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(54) **Titre : COMPOSITIONS COMPRENANT UNE CASEINE ET PROCEDES DE PRODUCTION DE CELLES-CI**
(54) **Title: COMPOSITIONS COMPRISING A CASEIN AND METHODS OF PRODUCING THE SAME**

(57) **Abrégé/Abstract:**
Disclosed herein are methods and compositions including casein, and methods for making these compositions.

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ABSTRACT

Disclosed herein are methods and compositions including casein, and methods for making these compositions.

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COMPOSITIONS COMPRISING A CASEIN AND METHODS OF PRODUCING THE SAME

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 62/040,393, filed on August 21, 2014.

FIELD OF THE INVENTION

The invention is directed to dairy substitutes, methods of manufacturing the same,and compositions comprising animal-free milk fats and proteins for food applications, such as milk, butter, cheese, yogurt, and cream.

BACKGROUND OF THE INVENTION

The global dairy market is estimated at $500 billion with an average annual growth rate of 4%. Bovine milk attributes to a significant portion of the market whereas plant-based alternatives account for $1 billion in the US and an estimated $700 million is estimated for lactose-intolerant milk. Bovine milk is known to have four specific caseins, α-s1-casein, α-s2-casein, β-casein, and κ-casein. Mammal- or mammalian-produced milk is a very complex fluid that includes several thousand components (e.g., if all triglycerides are identified). Mammal- or mammalin-produced milk includes water, variety of different lipids, sugar, a variety of different proteins, and a variety of different inorganic salts and compounds (see, e.g., Boland and Thompson (Eds), Milk Proteins from Expression to Food, Academic Press, 2014). Although mammal-produced milk, such as bovine milk, is considered by many to be an ideal source of nutrition, various milk alternatives to mammal- or mammalian-produced milk (e.g., bovine milk), such as plant- or nut-based milks, e.g., soy, almond, or coconut milk, have been pursued for reasons related to mammal- or mammalian-produced milk's allergenicity, lactose intolerance of certain components, personal preference, and the perceived environmental benefits of a reduced dairy industry.

For example, the environmental impact resulting from dairy effluent can result in significant levels of nitrate which has the potential to contaminate groundwater.

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Groundwater forms the main source of water supply for many towns and farms where surface water supplies are limited. In the US, half the population relies completely or partially on groundwater, and similar figures are available for Europe. The presence of foodborne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Outbreaks of disease in humans have been traced to the consumption of unpasteurized milk and have also been traced back to pasteurized milk. The major contaminants usually encountered in milk and milk products include pesticide residues, heavy metals, and aflatoxin M1 (Awasthi et al., *Indian J. Public Health* 56:95-99, 2012).

Existing dairy milk alternatives, such as soy, almond, or coconut milk fall short both in flavor and in functionality; moreover, a large part of the industrial and cultural significance of dairy milk stems from its usefulness in derivative products, such as cheese, yogurt, cream, or butter. Non-dairy plant-based milks, while addressing environmental and health concerns (and while providing adequate flavor for a small segment of the population), almost universally fail to form such derivative products when subjected to the same processes used for dairy milk.

What is needed, therefore, is a dairy substitute or composition that has desirable flavor and performance characteristics, e.g., a composition that replicates dairy flavors, minimizes foodborne pathogens, and has a lower environmental impact in production, while retaining the ability to be used for derivative or downstream applications of dairy milk and while providing a similar nutritional profile as a mammal- or mammalian-produced milk.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that only a subset of components in mammal-produced milk can be used to generate a composition that has a similar flavor, a similar appearance, a similar nutritional value, a similar aroma, and a similar mouth feel of mammal-produced milk.

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Provided herein are compositions including: about 0.3 g/L to about 1.1 g/L κ-casein protein; about 1.25 g/L to about 4.9 g/L β-casein protein; a final total concentration of one or more lipids of about 0 weight % to about 45 weight %; a final total concentration of one or more flavor compounds of about 0.01 weight % to about 6 weight %; a final total concentration of about 0.1 weight % to about 6 weight % of one or more sweetening agents; and a final total concentration of ash of about 0.15 weight % to about 1.5 weight %, where the composition does not include an animal-derived component.

Also provided are compositions that include: about 0.3 g/L to about 1.1 g/L κ-casein protein; about 1.25 g/L to about 4.9 g/L β-casein protein; a final total concentration of one or more lipids of about 0 weight % to about 45 weight %; a final total concentration of one or more flavor compounds of about 0.01 weight % to about 6 weight %; a final total concentration of about 0.1 weight % to about 6 weight % of one or more sweetening agents; and a final total concentration of ash of about 0.15 weight % to about 1.5 weight %, where the composition: does not include at least one component found in a mammal-produced milk; includes at least one component not present in a mammal-produced milk; and/or includes a higher or lower concentration of at least one component as compared to the concentration of the at least one component in a mammal-produced milk. In some embodiments of these compositions, the composition includes a higher concentration of at least one component selected from the group of: calcium, phosphate, B complex vitamins, vitamin A, vitamin D, vitamin E, and vitamin K, as compared to the concentration of the one or more components in a mammal-produced milk. In some embodiments of these compositions, the composition does not include at least one component found in a mammal-produced milk selected from the group of: lactose, bacteria, mycobacteria, allergens, viruses, prions, yeast, growth hormones, leukocytes, antibiotics, heavy metals, immunoglobulins, lactoferrin, lactoperoxidase, and lipase. In some embodiments of these compositions, wherein the composition includes at least one component not present in a mammal-produced milk selected from the group of an artificial sweetener, a plant-derived lipid, a β-casein protein that is non-glycosylated or has a non-mammalian glycosylation pattern, and a κ-casein protein that is non-glycosylated or has a non-mammalian glycosylation pattern.

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Also provided are compositions including: about 0.3 g/L to about 1.1 g/L κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern; about 1.25 g/L to about 4.9 g/L β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern; a final total concentration of one or more lipids of about 0 weight % to about 45 weight %; a final total concentration of one or more flavor compounds of about 0.01 weight % to about 6 weight %; a final total concentration of about 0.1 weight % to about 6 weight % of one or more sweetening agents; and a final total concentration of ash of about 0.15 weight % to about 1.5 weight %.

Also provided are composition including a micelle including a κ-casein protein and a β-casein protein, where the micelle has a diameter of about 50 nm to about 350 nm, and the κ-casein protein and the β-casein protein are unglycosylated or have a non-mammalian glycosylation pattern. In some embodiments of these methods, the compositions include a final concentration of micelles of about 2.0 weight % to about 6 weight %. In some embodiments of these compositions, the ratio of the β-casein protein to the κ-casein protein in the micelle is about 3.5:1 to about 5.5:1 (e.g., about about 4:1 to about 5:1). In some embodiments of these methods, the composition further includes: a final total concentration of one or more lipids of about 0 weight % to about 45 weight %; a final total concentration of one or more flavor compounds of about 0.01 weight % to about 6 weight %; a final total concentration of about 0.1 weight % to about 6 weight % of one or more sweetening agents; and a final total concentration of ash of about 0.15 weight % to about 1.5 weight %.

In some embodiments of any of the compositions described herein, the composition comprises about 0.27 weight % to about 0.75 weight % κ-casein protein and about 1.23 weight % to about 3.27 weight % β-casein. In some embodiments of any of the compositions described herein, the final total concentration of one or more lipids of about 0 weight % to about 4.5 weight %.

In some embodiments of any of the compositions described herein, the one or more lipids are selected from the group consisting of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. In some embodiments of any of the compositions described herein, the composition includes one of more of: a final concentration of sunflower oil of about 1 weight % to about 28 weight

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%; a final concentration of coconut oil of about 0.5 weight % to about 14 weight %; a final concentration of tributyrin of about 0.05 weight to about 1.0 weight %; a final total concentration of monoglycerides and diglycerides of about 0.08 weight % to about 1.2 weight %; a final total concentration of free fatty acids of about 0.02 weight % to about 0.28 weight %; and a final total concentration of phospholipids of about 0.02 weight % to about 0.3 weight percent. In some embodiments of any of the compositions described herein, the free fatty acids comprise at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. In some embodiments of any of the compositions described herein, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some embodiments of any of the compositions described herein, the monoglycerides and diglycerides are plant-derived monoglycerides and diglycerides, or are bacteria-derived monoglycerides and diglycerides.

In some embodiments of any of the compositions described herein, the flavor compounds include at least one flavor compound selected from the group consisting of: δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone. In some embodiments of any of the compositions described herein, the one or more sweetening agents is a saccharide. In some embodiments of any of the compositions described herein, the saccharide is selected from the group consisting of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. In some embodiments of any of the compositions described herein, the one or more sweetening agents is an artificial sweetener. In some embodiments of any of the compositions described herein, the artificial sweetener is selected from the group of: stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, and P-4000.

In some embodiments of any of the compositions described herein, the ash includes one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some embodiments of any of the compositions described herein, the ash comprises one

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or more (e.g., one, two, or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate. In some embodiments of any of the compositions described herein, the $CaCl_2$ has a final concentration of about 0.05 g/L to about 0.2 g/L; the KH_2PO_4 has a final concentration of about 0.2 g/L to about 0.4 g/L; and the Na_3 citrate has a final concentration of about 0.1 g/L to about 0.3 g/L.

In some embodiments of any of the compositions described herein, the κ-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth κ-casein protein. In some embodiments of any of the compositions described herein, the β-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-casein protein.

In some embodiments of any of the compositions described herein, the composition further includes: a final concentration of α-lactalbumin protein of about 0.4 g/L weight % to about 2.5 weight %; and/or a final concentration of β-lactoglobulin protein of about 2.5 weight % to about 4.5 weight %. In some embodiments of any of the methods described herein, the α-lactalbumin protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-lactalbumin protein. In some embodiments of any of the compositions described herein, the β-lactoglobulin protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-lactoglobulin protein.

In some embodiments of any of the compositions described herein, the composition further includes: a final concentration of α-S1-casein protein of about 11

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weight % to about 16 weight %; and/or a final concentration of α-S2-casein protein of about 2 weight % to about 5 weight %. In some embodiments of any of the compositions described herein, the α-S1-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S1-casein protein; and/or the α-S2-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S2-casein protein.

In some embodiments of any of the compositions described herein, the composition further includes one or more of: serum albumin, lactoferrin, and transferrin. In some embodiments of any of the compositions described herein, the serum albumin is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth serum albumin; the lactoferrin is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth lactoferrin; and/or the transferrin is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth transferrin protein.

Some embodiments of any of the compositions described herein, further include one or more color balancing agents. In some embodiments of any of the compositions described herein, the one or more color balancing agents is β-carotene or annatto. In

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some embodiments of any of the compositions described herein, the composition has a pH of about 6.2 to about 7.2 (e.g., about 6.2 to about 6.8).

Also provided are compositions including: a mammalian-produced milk or a processed mammal-produced milk; and one or both of a κ-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern, and a β-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern. In some embodiments of these methods, the final concentration of the κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern in the composition is 0.02 weight % to about 3.0 weight %. In some embodiments of these methods, the final concentration of the β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern in the composition is 0.02 weight % to about 3.0 weight %. In some embodiments of these methods, the final concentration of the κ-casein protein that is unglycosylated and/or has a non-mammalian glycosylation pattern in the composition is about 0.02 weight % to about 0.6 weight %; and the final concentration of the β-casein that is unglycosylated and/or has a non-mammalian glycosylation pattern in the composition is about 0.02 weight % to about 2.5 weight %.

Also provided are powder compositions that include: a final concentration of κ-casein protein of about 3.6 weight % to about 5.4 weight %; a final concentration of β-casein protein of about 16.3 weight % to about 24.5 weight %; a final concentration of a sweetening agent of about 35 weight % to about about 40 weight %; a final concentration of one or more lipids of about 25 weight % to about 30 weight %; a final concentration of ash of about 5 weight % to about 7 weight %; and a final concentration of water of about 2 weight % to about 5 weight %, where the κ-casein protein is an unglycosylated and/or has a non-mammalian glycosylation pattern, and/or the β-casein protein is an unglycosylated and/or has a non-mammalian glycosylation pattern.

Also provided are nucleic acids that include: a promoter; a sequence encoding a signal sequence; a sequence encoding a milk protein; and a yeast termination sequence, where the promoter is operably linked to the signal sequence, the signal sequence is operably linked to the sequence encoding the milk protein, and the terminal sequence is operably linked to the sequence encoding the milk protein. In some embodiments of these nucleic acids, the promoter is a constitutive promoter. In some embodiments of

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these nucleic acids, the promoter is an inducible promoter. In some embodiments of these nucleic acids, the signal sequence is a signal sequence from the encoded milk protein or a different milk protein, or is a signal sequence from a yeast mating factor. In some embodiments of these nucleic acids, the encoded milk protein is selected from the group consisting of: β-casein, κ-casein, α-S1-casein, α-S2-casein, α-lactalbumin, β-lactoglobulin, lactoferrin, or transferrin. In some embodiments of these nucleic acids, the nucleic comprises a bacterial origin of replication. In some embodiments of these nucleic acids, the nucleic acid further includes a selection marker. In some embodiments of these nucleic acids, the selection marker is an antibiotic resistance gene.

Some embodiments of these nucleic acids further include: an additional promoter sequence; an additional sequence encoding a signal sequence; a sequence encoding an additional milk protein; and an additional yeast termination sequence, where the additional promoter sequence is operably linked to the additional sequence encoding a signal sequence, the sequence encoding the signal sequence is operably linked to the sequence encoding the additional milk protein, and the sequence encoding the additional milk protein is operably linked to the additional yeast terminal sequence.

Also provided are host cells that include any of the nucleic acids described herein. In some embodiments of these host cells, the host cell is a yeast strain (e.g., a *Kluyveromyces* sp., *Pichia* sp., *Saccharomyces* sp., *Tetrahymena* sp., *Yarrowia* sp., *Hansenula* sp., *Blastobotrys* sp., *Candida* sp., *Zygosaccharomyces* sp., or *Debaryomyces* sp.).

Also provided herein are methods of producing a recombinant milk protein that is unglycosylated or has a non-mammalian glycosylation pattern, the method including: culturing any of the host cells described herein in a culture medium under conditions sufficient to allow for secretion of the milk protein that is unglycosylated or has a non-mammalian glycosylation pattern; and harvesting the milk protein that is unglycosylated or has a non-mammalian glycosylation pattern from the culture medium.

Also provided are methods of producing a micelle including a β-casein that is unglycosylated or has a non-mammalian glycosylation pattern and a κ-casein that is unglycosylated or has a non-mammalian glycosylation pattern, that include: culturing any of the host cells provided herein in a culture medium under conditions sufficient to allow

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for release of the micelle from the host cell, where the host cell includes nucleic acid including a sequence that encodes a β-casein and a sequence that encodes a κ-casein.

Also provided are methods of supplementing a mammal-produced milk that include: providing a mammalian-produced milk or a processed mammalian-produced milk; and mixing into the milk at least one of: a β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern; a κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern; and a micelle including a β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern, and a κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern.

Also provided are methods of producing a composition that include: sonicating a liquid including a protein mixture comprising β-casein protein and casein κ protein, or comprising micelles comprising β-casein protein and κ-casein protein; mixing ash into the liquid; adding to the liquid a mixture of one or more lipids, one or more flavor compounds, and one or more color balancing agents, and sonicating the liquid; and adding to the liquid one or more sweetening agents, thereby producing the composition. In some embodiments of these methods, the β-casein protein is unglycosylated or has a non-mammalian glycosylation pattern, and/or the κ-casein protein is unglycosylated or has a non-mammalian glycosylation pattern. In some embodiments of these methods, the ash includes one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some embodiments of these methods, the ash added includes one or more (e.g., one, two, or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate. In some embodiments of these methods, the one or more lipids comprises at least one of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. In some embodiments of these methods, the free fatty acids comprise at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. In some embodiments of these methods, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some embodiments of these methods, the monoglycerides and diglycerides are plant-derived monoglycerides and diglycerides, or are bacteria-derived monoglycerides and diglycerides. In some embodiments of these methods, the flavor compounds include at least one flavor compound selected from the group consisting of:

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δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone. In some embodiments of these methods, the one or more coloring balancing agent is β-carotene or annatto. In some embodiments of these methods, the one or more sweetening agents is a saccharide. In some embodiments of these methods, the saccharide is selected from the group consisting of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. In some embodiments of these methods, the one or more sweetening agents is an artificial sweetener. In some embodiments of these methods, the artificial sweetener is selected from the group consisting of: stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, and P-4000. In some embodiments of these methods, the pH of the liquid is between about 6.2 and about 7.4 (e.g., about 6.4 to about 6.8). In some embodiments of these methods, the β-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-casein protein; and/or the κ-casein protein is a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth κ-casein protein. In some embodiments of these methods, the protein mixture further includes one or more proteins selected from the group of: α-lactalbumin, β-lactoglobulin, α-S1-casein, α-S2-casein, lactoferrin, transferrin, and serum albumin.

Also provided is a composition produced by any of the methods described herein.

Also provided is a method of making butter, cheese, caseinate, or yogurt that include: providing any of the compositions described herein; and producing the butter, cheese, caseinate, or yogurt using any of the compositions described herein as a starting material.

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Also provided are kits that include: (a) a mixture of one or more milk proteins, one or more fats, and one or flavor compounds; and (b) a mixture of ash and at least one sweetening agent. In some embodiments of these kits, the one or more milk proteins are selected from the group of: β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, α-S1-casein, α-S2-casein, lactoferrin, transferrin, and serum albumin. In some embodiments of these kits, the one or more milk proteins are cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth milk proteins. In some embodiments of these kits, the one or more fats are selected from the group consisting of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. In some embodiments of these kits, the free fatty acids include at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. In some embodiments of these kits, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some embodiments of these kits, the monoglycerides and diglycerides are plant-derived monoglycerides and diglycerides, or are bacteria-derived monoglycerides and diglycerides. In some embodiments of these kits, the flavor compounds comprise at least one flavor compound selected from the group consisting of: δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone. In some embodiments of these kits, the mixture in (a) further includes one or more color balancing agent. In some embodiments of these kits, the one or more color balancing agent is β-carotene or annatto. In some embodiments of these kits, the one or more sweetening agents is a saccharide (e.g., a saccharide selected from the group of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose). In some embodiments of these kits, the one or more sweetening agents is an artificial sweetener (e.g., an artificial sweetener selected from the group of: stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame,

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sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, and P-4000). In some embodiments of any of these kits, the ash includes one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some embodiments of these kits, the ash includes one or more (e.g., one, two, or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate. Some embodiments of these kits further include instructions for making any of the compositions described herein.

Also provided are kits that include at least one of the nucleic acids described herein.

Also provided herein are dairy substitute food products including one or more isolated milk protein components, fats, carbohydrates, and ash. In some embodiments of these dairy substitute food products, the food product is non-animal derived. In some embodiments of these substitute food product, the food product includes milk, butter, cheese, caseinare, yogurt, and cream. In some embodiments of these dairy substitute food products, the isolated milk protein components include casein and whey proteins. In some embodiments of these dairy substitute food products, the casein protein further includes alpha-s1, alpha-s2, beta, and kappa-casein. In some embodiments of these dairy substitute food products, the casein protein further includes alpha-s1, beta, and kappa. In some embodiments of these dairy substitute food products, the casein protein further includes components for micelle formation. In some embodiments of these dairy substitute food products, the casein protein exhibits curdling properties at pH 4.0 – 6.0. In some embodiments of these dairy substitute food products, the casein protein is at least or equal to 2.5% (w/v) and less than or equal to 10% (w/v). In some embodiments of these dairy substitute food products, the whey protein further includes beta-lactoglobulin and alpha-lactalbumin. In some embodiments of these dairy substitute food products, the whey protein forms a polymer matrix gel. In some embodiments of these dairy substitute food products, the whey protein is at least 0.1 % (w/v) and less than or equal to 1% (w/v). In some embodiments of these dairy substitute food products, the one or more milk protein components is isolated from microbes. In some embodiments of any of these dairy substitute food products, the one or more milk protein components is isolated from recombinant microbes. In some embodiments of these dairy substitute food products,

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the one or more milk protein components is synthesized in eukaryotic microbes. In some embodiments of these dairy substitute food products, the eukaryotic microbes include yeast. In some embodiments of these dairy substitute food products, the yeast include *Kleuyveromyces* sp., *Pichia* sp., Saccharomyces sp. and *Tetrahymena* sp.

