEASISSSEIIVPISVEQKHIQKEDVPSEYLYGYLEQL LRLKKYNVPQLEIVPNLAEELHSMKEGIHAQ QKEPMIGVNQELAYF YPQLFRQFYQLDAYPSGAWWY VPLGTQYPDAPS FSDIPNPIGSENSEK TTMLW
30	Buffalo Alpha S1 Casein Mature Protein with Methionine	MRPKQPIKHQGLPQGVLENLLRFVVPFPEVFGKEKVNELSTDIGSE STEDQAMEDIKQMEASISSSEIIVPISVEQKHIQKEDVPSEYLYGYLE QLLRLKKYNVPQLEIVPNLAEELHSMKEGIHAQ QKEPMIGVNQELA YFYPQLFRQFYQLDAYPSGAWWY VPLGTQYPDAPS FSDIPNPIGSENS EKITMPLW
31	Equine Alpha S1 Casein	MKLLILTCLVAVALAR PKLPHRQPEIIQNEQDSREKVLKERKFPSFALE YINELNRQRELLKEKQKDEHKEYLIEDPEQQESSSTSSSEEVVPINTEQK RIPREDMLYQHTLEQLRRLSKYNQLQLQAIHAQEQLIRMKENSQRKP MRVVNQEAYFYLEPFQPSYQLDVYPYAAWFHPAQIMQHVAYS PFHDTAKLIASENSEKTDIPEW
32	Equine Alpha S1 Casein Mature Protein	RPKLPHRQPEIIQNEQDSREKVLKERKFPSFALEYINELNRQRELLKEK QKDEHKEYLIEDPEQQESSSTSSSEEVVPINTEQKRIPREDMLYQHTLE QLRRLSKYNQLQLQAIHAQEQLIRMKENSQRKPMRVVNQEAYFYLE PFQPSYQLDVYPYAAWFHPAQIMQHVAYS PFHDTAKLIASENSEK TDIPEW
33	Equine Alpha S1 Casein Mature Protein with Methionine	MRPKLPHRQPEIIQNEQDSREKVLKERKFPSFALEYINELNRQRELLKE KQKDEHKEYLIEDPEQQESSSTSSSEEVVPINTEQKRIPREDMLYQHTL EQLRRLSKYNQLQLQAIHAQEQLIRMKENSQRKPMRVVNQEAYFY

SEQ ID No.	Name	Sequence
		LEPFQPSYQLDVYPYAAWFHPAQIMQHVAYSPFHDTAKLIASENSEKTDIIPEW
34	Camel Alpha S1 casein	MKLLILTCLVAVALAR PKYPLRYPEVFQNEPDSIEEVLNKRKILELAVVSPIQFRQENIDELKDTRNEPTEDHIMEDTERKESGSSSSEEVVSSTTEQKDILKEDMPSQRYLEELHRLNKKYLLQLEAIRDQKLIPRVKLSHPYLEQLYRINEDNHPQLGEPVKVVTQEAYFHLPEFPQFFQLGASPYVAWYYPQVMQYIAHPSSYDTPEGIASEDGGKTDVMPQWW
35	Camel Alpha S1 casein Mature Protein	RPKYPLRYPEVFQNEPDSIEEVLNKRKILELAVVSPIQFRQENIDELKDTRNEPTEDHIMEDTERKESGSSSSEEVVSSTTEQKDILKEDMPSQRYLEELHRLNKKYLLQLEAIRDQKLIPRVKLSHPYLEQLYRINEDNHPQLGEPVKVVTQEAYFHLPEFPQFFQLGASPYVAWYYPQVMQYIAHPSSYDTPEGIASEDGGKTDVMPQWW
36	Camel Alpha S1 casein Mature Protein with Methionine	MRPKYPLRYPEVFQNEPDSIEEVLNKRKILELAVVSPIQFRQENIDELKDTRNEPTEDHIMEDTERKESGSSSSEEVVSSTTEQKDILKEDMPSQRYLEELHRLNKKYLLQLEAIRDQKLIPRVKLSHPYLEQLYRINEDNHPQLGEPVKVVTQEAYFHLPEFPQFFQLGASPYVAWYYPQVMQYIAHPSSYDTPEGIASEDGGKTDVMPQWW
37	Human Alpha S1 Casein	MRLILITCLVAVALAR PKLPLRYPERLQNPSESSEPIPLESREEYMNGMNRQRNIREKQTDIEIKDTRNESTQNCVVAEPEKMESSISSSSEEMSLSKCAEQFCRLNEYNLQQLQAAHAQEIQIRRMNENSHVQVPFQQLNQLAAPYAVWYYPQIMQYVFPFSDISNPTAHENYEKNNVMLQW
38	Human Alpha S1 Casein Mature Protein	RPKLPLRYPERLQNPSESSEPIPLESREEYMNGMNRQRNIREKQTDIEIKDTRNESTQNCVVAEPEKMESSISSSSEEMSLSKCAEQFCRLNEYNLQQLQAAHAQEIQIRRMNENSHVQVPFQQLNQLAAPYAVWYYPQIMQYVFPFSDISNPTAHENYEKNNVMLQW
39	Bovine Alpha S2 Casein	MKFFIFTCLLAVALAK NTMEHVSSEESIISQETKQKEKNMAINPSKENLCSTFCKEVVRNANEEYSIGSSSEESAEEVATEEVKITVDDKHQKALNEINQFYQKFPQYLQYLYQGPIVLPWDQVKRNPITPTLNREQLSTSEENSKKTVDMESTEVFTKTKLTEEEKNRLNFKKISQRYQKFALPQYLKTVYQHQAAMKPWIPKTKVIPYVRYL
40	Bovine Alpha S2 Casein Mature protein	KNTMEHVSSEESIISQETKQKEKNMAINPSKENLCSTFCKEVVRNANEEYSIGSSSEESAEEVATEEVKITVDDKHQKALNEINQFYQKFPQYLQYLYQGPIVLPWDQVKRNPITPTLNREQLSTSEENSKKTVDMESTEVFTKTKLTEEEKNRLNFKKISQRYQKFALPQYLKTVYQHQAAMKPWIPKTKVIPYVRYL
41	Bovine Alpha S2 Casein Mature protein with Methionine	MKNTMEHVSSEESIISQETKQKEKNMAINPSKENLCSTFCKEVVRNANEEYSIGSSSEESAEEVATEEVKITVDDKHQKALNEINQFYQKFPQYLQYLYQGPIVLPWDQVKRNPITPTLNREQLSTSEENSKKTVDMESTEVFTKTKLTEEEKNRLNFKKISQRYQKFALPQYLKTVYQHQAAMKPWIPKTKVIPYVRYL
42	Ovine Alpha S2 casein	MKFFIFTCLLAVALAK HKHMEHVSSEEPINISQEIYKQEKNAIHPRK EKLCTTSCEEVVRNADDEEYSIRSSSEESAEEVATEEVKITVDDKHQKALNEINQFYQKFPQYLQYLYQGPIVLPWDQVKRNPITPTLNREQLSTSEENSKKTVDMESTEVFTKTKLTEEEKNRLNFKKISQRYQKFALPQYLKTVYQHQAAMKPWIPKTKVIPYVRYL
43	Ovine Alpha S2 casein Mature protein	KHKHMEHVSSEEPINISQEIYKQEKNAIHPRK EKLCTTSCEEVVRNADDEEYSIRSSSEESAEEVATEEVKITVDDKHQKALNEINQFYQKFPQYLQYLYQGPIVLPWDQVKRNPITPTLNREQLSTSEENSKKTVDMESTEVFTKTKLTEEEKNRLNFKKISQRYQKFALPQYLKTVYQHQAAMKPWIPKTKVIPYVRYL

SEQ ID No.	Name	Sequence
		TEVFTKTKLTETEEKNRLNFKKISQYYQKFAWPQYLKTVDDQHQA KPWTQPKTNAIPYVRYL
44	Ovine Alpha S2 casein Mature protein with Methionine	MKHKMEHVSSEEPINISQEIYKQEKMAIHPRKEKLCTTSCEEVVRN ADEEEYSIRSSSESAEVAPEEVKITVDDKHYQKALNEINQFYQKFPQY LQYLYQGPIVLNPWDQVKRNAGPFTPTVNREQLSTSEENSKKTIDME STEVFTKTKLTETEEKNRLNFKKISQYYQKFAWPQYLKTVDDQHQA MKPWTQPKTNAIPYVRYL
45	Caprine Alpha S2 casein	MKFFIFTCLLAVALAK HKMEHVSSEEPINIFQEIYKQEKMAIHPRK EKLCTTSCEEVVRNANEEYSIRSSSESAEVAPEEIKITVDDKHYQKAL NEINQFYQKFPQYLQYPYQGPIVLNPWDQVKRNAGPFTPTVNREQL STSEENSKKTIDMESTEVFTKTKLTETEEKNRLNFKKISQYYQKFAWP QYLKTVDDQHQA MKPWTQPKTNAIPYVRYL
46	Caprine Alpha S2 casein Mature Protein	KHKMEHVSSEEPINIFQEIYKQEKMAIHPRKEKLCTTSCEEVVRN NEEYSIRSSSESAEVAPEEIKITVDDKHYQKALNEINQFYQKFPQYL QYPYQGPIVLNPWDQVKRNAGPFTPTVNREQLSTSEENSKKTIDMES TEVFTKTKLTETEEKNRLNFKKISQYYQKFAWPQYLKTVDDQHQA KPWTQPKTNAIPYVRYL
47	Caprine Alpha S2 casein Mature Protein with Methionine	MKHKMEHVSSEEPINIFQEIYKQEKMAIHPRKEKLCTTSCEEVVRN ANEEYSIRSSSESAEVAPEEIKITVDDKHYQKALNEINQFYQKFPQYL QYPYQGPIVLNPWDQVKRNAGPFTPTVNREQLSTSEENSKKTIDMES TEVFTKTKLTETEEKNRLNFKKISQYYQKFAWPQYLKTVDDQHQA KPWTQPKTNAIPYVRYL
48	Buffalo Alpha S2 Casein	MKFFIFTCLLAVALAK HKTMEHVSSEESIISQETKQEKMAIHPKSKE NLCSTFCKEVIRNANEEYSIGSSSESAEVAPEEVKITVDDKHYQKAL NEINQFYQKFPQYLQYLYQGPIVLNPWDQVKRNAVPITPTLNREQLS TSEENSKKTIDMESTEVFTKTKLTETEDKNRLNFKKISQHYQKFAWP QYLKTVYQYQKAMKPWTQPKTNVIPYVRYL
49	Buffalo Alpha S2 Casein Mature Protein	KHTMEHVSSEESIISQETKQEKMAIHPSKENLCSTFCKEVIRNAN EEYSIGSSSESAEVAPEEVKITVDDKHYQKALNEINQFYQKFPQYLQY LYQGPIVLNPWDQVKRNAVPITPTLNREQLSTSEENSKKTIDMESTE VFTKTKLTETEDKNRLNFKKISQHYQKFAWPQYLKTVYQYQKAMKP WTQPKTNVIPYVRYL
50	Buffalo Alpha S2 Casein Mature Protein with Methionine	MKHTMEHVSSEESIISQETKQEKMAIHPSKENLCSTFCKEVIRN NEEYSIGSSSESAEVAPEEVKITVDDKHYQKALNEINQFYQKFPQYL QYLYQGPIVLNPWDQVKRNAVPITPTLNREQLSTSEENSKKTIDMES TEVFTKTKLTETEDKNRLNFKKISQHYQKFAWPQYLKTVYQYQKAM KPWTQPKTNVIPYVRYL
51	Equine Alpha S2 Casein	MKFFIFTCLLAVALAK HKNMEHRSSSEDSVNISQEKFKQEKYVVIPTSK ESICSTSCEEATRININEMESAKFPTEREKEVEEKHHLKQLNKINQFY KLNFLQYLQALRQPRIVLTPWDQTKTGDSPIFIVNTEQLFTSEEIPKK TVDMESTEVVTEKTELTEEEKNYLKLLYEFKFTLPQYFKIVRQHQT MDPRSHRKTNSYQIIPVLRYP
52	Equine Alpha S2 Casein Mature Protein	KHNMEHRSSSEDSVNISQEKFKQEKYVVIPTSKESICSTSCEEATRIN INEMESAKFPTEREKEVEEKHHLKQLNKINQFYKLNFLQYLQALRQPR IVLTPWDQTKTGDSPIFIVNTEQLFTSEEIPKKTVDMESTEVEKTE LTEEEKNYLKLLYEFKFTLPQYFKIVRQHQTMDPRSHRKTNSYQIIPV LRYP

SEQ ID No.	Name	Sequence
53	Equine Alpha S2 Casein Mature Protein with Methionine	MKHNMEHRSSSEDSVNISQEKFKQEKYVVIPTSKESICSTSCEEATRNI NEMESAKFPTEREKEVEEKHHLKQLNKINQFYEKLNFLQYLQALRQP RIVLTPWDQTKTGDSPPFIPVNTQLFTSEEIPKKTVDMESTEVVTEKT ELTEEEKNYLKLLEYEKFTLPQYFKIVRQHQTMDPRSHRKTNSYQIIP VLRYF
54	Camel Alpha S2 casein	MKFFIFTCLLAVVLAK HEMDQGSSEESINVSQQKFKQVKKVAIHPSK EDICSTFCEEAVRNIKEVESAEVPTENKISQFYQKWKFLQYLQALHQQG QIVMNPWDQGKTRAYPFIPTVNTQLSISEESTEVPTTEESTEVFTKKTE LTEEEKDHQKFLNKIYQYYQTFLWPEYLKTVYQYQKTMTPWNHIKRY F
55	Camel Alpha S2 casein Mature Protein	KHEMDQGSSEESINVSQQKFKQVKKVAIHPSKEDICSTFCEEAVRNI KEVESAEVPTENKISQFYQKWKFLQYLQALHQQGQIVMNPWDQGKT RAYPFIPTVNTQLSISEESTEVPTTEESTEVFTKKTELTEEEKDHQKFLN KIYQYYQTFLWPEYLKTVYQYQKTMTPWNHIKRYF
56	Camel Alpha S2 casein Mature Protein with Methionine	MKHEMDQGSSEESINVSQQKFKQVKKVAIHPSKEDICSTFCEEAVR NIKEVESAEVPTENKISQFYQKWKFLQYLQALHQQGQIVMNPWDQGK TRAYPFIPTVNTQLSISEESTEVPTTEESTEVFTKKTELTEEEKDHQKFL NKIYQYYQTFLWPEYLKTVYQYQKTMTPWNHIKRYF

EMBODIMENTS

[00181] [Embodiment 1]: A consumable composition comprising a recombinant single variant of an alpha casein protein, wherein the single variant provides at least one dairy-like property selected from the group consisting of adhesiveness, stretch, texture, mouthfeel, melt, browning hardness, creaminess, taste, smell, and flexibility, wherein the single variant is not an animal-derived casein and has not been physically dissociated from a casein micelle and wherein the composition lacks any additional caseins.

[00182] [Embodiment 2]: The consumable composition of embodiment 1, wherein the single variant of the alpha casein protein is not derived from caseinate.

[00183] [Embodiment 3]: The consumable composition of embodiment 1 or embodiment 2, wherein the single variant of the alpha casein protein is an alpha S1 casein protein.

[00184] [Embodiment 4]: The consumable composition of embodiment 1 or embodiment 2, wherein the single variant of the alpha casein protein is an alpha S2 casein protein.

[00185] [Embodiment 5]: The consumable composition of any of embodiments 1-3, wherein the composition is free from any animal-produced proteins.

[00186] [Embodiment 6]: The consumable composition of embodiment 4, wherein the composition lacks any other animal-derived dairy proteins.

[00187] [Embodiment 7]: The consumable composition of any of embodiments 1-5, wherein at least one dairy-like property is improved as compared to a milk-derived cheese analogue.

[00188] [Embodiment 8]: The consumable composition of any of embodiments 1-5, wherein at least one dairy-like property is improved as compared to a caseinate-derived cheese analogue or improved as compared to a rennet casein-derived cheese analogue.

[00189] [Embodiment 9]: The consumable composition of any of embodiments 1-5, wherein at least one dairy-like property is improved as compared to a plant-derived cheese analogue.

[00190] [Embodiment 10]: The consumable composition of any of embodiments 1-9, wherein the single variant of the alpha casein protein comprises at least one non-native post-translational modification.

[00191] [Embodiment 11]: The consumable composition of embodiment 10, wherein the single variant of the alpha casein protein further comprises at least one native post-translational modification.

[00192] [Embodiment 12]: The consumable composition of any of embodiments 1-11, wherein the single variant of the alpha casein protein lacks one or more post-translational modifications of a native alpha casein protein.

[00193] [Embodiment 13]: The consumable composition of embodiment 12, wherein the single variant of the alpha casein protein further comprises at least one non-native post-translational modification.

[00194] [Embodiment 14]: The consumable composition of embodiment 13, wherein the single variant of the alpha casein protein is not post-translational modified.

[00195] [Embodiment 15]: The consumable composition of any of embodiments 1-14, wherein the composition comprises a full-length alpha casein protein.

[00196] [Embodiment 16]: The consumable composition of any of embodiments 1-14, wherein the single variant of the alpha casein protein comprises any one of SEQ ID Nos. 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.

[00197] [Embodiment 17]: The consumable composition of embodiment 16, wherein composition further comprises one or more truncated alpha casein proteins.

[00198] [Embodiment 18]: The consumable composition of embodiment 17, wherein the truncated alpha casein protein lacks 1 or more N-terminal amino acids of a mature native alpha casein protein.

[00199] [Embodiment 19]: The consumable composition of embodiment 18, wherein the truncated alpha casein protein is selected from the group consisting of an alpha casein lacking between 1-23 N-terminal amino acids of the native alpha casein protein or an alpha casein lacking between 1-59 N-terminal amino acids of the native alpha casein protein or a combination thereof.

[00200] [Embodiment 20]: The consumable composition of embodiment 19, wherein the truncated alpha casein protein comprises any one of SEQ ID NOs.: 4-12, 16-24.

[00201] [Embodiment 21]: The consumable composition of any of embodiments 17-20, wherein the truncated alpha casein protein lacks 1 or more C-terminal amino acids of a native alpha casein protein.

[00202] [Embodiment 22]: The consumable composition of embodiment 17, wherein between 0% and 20% wt/wt of the total recombinant alpha casein protein of the composition is the one or more truncated forms of the alpha casein protein.

[00203] [Embodiment 23]: The consumable composition of embodiment 22, wherein the one or more truncated forms of the alpha casein protein comprise between 1% and 20% wt/wt of the total recombinant alpha casein protein of the composition.

[00204] [Embodiment 24]: The consumable composition of any of embodiments 1-23, wherein the recombinant alpha casein protein comprises an amino acid sequence of a bovine, caprine or ovine alpha casein protein or any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56 or a sequence with at least 70%, 80%, 85% or 90% identity to any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.

[00205] [Embodiment 25]: The consumable composition of any of embodiments 1-24, wherein the single variant of an alpha casein protein comprises one or more non-native amino acids at the N-terminus.

[00206] [Embodiment 26]: The consumable composition of embodiment 25, wherein the single variant of an alpha casein protein comprises a non-native methionine at the N-terminal position.

[00207] [Embodiment 27]: The consumable composition of any of embodiments 1-26, wherein the single variant of an alpha casein protein is not derived from casein micelles.

[00208] [Embodiment 28]: A dairy product analogue comprising any of the consumable compositions of embodiments 1-27, wherein the analogue is selected from the group consisting of a cheese analogue, a yogurt analogue, a cream analogue, and an ice cream analogue.