In some embodiments of these substitute food products, the fats include triglycerides. In some embodiments of these dairy substitute food products, the fats comprise high-oleic oil. In some embodiments of these dairy substitute food products, the high-oleic oil further includes one or more of monounsaturates, oleic, linoleic, linolenic and saturates. In some embodiments of these dairy substitute food products, the fats comprise short chain fatty acids. In some embodiments of these dairy substitute food products, the short chain fatty acids include butanoic, hexanoic, octanoic, and decanoic acids. In some embodiments of these dairy substitute food products, one or more of the fats comprised trans-esterified fatty acids. In some embodiments of these dairy substitute food products, one or more of the fats are isolated from plants. In some embodiments of these dairy substitute food products, the plant is selected from one or more of the following: sunflower, corn, olive, soy, peanut, walnut, almond, sesame, cottonseed, canola, safflower, flax seed, palm, palm kernel, palm fruit, coconut, babassu, shea butter, mango butter, cocoa butter, wheat germ and rice bran oil. In some embodiments of these dairy substitute food products, the sugars comprise of galactose, sucrose, glucose, fructose and maltose. In some embodiments of these dairy substitute food products, the dairy substitute food product is essentially free of lactose. In some embodiments of these dairy substitute food products, the ash includes minerals. In some embodiments of these dairy substitute food products, the minerals further include one or more of the following: sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, chloride, manganese, selenium, iodine, retinol, carotene, vitamins, vitamin D, vitamin E, vitamin B12, thiamin and riboflavin. In some embodiments of these dairy substitute food products, the ash includes anions. In some embodiments of these dairy substitute food products, the minerals further include one or more of the following: phosphate, citrate, sulfate, carbonate, and chloride.

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Also provided are methods of making a dairy substitute food product including the step of contacting one or more isolated milk protein components, interesterified fats, carbohydrates and ash. Some embodiments of these methods, further include the step of isolating one or more milk protein components is from a lower eukaryote.

Also provided are methods of altering a flavor profile of a dairy substitute product that include modulating a combination of fatty acids in a mixture including milk protein components, carbohydrates, and ash. In some embodiments of these methods, the step of modulating includes triglyceride comprising three oleic acids and short-chain triglyceride comprising butyric, one hexanoic, and one octanoic acid. In some embodiments of these methods, the step of modulating comprises increasing or decreasing one or more fatty acids comprising butyric acid, caprioc acid, caprylic acid, and capric acid. In some embodiments of these methods, the flavor profile of a dairy substitute product mimics the flavor profile of one or more dairy product. In some embodiments of these methods, the flavor profile of one or more dairy food product includes bovine milk, goat milk, soy milk, almond milk and coconut milk. In some embodiments of these methods, the flavor profile includes one or more sensory impressions selected from: buttery, nutty, sweet, sour, fruity, floral, bitter, woody, earthy, beany, spicy, metallic, sweet, musty, oily and vinegary.

Disclosed herein are methods and compositions to produce dairy substitutes. In some embodiments, methods and compositions are provided for a dairy substitute food product comprising one or more isolated milk protein components, fats, carbohydrates and ash. In certain embodiments, methods and compositions are provided for dairy substitute composition comprising casein protein and whey protein wherein the composition is essentially free of animal products and wherein the casein protein to whey protein are in a preferred (w/v) ratio. In certain other embodiments, methods are provided to modulate a flavor profile of a dairy substitute food product comprising modulating a fatty acid content in a mixture comprising milk protein components, fats, carbohydrates, and ash. Preferred steps of modulating comprises increasing or decreasing one or more fatty acids comprising butyric acid, caproic acid, caprylic acid, and capric acid. In additional embodiments, methods and compositions of the present invention provide milk protein components and fats in a desired (w/v) ratio.

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In various aspects, the methods and compositions of the present invention provide for dairy substitutes that still retain their functional characteristics and organoleptic properties. In some embodiments, the core functionalities can be, but are not limited to achieving a nutritional profile similar to a conventional dairy product, and replicates one or more, if not all, of the core functionalities thereof.

In other embodiments, the core functionalities can be, but are not limited to replicating sensory characteristics that are identical or similar to the traditional dairy-based products, which include but are not limited to taste, appearance, handling and mouthfeel, desired density, structure, texture, elasticity, springiness, coagulation, binding, leavening, aeration, foaming, creaminess, and emulsification.

Preferred methods and compositions provide dairy substitute products such as milk, butter, cheese, yogurt, and cream. Provided herein are formulations for a non-dairy milk substitute comprising (3.3%) one or more isolated milk protein components, (4.0%) fats, (2.4%) carbohydrates and (0.7%) ash (w/v). Varying the fat content through modulating triglyceride levels and the fatty acid composition of the triglycerides enhances the flavor profile of the non-dairy milk substitute.

Advantages in the methods and dairy substitute compositions include reduction or removal of antibiotic residues, heavy metals, bacteria and adulterations commonly found in natural dairy products as well as reducing environmental impact.

Accordingly, certain aspects of the present invention provide animal-free dairy substitute that has desirable flavor characteristics, e.g., replicates dairy flavors, minimizes foodborne pathogens and has a lower environmental impact, while retaining the downstream applications of dairy milk.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art . Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, dairy processing, biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

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Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

The terminology and description used herein is for the purpose of describing particular embodiments only and is not intended to limit the invention. As used herein, the singular forms "a," "an," and "the" can be intended to include the plural forms as well, unless the context clearly indicates otherwise. The terms "including," "includes," "having," "has," "with," or variants thereof are intended to be inclusive in a manner similar to the term "comprising".

An "isolated" RNA, DNA or a mixed polymer is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its natural host cell, e.g., ribosomes, polymerases, and genomic sequences with which it is naturally associated.

As used herein, an "isolated" organic molecule (e.g., a fatty acid or a SCFA) is one which is substantially separated from the cellular components (membrane lipids, chromosomes, proteins) of the host cell from which it originated. As used herein, the term "isolated" with respect to protein indicates that the preparation of protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The term does not require that the biomolecule has been separated from all other chemicals, although certain isolated biomolecules may be purified to near homogeneity.

The term "polynucleotide" or "nucleic acid molecule" refers to a polymeric form of nucleotides of at least 10 bases in length. The term includes DNA molecules (e.g., cDNA or genomic or synthetic DNA) and RNA molecules (e.g., mRNA or synthetic RNA), as well as analogs of DNA or RNA containing non-natural nucleotide analogs,

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non-native internucleoside bonds, or both. The nucleic acid can be in any topological conformation. For instance, the nucleic acid can be single-stranded, or double-stranded, or circular.

The term "SCFA" is abbreviated for short-chain fatty acids, the term "HOSO" is abbreviated for high oleic sunflower oil, "SCTG" is abbreviated for short-chain triglycerides.

The term "milk protein component" refers to proteins or protein equivalents and variants found in milk such as casein, whey or the combination of casein and whey, including their subunits, which are derived from various sources and as further defined herein.

The term "milk protein" means a protein that is found in a mammal-produced milk or a protein having a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the sequence of a protein that is found in a mammal-produced milk. Non-limiting examples of milk proteins include: β-casein, κ-casein, α-S1-casein, α-S2-casein, α-lactalbumin, β-lactoglobulin, lactoferrin, transferrin, and serum albumin. Additional milk proteins are known in the art.

The term "casein protein" is art-known and represents a family of proteins that is present in mammal-produced milk and is capable of self-assembling with other proteins in the family to form micelles and/or precipitate out of an aqueous solution at an acidic pH. Non-limiting examples of casein proteins include: β-casein, κ-casein, α-S1-casein, and α-S2-casein. Non-limiting examples of sequences for casein protein from different mammals are provided herein. Additional sequences for other mammalian caseins are known in the art.

The term "mammal-produced milk" is art known and means a milk produced by a mammal.

The term "processed mammal-produced milk" means a mammal-produced milk that is processed using one or more steps known in the dairy industry (e.g., homogenization, pasteurization, irradiation, or supplementation).

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The term "mammal-derived component" means a molecule or compound (e.g., a protein, a lipid, or a nucleic acid) obtained from the body of a mammal or a molecule obtained from a fluid or solid produced by a mammal.

The term "component of milk" or "milk component" is a molecule, compound, element, or an ion present in a mammal-produced milk.

The term "non-mammalian glycosylation pattern" means one of a difference in one or more location(s) of glycosylation in a protein, and/or a difference in the amount of and/or type of glycosylation at one or more location(s) in a protein produced and post-translational modified in a non-mammalian cell (e.g., a yeast cell, an insect cell, or a bacterial cell) as compared to a reference protein (e.g., the same protein produced and post-translationally modified in a mammalian cell, e.g., a CHO cell, a MEK cell, or a mammalian udder cell).

The term "lipids" means one or more molecules (e.g., biomolecules) that include a fatty acyl group (e.g., saturated or unsaturated acyl chains). For example, the term lipids includes oils, phospholipids, free fatty acids, phospholipids, monoglycerides, diglycerides, and triglycerides. Non-limiting examples of lipids are described herein. Additional examples of lipids are known in the art.

The term "plant-derived lipid" means a lipid obtained from and/or produced by a plant (e.g., monocot or dicot).

The term "sweetening agent" means a saccharide (e.g., a monosaccharide, a disaccharide, or a polysaccharide) or an artificial sweetener (e.g., a small molecule artificial sweetener or a protein artificial sweetener) that, when added to a composition, makes the composition taste sweet when ingested by a mammal, such as a human. Non-limiting examples of sweetening agents are described herein. Additional examples of sweetening agents are known in the art.

The term "ash" is an art-known term and represents one or more ions, elements, minerals, and/or compounds that can be found in a mammal-produced milk. Non-limiting ions, elements, minerals, and compounds that are found in a mammal-produced milk are described herein. Additional ions, elements, minerals, and compounds that are found in a mammal-produced milk are also known in the art.

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The term "color balancing agent" or "coloring agent" means an agent added to a composition to modulate the color of the composition, e.g., to make the color of the composition appear more similar to a mammalian-produced milk. Non-limiting examples of color balancing agents or coloring agents include β-carotene and annatto. Other examples of coloring balancing agents are known in the art. A color balancing agent or a coloring agent can be produced by or obtained from a plant.

The term "micelle" means is a generally (or roughly) spherical supramolecular structure that exists as a dispersion within a composition. A micelle can have, e.g., a surface that is composed of a charged outer layer. A micelle can encapsulate one or more biomolecules. For example, a micelle can encapsulate two or more proteins (e.g., a β-casein protein and a κ-casein protein). A micelle can have diameter of between about 10 nm and about 350 nm. Additional aspects and characteristics of micelles are known in the art.

The phrase "concentration of a component in a mammal-produced milk" means the concentration of a component in the milk produced by a mammal or the mean concentration of a component in milk produced by a population of mammals of the same species.

The term "attenuate" as used herein generally refers to a functional deletion, including a mutation, partial or complete deletion, insertion, or other variation made to a gene sequence or a sequence controlling the transcription of a gene sequence, which reduces or inhibits production of the gene product, or renders the gene product non-functional. In some instances a functional deletion is described as a knockout mutation. Attenuation also includes amino acid sequence changes by altering the nucleic acid sequence, placing the gene under the control of a less active promoter, down-regulation, expressing interfering RNA, ribozymes or antisense sequences that target the gene of interest, or through any other technique known in the art. In one example, the sensitivity of a particular enzyme to feedback inhibition or inhibition caused by a composition that is not a product or a reactant (non-pathway specific feedback) is lessened such that the enzyme activity is not impacted by the presence of a compound. In other instances, an enzyme that has been altered to be less active can be referred to as attenuated.

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Deletion: The removal of one or more nucleotides from a nucleic acid molecule or one or more amino acids from a protein, the regions on either side being joined together.

Knock-Out: A gene whose level of expression or activity has been reduced to zero. In some examples, a gene is knocked-out via deletion of some or all of its coding sequence. In other examples, a gene is knocked-out via introduction of one or more nucleotides into its open reading frame, which results in translation of a non-sense or otherwise non-functional protein product.

The term "synthetic milk substitute" refers to a composition that resembles, is similar to, is to equivalent to, or is nearly identical to a dairy milk.

The term "flavor" refers to the taste and/or the aroma of a food or drink.

The term "recombinant" is an art known-term. When referring to a nucleic acid (e.g., a gene), the term "recombinant" can be used, e.g., to describe a nucleic acid that has been removed from its naturally occurring environment, a nucleic acid that is not associated with all or a portion of a nucleic acid abutting or proximal to the nucleic acid when it is found in nature, a nucleic acid that is operatively linked to a nucleic acid which it is not linked to in nature, or a nucleic acid that does not occur in nature. The term "recombinant" can be used, e.g., to describe cloned DNA isolates, or a nucleic acid including a chemically-synthesized nucleotide analog. When "recombinant" is used to describe a protein, it can refer to, e.g., a protein that is produced in a cell of a different species or type, as compared to the species or type of cell that produces the protein in nature.

As used herein, an endogenous nucleic acid sequence in the genome of an organism (or the encoded protein product of that sequence) is deemed "recombinant" herein if a heterologous sequence is placed adjacent to the endogenous nucleic acid sequence, such that the expression of this endogenous nucleic acid sequence is altered. In this context, a heterologous sequence is a sequence that is not naturally adjacent to the endogenous nucleic acid sequence, whether or not the heterologous sequence is itself endogenous (originating from the same host cell or progeny thereof) or exogenous (originating from a different host cell or progeny thereof). By way of example, a promoter sequence can be substituted (e.g., by homologous recombination) for the native promoter of a gene in the genome of a host cell, such that this gene has an altered

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expression pattern. This gene would now become "recombinant" because it is separated from at least some of the sequences that naturally flank it.

A nucleic acid is also considered "recombinant" if it contains any modifications that do not naturally occur to the corresponding nucleic acid in a genome. For instance, an endogenous coding sequence is considered "recombinant" if it contains an insertion, deletion, or a point mutation introduced artificially, e.g., by human intervention. A "recombinant nucleic acid" also includes a nucleic acid integrated into a host cell chromosome at a heterologous site and a nucleic acid construct present as an episome.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap, or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wis. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. See, e.g., Pearson, *Methods Enzymol.* 183:63-98, 1990. For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1. Alternatively, sequences can be compared using the computer program, BLAST (Altschul et al., *J. Mol. Biol.* 215:403-410, 1990; Gish and States, *Nature Genet.* 3:266-272, 1993; Madden et al., *Meth. Enzymol.* 266:131-141, 1996; Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997; Zhang and Madden, *Genome Res.* 7:649-656, 1997, especially blastp or tblastn (Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate

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nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 76%, 80%, 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

Alternatively, substantial homology or similarity exists when a nucleic acid or fragment thereof hybridizes to another nucleic acid, to a strand of another nucleic acid, or to the complementary strand thereof, under stringent hybridization conditions. "Stringent hybridization conditions" and "stringent wash conditions" in the context of nucleic acid hybridization experiments depend upon a number of different physical parameters. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, solvents, the base composition of the hybridizing species, length of the complementary regions, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. One having ordinary skill in the art knows how to vary these parameters to achieve a particular stringency of hybridization.

In general, "stringent hybridization" is performed at about 25 °C below the thermal melting point (Tm) for the specific DNA hybrid under a particular set of conditions. "Stringent washing" is performed at temperatures about 5 °C lower than the Tm for the specific DNA hybrid under a particular set of conditions. The Tm is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. See Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., page 9.51, 1989. For purposes herein, "stringent conditions" are defined for solution phase hybridization as aqueous hybridization (i.e., free of formamide) in 6xSSC (where 20xSSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% SDS at 65 °C for 8-12 hours, followed by two washes in 0.2xSSC, 0.1% SDS at 65 °C. for 20 minutes. It will be appreciated by the skilled worker that hybridization at 65 °C will occur at different rates depending on a number of factors including the length and percent identity of the sequences which are hybridizing.

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The nucleic acids (also referred to as polynucleotides) of this present invention may include both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. They may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.) Examples of modified nucleotides are described in Malyshev et al., *Nature* 509:385-388, 2014; and Li et al., *J. Am. Chem. Soc.* 136:826-829, 2014. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. Other modifications can include, for example, analogs in which the ribose ring contains a bridging moiety or other structure such as the modifications found in "locked" nucleic acids.

The term "mutated" when applied to nucleic acid sequences means that nucleotides in a nucleic acid sequence may be inserted, deleted, or changed compared to a reference nucleic acid sequence. A single alteration may be made at a locus (a point mutation) or multiple nucleotides may be inserted, deleted, or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid sequence. A nucleic acid sequence may be mutated by any method known in the art including but not limited to mutagenesis techniques such as "error-prone PCR" (a process for performing PCR under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product; see, e.g., Leung et al., *Technique* 1:11-15, 1989, and Caldwell and Joyce, *PCR Methods Applic.* 2:28-33, 1992); and "oligonucleotide-directed mutagenesis" (a

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process which enables the generation of site-specific mutations in any cloned DNA segment of interest; see, e.g., Reidhaar-Olson and Sauer, *Science* 241:53-57, 1988).

The term "vector" as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which generally refers to a circular double stranded DNA loop into which additional DNA segments may be ligated, but also includes linear double-stranded molecules such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme. Other vectors include cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC). Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome (discussed in more detail below). Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain preferred vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply "expression vectors").

Promoters useful for expressing the recombinant genes described herein include both constitutive and inducible/repressible promoters. Examples of inducible/repressible promoters include galactose-inducible promoters (e.g., PLAC4-PBI). Where multiple recombinant genes are expressed in an engineered yeast, the different genes can be controlled by different promoters or by identical promoters in separate operons, or the expression of two or more genes may be controlled by a single promoter as part of an operon.

The term "operably linked" expression control sequences refers to a linkage in which the expression control sequence is contiguous with the gene of interest to control the gene of interest, as well as expression control sequences that act in trans or at a distance to control the gene of interest.

The term "expression control sequence" or "regulatory sequences" are used interchangeably and as used herein refer to polynucleotide sequences which are necessary

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to affect the expression of coding sequences to which they are operably linked. Expression control sequences are sequences which control the transcription, post-transcriptional events, and translation of nucleic acid sequences. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals, such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "transfect", "transfection", "transfecting," and the like refer to the introduction of a heterologous nucleic acid into eukaryote cells, both higher and lower eukaryote cells. Historically, the term "transformation" has been used to describe the introduction of a nucleic acid into a yeast or fungal cell; however, herein the term "transfection" is used to refer to the introduction of a nucleic acid into any eukaryote cell, including yeast and fungal cells.

The term "recombinant host cell" ("expression host cell", "expression host system", "expression system" or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. A recombinant host cell may be an isolated cell or cell line grown in culture or may be a cell which resides in a living tissue or organism. Preferred host cells are yeasts and fungi.

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The term "yeast and filamentous fungi" include, but are not limited to any *Kluyveromyces* sp., such as *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Saccharomyces* sp., such as *Saccharomyces cerevisiae*, *Pichia* sp., such as *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Hansenula polymorpha*, *Candida albicans*, any *Aspergillus* sp., such as *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Physcomitrella patens*, and *Neurospora crassa*.

As used herein, the term "predominantly" or variations thereof will be understood to mean, for instance, a) in the context of fats the amount of a particular fatty acid composition relative to the total amount of fatty acid composition; b) in the context of protein the amount of a particular protein composition (e.g., β-casein) relative to the total amount of protein composition (e.g., α-, β-, and κ-casein).

The term "about," "approximately," or "similar to" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which can depend in part on how the value is measured or determined, or on the limitations of the measurement system. It should be understood that all ranges and quantities described below are approximations and are not intended to limit the invention. Where ranges and numbers are used these can be approximate to include statistical ranges or measurement errors or variation. In some embodiments, for instance, measurements could be plus or minus 10%.

The phrase "essentially free of" is used to indicate the indicated component, if present, is present in an amount that does not contribute, or contributes only in a *de minimus* fashion, to the properties of the composition. In various embodiments, where a composition is essentially free of a particular component, the component is present in less than a functional amount. In various embodiments, the component may be present in trace amounts. Particular limits will vary depending on the nature of the component, but may be, for example, selected from less than 10% by weight, less than 9% by weight, less than 8% by weight, less than 7% by weight, less than 6% by weight, less than 5% by

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weight, less than 4% by weight, less than 3% by weight, less than 2% by weight, less than 1% by weight, or less than 0.5% by weight.

As used herein, the term "essentially free of" a particular carbohydrate, such as lactose is used to indicate that the food composition is substantially devoid of carbohydrate residues. Expressed in terms of purity, essentially free means that the amount of carbohydrate residues do not exceed 10%, and preferably is below 5%, more preferably below 1%, most preferably below 0.5%, wherein the percentages are by weight or by mole percent. Thus, substantially all of the carbohydrate residues in a food composition according to the present invention are free of, for example, lactose.