- [00209] [Embodiment 29]: The dairy product analogue of embodiment 28, further comprising a fat or oil from a non-animal source.
- [00210] [Embodiment 30]: The dairy product analogue of embodiment 28 or embodiment 29, wherein the dairy product analogue lacks any animal-derived dairy proteins.
- [00211] [Embodiment 31]: The dairy product analogue of any of embodiments 28-30, wherein the dairy product analogue lacks any other casein proteins.
- [00212] [Embodiment 32]: The dairy product analogue of any of embodiments 28-31, wherein the single variant of an alpha casein protein is not comprised in a micellar form within the dairy product analogue.
- [00213] [Embodiment 33]: The dairy product analogue of any of embodiments 28-32, wherein the dairy product analogue is a cheese analogue.
- [00214] [Embodiment 34]: The dairy product analogue of embodiment 33, wherein the cheese analogue is a mozzarella analogue, a cheddar analogue or a parmesan analogue.
- [00215] [Embodiment 35]: The dairy product analogue of embodiment 33, wherein the cheese analogue is a mozzarella analogue and wherein the single variant of an alpha casein protein is an alpha S1 casein.
- [00216] [Embodiment 36]: The dairy product analogue of embodiment 35, wherein the alpha S1 casein is a bovine alpha S1 casein, and wherein the alpha S1 casein in the composition comprises between 0-20% of one or more truncated forms of alpha casein.
- [00217] [Embodiment 37]: The dairy product analogue of embodiment 35, wherein the alpha S1 casein in the composition comprises between 1-20% of one or more truncated forms of alpha S1 casein.
- [00218] [Embodiment 38]: The dairy product analogue of embodiment 36, or embodiment 37, comprising a N-terminal truncated form of alpha S1 casein.
- [00219] [Embodiment 39]: The dairy product analogue of embodiment 38, wherein the N-terminal truncated form is selected from any one of SEQ ID NOs: 4-12, 16-24 or a combination thereof.
- [00220] [Embodiment 40]: The dairy product analogue according to any of embodiments 28-39, further comprising one or more of (a) a plant-derived oil; (b) a plant-derived starch; (c) a sugar; and (d) a salt.

EXAMPLES

The following illustrative examples are representative of embodiments of the compositions and methods described herein and are not meant to be limiting in any way.

Example 1: Expression of casein proteins in *Lactococcus lactis* via nisin-inducible system (NICE)*Constructs design, cloning and transformation*

[00221] Bovine alpha S1 casein (variant C) protein coding sequence (without the native signal peptide) was codon-optimized for expression in *Lactococcus lactis* and a synthetic operon was constructed for co-expression and secretion of the two proteins under a nisin-inducible promoter. Signal peptide sequence from natively secreting lactococcal protein Usp45 was used to drive protein secretion. A synthetic operon was then cloned into an E. coli custom vector via restriction digest compatible sites and confirmed via Sanger sequencing, from which it was subcloned into nisin-inducible pNZ8149 vector via restriction digestion and ligation.

[00222] The vector was transformed into compatible *L. lactis* strain NZ3900 via electroporation and completely defined media (CDM) supplemented with lactose was used for selection. Positive clones were confirmed via colony PCR and 3 positive clones were taken forward for the protein expression induction and analysis.

Protein expression and analysis

[00223] Individual colonies were grown at 30 °C in liquid culture and protein production was induced with nisin for 2.5 hours (control samples left uninduced). Cells were then harvested by centrifugation and TCA-precipitated supernatants and lysed cell pellets were analysed by Coomassie gel staining (SDS-PAGE) and chemiluminescence (Western Blot against alpha S1 casein, LSBio primary antibodies).

Example 2: Expression in *L. lactis* via pH-inducible system

[00224] Similar to the constructions above, alpha casein protein constructions were created replacing the nisin promoter with the P170 promoter, a pH/lactate inducible promoter for *L. lactis*. Each of these constructs contained a secretion signal peptide.

[00225] Alpha S1 casein and its truncated forms were detected in *L. lactis* upon secretion on western blot. Unprocessed protein product accumulated intracellularly but secretion was detected for the mature protein and its truncated forms.

Example 3: Expression in *B. subtilis**Constructs design, cloning and transformation*

[00226] Bovine alpha S1 casein (variant C) protein coding sequence (without the native signal peptide) His-tagged C-terminally was codon-optimized for expression in *Bacillus subtilis*. Constructs were created with and without the codon-optimized signal peptide of

amyQ, alpha-amylase *Bacillus amyloliquefaciens* which has been reported for the efficient secretion of recombinant proteins. Constructs were cloned through *E. coli* via Gibson cloning into transformation and expression IPTG-inducible vector pHT01 and confirmed via Sanger sequencing. pHT01 is an *E. coli/B. subtilis* shuttle vector that provides ampicillin resistance to *E. coli* and chloramphenicol resistance to *B. subtilis*. Positive clones were further transformed into chemically competent *B. subtilis* WB800N. Positive clones were confirmed via colony PCR and 3 positive clones were taken forward for the protein expression induction and analysis.

Protein expression and analysis

[00227] Individual colonies were grown at 37°C in liquid culture and protein production was induced with IPTG for 1 hour, 2 hours and 6 hours (control samples were left uninduced). Cells were then harvested by centrifugation, and TCA-precipitated supernatants and lysed cell pellets were analysed by Coomassie gel staining (SDS-PAGE) and chemiluminescence (Western Blot against His tag and alpha S1 casein).

[00228] Western blotting showed expression of the alpha S1 casein in *B. subtilis*.

Example 4: Expression in *E. coli*

Constructs design, cloning and transformation

[00229] Bovine alpha S1 casein (variant C) or ovine alpha S1 casein protein coding sequence (without the native signal peptide) codon-optimized for *Escherichia coli* was cloned into IPTG-inducible commercially available pET vectors. Cloning was performed via Gibson reaction of DNA fragments and vector in such a way that only the protein coding sequence was left within the open reading frame. Gibson reactions were transformed into competent cells and confirmed by Sanger sequencing. Vectors were then transformed into chemically competent *E. coli* BL21(DE3) cells or their derivatives, or a wild-type like K12 strain or their derivatives, and several single colonies were screened for expression.

Protein expression, analysis and purification

[00230] Individual colonies were grown at 37 C in liquid culture, and protein production was induced with IPTG for 4 hours. Cells were then harvested by centrifugation, and lysed cell pellets were analysed by Coomassie gel staining (SDS-PAGE) and chemiluminescence (Western Blot against alpha S1 casein). Proteins were purified using phase separation. Purified product was analysed on a Coomassie stained gel similarly to explained above. Alpha S1 casein was expressed intracellularly in *E. coli*, successfully detected on Coomassie stained protein gel

and purified. An exemplary production of alpha casein is illustrated in FIG. 1 where the alpha S1 casein and 2 variants: N-terminally truncated F24-199 bovine alpha S1 casein and N-terminally truncated M60-199 bovine alpha S1 casein were also found.

Example 5: Mozzarella Cheese analogue from recombinant single variant alpha casein protein and its properties

[00231] Recombinant unphosphorylated bovine alpha S1 casein, was used to make non-micellar mozzarella cheese analogue termed NC mozzarella cheese. Casein, prewarmed coconut oil, trisodium citrate, disodium phosphate, salt and glucose were added in a beaker at concentrations specified in Table 2. To this, a mix of water, CaCl_2 , and modified potato starch were added at concentration specified in Table 2. The beaker was moved to a water bath preset at 85°C temperature and the contents were mixed using a mixing propeller at a speed of 300 rpm for 9 mins. Lactic acid was added and mixed for an additional 1 min. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 16-24 hrs. After incubation, the NC mozzarella cheese analogue was weighed to get yield estimation.

Table 2: Ingredient Composition and Concentration for Recombinant Single Casein Variant Cheese Analogue (NC1 mozzarella cheese analogue)

Ingredients	Concentration (% wt/wt)
Casein	18.5
Coconut oil	25.26
Trisodium Citrate	0.7
Disodium phosphate	1.29
Sodium chloride	1.49
Glucose	0.4
Water	48.32
CaCl_2	1
Modified potato starch	2.54

Lactic acid crystals	0.5
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[00232] The NC1 mozzarella cheese analogue samples were analyzed for qualitative and quantitative parameters such as pH, stretch, and texture profile. These parameters were compared to commercially available low-moisture mozzarella cheese, commercially available imitation mozzarella cheese and a commercially available plant-based vegan mozzarella-style cheese. The low-moisture mozzarella cheese is made from milk, where the caseins are micellar and come in their natural high complexity of variants (alpha S1, alpha S2, beta, kappa; phosphorylated and glycosylated). The imitation mozzarella cheese is made from milk casein blend with plant-based fats, where micellar caseins from milk are renneted and dried into a milk protein concentrate. The plant-based vegan mozzarella-style cheese has no casein proteins.

[00233] A pizza fork stretch test was performed to quantitate cheese extensibility by inserting a fork into the center of freshly baked pizza and lifted until all the cheese strands broke. For this, 6g of cheese was shredded on a 4" inch tortilla topped with 4g of tomato sauce. Samples were baked at 600°F for 90 seconds. A ruler was used to measure the stretch and the test was performed immediately after removing the pizza from the oven.

[00234] The texture profile was analyzed on TA-XT plus texture analyzer with TA-55 puncture probe. 1.5-1.9 g of cheese sample was cut in 1.5 cm l x 1.5 cm w x 1 cm h dimension. The tests were performed on samples stored at 4°C and at ambient temperatures at least for 30 mins.

[00235] The NC1 mozzarella cheese analogue, made from recombinant unphosphorylated alpha S1 casein and its truncated forms as the only protein ingredient in the cheese, showed animal-derived dairy-like melt, dairy-like stretch and dairy-like texture properties in the tests performed. The pH of set cheese was 5.9.

[00236] The pizza fork stretch test showed that NC1 mozzarella cheese analogue stretched >12 inches (Table 3, FIG. 2). In comparison, the low-moisture mozzarella stretched >18 inches but the imitation mozzarella stretched only <10 inches. The plant-based vegan mozzarella-style cheese didn't show stretch, it stretched <1 inch.

[00237] The texture profile analysis showed hardness, adhesiveness, resilience, cohesion, springiness and chewiness as shown in Table 3 (Table 3, FIG. 3). NC1 mozzarella cheese analogue showed nearly the same cohesion and springiness when compared to real dairy low-moisture mozzarella (< 10% deviation), and very similar hardness and chewiness profile

(< 30% deviation). It was consistently slightly harder, more resilient, more cohesive and chewier compared to both low-moisture mozzarella as well as the imitation mozzarella cheese. In comparison, the plant-based vegan mozzarella-style cheese deviates from dairy behavior, where it is extremely hard (~ 4.6x harder), chewy (~3.9x chewier) and adhesive (~19.6x more adhesive).

[00238] Interestingly, when compared to real dairy low-moisture mozzarella, NC1 mozzarella cheese analogue shows a different trend than imitation mozzarella for adhesiveness and springiness. While imitation mozzarella is more adhesive and springier than real dairy mozzarella, NC1 mozzarella cheese analogue is less adhesive and less springy than real dairy mozzarella. Reduced adhesiveness is a favourable cheese property as it represents the force required to remove the cheese from the probe (i.e. remove it sticking to teeth).

Table 3: Cheese Properties

Cheese types	Melt valu e (fold chan ge)	Stretch (inches)	Hardnes s g	Adhesiveness s g*sec	Resilienc e %	Cohesiveness s %	Springines s %	Chewiness g
Low-moisture mozzarella	2.4x	> 18	103.88	0.55	43.37	0.78	99.14	80.12
Imitation mozzarella	2.0x	<10	94.32	0.91	44.37	0.79	136.16	99.09
Plant-based vegan mozzarella- style cheese	0.8x	< 1	477.01	10.78	49.09	0.7	93.41	313.17
NC1	1.5x	> 12	126.16	0.16	60.25	0.86	98.85	106.72

mozzarella cheese analogue								
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Example 6: Properties of Mozzarella Cheese analogue from recombinant single variant alpha casein protein with an altered formulation lacking calcium chloride.

[00239] Recombinant unphosphorylated alpha S1 casein, was used to make non-micellar mozzarella cheese analogue termed New Culture (NC2) mozzarella cheese. Casein, prewarmed coconut oil, trisodium citrate, disodium phosphate, sodium chloride and glucose were added in a beaker at concentrations specified in Table 4. To this, a mix of water, and modified potato starch were added at concentration specified in Table 4. The beaker was moved to a water bath preset at 85°C temperature and the contents were mixed using a mixing propeller at a speed of 300 rpm for 9 mins. Lactic acid was added and mixed for an additional 1 min. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 3 days. After fridge storage, the NC mozzarella cheese analogue was weighed to get yield estimation.

Table 4: Ingredient Composition and Concentration for Recombinant Single Casein Variant Cheese Analogue (NC2 mozzarella cheese analogue).

Ingredient	Concentration (% wt/wt)
Casein	18.5
Coconut oil	24.2
Trisodium citrate	1
Disodium phosphate	2
Sodium chloride	1.49
Water	49.37
Modified potato starch	2.54
Glucose	0.4
Lactic acid crystals	0.5

[00240] The NC2 mozzarella cheese analogue samples were analyzed for qualitative and quantitative parameters such as pH, moisture, melt, stretch, and texture profile. These parameters were compared to a commercially available low-moisture mozzarella cheese, a

commercially available imitation mozzarella cheese and a commercially available plant-based vegan mozzarella-style cheese (see Example 5 for further descriptions on these cheeses).

[00241] The cheese melt was quantified by a modified Schreiber melt test that used a custom-built imaging station mounted on a black magnetic hotbed. 0.5g of cheese was melted at 95°C for 15 mins on a magnetic hotbed. A time lapse of melting was recorded to measure increase in the melt area by measuring the pixels. The melt value was calculated by dividing the melted area by the unmelted area prior to melting. A melt value greater than 1 indicates melting.

[00242] An extensibility test was performed on a Texture Analyzer to quantitate cheese extensibility by inserting a 6-pronged hook into a 6 g sample of hand-cut cheese shreds placed in an extensibility rig that had been melted for 10 min at 90°C in the oven and lifted until all the cheese strands broke. Distance to failure (breakage of all strands), representing extent of cheese stretch, and work to extend, representing tensile strength needed to stretch the cheese, were quantified. The tests were performed on samples taken out of the fridge and then kept at ambient temperatures for at least 30 mins.

Table 5: Melt and stretch (extensibility) of cheese

Cheese Type	Melt Value (Fold Change)	Extensibility - Work to Extend (g*sec)	Extensibility - Distance to Failure (mm)
Low-moisture mozzarella	2.5 X	105.10	224.12
Imitation mozzarella	2.0 X	148.91	189.36
Plant-based vegan mozzarella-style cheese	0.8 X	100.21	20.48
NC2 mozzarella cheese analogue	2.1X	128.41	227.66

[00243] NC2 mozzarella cheese analogue made from recombinant unphosphorylated alpha S1 casein with a formulation lacking calcium showed a dairy-like melt, dairy-like stretch and dairy-like extensibility properties. The moisture and pH of set NC2 mozzarella cheese analogue were 45.7% and 5.7 respectively.

[00244] Surprisingly, the NC2 mozzarella cheese analogue made using the compositions described herein showed a better melt than the formulation in Example 5 and 7 and reached a melt value of 2.1x (Table 5, FIG. 4). In comparison, low-moisture mozzarella and imitation mozzarella reached melt values of 2.5x and 2.0x respectively. Plant-based vegan mozzarella-style cheese reached a melt value of 0.8x suggesting a lack of melt (and even shrinkage).

[00245] The NC2 mozzarella cheese analogue melted at a slower rate than low-moisture mozzarella, reaching its peak melt somewhat later during the modified Schreiber melt test. Imitation mozzarella and low-moisture mozzarella needed ~4-6 minutes for maximal melt, whereas NC2 mozzarella cheese analogue melted fully in ~6.5 minutes (FIG.4).

[00246] NC2 mozzarella cheese analogue showed near identical extensibility to low-moisture mozzarella and imitation mozzarella while plant-based vegan mozzarella-style cheese failed to stretch entirely (Table 5, FIG. 5). The tensile strength (indicated by work to extend values, Table 5) needed to stretch NC2 mozzarella cheese analogue was in between the range needed to stretch the low-moisture mozzarella and imitation mozzarella. The NC2 cheese analogue stretched comparably to low-moisture mozzarella and significantly better than imitation mozzarella as indicated by the distance to failure values in Table 5. The NC2 mozzarella cheese analogue stretched to a length of ~228 mm. In comparison, low-moisture mozzarella imitation mozzarella stretched to length of ~ 224mm and ~189mm respectively (Table 5).

Example 7: Properties of Mozzarella Cheese analogue from recombinant single variant alpha casein protein with a different fat composition

[00247] Recombinant unphosphorylated alpha S1 casein, was used to make non-micellar mozzarella cheese analogue termed New Culture (NC3) mozzarella cheese. Casein, water, palm stearin, canola oil, trisodium citrate, disodium phosphate, modified potato starch, and sodium chloride was added in a beaker at concentrations specified in Table 6. To this CaCl_2 was added at the concentration specified in Table 6. The beaker was moved to a water bath preset at 85°C and the contents were mixed using a mixing propeller at a speed of 300 rpm for 9 minutes. Natural flavors and lactic acid were added, and the ingredients were mixed for an additional 1 minute. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 7 days. After incubation, the NC3 mozzarella cheese analogue was weighed to get yield estimation.

Table 6: Ingredient Composition and Concentration for Recombinant Single Casein Variant Cheese Analogue (NC3 mozzarella cheese analogue)

Ingredients	Concentration (% wt/wt)
Casein	18.5
Palm Stearin	17
Canola Oil	7

Trisodium citrate	0.7
Disodium phosphate	1.3
Sodium chloride	1.5
Water	49.7
CaCl ₂	0.5
Modified potato starch	2.5
Natural flavors	1.1
Lactic acid, 88% solution	0.2

[00248] The NC3 mozzarella cheese analogue samples were analyzed for qualitative and quantitative parameters such as pH, moisture, melt, stretch, and texture profile as set forth in Example 6. These parameters were compared to low-moisture mozzarella cheese, imitation mozzarella cheese and a plant-based vegan mozzarella-style cheese (see Example 5 for further descriptions).

[00249] The cheese melt was quantified by a modified Schreiber melt test as described in Example 6. An extensibility test was performed on a TA.XTPlus Texture Analyzer to quantitate cheese extensibility as described in Example 6. The tests were performed on samples stored at 4°C.

Table 7: Melt and stretch (extensibility) of cheese

Cheese Type	Melt Value (Fold Change)	Extensibility - Work to Extend (g*sec)	Extensibility - Distance to Failure (mm)
Low-moisture mozzarella	2.5 X	373.38	227.56
Imitation mozzarella	2.0 X	155.85	224.96
Plant-based vegan mozzarella-style cheese	0.8 X	8.25	22.3
NC3 mozzarella cheese analogue	1.6 X	319.18	229.96

[00250] The texture profile was analyzed on TA-XT plus texture analyzer with TA-55 puncture probe. 1.5-1.9 g of cheese sample was cut in 1.5 cm l x 1.5 cm w x 1 cm h dimension. The tests were performed on samples stored at 4°C.

Table 8: Cheese Properties

Cheese types	Hardness g	Adhesiveness g*sec	Resilience %	Cohesiveness %	Springiness %	Chewiness g
Low-moisture mozzarella	345.7	5.84	52.38	0.80	95.60	263.53
Imitation mozzarella	205.6	6.85	53.72	0.80	99.06	163.85
Plant-based vegan mozzarella-style cheese	636.7	4.14	54.71	0.66	101.37	431.50
NC3 mozzarella cheese analogue	729.9	0.26	55.12	0.29	214.19	184.14

[00251] NC3 mozzarella cheese analogue made from recombinant unphosphorylated alpha S1 casein with a different fat composition showed dairy-like melt, dairy-like stretch and dairy-like extensibility properties. The moisture and pH of set NC3 mozzarella cheese analogue were 44.8% and 5.7 respectively.