Unless indicated otherwise, percentage (%) of ingredients refer to total % by weight.

Unless otherwise indicated, and as an example for all sequences described herein under the general format "SEQ ID NO:", "nucleic acid comprising SEQ ID NO:1" refers to a nucleic acid, at least a portion of which has either (i) the sequence of SEQ ID NO:1, or (ii) a sequence complementary to SEQ ID NO:1. The choice between the two is dictated by the context. For instance, if the nucleic acid is used as a probe, the choice between the two is dictated by the requirement that the probe be complementary to the desired target.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents a flow diagram representative of an exemplary process to produce synthetic milk substitute.

Figure 2A represents a picture depicting precipitate of an exemplary milk protein component.

Figure 2B represents a picture depicting a pellet of an exemplary milk protein component.

Figure 3 represents an image of a silver stain SDS-PAGE gel to visualize the milk protein components.

Figure 4 is a SYPRO Ruby-stained SDS-PAGE gel showing the levels of secretion of α-lactalbumin mediated by the OST signal sequence, the native α-

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lactalbumin signal sequence, and the α mating factor signal sequence as described in Example 6.

Figure 5 is shows the levels of secretion of α-lactalbumin by wildtype yeast cells or yeast cells expressing α-lactalbumin using the native α-lactalbumin signal peptide or a OST1 signal peptide (as determined by an ELISA assay as described in Example 6).

Figure 6 is shows the levels of secretion of β-lactoglobulin by wildtype yeast cells and yeast cells including a vector as described in Example 6 (using SDS-PAGE).

Figure 7 is a Western blot showing the level of secretion of β-lactoglobulin from wildtype yeast and yeast cells including a vector as described in Example 6.

Figure 8 is a graph showing the level of secreted β-casein and secreted α-S1-casein produced by wildtype yeast and yeast cells including the vectors described in Example 6.

Figure 9 is a schematic showing the steps in the process described in Example 7.

Figure 10 is an image of a composition made by a method described herein.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the discovery that only a few components present in a mammal-produced milk provide for the texture and taste of a mammal-produced milk, and the development of compositions that have a similar taste, aroma, and mouth feel as compared to a mammal-produced milk. In view of this discovery, provided herein are such compositions, methods of making the compositions, and kits including these compositions and mixtures useful for making these compositions.

The compositions provided herein provide for compositions that have a similar taste, mouth feel, aroma, and nutritional value as compared to a mammal-produced milk, but lack one or more of the components of a mammal-produced milk that may be considered to be undesirable (e.g., allergens, lactose, antibiotics, hormones (e.g., stress hormones and/or growth hormones), heavy metals, bacteria (e.g., *E. coli*), viruses, and prions). The compositions provided herein also have an improved shelf-life as compared to mammal-produced milk, and can have an improved aroma profile as compared to a mammal-produced milk.

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Also provided herein are methods and compositions for dairy substitute food product comprising one or more isolated milk protein components, fats, carbohydrates and ash. In certain aspects the methods and compositions comprise milk or milk-like protein equivalents. Preferably, the milk protein component is essentially free of impurities. In some embodiments, the milk protein component comprises microbially derived or produced casein, whey or a combination thereof. More preferably, a method is provided to introduce an engineered nucleic acid sequence encoding one or more milk protein components. Even more preferably, the milk protein component is not animal derived. In other preferred embodiments, the recombinant milk protein component is modified to express the same phosphate groups or lack phosphate groups and/or carbohydrate groups attached to the casein proteins. By having recombinant β-casein and κ-casein having the same phosphate groups as the same proteins present in a mammal-produced milk, the recombinant β-casein and the recombinant κ-casein are able to form micelles.

The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates, 1992, and Supplements to 2002); Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1990; Taylor and Drickamer, Introduction to Glycobiology, Oxford Univ. Press, 2003; Worthington Enzyme Manual, Worthington Biochemical Corp., Freehold, N.J.; Handbook of Biochemistry: Section A Proteins, Vol I, CRC Press, 1976; Handbook of Biochemistry: Section A Proteins, Vol II, CRC Press, 1976; Essentials of Glycobiology, Cold Spring Harbor Laboratory Press, 1999.

Exemplary materials and methods for use in any of the methods and compositions are described below, and can be used in any combination. Additional materials and methods that can be used in any of the methods and compositions are also known in the art.

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Casein Proteins

Casein proteins include a variety of different proteins found in mammalian milk. Non-limiting examples of casein proteins include: β-casein, κ-casein, α-S2-casein, and α-S1-casein.

As an alternative to obtaining casein proteins from mammals or mammal-produced milk for us in dairy product manufacture, the present invention provides methods and composition for the production of recombinant casein proteins. In various aspects of the present invention, methods and compositions are provided for non-animal derived casein that has similar solubility and similar turbidity, and heat stability suitable for incorporation into various food products. Preferably, the non-animal derived casein has excellent solubility similar turbidity and heat stability suitable for incorporation into various dairy substitute products. Additionally, further characterization of the protein includes less or no aggregation or precipitation during such heat treatment and is suitable for procedures such as pasteurization, concentration, etc.

Difference in function of the non-animal derived casein in milk can be characterized in terms of viscosity of the liquid; the ability of the proteins to withstand heat; the ability of the proteins to form micelles; and the ability of the proteins to hold different minerals & vitamins.

B-casein

The primary structure of human β-casein as determined by Greenberg et al. (*J. Biol. Chem.* 259:5132-5138, 1984) was shown to be a phosphorylated protein with phosphorylation sites at specific seryl and threonyl residues located near the amino terminus. A comparison of human and bovine β-caseins showed 47% identity.

Non-limiting examples of β-casein proteins are SEQ ID Nos: 25, 27, 29, 31, 33, 35, 36, 38, 40, 42, 44, and 46. Non-limiting examples of nucleic acid sequences encoding a β-casein protein are SEQ ID NOs: 26, 28, 30, 32, 34, 37, 39, 41, 43, 45, 47, and 144. A β-casein protein can be a β-casein protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan,

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mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-casein protein. Additional sequences for different β-casein proteins and nucleic acids encoding different β-casein proteins are known in the art.

A β-casein protein can also be a proteins that is at least 50% (e.g., at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype β-casein protein (e.g., SEQ ID Nos: 25, 27, 29, 31, 33, 35, 36, 38, 40, 42, 44, or 46). A nucleic acid encoding a β-casein protein can encode a protein that is at least 50% (e.g., at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype β-casein protein (e.g., SEQ ID Nos: 25, 27, 29, 31, 33, 35, 36, 38, 40, 42, 44, or 46).

Methods known for isolating β-casein from genetically engineered bacterial cells typically involve precipitating the β-casein from a supernatant derived from lysed or fractionated cells. For example, Simons, et al., *Protein Eng.* 6: 763-770 (1993), used genetically engineered *E. coli* to express bovine β-casein. The protein, which accumulated in the periplasmic spaces of the bacteria, was released into a cell suspension by osmotic shock. After centrifugation of the suspension, the β-casein in the pellet was resuspended in a cold water wash and centrifuged again. The β-casein, present in the supernatant, was precipitated by acidification with acetic acid, filtered, and further purified by HPLC. Similarly, Hansson, et al., *Protein Express. Purif.* 4:373-381, 1993, used genetically engineered *E. coli* to express β-casein. The β-casein, present in a cell lysate, was precipitated with ammonium sulfate, dissolved in ethanolamine and 6M urea, and further purified by ion-exchange chromatography. See, e.g., U.S. Patent No. 6,121,421.

Additionally, methods for isolating recombinantly produced β-casein in yeast that are simpler and more effective than known techniques are also known. Choi et al., *J. Agric. Food Chem.* 49(4):1761-1766, 2001. Expression and purification of glycosylated bovine β-casein (L70S/P71S) in *Pichia pastoris*, resulted in the observation that the

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majority of bovine β-casein was not being hyperglycosylated in *P. pastoris*, and its molecular weight was estimated to be 33.6 kDa. Glycosylated bovine β-casein was normally phosphorylated to the same degree as native bovine β-casein.

K-Casein

Kappa-casein is both phosphorylated and glycosylated. The sequence of human κ-casein was determined by Brignon et al. (Fed. Eur. Biol. Soc. Lett. 188:48-54, 1985). See, e.g., U.S. Patent No. 5,710,044.

Non-limiting examples of κ-casein proteins are SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23. Non-limiting examples of nucleic acid sequences encoding a κ-casein protein are SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 148. A κ-casein protein can be a κ-casein protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth κ-casein protein. Additional sequences for different κ-casein proteins and nucleic acids encoding different κ-casein proteins are known in the art.

A κ-casein protein can also be a proteins that is at least 50% (e.g., at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype κ-casein protein (e.g., SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23). A nucleic acid encoding a κ-casein protein can encode a protein that is at least 50% (e.g., at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype κ-casein protein (e.g., SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23).

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α-S1-Casein

Non-limiting examples of α-S1-casein proteins are SEQ ID Nos: 48, 50, 52, 54, 56, 57, 59, 61, 63, 64, 66, 68, 70, 72, 74, and 76. Non-limiting examples of nucleic acid sequences encoding an α-S1-casein protein are SEQ ID NOs: 49, 51, 53, 55, 58, 60, 62, 65, 67, 69, 71, 73, 75, 77, and 147. A α-S1-casein protein can be an α-S1-casein protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S1-casein protein. Additional sequences for different α-S1-casein proteins and nucleic acids encoding different α-S1-casein proteins are known in the art.

An α-S1-casein protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α-S1-casein protein (e.g., SEQ ID Nos: 48, 50, 52, 54, 56, 57, 59, 61, 63, 64, 66, 68, 70, 72, 74, or 76). A nucleic acid encoding an α-S1-casein protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α-S1-casein protein (e.g., SEQ ID Nos: 48, 50, 52, 54, 56, 57, 59, 61, 63, 64, 66, 68, 70, 72, 74, or 76).

α-S2-Casein

Non-limiting examples of α-S2-casein proteins are SEQ ID Nos: 78, 80, 82, 84, 86, 88, and 90. Non-limiting examples of nucleic acid sequences encoding an α-S2-casein protein are SEQ ID NOs: 79, 81, 83, 85, 87, 89, 91, 145, and 146. A α-S2-casein protein can be an α-S2-casein protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S2-casein protein. Additional

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sequences for different α-S2-casein proteins and nucleic acids encoding different α-S2-casein proteins are known in the art.

An α-S2-casein protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α-S2-casein protein (e.g., SEQ ID Nos: 78, 80, 82, 84, 86, 88, or 90). A nucleic acid encoding an α-S2-casein protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α-S2-casein protein (e.g., SEQ ID Nos: 78, 80, 82, 84, 86, 88, or 90).

Micelles including Casein Proteins

In bovine milk, casein or casein micelles usually makes up 2.5% of the entire mixture in suspension. If sufficient casein is not present the micelles, which are very important for the optimum behavior of milk, will not form. Too much protein does not go into solution properly resulting in an undesirable mixture. The casein micelle can include water and salts - mainly calcium and phosphorous. Casein micelles are easily separated and removed by centrifugation. Separation from whey is easily done by precipitating casein with an acid to lower the pH to around 4.6.

In some embodiments, a micelle can include a β-casein protein (e.g., any of the β-casein proteins described herein) and κ-casein protein (e.g., any of the κ-casein proteins described herein). In some examples, the ratio of β-casein protein to κ-casein protein in the micelle is about 2.0:1 to about 5.5:1, 2.0:1 to about 5.0:1, 2.0:1 to about 4.5:1, about 2.0:1 to about 4.0:1, about 2.0:1 to about 3.5:1, about 2.0:1 to about 3.0:1, about 2.0:1 to about 2.5:1, about 2.5:1 to about 5.0:1, about 2.5:1 to about 4.5:1, about 2.5:1 to about 4.0:1, about 2.5:1 to about 3.5:1, about 2.5:1 to about 3.0:1, 3.0:1 to about 5.0:1, about 3.0:1 to about 4.5:1, about 3.0:1 to about 4.0:1, about 3.0:1 to about 3.5:1, about 3.5:1 to about 5.0:1, about 3.5:1 to about 4.5:1, about 3.5:1 to about 4.0:1, about 4.0:1 to about 5.0:1, about 4.0:1 to about 4.5:1, or about 4.5:1 to about 5.0:1.

In some examples, the micelle has a diameter (or a population of micelles have an average diameter) of about 20 nm to about 350 nm, about 320 nm, about 300 nm, about

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280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, about 140 nm, about 120 nm, about 100 nm, about 80 nm, about 60 nm, or about 40 nm; about 40 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, about 140 nm, about 120 nm, about 100 nm, about 80 nm, or about 60 nm; about 60 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, about 140 nm, about 120 nm, or about 100 nm; about 80 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, about 140 nm, about 120 nm, or about 100 nm; about 100 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, about 140 nm, or about 120 nm; about 120 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, about 160 nm, or about 140 nm; about 140 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, about 180 nm, or about 160 nm; about 160 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, about 200 nm, or about 180 nm; about 180 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, about 220 nm, or about 200 nm; about 200 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, about 240 nm, or about 220 nm; about 220 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, about 260 nm, or about 240 nm; about 240 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, about 280 nm, or about 260 nm; about 260 nm to about 350 nm, about 340 nm, about 320 nm, about 300 nm, or about 280 nm; about 280 nm to about 350 nm, about 340 nm, about 320 nm, or about 300 nm; about 300 nm to about 350 nm or about 325 nm; or about 325 nm to about 350 nm.

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Whey Proteins

Whey is commonly known as the by-product of cheese and is also known to be one cause for milk allergies. A typical whey composition comprises a mixture of β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and transferrin. Whey proteins do not contain phosphorus, and remain in solution at low pH whereas casein proteins do not. In one embodiment, a select combination of whey proteins comprising β-lactoglobulin and α-lactalbumin are used as the primary component or at least a part of the milk protein component or composition. Non-limiting examples of different whey proteins are provided below.

α-Lactalbumin

Non-limiting examples of α-lactalbumin proteins are SEQ ID Nos: 92, 94, 96, and 98. Non-limiting examples of nucleic acid sequences encoding an α-lactalbumin protein are SEQ ID NOs: 93, 95, 97, 99, and 157. An α-lactalbumin protein can be an α-lactalbumin protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-lactalbumin protein. Additional sequences for different α-lactalbumin proteins and nucleic acids encoding different α-lactalbumin proteins are known in the art.

An α-lactalbumin protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α-lactalbumin protein (e.g., SEQ ID Nos: 92, 94, 96, or 98). A nucleic acid encoding an α-lactalbumin protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype α- lactalbumin protein (e.g., SEQ ID Nos: 92, 94, 96, or 98).

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β-Lactoglobulin

Non-limiting examples of β-lactoglobulin proteins are SEQ ID Nos: 100, 102, 104, and 106. Non-limiting examples of nucleic acid sequences encoding a β-lactoglobulin protein are SEQ ID NOs: 101, 103, 105, 107, and 143. A β-lactoglobulin protein can be a β-lactoglobulin protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-lactoglobulin protein. Additional sequences for different β-lactoglobulin proteins and nucleic acids encoding different β-lactoglobulin proteins are known in the art.

A β-lactoglobulin protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype β-lactoglobulin protein (e.g., SEQ ID Nos: 100, 102, 104, or 106). A nucleic acid encoding a β-lactoglobulin protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype β-lactoglobulin protein (e.g., SEQ ID Nos: 100, 102, 104, or 106).

Lactoferrin

Non-limiting examples of lactoferrin proteins are SEQ ID Nos: 108, 110, 112, and 114. Non-limiting examples of nucleic acid sequences encoding a lactoferrin protein are SEQ ID NOs: 109, 111, 113, and 115. A lactoferrin protein can be a lactoferrin protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth lactoferrin protein. Additional sequences for different lactoferrin proteins and nucleic acids encoding different lactoferrin proteins are known in the art.

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A lactoferrin protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype lactoferrin protein (e.g., SEQ ID Nos: 108, 110, 112, or 114). A nucleic acid encoding a lactoferrin protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype lactoferrin protein (e.g., SEQ ID Nos: 108, 110, 112, or 114).

Transferrin

Non-limiting examples of transferrin proteins are SEQ ID Nos: 116 and 118. Non-limiting examples of nucleic acid sequences encoding a transferrin protein are SEQ ID NOs: 117 and 119. A transferrin protein can be a transferrin protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth transferrin protein. Additional sequences for different transferrin proteins and nucleic acids encoding different transferrin proteins are known in the art.

A transferrin protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype transferrin protein (e.g., SEQ ID Nos: 116 or 118). A nucleic acid encoding a transferrin protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype transferrin protein (e.g., SEQ ID Nos: 116 or 118).

Serum Albumin

Non-limiting examples of serum albumin proteins are SEQ ID Nos: 120, 122, 124, and 126. Non-limiting examples of nucleic acid sequences encoding a serum

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albumin protein are SEQ ID NOs: 121, 123, 125, and 127. A serum albumin protein can be a serum albumin protein from any mammalian species, e.g., a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth serum albumin protein. Additional sequences for different serum albumin proteins and nucleic acids encoding different serum albumin proteins are known in the art.

A serum albumin protein can also be a proteins that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype serum albumin protein (e.g., SEQ ID Nos: 20, 122, 124, or 126). A nucleic acid encoding a serum albumin protein can encode a protein that is at least 80% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%) identical to a wildtype serum albumin protein (e.g., SEQ ID Nos: 20, 122, 124, or 126).

Lipids in Mammal-Produced Milk

Milk fat contains approximately 400 different fatty acids, which makes it the most complex of all natural fats. The milk fatty acids are derived almost equally from two sources, the feed and the microbial activity in the rumen of the cow and the lipids in bovine milk are mainly present in globules as an oil-in-water emulsion. Fat is present in all natural dairy products and is critical for sensory characteristics such as flavor, mouthfeel and consistency. In addition, fats provide nutrition and health benefits. The milk fat consists mainly of triglycerides, approximately 98%, while other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) (about 0.1%) Jensen RG, Newburg DS. Bovine milk lipids, Handbook of milk composition. Jensen RG London: Academic Press; 1995. 543–75. In addition, there are trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, flavor compounds and compounds introduced by the feed (Lindmark Mansson H., Food & Nutrition Research 2008. DOI: 10.3402/fnr.v52i0.1821)

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Milk fat triglycerides are synthesized from more than 400 different fatty acids, which makes milk fat the most complex of all natural fats. Nearly all fatty acids in milk are present in trace quantities and only about 15 acids at the 1% level or higher. Many factors are associated with the variations in the amount and fatty acid composition of bovine milk lipids. They may be of animal origin, i.e. related to genetics (breed and selection), stage of lactation, mastitis and ruminal fermentation, or they may be feed-related factors, i.e. related to fibre and energy intake, dietary fats, and seasonal and regional effects. The fatty acids in the milk fat are arranged in the triglycerides in accordance with a pattern that appears to be universal among ruminants. The percent unsaturated fatty acids (e.g., oleic and linolenic) in goats do not differ from the average found for cow's milk. A major difference between the milk fat of the goat and the cow is the percentage distribution among specific short chain fatty acids. Goats have an appreciably higher proportion of capric, caprylic and caproic acids. The high amounts of these specific fatty acids are responsible for the characteristic flavor and odor associated with goat's milk. John C. Bruhn, FST, UC Davis, Davis, CA 95616-8598; Food Nutr Res. 2008; 52: 10.3402/fnr.v52i0.1821. Published online Jun 11, 2008. doi: 10.3402/fnr. v52i0.1821.

The milk fatty acids are derived almost equally from two sources, the feed and the microbial activity in the rumen of the cow. The fatty acid synthesizing system in the mammary gland of the cow produces fatty acids with even number of carbons of 4–16 carbons in length and accounts for approximately 60 and 45% of the fatty acids on a molar and weight basis, respectively. This *de novo* synthesis in the mammary gland is of the 4:0–14:0 acids together with about half of the 16:0 from acetate and β-hydroxybutyrate. Acetate and butyric acid are generated in the rumen by fermentation of feed components. The butyric acid is converted to β-hydroxybutyrate during absorption through the rumen epithelium.

Medium- and long-chain fatty acids, but mainly 18:0, may be desaturated in the mammary gland to form the corresponding monosaturated acids.

Fatty acids are not randomly esterified at the three positions of the triacylglycerol molecule (MacGibbon AHK, Taylor MW. Composition and structure of bovine milk

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lipidsAdvanced dairy chemistry. Fox PFMcSweeney PLHNew York: Springer; 2006. 1–42.). The short-chain acids butyric (4:0) and caproic (6:0) are esterified almost entirely at sn-3. Medium-chain fatty acids (8:0–14:0) as well as 16:0 are preferentially esterified at positions sn-1 and sn-2. Stearic acid (18:0) is selectively placed at position sn-1, whereas oleic acid (18:1) shows preference for positions sn-1 and sn-3 (Lindmark 2008).

Milk replacers with a fat component formulated to selected fatty acid profiles exist, however, such triglycerides are not interesterified into long-chain monounsaturated fatty acid triglycerides such as found in vegetable oils. U.S. Patent Appl. No. 20140147548 discloses milk replacers for young animals with by adding medium chain triglyceride, specifically caproic, caprylic, capric and lauric fatty acid or a combination thereof.

Lipids in the Present Compositions

The lipids in any of the compositions or used in any of the methods described herein can include: one or more fats, one or more oils, one or more monoglycerides, diglycerides, and/or triglycerides, one or more free fatty acids, and one or more phospholipids. Exemplary oils, monoglycerides, diglycerides, free fatty acids, and phospholipids are described below. Additional examples of fats, oils, monoglycerides, diglycerides, triglycerides, free fatty acids, and phospholipids are known in the art.

Oils

Oils used in the present compositions or methods can include, e.g., plant-derived oils. Non-limiting examples of plant-based oils include sunflower oil, coconut oil, peanut oil, corn oil, cottonseed oil, olive oil, palm oil, rapeseed oil, safflower oil, sesame oil, soybean oil, almond oil, beech nut oil, brazil nut oil, cashew oil, hazelnut oil, macadamia nut oil, mongongo nut oil, pecan oil, pine nut oil, pistachio nut oil, walnut oil, and avocado oil.

Monoglycerides and Diglycerides

Monoglycerides and diglycerides that can be used in the present invention can be plant-derived monoglycerides and diglycerides. For example, monoglycerides and

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diglycerides can be derived from sunflowers, coconuts, peanuts, cottonseed, olives, palm, rapeseed, safflowers, sesame seed, soybeans, almonds, beech nuts, brazil nuts, cashews, hazelnuts, macadameia nuts, mongongo nuts, pecans, pine nuts, pistachios, walnuts, and avocados. The monoglycerides and diglycerides can include the acyl chain of any of the free fatty acids listed herein. Additional examples of monoglycerides and diglycerides are known in the art.

Free Fatty Acids

The compositions described herein can include and the methods described herein can include the use of one or more free fatty acids. Non-limiting examples of free fatty acids include butyric acid, caproic acid, caprylic acid, and capric acid. Additional examples of fatty acids include lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, cerotic acid, myristoleic acid, pamitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, linoleic acid, linoelaidic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic acid, omega-3 fatty acids, and omega-6 fatty acids. In some examples, the free fatty acid is saturated. In some examples, the free fatty acid is unsaturated. In some embodiments, the free fatty acids are not derived from or produced by a mammal. Additional examples of free fatty acids are known in the art.

Phospholipids

The compositions described herein and the methods described herein can include the use of one or more phospholipids. Non-limiting examples of phospholipids include lecithin phospholipids (e.g., soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, rapeseed lecithin phospholipids. rice bran lecithin phospholipids, and corn lecithin phospholipids). In some embodiments, the phospholipids are not derived from or produced by a mammal. Additional aspects of phospholipids are known in the art.

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Flavor Compounds

Any of the compositions or methods described herein can include or include the use of one or more different flavor compounds. Non-limiting examples of flavor compounds include δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone. Additional examples of flavor compounds include artificial flavors, e.g., chocolate, coffee, strawberry, almond, hazelnut, vanilla, green tea, Irish cream, and coconut flavoring. Additional examples of flavor compounds are known in the art.

Ash

Any of the compositions or methods described herein can include or include the use of ash. Ash can, e.g., include one or more (two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen fifteen, sixteen, seventeen, eighteen, nineteen, or twenty) of: calcium, phosphorous, potassium, sodium, citrate, chloride, phosphate, magnesium, iron, molybdenum, manganese, copper, thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6 (pyridoxine), vitamin B12 (cobalamin), vitamin C, folate, vitamins A, vitamin D, vitamin E, and vitamin K. In some examples, the ash includes one or more (two or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate. Ash can be provided as a powder or as a solution. Additional components in and aspects of ash are known in the art. In some embodiments, the ash is not derived from or produced by mammal.

Color Balancing Agents

A variety of different color balancing agents are known in the art. For example, a color balancing agent can be a compound from obtained from a plant (e.g., a monocot or a dicot). In some examples, the color balancing agent is a synthetic compound. In some examples, the color balancing agent is not obtained from or produced by a mammal or a mammalian cell. Non-limiting examples of color balancing agents include β-carotene and annatto.

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Sweetening Agents

A sweetening agent can be a saccharide (e.g., a monosaccharide, a disaccharide, or a polysaccharide) or an artificial sweetener. Non-limiting examples of sweetening agents that are saccharides include glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. Additional examples of saccharides that can be used as a sweetening agent in any of the compositions or methods described herein are known in the art.

Non-limiting examples of sweetening agents that are artificial sweeteners include stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, and P-4000. Additional artificial sweeteners that can be used as sweetening agents in any of the compositions or methods described herein are known in the art.

Compositions

Provided herein are compositions including: about 0.3 g/L to about 1.1 g/L (e.g., about 0.3 g/L to about 1.0 g/L, about 0.3 g/L to about 0.9 g/L, about 0.3 g/L to about 0.8 g/L, about 0.3 g/L to about 0.7 g/L, about 0.3 g/L to about 0.6 g/L, about 0.3 g/L to about 0.5 g/L, about 0.3 g/L to about 0.4 g/L, about 0.4 g/L to about 1.1 g/L, about 0.4 g/L to about 1.0 g/L, about 0.4 g/L to about 0.9 g/L, about 0.4 g/L to about 0.8 g/L, about 0.4 g/L to about 0.7 g/L, about 0.4 g/L to about 0.6 g/L, about 0.4 g/L to about 0.5 g/L, about 0.5 g/L to about 1.1 g/L, about 0.5 g/L to about 1.0 g/L, about 0.5 g/L to about 0.9 g/L, about 0.5 g/L to about 0.8 g/L, about 0.5 g/L to about 0.7 g/L, about 0.5 g/L to about 0.6 g/L, about 0.6 g/L to about 1.1 g/L, about 0.6 g/L to about 1.0 g/L, about 0.6 g/L to about 0.9 g/L, about 0.6 g/L to about 0.8 g/L, about 0.6 g/L to about 0.7 g/L, about 0.7 g/L to about 1.1 g/L, about 0.7 g/L to about 1.0 g/L, about 0.7 g/L to about 0.9 g/L, about 0.7 g/L to about 0.8 g/L, about 0.8 g/L to about 1.1 g/L, about 0.8 g/L to about 1.0 g/L, about 0.8 g/L to about 0.9 g/L, about 0.9 g/L to about 1.1 g/L, about 0.9 g/L to about 1.0 g/L, about 1.0 g/L to about 1.1 g/L, or about 0.27 weight % to about 0.75 weight %) κ-casein

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protein (e.g., any of the κ-casein proteins described herein); about 1.25 g/L to about 4.9 g/L (e.g., about 1.25 g/L to about 4.6 g/L, about 1.25 g/L to about 4.4 g/L, about 1.25 g/L to about 4.2 g/L, about 1.25 g/L to about 4.0 g/L, about 1.25 g/L to about 3.8 g/L, about 1.25 g/L to about 3.6 g/L, about 1.25 g/L to about 3.4 g/L, about 1.25 g/L to about 3.2 g/L, about 1.25 g/L to about 3.0 g/L, about 1.25 g/L to about 2.8 g/L, about 1.25 g/L to about 2.6 g/L, about 1.25 g/L to about 2.4 g/L, about 1.25 g/L to about 2.2 g/L, about 1.25 g/L to about 2.0 g/L, about 1.25 g/L to about 1.8 g/L, about 1.25 g/L to about 1.6 g/L, about 1.25 g/L to about 1.4 g/L, about 1.4 g/L to about 4.9 g/L, about 1.4 g/L to about 4.6 g/L, about 1.4 g/L to about 4.4 g/L, about 1.4 g/L to about 4.2 g/L, about 1.4 g/L to about 4.0 g/L, about 1.4 g/L to about 3.8 g/L, about 1.4 g/L to about 3.6 g/L, about 1.4 g/L to about 3.4 g/L, about 1.4 g/L to about 3.2 g/L, about 1.4 g/L to about 3.0 g/L, about 1.4 g/L to about 2.8 g/L, 1.4 g/L to about 2.6 g/L, about 1.4 g/L to about 2.4 g/L, about 1.4 g/L to about 2.2 g/L, about 1.4 g/L to about 2.0 g/L, about 1.4 g/L to about 1.8 g/L, about 1.4 g/L to about 1.6 g/L, about 1.6 g/L to about 4.9 g/L, about 1.6 g/L to about 4.6 g/L, about 1.6 g/L to about 4.4 g/L, about 1.6 g/L to about 4.2 g/L, about 1.6 g/L to about 4.0 g/L, about 1.6 g/L to about 3.8 g/L, about 1.6 g/L to about 3.6 g/L, about 1.6 g/L to about 3.4 g/L, about 1.6 g/L to about 3.2 g/L, about 1.6 g/L to about 3.0 g/L, about 1.6 g/L to about 2.8 g/L, 1.6 g/L to about 2.6 g/L, about 1.6 g/L to about 2.4 g/L, about 1.6 g/L to about 2.2 g/L, about 1.6 g/L to about 2.0 g/L, about 1.6 g/L to about 1.8 g/L, about 1.8 g/L to about 4.9 g/L, about 1.8 g/L to about 4.6 g/L, about 1.8 g/L to about 4.4 g/L, about 1.8 g/L to about 4.2 g/L, about 1.8 g/L to about 4.0 g/L, about 1.8 g/L to about 3.8 g/L, about 1.8 g/L to about 3.6 g/L, about 1.8 g/L to about 3.4 g/L, about 1.8 g/L to about 3.2 g/L, about 1.8 g/L to about 3.0 g/L, about 1.8 g/L to about 2.8 g/L, 1.8 g/L to about 2.6 g/L, about 1.8 g/L to about 2.4 g/L, about 1.8 g/L to about 2.2 g/L, about 1.8 g/L to about 2.0 g/L, about 2.0 g/L to about 4.9 g/L, about 2.0 g/L to about 4.6 g/L, about 2.0 g/L to about 4.4 g/L, about 2.0 g/L to about 4.2 g/L, about 2.0 g/L to about 4.0 g/L, about 2.0 g/L to about 3.8 g/L, about 2.0 g/L to about 3.6 g/L, about 2.0 g/L to about 3.4 g/L, about 2.0 g/L to about 3.2 g/L, about 2.0 g/L to about 3.0 g/L, about 2.0 g/L to about 2.8 g/L, 2.0 g/L to about 2.6 g/L, about 2.0 g/L to about 2.4 g/L, about 2.0 g/L to about 2.2 g/L, about 2.2 g/L to about 4.9 g/L, about 2.2 g/L to about 4.6 g/L, about 2.2 g/L to about 4.4 g/L, about 2.2 g/L to about 4.2 g/L, about 2.2 g/L to about 4.0 g/L, about 2.2

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g/L to about 3.8 g/L, about 2.2 g/L to about 3.6 g/L, about 2.2 g/L to about 3.4 g/L, about 2.2 g/L to about 3.2 g/L, about 2.2 g/L to about 3.0 g/L, about 2.2 g/L to about 2.8 g/L, 2.2 g/L to about 2.6 g/L, about 2.2 g/L to about 2.4 g/L, about 2.4 g/L to about 4.9 g/L, about 2.4 g/L to about 4.6 g/L, about 2.4 g/L to about 4.4 g/L, about 2.4 g/L to about 4.2 g/L, about 2.4 g/L to about 4.0 g/L, about 2.4 g/L to about 3.8 g/L, about 2.4 g/L to about 3.6 g/L, about 2.4 g/L to about 3.4 g/L, about 2.4 g/L to about 3.2 g/L, about 2.4 g/L to about 3.0 g/L, about 2.4 g/L to about 2.8 g/L, 2.4 g/L to about 2.6 g/L, about 2.6 g/L to about 4.9 g/L, about 2.6 g/L to about 4.6 g/L, about 2.6 g/L to about 4.4 g/L, about 2.6 g/L to about 4.2 g/L, about 2.6 g/L to about 4.0 g/L, about 2.6 g/L to about 3.8 g/L, about 2.6 g/L to about 3.6 g/L, about 2.6 g/L to about 3.4 g/L, about 2.6 g/L to about 3.2 g/L, about 2.6 g/L to about 3.0 g/L, about 2.6 g/L to about 2.8 g/L, about 2.8 g/L to about 4.9 g/L, about 2.8 g/L to about 4.6 g/L, about 2.8 g/L to about 4.4 g/L, about 2.8 g/L to about 4.2 g/L, about 2.8 g/L to about 4.0 g/L, about 2.8 g/L to about 3.8 g/L, about 2.8 g/L to about 3.6 g/L, about 2.8 g/L to about 3.4 g/L, about 2.8 g/L to about 3.2 g/L, about 2.8 g/L to about 3.0 g/L, about 3.0 g/L to about 4.9 g/L, about 3.0 g/L to about 4.6 g/L, about 3.0 g/L to about 4.4 g/L, about 3.0 g/L to about 4.2 g/L, about 3.0 g/L to about 4.0 g/L, about 3.0 g/L to about 3.8 g/L, about 3.0 g/L to about 3.6 g/L, about 3.0 g/L to about 3.4 g/L, about 3.0 g/L to about 3.2 g/L, about 3.2 g/L to about 4.9 g/L, about 3.2 g/L to about 4.6 g/L, about 3.2 g/L to about 4.4 g/L, about 3.2 g/L to about 4.2 g/L, about 3.2 g/L to about 4.0 g/L, about 3.2 g/L to about 3.8 g/L, about 3.2 g/L to about 3.6 g/L, about 3.2 g/L to about 3.4 g/L, about 3.4 g/L to about 4.9 g/L, about 3.4 g/L to about 4.6 g/L, about 3.4 g/L to about 4.4 g/L, about 3.4 g/L to about 4.2 g/L, about 3.4 g/L to about 4.0 g/L, about 3.4 g/L to about 3.8 g/L, about 3.4 g/L to about 3.6 g/L, about 3.6 g/L to about 4.9 g/L, about 3.6 g/L to about 4.6 g/L, about 3.6 g/L to about 4.4 g/L, about 3.6 g/L to about 4.2 g/L, about 3.6 g/L to about 4.0 g/L, about 3.6 g/L to about 3.8 g/L, about 3.8 g/L to about 4.9 g/L, about 3.8 g/L to about 4.6 g/L, about 3.8 g/L to about 4.4 g/L, about 3.8 g/L to about 4.2 g/L, about 3.8 g/L to about 4.0 g/L, about 4.0 g/L to about 4.9 g/L, about 4.0 g/L to about 4.6 g/L, about 4.0 g/L to about 4.4 g/L, about 4.0 g/L to about 4.2 g/L, about 4.2 g/L to about 4.9 g/L, about 4.2 g/L to about 4.6 g/L, about 4.2 g/L to about 4.4 g/L, about 4.4 g/L to about 4.9 g/L, about 4.4 g/L to about 4.6 g/L, about 4,6 g/L to about 4.9 g/L, or about 1.23 weight % to about 3.27 weight %) β-casein protein (e.g., any of the

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β-casein proteins described herein); a final total concentration of one or more lipids (e.g., any one or more of the lipids described herein) of about 0 weight % to about 45 weight % (e.g., 0 weight %; about 0 weight % to about 4.5 weight %; about 0.5 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 5 weight %, about 4 weight %, about 3 weight %, about 2 weight %, or about 1 weight %; about 1.0 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 5 weight %, about 4 weight %, about 3 weight %, or about 2 weight %; about 2 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 5 weight %, about 4 weight %, or about 3 weight %; about 3 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 5 weight %, or about 4 weight %; about 4 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, about 6 weight %, or about 5 weight %; about 5 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, about 8 weight %, or about 6 weight %; about 6 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, about 10 weight %, or about 8 weight %; about 8 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, about 15 weight %, or about 10 weight %; about 10 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, about 20 weight %, or about 15 weight %; about 15 weight % to about 40 weight %, about 35 weight %, about 30 weight %, about 25 weight %, or about 20 weight %; about 20 weight % to about 40 weight %, about 35 weight %, about 30 weight %, or about 25 weight %; about 25 weight % to about 40 weight %, about 35 weight %, or about 30 weight %; about 30 weight % to about 40 weight %, or about 35 weight %; or about 35 weight % to about 40 weight %); a final total concentration of one or more flavor compounds (e.g., any of one or more of the

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flavor compounds described herein) of about 0.01 weight % to about 6 weight % (e.g., about 0.1 weight % to about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, about 1.5 weight %, about 1.0 weight %, or about 0.5 weight %; about 0.5 weight % to about 6 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, about 1.5 weight %, or about 1.0 weight %; about 1.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, or about 1.5 weight %; about 1.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, or about 2.0 weight %; about 2.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, or about 2.5 weight %; about 2.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, or about 3.0 weight %; about 3.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, or about 3.5 weight %; about 3.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, or about 4.0 weight %; about 4.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %; about 4.5 weight % to about 6.0 weight %, about 5.5 weight %, or about 5.0 weight %; about 5.0 weight % to about 6.0 weight % or about 5.5 weight %; or about 5.5 weight % to about 6.0 weight %); a final total concentration of about 0.1 weight % to about 6 weight % (e.g., about 0.1 weight % to about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, about 1.5 weight %, about 1.0 weight %, or about 0.5 weight %; about 0.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, about 1.5 weight %, or about 1.0 weight %; about 1.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about

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3.5 weight %, about 3.0 weight %, about 2.5 weight %, about 2.0 weight %, or about 1.5 weight %; about 1.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, about 2.5 weight %, or about 2.0 weight %; about 2.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, or about 2.5 weight %; about 2.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, or about 3.0 weight %; about 3.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, or about 3.5 weight %; about 3.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, or about 4.0 weight %; about 4.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, or about 4.5 weight %; about 4.5 weight % to about 6.0 weight %, about 5.5 weight %, or about 5.0 weight %; about 5.0 weight % to about 6.0 weight %, or about 5.5 weight %; or about 5.5 weight % to about 6.0 weight %) of one or more sweetening agents (e.g., any one or more of the sweetening agents described herein); and a final total concentration of ash of about 0.15 weight % to about 1.5 weight % (e.g., about 0.15 weight % to about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.6 weight %, about 0.5 weight %, about 0.4 weight %, about 0.3 weight %, or about 0.2 weight %; about 0.2 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.6 weight %, about 0.5 weight %, about 0.4 weight %, or about 0.3 weight %; about 0.3 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.6 weight %, about 0.5 weight %, or about 0.4 weight %; about 0.4 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.6 weight %, or about 0.5 weight %; about 0.5 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, or about 0.6 weight %; about 0.6 weight % to about

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1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, about 0.9 weight %, or about 0.8 weight %; about 0.8 weight % to about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, about 1.0 weight %, or about 0.9 weight %; about 0.9 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, about 1.1 weight %, or about 1.0 weight %; about 1.0 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, about 1.2 weight %, or about 1.1 weight %; about 1.1 weight % to about 1.5 weight %, about 1.4 weight %, about 1.3 weight %, or about 1.2 weight %; about 1.2 weight % to about 1.5 weight %, about 1.4 weight %, or about 1.3 weight %; about 1.3 weight % to about 1.5 weight % or about 1.4 weight %; or about 1.4 weight % to about 1.5 weight %), where the composition does not comprise an animal-derived component.