[00252] The NC3 mozzarella cheese analogue made using the compositions described herein reached a melt value of 1.6x (Table 7, FIG. 6). In comparison, low-moisture mozzarella and imitation mozzarella reached melt values of 2.5x and 2x respectively. The plant-based vegan mozzarella-style cheese reached a melt value of 0.8x suggesting a lack of melt (and even shrinkage).

[00253] Surprisingly, NC3 mozzarella cheese analogue melts at a quicker rate than low-moisture mozzarella, reaching its peak melt sooner during the modified Schreiber melt test. Imitation mozzarella and low-moisture mozzarella needed ~4-6 minutes for maximal melt, whereas NC3 mozzarella cheese analogue melted fully in ~2 minutes (FIG.6).

[00254] This is likely due to the substitution of the coconut oil with a combination of palm stearin and canola oil.

[00255] NC3 mozzarella cheese analogue showed near identical extensibility to low-moisture mozzarella and imitation mozzarella while plant-based vegan mozzarella-style cheese failed to stretch entirely (Table 7, FIG. 7). The tensile strength (indicated by work to extend values, Table 7) needed to stretch NC1 mozzarella cheese analogue was similar to that needed to stretch the low-moisture mozzarella whereas imitation mozzarella needed significantly lower tensile strength. The NC3 cheese analogue stretched to the same extent as low-moisture mozzarella and imitation mozzarella as indicated by the distance to failure values in Table 7.

The NC3 mozzarella cheese analogue stretched to a length of ~230 mm. In comparison, low-moisture mozzarella imitation mozzarella stretched to length of ~ 228mm and ~225mm respectively (Table 7).

[00256] The texture profile analysis showed hardness, adhesiveness, resilience, cohesion, springiness and chewiness (as shown in Table 8, FIG. 8). The NC3 mozzarella cheese analogue showed nearly the same chewiness (< 10% deviation) when compared to imitation mozzarella. In comparison, plant-based vegan mozzarella-style cheese has higher chewiness (>1.5X) compared to low-moisture mozzarella and imitation mozzarella. The NC3 mozzarella cheese shows similar resilience (<10% deviation), higher hardness, springiness, and lower adhesiveness and cohesiveness compared to other commercial cheeses.

[00257] Interestingly, when compared to animal-derived dairy low-moisture mozzarella, NC3 mozzarella cheese analogue shows a different trend than imitation mozzarella for adhesiveness. While imitation mozzarella is more adhesive than real dairy mozzarella, NC3 mozzarella cheese analogue is less adhesive. Reduced adhesiveness is a favorable cheese property as it represents the force required to remove the cheese from the probe, which corresponds to stickiness in consumer's mouth (i.e. sticking to teeth when chewing).

Example 8: Properties of Mozzarella Cheese analogue from recombinant single variant alpha casein protein with an altered formulation and altered processing parameters

[00258] Recombinant unphosphorylated alpha S1 casein, was used to make non-micellar mozzarella cheese analogue termed New Culture (NC4) mozzarella cheese. Casein, water, palm stearin, canola oil, modified potato starch sodium chloride, and CaCl₂ were added in a beaker at concentrations specified in Table 9. To this, trisodium citrate and dipotassium phosphate were added at the concentration specified in Table 9. The beaker was moved to a water bath preset at 85°C and the contents were mixed using a mixing propeller at a speed of 500 rpm for 4 minutes, followed by 1 minute at 300 rpm, at which point natural flavors were also added. Lactic acid was added and the ingredients were mixed for an additional 1 minute at 300 rpm. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 7 days. After incubation, the NC4 mozzarella cheese analogue was weighed to get yield estimation.

Table 9: Ingredient Composition and Concentration for Recombinant Single Casein Variant Cheese Analogue (NC4 mozzarella cheese).

Ingredients	Concentration (% wt/wt)
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Casein	18.5
Palm Stearin	17
Canola Oil	7
Trisodium citrate	0.7
Disodium phosphate	1.3
Sodium chloride	1.5
Water	49.7
CaCl ₂	0.5
Modified potato starch	2.5
Natural flavors	1.1
Lactic acid, 88% solution	0.2

[00259] The NC4 mozzarella cheese analogue samples were analyzed for qualitative and quantitative parameters such as pH, moisture, melt, stretch, and texture profile. These parameters were compared to low-moisture mozzarella cheese, imitation mozzarella cheese and plant-based vegan mozzarella-style cheese (see Example 5 for further descriptions).

[00260] The cheese melt was quantified by a modified Schreiber melt test as described in Example 6. An extensibility test was performed on a TA.XTPlus Texture Analyzer to quantitate cheese extensibility as described in Example 6. The tests were performed on samples stored at 4°C.

Table 10: Melt and stretch (extensibility) of cheese

Cheese Type	Melt Value (Fold Change)	Extensibility - Work to Extend (g*sec)	Extensibility - Distance to Failure (mm)
Low-moisture mozzarella	2.5 X	373.37	227.56
Imitation mozzarella	2.0 X	155.85	224.96
Plant-based vegan mozzarella-style cheese	0.8 X	8.24	22.30
NC4 mozzarella cheese analogue	1.5 X	268.80	241.46

[00261] NC4 mozzarella cheese analogue made from recombinant unphosphorylated alpha S1 casein and its truncated forms as the only protein ingredient in the cheese, using a

different formulation and different processing parameters compared to examples 5, 6, and 7, also showed dairy-like melt, dairy-like stretch and dairy-like extensibility properties. The moisture and pH of set NC4 mozzarella cheese analogue were 48.1% and 5.7 respectively.

[00262] The NC4 mozzarella cheese analogue using the compositions described herein reached a melt value of 1.5x (Table 10, FIG.9). In comparison, low-moisture mozzarella and imitation mozzarella reached melt values of 2.5x and 2x respectively. The plant-based vegan mozzarella-style cheese reached a melt value of 0.8x suggesting a lack of melt (and even shrinkage).

[00263] More notably, NC4 mozzarella cheese analogue melted at a quicker rate than low-moisture mozzarella, reaching its peak melt sooner during the modified Schreiber melt test. Imitation mozzarella and low-moisture mozzarella needed ~4-6 minutes for maximal melt, whereas NC4 mozzarella cheese analogue melted fully in ~2 minutes (FIG.9).

[00264] NC4 mozzarella cheese analogue showed similar extensibility to low-moisture mozzarella and imitation mozzarella while plant-based vegan mozzarella-style cheese failed to stretch entirely (Table 10, FIG. 10). The tensile strength (indicated by work to extend values, Table 10) needed to stretch NC4 mozzarella cheese analogue was in the range of tensile strength of low-moisture mozzarella and imitation mozzarella. The NC4 cheese analogue stretched better than low-moisture mozzarella and imitation mozzarella as indicated by the distance to failure values in Table 10. The NC4 mozzarella cheese analogue stretched to a length of ~241 mm. In comparison, low-moisture mozzarella and imitation mozzarella stretched to length of ~228mm and ~225mm respectively (Table 10, FIG.10).

Example 9: Properties of mozzarella cheese analogue from recombinant single variant alpha casein protein from a different species

[00265] Recombinant unphosphorylated ovine alpha S1 casein was used to make non-micellar mozzarella cheese analogue termed New Culture (NC5) mozzarella cheese. Casein, water, palm stearin, canola oil, trisodium citrate, disodium phosphate, modified potato starch, and sodium chloride were added to a beaker at concentrations specified in Table 11. The beaker was moved to a water bath preset at 85°C, and the contents were mixed using a mixing propeller at a speed of 300 rpm for 9 minutes. Natural flavors were added, and the ingredients were mixed for an additional 1 minute. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 5 days. After incubation, the NC5 mozzarella cheese analogue was weighed for yield estimation.

Table 11: Ingredient composition and concentration for recombinant single casein variant cheese analogue (NC5 mozzarella cheese).

Ingredients	Final concentration (%)
Casein	22.1
Palm Stearin	16.9
Canola Oil	7.2
Trisodium citrate	0.7
Disodium phosphate	1.3
Sodium chloride	1.5
Water	47.25
Modified potato starch	2.5
Natural flavors	0.55

[00266] The NC5 mozzarella cheese analogue sample was analyzed for qualitative and quantitative parameters such as pH, moisture, melt, stretch, and texture profile as described in example 7. These parameters were compared to low-moisture mozzarella, imitation mozzarella, and plant-based vegan mozzarella-style cheese (see example 7 for further descriptions).

Table 12: Melt and stretch (extensibility) of cheese

Cheese Type	Melt Value (Fold Change)	Extensibility - Work to Extend (g*sec)	Extensibility - Distance to Failure (mm)
Low-moisture mozzarella	2.3x	338	224
Imitation mozzarella	1.8x	159	222
Plant-based vegan mozzarella cheese slices	0.8x	12	20
NC5 mozzarella cheese analogue	1.3x	59	191

[00267] The NC5 mozzarella cheese analogue made from recombinant unphosphorylated ovine alpha S1 casein as the only protein ingredient in the cheese showed dairy-like melt, dairy-like stretch and dairy-like extensibility properties of a mozzarella cheese. The moisture and pH of the NC5 mozzarella cheese analogue were 45.6% and 6.85 respectively.

[00268] The NC5 mozzarella cheese analogue had a higher melt value than plant-based mozzarella cheese which exhibited a lack of melt (and even shrinkage) (Table 12, FIG.11). The melt rate of NC5 mozzarella cheese analogue was slightly faster compared to the melt of low-moisture mozzarella and imitation mozzarella, reaching its peak melt sooner during the modified Schreiber melt test. Imitation mozzarella and low-moisture mozzarella needed >10 minutes for maximal melt, whereas NC5 mozzarella cheese analogue melted fully in ~7 minutes (FIG.11).

[00269] NC5 mozzarella cheese analogue showed similar extensibility to low-moisture mozzarella and imitation mozzarella (19.1 cm compared to 22.4 and 22.2 cm respectively), while plant-based mozzarella failed to stretch entirely (< 2.5 cm) (Table 12, FIG. 12). The stretch and melt of NC5 cheese analogue were inferior to the stretch and melt of NC 1, 2, and 4 cheese analogues.