Also provided are compositions including: about 0.3 g/L to about 1.1 g/L (e.g., any of the subranges of about 0.3 g/L to about 1.1 g/L described in the above paragraph) κ-casein protein (e.g., any of the κ-casein proteins described herein); about 1.25 g/L to about 4.9 g/L (e.g., any of the subranges of about 1.25 g/L to about 4.9 g/L described in the above paragraph) β-casein protein (e.g., any of the β-casein proteins described herein); a final total concentration of one or more lipids (e.g., any of the one or more lipids described herein) of about 0 weight % to about 45 weight % (e.g., any of the subranges of about 0 weight % to about 45 weight % described in the above paragraph); a final total concentration of one or more flavor compounds (e.g., any of the one or more flavor compounds described herein) of about 0.01 weight % to about 6 weight % (e.g., any of the subranges of about 0.01 weight % to about 6 weight % described in the above paragraph); a final total concentration of about 0.1 weight % to about 6 weight % (e.g., any of the subranges of about 0.1 weight % to about 6 weight % described herein) of one or more sweetening agents (e.g., any one or more sweetening agents described herein); and a final total concentration of ash (e.g., any of the exemplary ash described herein) of about 0.15 weight % to about 1.5 weight % (e.g., any of the subranges of about 0.15 weight % to about 1.5 weight % described in the above paragraph), where: the composition: does not include at least one component found in a mammal-produced milk; includes at least one component not present in a mammal-produced milk; and/or includes

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a higher or lower concentration of at least one component as compared to the concentration of the at least one component in a mammal-produced milk. In some examples of these compositions, the composition includes a higher concentration of at least one component selected from the group of: calcium, phosphate, B complex vitamins, vitamin A, vitamin D, vitamin E, and vitamin K, as compared to the concentration of the one or more components in a mammal-produced milk. In some embodiments of these compositions, the composition does not include at least one component found in a mammal-produced milk selected from the group of: lactose, bacteria, mycobacteria, allergens, viruses, prions, yeast, growth hormones, leukocytes, antibiotics, heavy metals, immunoglobulins, lactoferrin, lactoperoxidase, and lipase. In some examples of these compositions, the composition includes at least one component not present in a mammal-produced milk selected from the group of an artificial sweetener, a plant-derived lipid, a β-casein protein that is non-glycosylated or has a non-mammalian glycosylation pattern, and a κ-casein protein that is non-glycosylated or has a non-mammalian glycosylation pattern.

Also provided are compositions including: about 0.3 g/L to about 1.1 g/L (e.g., any of the subranges of about 0.3 g/L to about 1.1 g/L described in this section) κ-casein protein (e.g., any of the κ-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern; about 1.25 g/L to about 4.9 g/L (e.g., any of the subranges of about 1.25 g/L to about 4.9 g/L described in this section) β-casein protein (e.g., any of the β-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern; a final total concentration of one or more lipids (e.g., any of the one or more lipids described herein) of about 0 weight % to about 45 weight % (e.g., any of the subranges of about 0 weight % to about 45 weight % described in this section); a final total concentration of one or more flavor compounds (e.g., any of the one or more flavor compounds described herein) of about 0.01 weight % to about 6 weight % (e.g., any of the subranges of about 0.01 weight % to about 6 weight % described in this section); a final total concentration of about 0.1 weight % to about 6 weight % (e.g., any of the subranges of about 0.1 weight % to about 6 weight % described in this section) of one or more sweetening agents (e.g., any of the one or more sweetening agents described herein); and a final total concentration of ash (e.g., any of the ash described herein) of

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about 0.15 weight % to about 1.5 weight % (e.g., any of the subranges of about 0.15 weight % to about 1.5 weight % described in this section).

Also provided are compositions including a micelle including a κ-casein protein (e.g., any of the κ-casein proteins described herein) and a β-casein protein (e.g., any of the β-casein proteins described herein), where the micelle has a diameter of about 50 nm to about 350 nm (e.g., any of the subranges of the diameter of a micelle described herein), and the κ-casein protein and the β-casein protein are unglycosylated or have a non-mammalian glycosylation pattern. In some embodiments, the composition includes a final concentration of micelles of about 2.0 weight % to about 6 weight % (e.g., about 2.0 weight % to about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, or about 2.5 weight %; about 2.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, or about 3.0 weight %; about 3.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, or about 3.5 weight %; about 3.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, or about 3.5 weight %; about 3.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, or about 4.0 weight %; about 4.5 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, about 4.5 weight %, or about 4.0 weight %; about 4.0 weight % to about 6.0 weight %, about 5.5 weight %, about 5.0 weight %, or about 4.5 weight %; about 4.5 weight % to about 5.5 weight %, or about 5.0 weight %; about 5.0 weight % to about 6.0 weight % or 5.5 weight %; or about 5.5 weight % to about 6.0 weight %). In some embodiments of these compositions, the ratio of the β-casein protein to the κ-casein protein in the micelle is about 2.0:1 to about 5.5:1 (e.g., any of the subranges of the ratios about 2.0:1 to about 5.5:1 described for the micelle herein). In some embodiments, these compositions further include: a final total concentration of one or more lipids (e.g., any of the one or more lipids described herein) of about 0 weight % to about 45 weight % (e.g., any of the subranges of about 0 weight % to about 45 weight percent described in this section); a final total concentration of one or more flavor compounds (e.g., any of the one or more flavor compounds described herein) of about 0.01 weight % to about 6 weight % (e.g., any of the subranges of 0.01

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weight % to about 6 weight % described in this section); a final total concentration of about 0.1 weight % to about 6 weight % (e.g., any of the subranges of about 0.1 weight % to about 6 weight % described in this section) of one or more sweetening agents (e.g., any one or more of the sweetening agents described herein); and a final total concentration of ash (e.g., any of the ash described herein) of about 0.15 weight % to about 1.5 weight % (e.g., any of the subranges of about 0.15 weight % to about 1.5 weight % described in this section).

In some embodiments of any of the compositions described herein, the one or more lipids are selected from the group consisting of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. Some examples of any of the compositions described herein further include one or more of: a final concentration of sunflower oil of about 1 weight % to about 28 weight % (e.g., about 1 weight % to about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, about 12 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 4 weight %, or about 2 weight %; about 2 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, about 12 weight %, about 10 weight %, about 8 weight %, about 6 weight %, or about 4 weight %; about 4 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, about 12 weight %, about 10 weight %, about 8 weight %, or about 6 weight %; about 6 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, about 12 weight %, about 10 weight %, or about 8 weight %; about 8 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, about 12 weight %, or about 10 weight %; about 10 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, about 14 weight%, or about 12 weight %; about 12 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, about 16 weight %, or about 14 weight%; about 14 weight

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% to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %, or about 16 weight %; about 16 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, about 20 weight %, about 18 weight %; about 18 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %, or about 20 weight %; about 20 weight % to about 28 weight %, about 26 weight %, about 24 weight %, about 22 weight %; about 22 weight % to about 28 weight %, about 26 weight %, about 24 weight %; about 24 weight % to about 28 weight % or about 26 weight %; or about 28 weight % to about 30 weight %); a final concentration of coconut oil of about 0.5 weight % to about 14 weight % (e.g., about 0.5 weight % to about 12 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 4 weight %, about 2 weight %, or about 1 weight %; about 1 weight % to about 14 weight %, about 12 weight %, about 10 weight %, about 8 weight %, about 6 weight %, about 4 weight %, or about 2 weight %; about 2 weight % to about 12 weight %, about 10 weight %, about 8 weight %, about 6 weight %, or about 4 weight %; about 4 weight % to about 14 weight %, about 12 weight %, about 10 weight %, about 8 weight %, or about 6 weight %; about 6 weight % to about 14 weight %, about 12 weight %, about 10 weight %, or about 8 weight %; about 8 weight % to about 14 weight %, about 12 weight %, or about 10 weight %; about 10 weight % to about 14 weight % or 12 weight %; or about 12 weight % to about 14 weight %); a final concentration of tributyrin of about 0.05 weight to about 1.0 weight % (e.g., between about 0.05 weight % to about 0.9 weight %, about 0.8 weight %, about 0.7 weight %, about 0.6 weight %, about 0.5 weight %, about 0.4 weight %, about 0.3 weight %, or about 0.2 weight %; 0.1 weight % to about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.7 weight %, about 0.6 weight %, about 0.5 weight %, about 0.4 weight %, about 0.3 weight %, or about 0.2 weight %; about 0.2 weight % to about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.7 weight %, about 0.6 weight %, about 0.5 weight %, or about 0.4 weight %; about 0.4 weight % to about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.7 weight %, about 0.6 weight %, or about 0.5 weight %; about 0.5 weight % to about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, about 0.7 weight %, or about 0.6 weight %; about 0.6 weight % to about 1.0 weight %, about 0.9 weight %, about 0.8 weight %, or about 0.7 weight %; about 0.7 weight % to

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about 1.0 weight %, about 0.9 weight %, or about 0.8 weight %; about 0.8 weight % to about 1.0 weight % or about 0.9 weight %; or about 0.9 weight % to about 1.0 weight %); a final total concentration of monoglycerides and diglycerides (e.g., any one or more of the monoglycerides or diglycerides described herein) of about 0.08 weight % to about 1.2 weight % (e.g., 0.08 weight % to about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, or about 0.2 weight %; about 0.2 weight % to about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, or about 0.4 weight %; about 0.4 weight % to about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, or about 0.6 weight %; about 0.6 weight % to about 1.2 weight %, about 1.0 weight %, or about 0.8 weight %; about 0.8 weight % to about 1.2 weight % or about 1.0 weight %; or about 1.0 weight % to about 1.2 weight %); and a final total concentration of free fatty acids of about 0.02 weight % to about 0.28 weight %; and a final total concentration of phospholipids (e.g., any one or more of the phospholipids described herein) of about 0.02 weight % to about 0.3 weight % (e.g., about 0.02 weight % to about 0.25 weight %, about 0.20 weight %, about 0.15 weight %, or about 0.10 weight %; about 0.05 weight % to about 0.3 weight %, about 0.25 weight %, about 0.20 weight %, about 0.15 weight %, or about 0.10 weight %; about 0.10 weight % to about 0.30 weight %, about 0.25 weight %, about 0.20 weight %, or about 0.15 weight %; about 0.15 weight % to about 0.30 weight %, about 0.25 weight %, or about 0.20 weight %; about 0.20 weight % to about 0.30 weight % or about 0.25 weight %; or about 0.25 weight % to about 0.30 weight %).

In some embodiments of any of the compositions, the free fatty acids include at least one (e.g., two, three, or four) fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. In some embodiments of any of the compositions, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some examples of any of the compositions described herein, the flavor compounds include at least one flavor compound selected from the group of: δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone. In some embodiments of any of the compositions described herein, the one or more sweetening agents is a saccharide (e.g., glucose, mannose, maltose, fructose, galactose, lactose,

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sucrose, monatin, or tagatose). In some examples of any of the compositions described herein, the

one or more sweetening agents is an artificial sweetener (e.g., stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, or P-4000).

In some examples of any of the compositions described herein, the ash includes one or more (e.g., two, three, four, five, or six) of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some embodiments of any of the compositions described herein, the ash comprises one or more (e.g., two or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate. Some embodiments of the compositions described herein include: a final concentration of $CaCl_2$ of about 0.05 g/L to about 0.2 g/L (e.g., about 0.05 g/L to about 0.15 g/L, about 0.05 g/L to about 0.10 g/L, about 0.10 g/L to about 0.20 g/L, about 0.10 g/L to about 0.15 g/L, or about 0.15 g/L to about 0.2 g/L); a final concentration of KH_2PO_4 of about 0.2 g/L to about 0.4 g/L (e.g., about 0.2 g/L to about 0.35 g/L, about 0.2 g/L to about 0.30 g/L, about 0.2 g/L to about 0.25 g/L, about 0.25 g/L to about 0.4 g/L, about 0.25 g/L to about 0.30 g/L, about 0.30 g/L to about 0.40 g/L, or about 0.30 g/L to about 0.35 g/L, or about 0.35 g/L to about 0.40 g/L); and/or a final concentration of Na_3 citrate of about 0.1 g/L to about 0.3 g/L (e.g., 0.1 g/L to about 0.25 g/L, about 0.1 g/L to about 0.20 g/L, about 0.1 g/L to about 0.15 g/L, about 0.15 g/L to about 0.30 g/L, about 0.15 g/L to about 0.25 g/L, about 0.15 g/L to about 0.20 g/L, about 0.20 g/L to about 0.30 g/L, about 0.20 g/L to about 0.25 g/L, or about 0.25 g/L to about 0.30 g/L).

In any of the composition described herein, the κ-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth κ-casein protein. In any of the compositions described herein, the β-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla,

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chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-casein protein.

In some examples of any of the compositions described herein can further include: a final concentration of α-lactalbumin protein (e.g., any of the α-lactalbumin proteins described herein) of about 0.4 weight % to about 2.5 weight % (e.g., about 0.4 weight % to about 2.0 weight %, about 1.5 weight %, or about 1.0 weight %; about 1.0 weight % to about 2.5 weight %, about 2.0 weight %, or about 1.5 weight %, about 1.5 weight % to about 2.5 weight % or 2.0 weight %; or about 2.0 weight % to about 2.5 weight %), and/or a final concentration of β-lactoglobulin protein (e.g., any of the β-lactoglobulin proteins described herein) of about 2.5 weight % to about 4.5 weight %. In some embodiments of any of the compositions described herein, the α-lactalbumin protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-lactalbumin protein. In some embodiments of any of the compositions described herein, the β-lactoglobulin protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-lactoglobulin protein.

Some embodiments of any of the compositions described herein further include: a final concentration of α-S1-casein protein (e.g., any of the α-S1-casein proteins described herein) of about 11 weight % to about 16 weight % (e.g., about 11 weight % to about 15 weight %, about 14 weight %, about 13 weight %, or about 12 weight %; about 12 weight % to about 16 weight %, about 15 weight %, about 14 weight %, or about 13 weight %; about 13 weight % to about 16 weight %, about 15 weight %, or about 14 weight %; about 14 weight % to about 16 weight % or 15 weight %; or about 15 weight % to about 16 weight %); and/or a final concentration of α-S2-casein protein (e.g., any of the α-S2-casein proteins described herein) of about 2 weight % to about 5 weight % (e.g., about 2

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weight % to about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, about 3.0 weight %, or about 2.5 weight %; about 2.5 weight % to about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, about 3.5 weight %, or about 3.0 weight %; about 3.0 weight % to about 5.0 weight %, about 4.5 weight %, about 4.0 weight %, or about 3.5 weight %; about 3.5 weight % to about 5 weight %, about 4.5 weight %, or about 4.0 weight %; about 4.0 weight % to about 5.0 weight % or 4.5 weight %; or about 4.5 weight % to about 5.0 weight %).

In some examples of any of the compositions described herein, the α-S1-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S1-casein protein; and/or the α-S2-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth α-S2-casein protein.

Some examples of any of the compositions described herein further include one or more (e.g., two or three) of serum albumin (e.g., any of the serum albumin proteins described herein), lactoferrin (e.g., any of the lactoferrin proteins described herein), and transferrin (e.g., any of the transferrin proteins described herein). In some examples of any of the compositions described herein, the serum albumin can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth serum albumin; the lactoferrin can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth lactoferrin; and/or the transferrin can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur,

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panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth transferrin protein.

In some examples of any of the compositions described herein, the composition further includes one or more color balancing agents (e.g., any of the coloring agents described herein, e.g., β-carotene or annatto).

Any of the compositions described herein can have a pH of about 6.2 to about 7.2 (e.g., about 6.2 to about 7.0, about 6.2 to about 6.8, about 6.2 to about 6.6, about 6.2 to about 6.4, about 6.4 to about 7.2, about 6.4 to about 7.0, about 6.4 to about 6.8, about 6.4 to about 6.6, about 6.6 to about 7.2, about 6.6 to about 7.0, about 6.6 to about 6.8, about 6.8 to about 7.2, about 6.8 to about 7.0, or about 7.0 to about 7.2).

In various embodiments, the milk protein components comprise about 0.5% about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6% milk protein by dry weight or total weight. In some embodiments, the compositions can comprise about 0.5-2.5%, about 1-2%, about 2-3%, or about 4-10% protein by dry weight or total weight. In particular embodiments, the compositions can comprise about 10-15% protein by dry weight or total weight.

A wide range of caseins including casein with substantial homology to the wild-type casein, variants, mutants of casein are expressed and incorporated as a component of milk protein.

Dry Compositions

Also provided are powder compositions including: a final concentration of κ-casein protein (e.g., any of the α-casein proteins described herein) of about 3.6 weight % to about 5.4 weight % (e.g., about 3.6 weight % to about 5.2 weight %, about 5.0 weight %, about 4.8 weight %, about 4.6 weight %, about 4.4 weight %, about 4.2 weight %, about 4.0 weight %, or about 3.8 weight %; about 3.8 weight % to about 5.4 weight %, about 5.2 weight %, about 5.0 weight %, about 4.8 weight %, about 4.6 weight %, about 4.4 weight %, about 4.2 weight %, or about 4.0 weight %; about 4.0 weight % to about 5.4 weight %, about 5.2 weight %, about 5.0 weight %, about 4.8 weight %, about 4.6

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weight %, about 4.4 weight %, or about 4.2 weight %; about 4.2 weight % to about 5.2 weight %, about 5.2 weight %, about 5.0 weight %, about 4.8 weight %, about 4.6 weight %, or about 4.4 weight %; about 4.8 weight % to about 5.4 weight %, about 5.2 weight %, or about 5.0 weight %; about 5.0 weight % to about 5.4 weight % or about 5.2 weight %; or about 5.2 weight % to about 5.4 weight %); a final concentration of β-casein protein (e.g., any of the β-casein proteins described herein) of about 16.3 weight % to about 24.5 weight %; 16.3 weight % to about 22 weight %, about 20 weight %, or about 18 weight %; about 18 weight % to about 24.5 weight %, about 22 weight %, or about 20 weight %; about 20 weight % to about 24.5 weight % to about 22 weight %; or about 22 weight % to about 24.5 weight %); a final concentration of a sweetening agent (e.g., any one or more of the sweetening agents described herein) of about 35 weight % to about 40 weight % (e..g, about 35 weight % to about 39 weight %, about 38 weight %, about 37 weight %, or about 36 weight %; about 36 weight % to about 40 weight %, about 39 weight %, about 38 weight %, or about 37 weight %; about 37 weight % to about 40 weight %, about 39 weight %, or about 38 weight %; about 38 weight % to about 40 weight % or 39 weight %; or about 39 weight % to about 40 weight %); a final concentration of one or more lipids (e.g., any of the one or more lipids described herein) of about 25 weight % to about 30 weight % (e.g., about 25 weight % to about 29 weight %, about 28 weight %, about 27 weight %, or about 26 weight %; about 26 weight % to about 30 weight %, about 29 weight %, about 28 weight %, or about 27 weight %; about 27 weight % to about 30 weight %, about 29 weight %, or about 28 weight %; about 28 weight % to about 30 weight % or about 29 weight %; or about 29 weight % to about 30 weight %); a final concentration of ash (e.g., any of the ash described herein) of about 5 weight % to about 7 weight % (e.g., about 5 weight % to about 6.5 weight %, about 6.0 weight %, or about 5.5 weight %; about 5.5 weight % to about 7.0 weight %, about 6.5 weight %, or about 6.0 weight %; about 6.0 weight % to about 7.0 weight % or about 6.5 weight %; or about 6.5 weight % to about 7.0 weight %); and a final concentration of water of about 2 weight % to about 5 weight % (e.g., about 2 weight % to about 4 weight % or about 3 weight %; about 3 weight % to about 5 weight % or about 4 weight %; or about 4 weight % to about 5 weight %), where the κ-casein protein is an unglycosylated

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and/or has a non-mammalian glycosylation pattern, and/or the β-casein protein is an unglycosylated and/or has a non-mammalian glycosylation pattern.

Any of the powder compositions can contain any of the components described in any of the compositions described herein (e.g., one or more of any of the color matching agents, α-S1-casein proteins, α-S2-casein proteins, α-lactalbumin proteins, β-lactoglobulin proteins, lactoferrin proteins, transferrin proteins, and serum albumin protein described herein at any of the concentrations described herein for each component, respectively).

Supplemented Milk Compositions

Also provided herein are compositions including: a mammalian-produced milk or a processed mammal-produced milk; and one or more (e.g., two or three) of a κ-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern; a β-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern; or a micelle including a κ-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern and a β-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern.

In some examples, the composition includes a mammal-produced milk or a processed mammalian-produced milk and a κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern. In some examples, the composition includes a mammal-produced milk or a processed mammalian-produced milk and a β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern. In other examples, the composition includes a mammal-produced milk or a processed mammalian-produced milk and a micelle including a κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern and a β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern.

In some examples, the final concentration of the κ-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern or the final concentration of the β-casein protein that is unglycosylated or has a non-mammalian glycosylation pattern in the composition is: 0.02 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about

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1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, about 0.2 weight %, or about 0.1 weight %; about 0.1 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, or about 0.2 weight %; about 0.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, or about 0.4 weight %; about 0.8 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, or about 1.0 weight %; about 1.0 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, or about 1.2 weight %; about 1.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, or about 1.4 weight %; about 1.4 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, or about 1.6 weight %; about 1.6 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, or about 1.8 weight %; about 1.8 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, or about 2.0 weight %; about 2.0 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, or about 2.2 weight %; about 2.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, or about 2.4 weight %; about 2.4 weight % to about 3.0 weight %, about 2.8 weight %, or about 2.6 weight %; about 2.6 weight % to about 3.0 weight % or about 2.8 weight %; or about 2.8 weight % to about 3.0 weight % (of the final composition).

In some compositions, the final concentration of the κ-casein protein that is unglycosylated and/or has a non-mammalian glycosylation pattern in the composition is

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about 0.02 weight % to about 0.6 weight % (e.g., about 0.02 weight % to about 0.5 weight %, about 0.02 weight % to about 0.4 weight %, about 0.02 weight % to about 0.3 weight %, about 0.02 weight % to about 0.2 weight %, about 0.02 weight % to about 0.1 weight %, about 0.1 weight % to about 0.5 weight %, about 0.1 weight %, to about 0.4 weight %, about 0.1 weight % to about 0.3 weight %, about 0.1 weight % to about 0.2 weight %, about 0.2 weight % to about 0.5 weight %, about 0.2 weight % to about 0.4 weight %, about 0.2 weight % to about 0.3 weight %, about 0.3 weight % to about 0.5 weight %, about 0.3 weight % to about 0.4 weight %, or about 0.4 weight % to about 0.5 weight %); and the final concentration of β-casein that is unglycosylated and/or has a non-mammalian glycosylation pattern in the composition is about 0.02 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, or about 0.2 weight %; about 0.2 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, or about 0.4 weight %; about 0.4 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, or about 0.6 weight %; about 0.6 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, or about 0.8 weight %; about 0.8 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about

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2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, or about 1.0 weight %; about 1.0 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, or about 1.2 weight %; about 1.2 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, or about 1.4 weight %; about 1.4 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, or about 1.6 weight %; about 1.6 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, or about 1.8 weight %; about 1.8 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, or about 2.0 weight %; about 1.8 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, or about 2.0 weight %; about 2.0 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, or about 2.2 weight %; about 2.2 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, or about 2.4 weight %; about 2.4 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, about 2.8 weight %, or about 2.6 weight %; about 2.6 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, about 3.0 weight %, or about 2.8 weight %; about 2.8 weight % to about 4.0 weight %, about 3.8

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weight %, about 3.6 weight %, about 3.4 weight %, about 3.2 weight %, or about 3.0 weight %; about 3.0 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, about 3.4 weight %, or about 3.2 weight %; about 3.2 weight % to about 4.0 weight %, about 3.8 weight %, about 3.6 weight %, or about 3.4 weight %; about 3.4 weight % to about 4.0 weight %, about 3.8 weight %, or about 3.6 weight %; about 3.6 weight % to about 4.0 weight % or about 3.8 weight %; or about 3.8 weight % to about 4.0 weight %.

In some examples, the final concentration of micelles including a κ-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern and a β-casein protein that is unglycosylated or has an non-mammalian glycosylation pattern in the composition is: 0.02 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, about 0.2 weight %, or about 0.1 weight %; about 0.1 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, about 0.4 weight %, or about 0.2 weight %; about 0.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, about 1.0 weight %, about 0.8 weight %, about 0.6 weight %, or about 0.4 weight %; about 0.8 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, about 1.2 weight %, or about 1.0 weight %; about 1.0 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, about 1.4 weight %, or about 1.2 weight %; about 1.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, about 1.8 weight %, about 1.6 weight %, or about 1.4 weight %; about 1.4 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2

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weight %, about 2.0 weight %, about 1.8 weight %, or about 1.6 weight %; about 1.6 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, about 2.0 weight %, or about 1.8 weight %; about 1.8 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, about 2.2 weight %, or about 2.0 weight %; about 2.0 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, about 2.4 weight %, or about 2.2 weight %; about 2.2 weight % to about 3.0 weight %, about 2.8 weight %, about 2.6 weight %, or about 2.4 weight %; about 2.4 weight % to about 3.0 weight %, about 2.8 weight %, or about 2.6 weight %; about 2.6 weight % to about 3.0 weight % or about 2.8 weight %; or about 2.8 weight % to about 3.0 weight % (of the final composition).

Nucleic Acids and Vectors

Also provided are nucleic acids (e.g., vectors) that include: a promoter (e.g., a yeast, bacterial, or a mammalian promoter); a sequence encoding a signal sequence; a sequence encoding a milk protein (e.g., any of the exemplary sequences described herein); and a yeast termination sequence, where the promoter is operably linked to the signal sequence, the signal sequence is operably linked to the sequence encoding the milk protein, and the terminal sequence is operably linked to the sequence encoding the milk protein. In some examples of these nucleic acids, the promoter is a constitutive promoter or an inducible promoter. Non-limiting examples of promoters are described herein. Additional promoters that can be used in these nucleic acids are known in the art.

The signal sequence in any of the vectors described herein can be a signal sequence from the encoded milk protein or a different milk protein, or is a signal sequence from a yeast mating factor (e.g., any alpha mating factor). In some examples, the encoded milk protein is selected from the group of: β-casein (e.g., any of the β-casein proteins described herein), κ-casein (e.g., any of the κ-casein proteins described herein), α-S1-casein (e.g., any of the α-S1-casein proteins described herein), α-S2-casein (e.g., any of the α-S2-casein proteins described herein), α-lactalbumin (e.g., any of the α-lactalbumin proteins described herein), β-lactoglobulin (e.g., any of the β-lactoglobulin proteins described herein), lactoferrin (e.g., any of the lactoferrin proteins described

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herein), or transferrin (e.g., any of the transferrin proteins described herein). Additional signal sequences that can be used in the present vectors are known in the art.

Any of the nucleic acids described herein can further include a bacterial origin of replication. Any of the nucleic acids described herein can further include a selection marker (e.g., an antibiotic resistance gene). The sequences of bacterial origin of replication are known in the art. Non-limiting examples of antibiotic resistance genes are described herein. Additional examples of resistance genes are known in the art.

Non-limiting examples of termination sequences are described herein. Additional examples of termination sequences are known in the art.

Some embodiments of the nucleic acids provided herein further include: an additional promoter sequence (e.g., any of the exemplary promoters described herein); an additional sequence encoding a signal sequence (e.g., any of the exemplary signal sequences described herein); a sequence encoding an additional milk protein (e.g., any of the exemplary sequences encoding a milk protein described herein); and an additional yeast termination sequence (e.g.. any of the exemplary yeast termination sequences described herein), where the additional promoter sequence is operably linked to the additional sequence encoding a signal sequence, the sequence encoding the signal sequence is operably linked to the sequence encoding the additional milk protein, and the sequence encoding the additional milk protein is operably linked to the additional yeast terminal sequence. The additional milk protein can be, e.g., β-casein (e.g., any of the β-casein proteins described herein), κ-casein (e.g., any of the κ-casein proteins described herein), α-S1-casein (e.g., any of the α-S1-casein proteins described herein), α-S2-casein (e.g., any of the α-S2-casein proteins described herein), α-lactalbumin (e.g., any of the α-lactalbumin proteins described herein), β-lactoglobulin (e.g., any of the β-lactoglobulin proteins described herein), lactoferrin (e.g., any of the lactoferrin proteins described herein), or transferrin (e.g., any of the transferrin proteins described herein). In some embodiments, the nucleic acid includes a sequence encoding a β-casein and a sequence encoding a κ-casein. The promoter and the additional promoter can be the same or different. The yeast termination sequence and the additional yeast terminal sequence can be the same or different. The signal sequence and the additional signal sequence can be the same or different.

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The present invention also encompasses a vector containing the isolated DNA sequence encoding casein or whey polypeptide and host cells comprising the vector. The vector may further comprise an isolated DNA sequence comprising a nucleotide sequence encoding a casein, wherein the nucleotide sequence is operably linked to a promoter, a nucleotide sequence encoding an alpha mating factor, or a variant thereof, a nucleotide sequence encoding a bacterial resistance marker and a transcription terminator. One or more of suitable promoters are utilized for expression of the genes encoding casein or whey proteins may be any promoter which is functional in the host cell and is able to elicit expression of the product encoded by the gene. Suitable promoters include, for example, $P_{LAC4\text{-}PBI}$, T7, Ptac, Pgal, λPL, λPR, bla, spa, Adh, CYC, TDH3, ADH1 and CLB1.

Introducing Nucleic Acids into a Cell

Methods of introducing nucleic acids (e.g., any of the nucleic acids described herein) into a cell to generate a host cell are well-known in the art. Non-limiting examples of techniques that can be used to introduce a nucleic acid into a cell include: calcium phosphate transfection, dendrimer transfection, liposome transfection (e.g., cationic liposome transfection), cationic polymer transfection, electroporation, cell squeezing, sonoporation, optical transfection, protoplast fusion, impalefection, hyrodynamic delivery, gene gun, magnetofection, and viral transduction.

One skilled in the art would be able to select one or more suitable techniques for introducing the nucleic acids into a cell based on the knowledge in the art that certain techniques for introducing a nucleic acid into a cell work better for different types of host cells. Exemplary methods for introducing a nucleic acid into a yeast cell are described in Kawai et al., *Bioeng. Bugs* 1:395-403, 2010.

Host Cells

Also provided herein a host cells including any of the nucleic acids (e.g., vectors) described herein. In some examples, the nucleic acid described herein is stably integrated within the genome (e.g., a chromosome) of the host cell. In other examples, the nucleic acid described herein is not stably integrated within the genome of the host cell.

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In some embodiments, the host cell is a yeast strain or a bacterial strain. In some embodiments, the host cell can be, e.g., a yeast strain selected from the group of: a *Kluyveromyces* sp., *Pichia* sp., *Saccharomyces* sp., *Tetrahymena* sp., *Yarrowia* sp., *Hansenula* sp., *Blastobotrys* sp., *Candida* sp., *Zygosaccharomyces* sp., and *Debaryomyces* sp. Additional non-limiting examples of yeast strains that can be used as the host cell are *Kluyveromyces lactis*, *Kluyveromyces marxianus, Saccharomyces cerevisiae*, and *Pichia pastoris*. Additional species of yeast strains that can be used as host cells are known in the art.

In some examples, the host cell can be a protozoa, such as, e.g., *Tetrahymena thermophile*, *T. hegewischi*, *T. hyperangularis*, *T. malaccensis*, *T. pigmentosa*, *T. pyriformis*, and *T. vorax*.

It is an object of the invention to isolate milk protein components by recombinantly expressing them in any of the host cells provided herein.

Methods of Producing a Recombinant Milk Protein and Methods of Making a Micelle

Also provided are methods of producing a recombinant milk protein (e.g., one or more of any of the milk proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern that include: culturing any of the host cells described herein in a culture medium under conditions sufficient to allow for secretion of the milk protein that is unglycosylated or has a non-mammalian glycosylation pattern; and harvesting the milk protein that is unglycosylated or has a non-mammalian glycosylation pattern from the culture medium. Suitable culture medium for use in these methods are known in the art. Culture conditions sufficient to allow for secretion of a milk protein are also known in the art. The host cells used in these methods can be any of the host cells described herein. The host cells can include any of the nucleic acids described herein. The recombinant milk protein produced can be one or more of: β-casein (e.g., any of the β-casein proteins described herein), κ-casein (e.g., any of the κ-casein proteins described herein), α-S1-casein (e.g., any of the α-S1 caseins described herein), α-S2-casein (e.g., any of the α-S2-caseins described herein), α-lactalbumin (e.g., any of the α-lactalbumin proteins described herein), β-lactoglobulin (e.g., any of the β-lactoglobulin proteins

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described herein), lactoferrin (e.g., any of the lactoferrin proteins described herein), transferrin (e.g., any of the transferrin proteins described herein), and serum albumin (e.g., any of the serum albumin proteins described herein). Some of these methods further include isolating (e.g., purifying) the recombinant milk protein from the culture medium. Methods of isolating (e.g., purifying) a recombinant milk protein from a liquid are well-known in the art. Exemplary methods for isolating (e.g., purifying) recombinant milk proteins are described in Imafidon et al., *Crit. Rev. Food Sci. Nutrition* 37:663-669, 1997),

Also provided are methods of producing a micelle including a β-casein (e.g., any of the β-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern and a κ-casein (e.g., any of the κ-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern, that include: culturing any of the host cells described herein in a culture medium under conditions sufficient to allow for release of the micelle from the host cell, where the host cell comprises nucleic acid including a sequence that encodes a β-casein and a sequence that encodes a κ-casein; and harvesting the micelle from the culture medium. Suitable culture medium for use in these methods are known in the art. The host cells used in these methods can be any of the host cells described herein. The host cells can include any of the nucleic acids described herein. The micelles produced can be any of the micelles described herein (and can have any of the physical characteristics of micelles described herein). Some of these methods further include isolating (e.g., purifying) the micelle from the culture medium. Methods of isolating (e.g., purifying) a micelle from a liquid are well-known in the art (e.g., ultracentrifigation).

Exemplary details of culturing yeast host cells are described in Idiris et al., *Appl. Microbiol. Biotechnol.* 86:403-417, 2010; Zhang et al., *Biotechnol. Bioprocess. Eng.* 5:275-287, 2000; Zhu, *Biotechnol. Adv.* 30:1158-1170, 2012; Li et al., *MAbs* 2:466-477, 2010.

It is an object of the invention to express one or more different forms of casein for application into various types of dairy substitute products. Casein subunits such as α-s1-casein, α-s2-casein, β-casein and κ-casein differ by one or more amino acid changes. In certain embodiments, the methods and compositions comprise incorporation of bovine

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casein such as α-s1-casein, α-s2-casein, β-casein and κ-casein. In other embodiments, the methods and compositions comprise incorporation of human casein such as β-casein and κ-casein. See U.S. Patent No. 5,942,274. In alternative embodiments, casein is selected from one or more following sources including but not limited to: bovine, human, buffalo, camel, goat, sheep, horse, dolphin, whale, mountain goat and pig.

Also provided are methods for producing the milk protein components that can include, e.g., using a plasmid or construct of the invention as described in Example 1. This method comprises preparing the plasmid of interest, inserting the plasmid into an appropriate host cell, culturing the host cell for a suitable time and under suitable conditions such that the protein of interest is expressed, and then purifying the protein.

Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the protein, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein.

Generally, centrifugation at an optimum pH yields purification efficiency >95%. Isoelectric point for the native caseins and whey proteins are known. In nature, the pH is 4.91 for bovine α-s1-casein, pH 4.1 for bovine α-s2-casein, pH 4.5 for bovine β-casein, pH 4.1 for bovine κ-casein, pH 4.2 for bovine α-lactalbumin, and pH 5.2 for bovine β-lactoglobulin. The recombinantly produced casein and whey can differ in terms of its phosphate groups and sugar groups. Other methods for protein purification include membrane filtration to remove any potential bacteria or contaminants, followed by lyophilization for protein isolation.

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Preferably, the methods and compositions provide for a production cost that is competitive at or below $1,000/kg, $500/kg, $10/kg, $1.0/kg, $0.10/kg, $0.010/kg or $0.0010/kg of milk protein component. In more preferred embodiments, the cost is below $0.009, $0.007, $0.006, $0.005/kg of milk protein component.

Methods of Supplementing a Mammal-Produced Milk

Also provided herein are methods of supplementing a mammal-produced milk that include providing a mammalian-produced milk or a processed mammalian-produced milk; and mixing into the milk at least one of: a β-casein protein (e.g., any of the β-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern; a κ-casein protein (e.g., any of the κ-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern; and a micelle (e.g., any of the micelles described herein) comprising a β-casein protein (e.g., any of the β-casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern, and a κ-casein protein (e.g., any of the casein proteins described herein) that is unglycosylated or has a non-mammalian glycosylation pattern.

One or more of the β-casein protein, the κ-casein protein, and the micelles can be mixed into the milk to achieve any of the exemplary final concentrations of the β-casein protein, the κ-casein protein, and the micelles in a composition described in the section called "Supplemented Milk Compositions" herein. Methods of mixing are well known in the art. As one of skill in the art can appreciate, additional components described herein can also be mixed into the milk (e.g., any component described herein without limitation).

Methods of Making a Composition

Also provided are methods of producing a composition that include: sonicating a liquid including a protein mixture comprising β-casein protein (e.g., any of the β-casein proteins described herein) and casein κ protein (e.g., any of the κ-casein proteins described herein), or including micelles comprising β-casein protein (e.g., any of the β-casein proteins described herein) and κ-casein protein (e.g., any of the κ-casein proteins described herein); mixing ash (e.g., any of the ash described herein) into the liquid;

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adding to the liquid a mixture of one or more lipids (e.g., any of the one or more liquids described herein), one or more flavor compounds (e.g., any of the one or more flavor compounds described herein), and one or more color balancing agents (e.g., any of the one or more color balancing agents described herein), and sonicating the liquid; and adding to the liquid one or more sweetening agents (e.g., one or more of any of the sweetening agents described herein), thereby producing the composition.

In some examples of these methods, the β-casein protein is unglycosylated or has a non-mammalian glycosylation pattern, and/or the κ-casein protein is unglycosylated or has a non-mammalian glycosylation pattern. In some examples of these methods, the ash includes one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some examples of any of these methods, the ash added includes one or more (e.g., two or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate.

In some examples of these methods, the one or more lipids comprises at least one (e.g., two, three, four, five, six, or seven) of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. In some examples of these methods, the free fatty acids comprise at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. In some examples of these methods, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some embodiments of these methods, the flavor compounds include at least one (e.g., two, three, four, five, or six) flavor compound selected from the group of: δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone.

In some examples of these methods, the one or more coloring balancing agent is β-carotene or annatto. In some embodiments of these methods, the one or more sweetening agents is a saccharide (e.g., glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, or tagatose) or an artificial sweetener (e.g., stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, or P-4000).

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The pH of the resulting composition can be between about pH 6.2 and about pH 7.4 (e.g., about 6.2 to about 7.2; about 6.2 to about 7.0, about 6.2 to about 6.8, about 6.2 to about 6.6, about 6.2 to about 6.4, about 6.4 to about 7.2, about 6.4 to about 7.0, about 6.4 to about 6.8, about 6.4 to about 6.6, about 6.6 to about 7.2, about 6.6 to about 7.0, about 6.6 to about 6.8, about 6.8 to about 7.2, about 6.8 to about 7.0, or about 7.0 to about 7.2).

In any of these methods, the β-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth β-casein protein; and/or the κ-casein protein can be a cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth κ-casein protein.

In some embodiments of these methods, the protein mixture further comprises one or more proteins selected from the group of: α-lactalbumin (e.g., any of the α-lactalbumin proteins described herein), β-lactoglobulin (e.g., any of the β-lactoglobulin proteins described herein), α-S1-casein (e.g., any of the α-S1-casein proteins described herein), α-S2-casein (e.g., any of the α-S2-casein proteins described herein), lactoferrin (e.g., any of the lactoferrin proteins described herein), transferrin (e.g., any of the transferrin proteins described herein), and serum albumin (e.g., any of the serum albumin proteins described herein).

As one of skill in the art can appreciate, the amount of each component used in these methods can be calculated in order to produce any of the compositions described herein.

Methods of Making Butter, Cheese, Caseinate, or Yogurt

Also provided herein are methods of making butter, cheese, caseinate, or yogurt that include providing any of the compositions provided herein; and producing the butter,

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cheese, caseinate, or yogurt using any of the composition provided herein as a starting material.

Methods for making butter, cheese, caseinate, or yogurt are well-known in the art. See, e.g., Scott, Cheesemaking Practice, Kluwer Academic/Plenum Publishers, New York, NY, 1998; U.S. Patent No. 4,360,535 (which describes methods of making creams); U.S 285,878 (which described methods of making butter);

Kits

Also provided are kits that include: (a) a mixture of one or more milk proteins (e.g., any of the milk proteins described herein, including any one or more of the β-casein proteins, κ-casein proteins, α-S1-proteins, α-S2-proteins, α-lactalbumin proteins, β-lactoglobulin proteins, lactoferrin proteins, transferrin proteins, and serum albumin proteins described herein), one or more lipids (e.g., any of one or more of the lipids described herein), and one or flavor compounds (e.g., any one or more of the flavor compounds described herein); and (b) a mixture of ash (e.g., any of the ash described herein) and at least one sweetening agent (e.g., any one or more of the sweetening agents described herein). In some examples of these kits, the one or more milk proteins are cow, human, sheep, goat, buffalo, camel, horse, donkey, lemur, panda, guinea pig, squirrel, bear, macaque, gorilla, chimpanzee, mountain goat, monkey, ape, cat, dog, wallaby, rat, mouse, elephant, opossum, rabbit, whale, baboons, gibbons, orangutan, mandrill, pig, wolf, fox, lion, tiger, echidna, or woolly mammoth milk proteins.