[00270] The texture profile analysis results for hardness, adhesiveness, resilience, cohesion, springiness, and chewiness are shown in Table 13. The NC5 mozzarella cheese analogue showed hardness and chewiness in the range of low-moisture mozzarella and imitation mozzarella. In comparison, the plant-based mozzarella cheese deviated from dairy behavior, and it exhibited more chewiness (~1.6x) and higher adhesiveness (~1.5x) when compared to the low-moisture mozzarella.

[00271] Interestingly, the NC5 mozzarella cheese analogue showed an improvement in adhesiveness that was not found with imitation mozzarella. While imitation mozzarella is more adhesive than low-moisture mozzarella, the NC5 mozzarella cheese analogue is less adhesive. Reduced adhesiveness is a favorable cheese property. Adhesiveness represents the force required to remove the cheese from the probe, and can be used as a proxy for whether the cheese will be adhesive to teeth when eaten.

Table 13: Cheese properties

Cheese types	Hardness g	Adhesiveness g*sec	Resilience %	Cohesiveness %	Springiness %	Chewiness
Low-moisture mozzarella	380	4.1	54.5	0.7	97.2	245.9
Imitation mozzarella	240	5.8	54.5	0.7	97.7	160.1
Plant-based vegan mozzarella cheese slices	649	6.2	52.6	0.7	103.1	394.7

NC5 mozzarella cheese analogue	310	2.3	52	0.45	95	220
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Example 10: Properties of yogurt and yogurt drink made from recombinant single variant alpha casein

[00272] Recombinant unphosphorylated bovine alpha S1 casein, native alpha casein (a mix of alpha S1 and alpha S2, with native phosphorylation, purified from cow's milk) and commercially available micellar casein were used to make yogurt analogue and yogurt-drink analogue. For comparison, yogurt and yogurt drink were also made from commercially available homogenized milk. Protein, trisodium citrate, disodium phosphate, calcium chloride, and carbohydrate (sugar) were mixed in water at concentrations specified in Table 14. To this solution, lecithin and prewarmed fat were added at the concentrations listed in Table 14. The mixture was homogenized using an ultrasonic sonicator with a 16 mm probe at 90% power for 4 mins. The mixture was pasteurized at 90°C for 10 min in a water bath and then cooled to 40 C. 0.1 g of standard lactic acid bacterial culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was added. The milk-like colloid was fermented for 14 hrs. The yogurt making process for whole milk consisted only of the pasteurization and fermentation steps described above.

Table 14: Yogurt and Yogurt analogue formulation

Ingredients %	*Whole milk (g)	No protein (g)	Micellar casein/ dairy alpha/ Recombinant alpha S1 (g)
Protein	3.33	0	3.3
Fat	3.33	4.3	4.3
Phosphates	0.100	0.113	0.113
Citrate	0.08	0.115	0.115
Calcium	0.129	0.036	0.036
Lecithin	0	1	1
Carbohydrate	5	5	5
Water	87.5	89.436	86.136

*Ingredients in milk were derived from nutritional fact panel

[00273] The yogurts and yogurt analogues were analyzed for qualitative and quantitative parameters such as pH, smell, appearance, relative viscosity, and texture.

[00274] The recombinant alpha S1 casein yogurt analogue had a milk yogurt-like smell and appearance, fermented well and showed milk yogurt-like textural properties as shown in Table 15. The starting pH of the milk and milk-like solutions were in the range of 6.8 -7.4 which dropped to 4 - 4.8 after 14 hrs of fermentation (Table 15). All the yogurt analogues except the one with no protein had the typical smell of fermented yogurt made from milk.

[00275] The yogurt analogues made from recombinant alpha S1 casein, micellar casein, and native alpha casein turned to gel-like colloid that had stable emulsion after the fermentation (FIG. 13). In comparison, the no-protein milk-like solution resulted in phase separation upon fermentation and remained in the liquid form (FIG. 13). The yogurt analogue formulation does not include any added thickeners, instead relying on protein to form a yogurt-like product. All yogurt analogues showed some level of syneresis.

[00276] Yogurt structure was analyzed using the cyclic test on the TA.XT Plus Texture Analyzer equipped with a TA-18 ½” ball probe. This method breaks down yogurt structure through a series of 10 consecutive sample insertions that give insight into extent of deformation over time. Firmness (g) is quantified as the probe penetrates the sample, as well as tackiness (g) and adhesiveness (g*sec). This test also makes it possible to infer relative viscosity through the work required to perform each cycle (insertion and removal of probe from sample). It can be assumed that the more work required to complete each cycle, the more viscous and robust the yogurt is. The test was performed on the set, undisturbed and cooled (4 °C) yogurt or yogurt analogue. Since the no-protein sample remained liquid, texture analysis couldn't be performed on that sample.

[00277] The recombinant alpha S1 yogurt analogue had nearly identical relative viscosity to milk yogurt (Table 15). The recombinant alpha S1 casein was only 15% less sticky than milk yogurt. In comparison, native alpha casein and micellar casein yogurt analogues were 26% and 75% less sticky than milk yogurt (Table 15).

Table 15: Yogurt and yogurt analogue properties made from different proteins.

	pH at 0 hrs	pH after 14 hrs	Firmness (g)	Work (g.sec)	Tack (g)	Work of Stickiness (g.sec)	Relative viscosity (slope of work (g.sec))
Milk	6.8	4	25.355	44.685	7.5	6.521	2.65
Micellar casein	7.1	4.3	10.65	21.261	2.4	1.636	3.02
Native alpha casein	7.1	4.5	142	216.753	8.4	4.809	2.39

Recombinant alpha S1 casein	6.9	4.4	74.14	119.625	4.9	5.52	2.66
No protein	7.4	4.2	-	-	-	-	-

[00278] The tackiness of native alpha casein yogurt analogue was 12% higher than for milk-derived yogurt. In comparison, micellar casein and recombinant alpha S1 casein yogurt analogues were less tacky than milk-derived yogurt, 36% and 68% less tacky respectively. While micellar casein yogurt analogues were less firm, native alpha casein and recombinant alpha S1 casein yogurt analogues were firmer compared to milk yogurt (Table 15).

[00279] The yogurt drink analogue was made by mixing the yogurt or yogurt analogue using a handheld homogenizer for 15 sec. The drink analogues were analyzed for smoothness, texture, appearance, and smell in comparison to the yogurt drink made from milk. The recombinant alpha S1 casein and native alpha casein yogurt drinks were similar in smoothness, texture, appearance, and smell (aroma) to milk yogurt-drink and micellar casein yogurt-drink analogue (FIG. 14).

Example 11: Properties of mozzarella cheese analogue made from alpha casein with varying phosphorylation levels and varying calcium in the formulation

[00280] Recombinant unphosphorylated bovine alpha S1 casein, native alpha casein (a mix of alpha S1 and alpha S2, with native phosphorylation, purified from cow's milk), and 70% dephosphorylated alpha casein (a mix of alpha S1 and alpha S2 casein enzymatically 70% dephosphorylated after being purified from milk, sourced from Sigma Aldrich) were used to make non-micellar mozzarella cheese analogue termed as NC6-8 with varying amount of CaCl₂ outlined in Table 16. Casein, water, palm stearin, canola oil, trisodium citrate, disodium phosphate, CaCl₂, starch, and sodium chloride were added to a beaker at concentrations specified in Table 17. The formulations in Table 17 vary by the amount of calcium chloride added. The beaker was moved to a water bath preset at 85°C, and the contents were mixed using a mixing propeller at a speed of 300 rpm for 9 minutes. Natural flavors were added, and the ingredients were mixed for an additional 1 minute. The resulting mixture turned to a homogeneous non-micellar mass, transferred to standard molds, and allowed to sit in the fridge for 5 days. After incubation, the NC6-8 mozzarella cheese analogues were weighed for yield estimation.

Table 16: List of New Culture alpha casein cheese analogues

NC cheeses	Protein	Calcium chloride (% w/w)
NC 6.1	Recombinant unphosphorylated alpha S1 casein	0
NC 6.2		0.5 (10mg of calcium/gram of casein)
NC 6.3		1 (20mg of calcium/gram of casein)
NC 7.1	70% dephosphorylated alpha casein (alpha S1+ alpha S2 casein)	0
NC 7.2		0.5 (10mg of calcium/gram of casein)
NC 7.3		1 (20mg of calcium/gram of casein)
NC 8.1	Native alpha casein (alpha S1 + alpha S2 casein)	0
NC 8.2		0.5 (10mg of calcium/gram of casein)
NC 8.3		1 (20mg of calcium/gram of casein)

Table 17: Ingredient composition of NC 6-8 mozzarella cheese analogues.

	Formulation 1 0% CaCl ₂	Formulation 2 0.5% CaCl ₂ (10mg of calcium/gram of casein)	Formulation 3 1% CaCl ₂ (20 mg of calcium/g of casein)
Ingredients	Final concentration (% w/w)		
Casein	18.5	18.5	18.5
Palm Stearin	16.7	16.7	16.7
Canola Oil	7.2	7.2	7.2
Trisodium citrate	0.7	0.7	0.7
Disodium phosphate	1.3	1.3	1.3
Sodium chloride	1.5	1.5	1.5
Water	50.9	50.4	49.9
Modified potato starch	2.5	2.5	2.5
Natural flavors	0.6	0.6	0.6
8.8% lactic acid	0.1	0.1	0.1
CaCl ₂	0	0.5	1

[00281] The NC6-8 mozzarella cheese analogue samples were analyzed for qualitative and quantitative parameters such as pH, moisture, melt, stretch, and texture profile as described in example 7. Additional qualitative properties such as the appearance of cheese/cheese

analogues after melt and cheese/cheese analogues' strand quality were noted as shown in Table 18. These parameters were compared between the tested cheese analogues, animal-derived low-moisture mozzarella, animal-derived imitation mozzarella, and plant-based vegan mozzarella-style cheese.