In some examples of these kits, the one or more fats are selected from the group of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. The fatty acids present in the kit can include at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. The phospholipids in the kit can be soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids.

The flavor compounds in the kit can include at least one flavor compound selected from the group of: δ-decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, and δ-undecalactone.

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In some embodiments of the kit, the mixture in (a) further includes one or more color balancing agent (e.g., any of the color balancing agents described herein, e.g., β-carotene or annatto).

In some examples of the kits, the one or more sweetening agents is a saccharide (e.g., glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, or tagatose) or an artificial sweetener (e.g., stevia, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacititol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihyrdochalcone, neotame, or P-4000).

The kits can include an ash including one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some examples, the ash in the kit includes one or more (e.g., two or three) of $CaCl_2$, KH_2PO_4, and Na_3 citrate.

In some embodiments of the kits, the mixture in (a) is provided in a light-sealed and airtight package (e.g., a metal foil, e.g., an aluminum foil), and/or the mixture in (b) is provided in an airtight package (e.g., a sealed plastic bag).

Some examples of the kits further include instructions for making any of the compositions described herein.

Also provided herein are kits including at least one nucleic acid described herein.

Modulating Flavor Profiles

Sensory impressions such as "feed," "barny," or "unclean," are described as flavor descriptions that are absorbed from the food ingested by the cow and from the odours in its surroundings. Others develop through microbial action due to growth of bacteria in large numbers. Chemical changes can also take place through enzyme action, contact with metals (such as copper), or exposure to sunlight or strong fluorescent light. Quality-control directors are constantly striving to avoid off-flavors in milk and other dairy foods. It is, therefore, an object of the invention to reduce, eliminate or even mask the undesirable flavors and odor of various dairy products.

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In certain preferred aspects of the present invention, varying the fat content can alter the flavors and odor of various dairy substitute products. For example, increasing the butyric acid content can change a flavor profile of a non-dairy cheese to a flavor profile similar to parmesan cheese. In other embodiments, modulating the triglycerides such caproic, capric, and/or caprylic acid results in a flavor profile similar to goat cheese. Accordingly, modulating the triglycerides with the ratios of fatty acid components provides different flavor profiles that can be fine-tuned to resemble those of various desirable dairy-food products.

Similarly, the methods and compositions provide for minimizing one or more undesirable aromas by modulating various triglycerides incorporated into the dairy substitute products.

In certain aspects flavor profile is modulated by incorporating synthetic short-chain triglycerides combined with plant-based oils e.g., sunflower oil, in desired combinations. For example a mixture of [C18 C18 C6] and [C18 C6 C18] provides a different flavor profile than a mixture of [C18 C4 C4] and [C18 C10 C10].

Dairy Substitute Products

A wide variety of dairy substitute products can be made using the methods and compositions of the present invention. Such products include without limitation, milk, whole milk, buttermilk, skim milk, infant formula, condensed milk, dried milk, evaporated milk, butter, clarified butter, cream and various types of cheese.

The dairy substitute products can also be incorporated into various food applications as a replacement for dairy products, which include the following ice cream, frozen custard, frozen yogurt, cookies, cakes, cottage cheese, cream cheese, crème fraiche, curds and yogurt.

In certain aspects, the present invention provides one or more subunits of casein selected from α-s1-casein, α-s2-casein, β-casein and κ-casein for the milk protein component in a dairy substitute product. A select combination of casein subunits are used as the primary or at least a part of the milk protein component. In preferred embodiments, the casein composition comprises the following amounts of casein

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subunits such that about 12-15g/L α-s1-casein, about 3-4g/L α-s2-casein, about 9-11g/L β-casein and about 2-4g/L κ-casein represent the total casein in a synthetic milk product..

In various embodiments, the casein compositions can comprise about 0.5% about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6%, protein by dry weight or total weight. In some embodiments, the casein compositions can comprise about 0.5-2.5%, about 1-2%, about 2-3%, or about 4-10% casein protein by dry weight or total weight. In particular embodiments, the casein compositions can comprise about 1.5-10% protein by dry weight or total weight.

In certain aspects, the methods and compositions of the dairy substitute products are essentially free of one or more serum proteins. Serum proteins typically comprise, among other proteins, enzymes, hormones, growth factors, nutrient transporters and disease resistance factors. In additional embodiments, the methods and compositions of the dairy substitute products are essentially free of one or more immunoglobulins, which may induce an undesirable immune response.

In some embodiments, whey compositions can comprise about 0.001%, about 0.05%, about 0.1%, about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4% whey protein by dry weight or total weight. In some embodiments, the compositions can comprise about 0.1-1%, about 1-2%, about 2-3%, or about 0.1-2.3% protein by dry weight or total weight. In particular embodiments, the compositions can comprise about 10-15% protein by dry weight or total weight.

In various embodiments, carbohydrates are incorporated into the dairy substitute products. These carbohydrates provide a bland sweetness to the flavor profile of the product and additionally serve as a fast-acting energy and nutrition source. Carbohydrates include but are not limited to sugars such as galactose, sucrose, glucose, fructose and maltose. Dairy-free sources of sugars include but are not limited to sugar beet and other plants such as celery, basil, honey, cherries, corn, spinach, plums, kiwis and peas.

Lactose intolerance is common for many milk consumers. Accordingly, in preferred embodiments, carbohydrates such as lactose are omitted from the dairy substitute composition. In preferred embodiments, methods and compositions of the dairy substitute composition essentially free of lactose.

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In some embodiments, the carbohydrate compositions can comprise about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5% carbohydrate by dry weight or total weight. In some embodiments, the compositions can comprise about 1-3%, about 2-4%, or about 10-30% carbohydrate by dry weight or total weight. In particular embodiments, the compositions can comprise about 2-5% carbohydrate by dry weight or total weight.

Ash attributes to the structure and stability of casein micelles. Ash is important for holding the emulsion that is milk or cream together. The calcium and phosphate present in the ash interact with the fat globules and the casein micelles to maintain an emulsified mixture.

The ash also affects the sensory characteristics such as mouthfeel, consistency, and to a certain extent, the flavor of the milk.

In some embodiments, the ash compositions can comprise about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 2% or about 3% ash by dry weight or total weight. In some embodiments, the compositions can comprise about 0.1-0.3%, about 0.5-0.7%, about 0.7-1%, or about 1-2% ash by dry weight or total weight. In particular embodiments, the compositions can comprise about 0.6-0.8% protein by dry weight or total weight.

Additional ingredients for various animal-free dairy products include vitamins, flavoring agents, natural or artificial sweeteners, coloring agents, salt, pH adjustment agents, binders, buffers, stabilizers, essential amino acids, anti-caking agents, anti-foaming agents, and mixtures thereof.

In some embodiments, the remaining ingredient compositions can comprise about 0%, about 0.01%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4% or about 5% additives by dry weight or total weight. In some embodiments, the compositions can comprise about 0.001-0.01%, about 0.01-1%, about 0.01-2%, or about 1-5% additives by dry weight or total weight. In particular embodiments, the compositions can comprise about 0-10% additives by dry weight or total weight.

In some aspects, the present invention provides methods and compositions for dairy substitutes with fat comprising varying levels of triglyceride content. In preferred embodiments, isolated triglycerides from various plant sources are incorporated with

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milk protein components, carbohydrates and ash. It is an object of the present invention to modulate the fatty acids isolated in plants and transesterified in a dairy substitute to resemble the percentage of fatty acids found in natural dairy products, and/or to develop novel flavor profiles with improved flavor not found in nature. In some embodiments, modulating specific short-to-medium chain fatty acids including but not limited to s butyric, capric, caprylic, caproic and lauric acids provides the desired flavor profile in a dairy substitute.

In some embodiments, the fat compositions in synthetic milk comprises about 0%, about 1%, about 2%, about 3%, about 3.5%, about 4% fat by dry weight or total weight. In some embodiments, the compositions can comprise about 1-2%, about 2-3%, about 3-4% fat by dry weight or total weight. In particular embodiments, the compositions can comprise about 3-4% fat by dry weight or total weight. In alternative embodiments, fat compositions in cream can comprise about 10%, about 20%, about 30%, about 40%, about 50% or even 60%. Preferably, fat compositions in cream is typically about 40 to about 50%.

In some aspects, the short-chain triglycerides are combined with longer chain oil to produce transesterified fatty acid esters. Preferably, the longer chain oils are selected from: sunflower, corn, olive, soy, peanut, walnut, almond, sesame, cottonseed, canola, safflower, flax seed, palm, palm kernel, palm fruit, coconut, babassu, shea butter, mango butter, cocoa butter, wheat germ and rice bran oil. More preferably, the longer chain oils comprise engineered sunflower varieties, which overexpress oleic acid by 400%.

Longer chain oil can also provide to the flavor profile, for example, reduce or even remove sharpness and mellow out the overall flavor profile of the desired end product.

In some embodiments, the fat component of the dairy substitute comprises select triglycerides that are transesterified into longer chain oil such as high-oleic sunflower oil (Example 2). It is contemplated that the same four short chain fatty acids give milk and derivative products such as cheese their particular flavors such as robustness and richness. Various combinations of triglycerides and longer chain oils are incorporated to create a number of different flavor profiles. In one embodiment, triglyceride with three oleic acids and synthetic short-chain triglyceride with, in this case, one butyric, one

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hexanoic, and one octanoic acid, yields a desired synthetic "milk fat" triglyceride. Additional embodiments include incorporating various short-chain triglycerides to tune slightly different flavor profiles, for instance, short-chain triglyceride comprising hexanoic acid; short-chain triglyceride comprising hexanoic acid and butyric acid; short-chain triglyceride comprising hexanoic acid and decanoic acid. Accordingly, methods and compositions provide for various combinations of synthetic short-chain triglycerides with the sunflower oil triglycerides resulting in different flavor profiles.

Synthetic Milk

An exemplary embodiment of synthetic milk formulation comprising microbially derived proteins of the present invention is illustrated in Example 4. For example, the present formulation incorporates all four subunits of bovine casein: α-s1-casein, α-s2-casein, β-casein and κ-casein and two whey proteins α-lactalbumin and β-lactoglobulin as the predominant milk protein components in the formulation. The exemplary synthetic milk formulation further comprises plant-based interesterified fats as shown in Figure 1. Additional components include carbohydrates and ash. The resulting milk substitute exhibits characteristics that looks, functions, tastes, smells, and feels like natural milk. As one of key facets of the present invention, modifying the formulations for synthetic milk can exhibit different sensory impressions such as flavoring by modulating the oil content, namely the types of triglycerides added to mimic milk of different flavors.

As described in Young W. Park, Bioactive Components in Milk and Dairy Products, Technology & Engineering, pp 60, 2009, sterols are a minor fraction of total lipids in milk, the main sterol being cholesterol (300 mg/100 g fat, equivalent to 10 mg/100 mL bovine milk) (Park et al., *Small Rumin. Res.* 68: 88-113, 2007). Goat milk has been shown to contain less cholesterol than other milk but generally contains higher total fat than cow milk. See, Posati et al., 1976. Composition of Foods. Agric. Handbook No. 8 - 1. ARS, USDA, Washington, D.C., 1976; Jenness, *J. Dairy Sci.* 63:1605 – 1630, 1980; and Juarez et al., Intl. Dairy Fed. Bull. No. 202. pp. 54-67, 1986, have shown that goat milk has greater palmitic and oleic acid fractions than cows. Cholesterol content was significantly varied among different breeds and most cholesterol in goat milk was in

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free state, with only a small fraction in ester form 52 mg/100 g fat. See, e.g., Arora et al., Ind. J. Dairy Sci. 29: 191.

In certain embodiments, the methods and composition of the present invention provide synthetic milk product that has less cholesterol, or is cholesterol free or has the same cholesterol content in comparison to the dairy milk by modulating the oil content, namely the types of triglycerides. In other embodiments, the amount of saturated and unsaturated fats is also modulated in dairy substitutes to at least less or the same amount of fats in comparison to the dairy milk. In preferred embodiments the synthetic milk product of the present invention is very low in saturated fat but smells and tastes like dairy milk. The long chain fatty acids, which are typically saturated fatty acids in milk, are instead monounsaturated acids such as oleic acid, in the preferred embodiments of the invention.

The present invention may not require or at least minimizes pasteurization, as each component can be rendered sterile separately, before combining through the formulation process. In other embodiments of the invention, synthetic milk product of the present invention can undergo pasteurization.

Homogenization is optional for the methods and compositions of the present invention as is the case for natural milk. When sold as a standalone liquid beverage, the synthetic milk product of the present invention can be sold in homogenized form.

Differences between the milk substitute of the present invention with dairy milk include flavor, nutritional value and storage stability. Flavorings can be adjusted to a desired sensory impression based on triglycerides as well as other natural or artificial flavors that can impart in blandness or sharpness or a different aroma such as cow, goat, coconut, almond or soy.

Synthetic Cheese

In other aspects of the present invention, methods and compositions comprising one or more isolated milk protein components, fats, carbohydrates and ash are provided to produce various types of cheese products. Generally, the cheese is made from the milk protein components of the present invention. One or more sensory impressions are incorporated into the cheese product through modulating the triglycerides. Accordingly,

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cheese with desired organoleptic characteristics with distinct appearance, aroma, taste and texture can be produced. For some cheese varieties, in addition to modulating the triglycerides, one or more bacteria is employed in the cheese making process for fermentation where fermentative products and by-products such as lactic acid, carbon dioxide, alcohols, aldehydes and ketones are produced. Types of cheese include whey cheese such as ricotta and mozzarella, semi-soft cheese include Havarti and Munster, medium-hard cheese such as Swiss and Jarlsberg, hard cheese such as Cheddar and soft ripened cheese such as Brie and Camembert.

Synthetic Cream

Directly usable cream substitutes should preferably comprise from about 50 to 90% by weight water, and more preferably from about 65 to 80% by weight water, with the base being dispersed within the water. The base for a substitute cream should advantageously contain (all percentages computed using the total weight of the base taken as 100%) from about 22 to 87% by weight carbohydrate (more preferably from about 30 to 64%), from about 12 to 70% by weight of particulate fat (most preferably from about 28 to 60%), and from about 0.4 to 8% by weight of a selected emulsifier or group thereof (most preferably from about 1 to 4%).

In preferred embodiments, the products of the invention are stable in aqueous emulsion. As used herein, a dried, liquid fat-containing non-dairy food product is said to be "stable" when the following minimum criteria are met: reconstituted emulsion stability, whitening capability, oiling or oil separation, feathering-precipitation. See U.S. Patent No. 4,310,561.

Synthetic Butter

Commercial butter is 80–82 % milk fat, 16–17 % water, and 1–2 % milk solids other than fat (sometimes referred to as curd).

Advantages of Dairy Substitute Products or the Compositions Provided Herein

Desirable advantages of the present invention are environmental in nature such as 8 times more energy efficient, 260 times more water efficient than conventional milk

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product. Other environmental advantages include less water usage than conventional milk production, which is estimated to be about 1000 L/L and reduced land usage for conventional milk production typically requires grazing, crop land, ability to reduce the 600 billion kg of carbon dioxide per year that is emitted from conventional milk production. The present invention also provides reduction or elimination of costs of feed, operations, labor, animal and marketing. Preferably, substantially reduce feed cost by a factor of 8.

Advantages in food safety include reduction or removal of antibiotic residues, heavy metals, bacteria, adulterations. Accordingly, certain aspects of the present invention provide animal-free milk that is bacteria-free, requires no pasteurization or cold shipping yet has an increased shelf-life and exhibit a number of characteristics such as taste, appearance, handling and mouth feel properties which are identical or at least closely similar to their traditional dairy counterparts. Preferably, the dairy substitute products are essentially free of bacteria such as *Brucella, Camplyobacter, Listeria, Mycobacterium, Salmonella, Shigella, Yersinia, Giardia* and noroviruses, and, thus are safer for consumption. Further advantage include minimal or no pasteurization and/or homogenization. More preferably, the dairy substitute is shelf stable for relatively long periods (e.g., at least three weeks and preferably longer) for production and distribution. Even more preferably, the dairy substitute products has a lower environmental impact.

Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods.

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EXAMPLES

Example 1

Vectors

Protein sequences bovine α-S1 casein (UniProt accession #P02662), bovine α-2 casein (UniProt accession #P02663), bovine β-casein (UniProt accession #P02666), bovine κ-casein (UniProt accession #P02668), bovine α-lactalbumin (UniProt accession #B6V3I5) and bovine β-lactoglobulin (UniProt accession #P02754) were obtained on Uniprot.org and altered with the following changes: removed 15 or 21-residue signal peptide from N-terminal end; added *XhoI* (CTC GAG) endonuclease recognition sequence and KEX endopeptidase recognition sequence (AAA AGA) to 5' end of DNA; and added *SalI* (GTC GAC) endonuclease recognition sequence to 3' end of DNA. An additional combination sequence was made by combining the sequences for the four caseins in the order shown above, separating each sequence with the following DNA phrase:

[0001] GGC TCA GGA TCA GGG TCG AAA AGA GGC TCA GGA TCA GGG TCG (SEQ ID NO: 128).

[0002] Here the non-underlined segments encode a $(GS)_6$ linker sequence for adequate posttranslational spacing and accessibility to the KEX protease, and the underlined segment encodes the KEX endopeptidase sequence which cleaves the proteins apart post-translation. As above, the entire cassette is flanked on the 5' end by *XhoI* and on the 3' end by *SalI* for ligation into pKLAC2 (New England Biolabs, Beverly, MA). DNA was synthesized by either Gen9, Inc. (Cambridge, MA) or IDT (Coralville, IA). The plasmid used had, among other things, a multiple cloning site, a Lac promoter, an Acetamide based reporter gene and the alpha-mating factor gene, used as a fusion protein for secretion of exogenous proteins.

Yeast Transfection

Transfection of the yeast was accomplished by thawing a tube of 0.5 mL competent cells containing 25% glycerol on ice and adding 0.62 mL yeast transfection reagent. The mixture was then warmed at 30°C for 30 minutes, heat shocked at 37°C for 1 hour. The cells were then pelleted at 7000 rpm & washed twice with 1.0mL of YPGal medium. The cell mixture was then transferred to a sterile culture tube and incubated at

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30°C for 3 hours, with constant shaking at 300 rpm. The cell mixture was then transferred to a sterile 1.5mL microcentrifuge tube and pelleted the cells at 7000 rpm for 2 minutes, and resuspended in 1 mL sterile 1X PBS. 10, 50 and 100 μL of the cell suspension was placed into separate fresh sterile 1.5 mL microcentrifuge tubes each containing 50 μL of sterile deionized water. Tubes were mixed briefly and spread onto separate yeast carbon base agar (YCB Agar) plates containing 5 mM acetamide for selection. Plates were then incubated, inverted, at 30°C for 4 days until colonies form. 15 individual colonies were then streaked onto fresh YCB Agar plates containing 5 mM acetamide and incubated at 30°C for 2 days.

DNA encoding alpha-lactalbumin and beta-lactoglobulin, two key whey proteins, was designed in-house and ordered for synthesis from IDT and was transfected into competent *K. lactis* cells from the New England Biolabs kit (Catalog #E1000S) according to the vendor-supplied protocol.

High-Throughput Transfectant Selection

From each YCB Agar plate, once the colonies had grown sufficiently, each of the 30 plates was tested for successful integration of the vector plasmid. This was followed by PCR analysis of each plate to test for special cells with multiple integrants of the vector. Once isolated, the highest producing individual culture was used for scale up. This process can be iterated with successively higher concentrations of selective pressure in order to force colonies to develop higher copy numbers of our engineered plasmid.