Table 18: Cheese and cheese analogue properties

	Recombinant unphosphorylated alpha S1 casein			70% dephosphorylated alpha casein			Native alpha casein			Benchmark cheeses		
	NC 6.1 (no CaCl ₂)	NC 6.2 (0.5% CaCl ₂)	NC 6.3 (1% CaCl ₂)	NC 7.1 (no CaCl ₂)	NC 7.2 (0.5% CaCl ₂)	NC 7.3 (1% CaCl ₂)	NC 8.1 (no CaCl ₂)	NC 8.2 (0.5% CaCl ₂)	NC 8.3 (1% CaCl ₂)	Low-moisture mozzarella	Imitation mozzarella	Plant-based vegan mozzarella cheese slices
Melt (fold increase)	1.81	1.6	1.27	2.06	2.15	2.31	4.58	1.76	1.34	2.42	1.77	1.01
Cheese appearance after melt	opaque	opaque	opaque	translucent edges	less translucent edges	opaque	transparent	translucent edges	opaque	opaque	opaque	opaque
Stretch (mm)	227.46	240.36	238.86	227.56	240.46	226.73	228.26	227.56	248.26	223.86	223.48	20.08
Stretch quality based on strand robustness (0-5)	1	4	4.5	1	1	2	2.5	2.5	3.5	5	4	0
Hardness (g)	367.95	363.72	488.45	379.13	408.13	470.95	368.96	353.03	361.8	380.1	239.6	649.1
Adhesiveness (g*sec)	0.92	0.25	0.42	2.54	1.47	1.03	3.81	3.34	2.62	4.1	5.8	6.2

*0.5% CaCl₂ and 1% CaCl₂ is equivalent to using 10 mg and 20 mg of calcium ion per gram of casein respectively.

[00282] The moisture and pH of the NC6-8 mozzarella cheese analogue were 47.2-49.4% and 5.7- 6.7, respectively.

[00283] Cheese analogues made from native alpha casein showed a melt response to varying amounts of added calcium, where they melted better and spread more under reduced amounts of calcium; however, the cheese analogues with reduced calcium upon melting turned to a transparent/translucent liquid suggesting the elimination of a cheese-like emulsion. Surprisingly, cheese analogues made from recombinant unphosphorylated alpha S1 casein showed a similar trend of melt and spread responsiveness to reduced calcium amounts, while preserving the cheese-like emulsion properties in lower calcium conditions (Table 18). In comparison, cheese analogues made from 70% dephosphorylated alpha casein didn't show a melt response to varied calcium amounts, and upon melting, reduced calcium samples also formed translucent edges indicating instability of cheese-like emulsions.

[00284] All the tested cheese analogues stretched comparable to dairy-like cheeses in terms of length of stretch. Table 18 shows qualitatively assigned stretch quality metrics from 0-5, where 0 indicates the poorest quality and 5 indicates the highest quality stretch. These values are based on strand robustness and thickness observed while stretching the cheese analogues using TA.XT texture analyzer. The cheese analogues made from 70% dephosphorylated alpha casein and native alpha casein showed mild improvement in stretch quality upon calcium addition. Surprisingly, the cheese analogues made from recombinant unphosphorylated alpha S1 casein showed significant improvement in stretch quality, with the high calcium condition leading to stretch quality comparable to animal-derived mozzarella, and with even the low/medium calcium condition leading to stretch quality comparable to milk-derived micellar imitation mozzarella. This strong dose-response to calcium amount was observed uniquely in cheese analogues made from recombinant unphosphorylated alpha S1 casein.

[00285] The textural properties of cheese analogues are outlined in Table 18. The cheese analogue made from recombinant alpha S1 casein showed a significant reduction in adhesiveness. In comparison, the cheese analogues made from native and 70% dephosphorylated alpha casein had only slightly reduced adhesiveness. The adhesiveness of analogue cheeses also decreased upon increasing the calcium amount in the formulation.

[00286] The cheese analogues made from native alpha casein were similarly hard/firm (~5% softer) as low-moisture mozzarella from milk, no matter the calcium dosage. Surprisingly, recombinant unphosphorylated alpha S1 casein cheese analogues were also similarly hard (~5% softer compared to low-moisture mozzarella) for no-calcium as well as for low/medium calcium conditions. In comparison, analogues with 70% dephosphorylated alpha casein showed increased hardness for even low/medium calcium conditions (>5% harder compared to low-moisture mozzarella and >10% harder compared to cheese analogues made from native alpha casein or recombinant unphosphorylated alpha S1 casein). Increased hardness, as typically noted for plant-based cheese analogues, is an undesirable property of cheese analogues.

[00287] Other textural properties like chewiness, cohesiveness, springiness, and resilience were also measured, which didn't show notable differences among the tested conditions.

[00288] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now

occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A consumable composition comprising a recombinant single variant of an alpha casein protein, wherein the single variant provides at least one dairy-like property to the consumable composition selected from the group consisting of adhesiveness, stretch, texture, mouthfeel, melt, browning, hardness, creaminess, taste, smell, and flexibility, wherein the recombinant single variant is not an animal-derived casein and has not been physically dissociated from a casein micelle; wherein the dairy-like property is provided substantially from the recombinant single variant casein protein; and wherein the consumable composition is comparable to a dairy-derived consumable composition in at least one of the dairy-like properties.
2. The consumable composition of claim 1, wherein the consumable composition comprises one or more dairy-like properties selected from the group consisting of adhesiveness, stretch, firmness, viscosity, tackiness, chewiness, resilience, springiness, mouthfeel, melt, hardness, creaminess, and flexibility.
3. The consumable composition of any one of claims 1-2, wherein the casein content of the consumable composition comprises substantially the recombinant single variant of the alpha casein protein.
4. The consumable composition of any one of claims 1-3, wherein the casein content of the consumable composition comprises only the recombinant single variant of the alpha casein protein.
5. The consumable composition of any one of claims 1-4, wherein the recombinant single variant of the alpha casein protein comprises at least 95% or at least 97% of the casein content in the consumable composition.
6. The consumable composition of any one of claims 1-5, wherein the recombinant single variant of the alpha casein protein comprises at least 99% of the casein content in the consumable composition.
7. The consumable composition of any one of claims 1-6, wherein the consumable composition lacks any additional caseins other than the recombinant single variant of the alpha casein protein.
8. The consumable composition of any one of claims 1-7, wherein the single variant of the alpha casein protein is not derived from caseinate.
9. The consumable composition of any of claims 1-8, wherein the composition is free from any animal-produced proteins.

10. The consumable composition of any one of claims 1-9, wherein the composition lacks any other animal-derived dairy proteins.
11. The consumable composition of any of claims 1-10, wherein at least one dairy-like property is comparable to or improved as compared to a milk-derived consumable composition; wherein the milk-derived consumable composition is identical to the consumable composition in all ingredients except the milk-derived consumable composition comprises milk, one or more milk-derived proteins or a milk-derived ingredient instead of or in addition to the recombinant single variant of the alpha casein protein.
12. The consumable composition of any of claims 1-11, wherein at least one dairy-like property is comparable to or improved as compared to a micellar casein composition wherein the micellar casein composition is identical to the consumable composition in all ingredients except the micellar casein composition comprises micellar casein isolated from milk instead of or in addition to the recombinant single variant of the alpha casein protein.
13. The consumable composition of any of claims 1-12, wherein the recombinant single variant is an alpha casein protein that comprises at least one non-native post-translational modification.
14. The consumable composition of claim 13, wherein the recombinant single variant of the alpha casein protein further comprises at least one native post-translational modification.
15. The consumable composition of any of claims 1-14, wherein the recombinant single variant is an alpha casein protein lacks one or more post-translational modifications of a native alpha casein protein.
16. The consumable composition of claim 15, wherein the single variant of the alpha casein protein further comprises at least one non-native post-translational modification.
17. The consumable composition of claim 16, wherein the single variant of the alpha casein protein is not post-translational modified.
18. The consumable composition of claim 17, wherein the single variant of the alpha casein protein is not phosphorylated.
19. The consumable composition of any one of claims 1-18, wherein the single variant of the alpha casein protein is an alpha-s1 casein protein.
20. The consumable composition of any one of claims 1-19, wherein the single variant of the alpha casein protein is an alpha-s2 casein protein.
21. The consumable composition of any of claims 1-20, wherein the composition comprises a full-length alpha casein protein.

22. The consumable composition of any of claims 1-21, wherein the single variant of the alpha casein protein comprises any one of SEQ ID Nos. 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.
23. The consumable composition of any of claims 1-22, wherein the single variant of the alpha casein protein comprises an amino acid sequence of a bovine, caprine or ovine alpha casein protein or any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56 or a sequence with at least 70%, 80%, 85% or 90% identity to any one of SEQ ID NOs.: 2, 3, 14, 15, 26, 27, 29, 30, 32, 33, 35, 36, 38, 40, 41, 43, 44, 46, 47, 49, 50, 52, 53, 55 or 56.
24. The consumable composition of any of claims 1-23, wherein the single variant of the alpha casein protein comprises one or more non-native amino acids at the N-terminus.
25. The consumable composition of claim 24, wherein the single variant of the alpha casein protein comprises a non-native methionine at the N-terminal position.
26. The consumable composition of any one of claims 1-25, wherein the single variant of the alpha casein protein is not derived from casein micelles.
27. A dairy product analogue comprising any of the consumable compositions of claims 1-26, wherein the analogue is selected from the group consisting of a cheese analogue, a yogurt analogue, a cream analogue, and an ice cream analogue.
28. The dairy product analogue of claim 27, wherein the consumable composition comprises the recombinant single variant alpha casein.
29. The dairy product analogue of claim 27 or 28, further comprising a fat or oil from a non-animal source.
30. The dairy product analogue of any one of claims 27-29, wherein the dairy product analogue lacks any animal-derived dairy proteins.
31. The dairy product analogue of any of claims 27-30, wherein the dairy product analogue lacks any other casein proteins.
32. The dairy product analogue of any of claims 27-31, wherein the recombinant single variant of the alpha casein protein is not comprised in a micellar form within the dairy product analogue.
33. The dairy product analogue according to any of claims 27-32, further comprising one or more of (a) a plant-derived oil; (b) a plant-derived starch; (c) a sugar; and (d) a salt.
34. The dairy product analogue of any of claims 27-33, wherein the dairy product analogue is a cheese analogue.

35. The dairy product analogue of claim 34, wherein the cheese analogue is a mozzarella analogue, a cheddar analogue or a parmesan analogue.
36. The dairy product analogue of claim 34 or claim 35, wherein the cheese analogue is a low-moisture cheese analogue.
37. The dairy product analogue of claim 34 or claim 35, wherein the cheese analogue is a soft cheese analogue.
38. The dairy product analogue of claim 34 or claim 35, wherein the cheese analogue is a hard cheese analogue.
39. The dairy product analogue of any one of claims 34-38, wherein the cheese analogue is a mozzarella analogue and wherein the single variant of the casein protein is an alpha casein.
40. The dairy product of analogue of any one of claims 34-39, wherein the alpha casein is an alpha S1 casein.
41. The dairy product analogue of claim 40, wherein the alpha S1 casein is a bovine alpha S1 casein.
42. The dairy product analogue of claim 40 or 41, wherein the alpha S1 casein is a full-length casein.
43. The dairy product of analogue of any one of claims 34-39, wherein the alpha casein is an alpha S2 casein.
44. The dairy product analogue of claim 43, wherein the alpha S2 casein is a bovine alpha S2 casein.
45. The dairy product analogue of claim 44, wherein the alpha S2 casein is a full-length casein.
46. The dairy product analogue of any one of claims 34-45, wherein a stretch of the cheese analogue is comparable to or improved relative to a stretch of a dairy-derived cheese or dairy-derived cheese analogue.
47. The dairy product analogue of any one of claims 34-46, wherein the cheese analogue has a comparable or greater melt area/time as compared to the melt area/time of a dairy-derived cheese or dairy-derived cheese analogue.
48. The dairy product analogue of any one of claims 34-47, wherein an extensibility of the cheese analogue is comparable to or improved relative to an extensibility of a dairy-derived cheese or dairy-derived cheese analogue.
49. The dairy product analogue of any one of claims 34-48, wherein a texture of the cheese analogue is comparable to or improved relative to a texture of a dairy-derived cheese or dairy-derived cheese analogue.

50. The dairy product analogue of any one of claims 34-49, wherein an adhesiveness of the cheese analogue is reduced relative to an adhesiveness of a dairy-derived cheese or dairy-derived cheese analogue.
51. The dairy product analogue of any one of claims 34-50, wherein the dairy-derived cheese or dairy-derived cheese analogue comprises micellar casein.
52. The dairy product analogue of any one of claims 34-51, wherein a stretch of the cheese analogue is improved relative to a stretch of a plant-derived cheese analogue.
53. The dairy product analogue of any one of claims 34-52, wherein a melt of the cheese analogue is improved relative to a melt of a plant-derived cheese analogue.
54. The dairy product analogue of any one of claims 34-53, wherein a texture of the cheese analogue is improved relative to a texture of a plant-derived cheese analogue.
55. The dairy product analogue of any one of claims 34-54, wherein an adhesiveness of the cheese analogue is reduced relative to an adhesiveness of a plant-derived cheese analogue.
56. The dairy product analogue of any one of claims 34-55, wherein the cheese analogue comprises from at least 5% single variant of the casein protein w/w.
57. The dairy product analogue of any one of claims 34-56, wherein the cheese analogue comprises from about 5% to about 30% single variant of the casein protein w/w.
58. The dairy product analogue of any one of claims 34-57, wherein the cheese analogue comprises from about 10% to about 25% single variant of the casein protein w/w.
59. The dairy product analogue of any one of claims 34-58, wherein the cheese analogue comprises from about 15% to about 25% single variant of the casein protein w/w.
60. The dairy product analogue of any one of claims 34-59, wherein the cheese analogue comprises at most 25mg of calcium per gram of casein.
61. The dairy product analogue of any one of claims 34-60, wherein the cheese analogue comprises from 0mg to 25mg of calcium per gram of casein.
62. The dairy product analogue of any one of claims 34-61, wherein the cheese analogue comprises from 0mg to 20mg of calcium per gram of casein.
63. The dairy product analogue of any one of claims 34-62, wherein the cheese analogue comprises from 0mg to 10mg of calcium per gram of casein.
64. The dairy product analogue of any one of claims 34-62, wherein the cheese analogue comprises from 5mg to 15mg of calcium per gram of casein.
65. The dairy product analogue of any one of claims 34-64, wherein the cheese analogue comprises about 0mg of calcium per gram of casein.

66. The dairy product analogue of any one of claims 34-64, wherein the cheese analogue comprises about 10mg of calcium per gram of casein.
67. The dairy product analogue of any one of claims 34-66, wherein the cheese analogue comprises from about 15% to about 30% fats w/w.
68. The dairy product analogue of any one of claims 34-67, wherein the cheese analogue comprises from about 18% to about 28% fats w/w.
69. The dairy product analogue of any one of claims 34-68, wherein the cheese analogue comprises from about 20% to about 25% fats w/w.
70. The dairy product analogue of any one of claims 34-69, wherein the cheese analogue comprises from about 0.5% to about 4% starch w/w.
71. The dairy product analogue of any one of claims 34-70, wherein the cheese analogue comprises from about 1% to about 3% starch w/w.
72. The dairy product analogue of any one of claims 34-71, wherein the cheese analogue comprises from about 2% to about 3% starch w/w.
73. The dairy product analogue of any one of claims 34-72, wherein the cheese analogue comprises at most 10% starch w/w.
74. The dairy product analogue of any one of claims 34-73, wherein the cheese analogue comprises at most 5% starch w/w.
75. The dairy product analogue of any one of claims 34-74, wherein a ratio of the recombinant single variant alpha casein to emulsifying salts is from 12:1 to 6:1.
76. The dairy product analogue of any one of claims 34-75, wherein the cheese analogue does not comprise emulsifiers other than the emulsifying salts.
77. The dairy product analogue of any of claims 27-33, wherein the dairy product analogue is a yogurt analogue.
78. The dairy product analogue of claim 77, wherein the single variant of the casein protein is an alpha casein.
79. The dairy product of analogue of claim 78, wherein the alpha casein is an alpha S1 casein.
80. The dairy product analogue of claim 79, wherein the alpha S1 casein is a bovine alpha S1 casein.
81. The dairy product analogue of any one of claims 77-80, wherein the alpha S1 casein is a full-length casein.
82. The dairy product of analogue of claim 78, wherein the alpha casein is an alpha S2 casein.

83. The dairy product analogue of claim 82, wherein the alpha S2 casein is a bovine alpha S2 casein.
84. The dairy product analogue of any one of claims 82-83, wherein the alpha S2 casein is a full-length casein.
85. The dairy product analogue of any one of claims 77-84, wherein an emulsification of the yogurt analogue is comparable to or improved relative to an emulsification of a dairy-derived yogurt or dairy-derived yogurt analogue.
86. The dairy product analogue of any one of claims 77-85, wherein firmness, tackiness, or viscosity of the yogurt analogue is comparable to or improved relative to a dairy-derived yogurt or dairy-derived yogurt analogue.
87. The dairy product analogue of any one of claims 77-86, wherein adhesiveness of the yogurt analogue is reduced relative to a dairy-derived yogurt or dairy-derived yogurt analogue.
88. The dairy product analogue of any one of claims 85-87, wherein the dairy-derived yogurt or dairy-derived yogurt analogue comprises micellar casein.
89. The dairy product analogue of any one of claims 85-87, wherein the dairy-derived yogurt or dairy-derived yogurt analogue is identical to the dairy product analogue in all ingredients except the dairy-derived yogurt or dairy-derived yogurt analogue comprises micellar casein instead of the recombinant single variant of the alpha casein protein.
90. The dairy product analogue of any one of claims 77-89, wherein an emulsification of the yogurt analogue is comparable to or improved relative to an emulsification of a plant-derived yogurt analogue.
91. The dairy product analogue of any one of claims 77-90, wherein firmness, tackiness, or viscosity of the yogurt analogue is comparable to or improved relative to a plant-derived yogurt analogue.
92. The dairy product analogue of any one of claims 77-91, wherein adhesiveness of the yogurt analogue is reduced relative to a plant-derived yogurt analogue.
93. The dairy product analogue of any one of claims 90-92, wherein the plant-derived yogurt analogue lacks any dairy-proteins.
94. The dairy product analogue of any one of claims 77-93, wherein the yogurt analogue comprises from about 1% to about 4% single variant of the casein protein w/w.
95. The dairy product analogue of any one of claims 77-94, wherein the yogurt analogue comprises from about 2% to about 4% single variant of the casein protein w/w.
96. The dairy product analogue of any one of claims 77-95, wherein the yogurt analogue comprises from about 2% to about 6% fats w/w.

97. The dairy product analogue of any one of claims 77-96, wherein the yogurt analogue comprises from about 4% to about 8% carbohydrates w/w.
98. The dairy product analogue of any of claims 27-33, wherein the dairy product analogue is a beverage.
99. The dairy product analogue of claim 98, wherein the single variant of the casein protein is an alpha casein.
100. The dairy product analogue of any one of claims 98-99, wherein a smoothness of the beverage is comparable to or improved relative to a smoothness of a dairy-derived beverage.
101. The dairy product analogue of any one of claims 98-100, wherein a texture of the beverage is comparable to or improved relative to a texture of a dairy-derived beverage.
102. The dairy product analogue of any one of claims 98-101, wherein emulsification of the beverage is comparable to or improved relative to a dairy-derived beverage.
103. The dairy product analogue of any one of claims 98-102, wherein the beverage comprises from about 0.5% to about 10% single variant of the casein protein w/w.
104. The dairy product analogue of any one of claims 98-104, wherein the beverage comprises from about 0.1% to about 6% fats w/w.