Five transfection events were performed and plated on 5 separate plates consisting of nitrogen-free yeast carbon base medium. (Any observed growth on these plates therefore implied successful uptake of the plasmid, if not uptake of the exogenous DNA itself). Of these 5 plates, 100% showed positive growth. 30 individual colonies from the 5 plates were chosen for scale-up, and each was grown in a separate YCB agar plate to create a homozygous culture plate to allow for easy characterization and management. After a 3 day growth period, a single colony from each plate was initially added to a 10 ml glass culture tube, containing 2ml YPGal media, to test for protein expression. After a growth period of two days, the cells were pelleted out and the supernatant was run on an SDS PAGE gel to check for protein expression. The strains which provided the best protein expression were scaled up to a 10 ml, 100 ml, 500 ml,

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and ultimately 1L culture vessel. From each whey protein, two liters of culture were grown. Approximately one gram of protein was harvested from the total, suggesting a non-optimized yield/productivity of 0.5 g/L.

Scale-Up in 1L Shake Flask Culture

Cultures are scaled up and seeded in a 1L shake flask at split ratios of at least 1:10. Prior to seeding, inoculation flasks are grown for 24 hours in production media without acetamide supplementation. On the starting day of a fedbatch production run, the reactor is charged with 90% of the target starting volume and heated to the run temperature. For now, the temperature is set at 30°C in order to save on energy costs associated with heating the reactor. Additional parameters can be explored in the process optimization phase. When the reactor reaches 30°C, the inoculation flask is added to the reaction vessel dropwise using a peristaltic pump. The reactor is maintained using vendor supplied software at a target pH. Twice daily samples are taken of the reactor broth in order to quantify the amount of glucose and electrolyte usage by the cells, and as a doublecheck for the reactor's pH and dissolved gas measurements. After each measurement, bolus glucose is added to maintain a target glucose concentration 10% to start, although this may also be altered in process development. When cells reach maximum density, protein production is triggered by the addition of galactose, which triggers the promoter on our pKLAC2 plasmid. Galactose is supplemented until the end of the run. Optimum run length can be determined in process development as well, but is set as a 5-day fedbatch. After a full run, yeast cells are removed from the reactor and the proteins are purified as discussed below.

Casein Protein Purification

The following casein proteins α-s1casein, α-s2casein, and β-casein are inherently hydrophobic, which precipitate out when secreted from the yeast and come into contact with water. Purification from the reactor media involves collection of the protein from the surface of the media, followed by drying to isolate pure protein. Kappa-casein is inherently hydrophilic and purification of the κ-caseins involves the change in pH of the solution to 4.6, followed by centrifugation at 10,000 rcf. Combined casein cassette works the same way as κ-casein.

Whey Protein Purification

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Alpha-lactalbumin: The isoelectric point of alpha-lactalbumin is 4.2. When the pH of the bioreactor media solution is lowered to 4.2, the solubility of the protein is at its lowest. This knocks the protein out of solution and allows for collection by centrifugation. Beta-lactoglobulin: Similar to the purification of the alpha-lactalbumin, the pH of the solution is lowered to 5.2 the isoelectric point of beta-lactoglobulin. This neutralizes the charge of the protein and allows its collection by centrifugation at 14,000 rcf.

Protein Purification

The 2L of culture media was spun at 3,000g in a floor centrifuge to pellet out the yeast cells. The pellet was discarded, and the supernatant was transferred into a new vessel & the pH of the solution was lowered to 4.2 for the alpha-lactalbumin and 5.2 for the beta-lactoglobulin (Figure 2A). This was followed by incubation of the supernatant at 35°C for 30 mins in a shaker flask, centrifugation at 14,000g in a floor centrifuge to pellet out the protein mixture (Figure 2B).

Protein Characterization

After separation of the protein by centrifugation, the solid pellet and the supernatant solution were run on a 14% SDA-PAGE gel to check for protein expression. A positive band was observed at 14 kDa and at 18 kDa (Figure 3), which correlates to the size of alpha-lactalbumin and beta-lactoglobulin of bovine origin, respectively. Further characterization is done to confirm equivalence in terms of primary sequence, glycosylation and phosphorylation.

Example 2

Triglyceride Synthesis

Milk fat triglycerides were made by transesterifying short-chain triglycerides into high oleic sunflower oil, the oil from a custom engineered variant of sunflowers which express the following ratios of fatty acid esters as described in Table 1:

Table 1:

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Table 1:

Fatty Acids	Sunflower†	NuSun Mid-Oleic Sunflower‡	High-Oleic Sunflower†
C6:0	ND	ND	ND
C8:0	ND	ND	ND
C10:0	ND	ND	ND
C12:0	ND-0.1	ND	ND
C14:0	ND-0.2	0.4-0.8	ND-0.1
C16:0	2.0-7.6	4.0-5.5	2.6-5.0
C16:1	ND-0.3	ND-0.05	ND-0.1
C17:0	ND-0.2	ND-0.05	ND-0.1
C17:1	ND-0.1	ND-0.06	ND-0.1
C18:0	1.0-6.5	2.1-5.0	2.9-6.2
C18:1	14-39.4	43.1-71.8	75-90.7
C18:2	48.3-74.0	18.7-45.3	2.1-17.0
C18:3	ND-0.3	ND-0.1	ND-0.3
C20:0	0.1-0.5	0.2-0.4	0.2-0.5
C20:1	ND-0.3	0.2-0.3	0.1-0.5
C20:2	ND	ND	ND
C22:0	0.3-1.5	0.6-1.1	0.5-1.6
C22:1	ND-0.3	ND	ND-0.3
C22:2	ND-0.3	ND-0.09	ND
C24:0	ND-0.5	0.3-0.4	ND-0.5
C24:1	ND	ND	ND
ND=not detectable (ND defined as <0.05%)			

† From Codex Alimentarius (2001)
‡ From Table 3

Short-chain triglyceride preparation

The short-chain fatty acids which are principally responsible for rich flavor in milk and cream are the molecules with even numbers of carbons between 4 and 10, and are mixed in the following ratios as described in Table 2:

Table 2:

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Table 1. Fatty acid composition expressed as percent by weight of total fatty acids in Swedish dairy milk in 2001, given as weighted means with standard deviations (SD) and as the minimum and maximum weighted means. The estimation of the weighted mean values was based on the proportion of milk delivered to each dairy or dairy company at each sampling occasion (seven dairies at four sampling occasions during 2001). The lowest and highest values observed and *p*-values for geographical and seasonal variation are also given

Fatty acid	Weighted mean 2001	SD	Lowest value observed	Highest value observed	Seasonal variation
4:0	4.4	0.1	4.0	5.1	n.s.
6:0	2.4	0.1	2.1	2.9	n.s.
8:0	1.4	0.1	1.2	1.9	n.s.
10:0	2.7	0.2	2.4	3.5	*
12:0	3.3	0.2	3.0	4.1	**
14:0	10.9	0.5	10.0	12.1	***
15:0	0.9	0.0	0.8	1.1	n.s.
16:0	30.6	0.9	28.7	34.1	**
17:0	0.4	0.0	0.4	0.5	**
18:0	12.2	0.4	10.3	13.3	n.s.
20:0	0.2	0.0	0.2	0.2	n.s.
Saturated fatty acids total	*69.4*	*1.7*	*67.1*	*74.4*	***
10:1	0.3	0.0	0.2	0.4	n.s.
14:1	0.8	0.4	0.4	1.3	**
16:1	1.0	0.0	0.9	1.8	n.s.
17:1	0.1	0.0	<0.1	0.3	n.s.
18:1	22.8	1.0	19.7	24.7	***
Mono-unsaturated fatty acids, cis, total	*25.0*	*1.0*	*22.2*	*26.7*	**
18:2	1.6	0.1	1.4	1.8	n.s.
18:3	0.7	0.0	0.6	0.9	**
Poly-unsaturated fatty acids, cis, total	*2.3*	*0.1*	*2.0*	*2.5*	n.s.
16:1t	0.4	0.1	0.3	0.4	***
18:1t	2.1	0.7	2.0	3.3	***
18:2t	0.2	0.0	0.1	0.5	n.s.
Trans fatty acids total	*2.7*	*0.7*	*0.6*	*3.9*	***
CLA	0.4	0.1	0.3	0.5	***

n.s.: Not significant; *p <0.05; **p <0.01; ***p <0.001.

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Table 3:

Chain Length	Names	Mass Fraction in Mixture (%)
4	Butanoic / butyric acid	40
6	Hexanoic / caproic acid	26
8	Octanoic / caprylic acid	11
10	Decanoic / capric acid	22

The fractions in Table 3 are based upon the relative prevalence of these species in cow's milk, but can be altered during process development both in order to design a better tasting product and in order to design milks of other species, such as buffalo or goat. Short-chain fatty acids in the mass ratios shown above are combined with toluene, paratoluenesulfonic acid, and glycerol in a Dean-Stark water trap, commonly used for esterification reactions in order to remove water produced in the condensation reaction. The reaction is carried out in a fume hood for several hours, until the level of water entering the water trap is observed as unchanging for more than 30 minutes. The vessel is allowed to cool and the mixture is removed from the reaction flask. The mixture is washed twice with a 5% sodium carbonate solution and five times with plain water. Brine (a 10% solution of NaCl in water) is added periodically in order to disrupt an emulsion which forms in the separating funnel. The washed mixture of short-chain triglycerides, water, toluene, and impurities is dried in a rotary evaporator at 90°C and under a 54 mbar atmosphere for one hour, until it has proceeded well past excess in order to minimize the chance of food contamination.

Transesterification

The short-chain triglyceride mixture is combined with high-oleic sunflower oil at a volumetric ratio of 1:8. A mass of sodium methoxide equal to 1% of the oil mixture mass is added in order to catalyze the transesterification, and the reaction vessel is heated to 65°C, stirring continuously, under an inert Argon atmosphere, for six hours. A 5% acetic acid mixture is added to quench the reaction, then the oil is washed five times with deionized water and dried in a rotary evaporator for one hour at >90°C. The finished milk

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fat is autoclaved to ensure sterility and is thence suitable for use in milk or cream as described above.

Example 3

Milk Formulation

One non-limiting milk composition formulation is described below.

Table 4:

Components	% (w/v) Range	Amount (g/L)
Casein proteins	1 – 10	10 – 100
Whey proteins	0 – 1	0 – 10
Plant-based milk fats	0 – 8	0 – 80 ml/L
Sugar	0 – 5	0 – 50
Ash	0.1 – 1	1 – 10
Calcium	0.1 – 0.5	1 – L
X (Functional additive)	0 - 1	0 – 10L

Following Table 4, milk formulation is achieved through the following procedure, per 1 liter of milk. 26 grams of casein, 3.5 grams of whey and 5 grams of ash are combined and mixed well. 40 mL of triglycerides are thawed & heated to 55°C. Protein mixture is poured slowly into triglycerides and vortexed at high speed for five minutes. In the meantime, 3.5 grams of whey and 24 grams of galactose are added to 850 mL deionized water; mixture is heated to 37°C. Triglyceride/protein/ash mixture is moved into Waring commercial blender and blended at low speed. Whey/galactose/water mixture is poured slowly into blender; cap placed on blender. Mixture is blended at high speed for ten minutes. Deionized water is added to a final volume of 1000 mL. Milk can optionally be homogenized using existing methods. The above protocol can be altered for cream or arbitrary milk formulations by altering the ratios of solids; however, our preliminary research suggests that the presence of ash in the protein mixture and the separation of a significant proportion of the whey can greatly affect the quality of the emulsion.

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Example 4

Synthetic Milk Formulation

As a preliminary proof of concept, in order to determine whether the key components of milk could be recombined to form milk, dry food-grade purified casein and research grade whey was purchased. Irish cream was obtained from a local source and pure fat was isolated from it by centrifuging the cream at 14,000g. Finally, all minerals used were purchased from Sigma Aldrich.

Terms:

C-roux= roux made by mixing casein proteins & fat together while maintaining the temperature of the mixture at 37°C.

W-roux= roux made by mixing whey proteins & fat together while maintaining the temperature of the mixture at 37°C.

CW-roux= roux made by mixing casein & whey proteins together in a mixture first, adding fat and mixing at 37°C.

Table 5:

Experiment	Result
Casein + Fat + Water	A pale yellow liquid with bad taste, precipitation of protein, and bad mouthfeel (watery).
Casein + Water + Fat	A pale yellow liquid with bad taste, precipitation of protein, and bad mouthfeel (watery).
(Casein + Fat) to make a roux. roux + Water	A pale yellow liquid with average taste and bad mouthfeel (watery). Low protein precipitation was observed.

Hypothesized that the bad mouthfeel (e.g., wateriness) was due to the lack of whey protein.

Table 6:

Experiment	Result

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Casein + Whey + Fat + Water	Pale yellow-white liquid with bad taste, precipitation of protein, and bad mouthfeel.
C-roux + Whey + Water	Pale yellow-white liquid with average taste, low precipitation of protein, and bad mouthfeel
W-roux + Casein + Water	Pale yellow-white liquid with average taste, low precipitation of protein, and bad mouthfeel.
CW-roux + Water	Pale yellow-white liquid with average taste and bad mouthfeel. Zero protein precipitation.

Hypothesized that bad mouth feel was because of bad casein micelle formation, that addition of Ca would allow the micelle to reform.

Table 7:

Experiment	**Result**
CW-roux + Water + Calcium phosphate (optimum amount of Ca was figured out by trial & error)	White liquid with normal mouth feel. Zero protein precipitation. Average taste

5

To improve taste, different sugars were added in different concentrations to the above mixture.

Table 8:

Sugar	**2.4%**	**3.0%**	**3.6%**	**4.2%**	**4.8%**
Glucose	Good	Too Sweet	Too Sweet	Too Sweet	Too Sweet
Galactose	Bland	Excellent	Average	Excellent	Too Sweet
Sucrose	Bad	Bad	Bad	Bad	Bad
Maltose	Bland	Excellent	Excellent	Too Sweet	Too Sweet

10 All additional ions found in cow milk was incorporated to recreate the ionic environment found in nature.

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Reference: R. Rosmaninho, L.F. Melo / Journal of Food Engineering 73 (2006) 379–387

Table 9:

Reagent	Composition (mM)
KH_2PO_4	11.60
K_3 Citrate H_2O^a	3.7
Na_3 Citrate $2H_2O$	6.1
K_2SO_4	1.03
K_2CO_3	2.17
KCL	8.0
$CaCL_2$. $2H_2O$	8.98

End result was a liquid which was bright white in color, likely because the ionic environment kept the solids present in milk from joining together and increased the overall refractive index of the solution. Taste was excellent, but it had an average mouthfeel (e.g., a certain amount of chalkiness was observed in the liquid). Exact mineral composition as described in Table 9 can provide excellent mouthfeel.

Milk Fat Synthesis

Synthetic milk fat was made by interesterifying short-chain fatty acids among the large-chain fatty acids present in high-oleic sunflower oil triglycerides. The four short-chains used were:

40% C4: Butyric acid. found in milk, especially goat, sheep and buffalo milk, butter, Parmesan cheese, and as a product of anaerobic fermentation (including in the colon and as body odor). It has an unpleasant smell and acrid taste, with a sweetish aftertaste (similar to ether). Butyric acid is present in, and is the main distinctive smell of, human vomit.

26% C6: Caproic acid. a colorless oily liquid with an odor that is fatty, cheesy, waxy, and like that of goats or other barnyard animals.

11% C8: Caprylic acid. It is an oily liquid that is minimally soluble in water with a slightly unpleasant rancid-like smell and taste.

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22% C10: Capric acid. Not much said about the flavor, and with longer carbon chains you start to get less flavors. This is in coconut oil so it is not a milk fat flavor *per se* as much as the other ones.

Iterations include lauric acid (C12), as it is present at 2.9% of total fatty acid content in cow's milk (Beare-Rogers, J.; Dieffenbacher, A.; Holm, J.V. (2001). "Lexicon of lipid nutrition (IUPAC Technical Report)". Pure and Applied Chemistry 73 (4): 685–744. doi:10.1351/pac200173040685.)

The following procedure as described Yu et al., The modification an analysis of vegetable oil for cheese making. *J. Am. Oil Chem. Soc.*, 77:911 (2000) was followed in, at quarter of the amounts specified below:

A mixture of butyric, caproic, caprylic, and capric acids (Sigma Chemical Co., St. Louis, MO) at the same ratios found for a milk fat sample [see above] and totaling 7.26 mol, 21.42 g of p-toluenesulfonic acid (Sigma Chemical Co.), 2.305 mol of glycerol (Sigma Chemical Co.), and 458 mL of toluene (Fisher Scientific) was refluxed with a Dean-Stark water trap for 6 h. The reaction was considered complete when no more water dripped into the trap. The SCTG were washed once with 5% sodium carbonate solution and several times with water. Then, the SCTG were heated at 85°C in a rotary evaporator to remove water and toluene.

SCTG from both commercial and natural sources are interesterified with HOSO (Trisun 80, RBD; AC Humko, Memphis, TN) at a SCTG/HOSO ratio of 1:8.82 in order to produce a fat that has the same percentage of SCFA as that of milk fat. SCTG from the commercial source are also interesterified at a SCTG/HOSO ratio of 1:7.19 to produce a fat that has a level of SCFA equal to 120% of that in milk fat. Sodium methoxide (Aldrich Chemical Company, St. Louis, MO) is used as a catalyst at 0.5% of total oil weight. The reaction is carried out at 65°C under nitrogen with stirring for 6 h. Next, 5% acetic acid (Fisher Scientific) is added to neutralize the catalyst, and the oil is then washed several times with distilled water and dried on a rotary evaporator for 30 min at 90°C.

A pilot-scale continuous deodorizer similar to the one described by Smouse (Smouse, T.H., A Laboratory Continuous Deodorizer, inform 8:1176–1181 (1997).) is used to deodorize the interesterified oils. The oil flow rate is 600 mL/h, the column

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temperature is 180°C, pressure at 0.5 Torr, and the steam rate 12.6 mL/h. Each batch of deodorized oil is tasted by to ensure the flavor. The deodorized oil is stored at 4°C until used for cheese making.

Example 5

Modulation of Fatty Acids

Sunflower oil triglycerides with three oleic acids are transesterified with four short chain fatty acids containing one butyric acid, one hexanoic acid, and one octanoic acid as part of the fat composition in a mixture of synthetic milk product. This array or combination of fat is expected to result in a synthetic milk fat providing its rich flavor as compared to natural dairy milk. The ability to control the composition of one or more triglycerides is likely to enhance or change flavor profiles of synthetic dairy products. Accordingly, a matrix of long-chain and short-chain can yield in flavor profiles including, but not limited to, multiple aromatic compounds associated with buttery, nutty, sweet, sour, fruity, floral, bitter, woody, earthy, beany, spicy, metallic, sweet, musty, oily and vinegary sensory impressions. Additionally, increase in texture such as creaminess, improvements in melting characteristics or tolerance and increase in stretching ability relative to a corresponding dairy product can be exhibited.

Example 6. Recombinant Production of Milk Proteins

Alpha-lactalbumin, β-lactoglobulin, α-S1-casein, α-S2-casein, β-casein, and κ-casein were produced in recombinant yeast strain (*Pichia pastoris*) strains. As the glycosylation enzymes in yeast are different than mammalian cells, the proteins producted by the yeast will either be non-glucosylated or have a non-mammalian glycosylation pattern. The produced proteins can be used as a component in any of the compositions described herein.

Plasmids

Plasmids were constructed for the expression of each protein. Each plasmid included the following components: an inducible promoter (e.g., AOX1 promoter) or a constuitive (GAP promoter or PGK promoter) promoter, for each protein being

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expressed; a sequence encoding a signal peptide for each protein being expressed, derived either from the native bovine protein sequence or one from a yeast protein sequence (alpha mating factor or OST1); a sequence encoding the milk protein(s) to be expressed; a yeast transcription terminator sequence (e.g., AOX1, AOD, or CYC1) for each protein being expressed; a bacterial origin of replication from pUC19 to enable replication of the plasmid in *E. coli*; and a selectable marker cassette (e.g., kanR or zeocinR) to enable selection in bacteria and yeast with antibiotics.

The different plasmids used to produce the different proteins are listed in Table 10 below.

Table 10. Expression Plasmids (SEQ ID NO)

Plasmid name	Select marker	Prom 1	Signal peptide 1	ORF 1	Terminat 1	Prom 2	Signal pept 2	OR F 2	Term 2
pJAG-nat-LAA	Amp (bacteria), G418 (yeast) (159)[1]	P_AOX1 (153)	SP_lactalbumin (156)	α-lactalbumin (157)	TT_AOX1 (158)				
pJAG-MFa-LAA	Ampicillin (bacteria), G418 (yeast) (159)	P_AOX1 (153)	SP_MFα (154)	α-lactalbumin (157)	TT_AOX1 (158)				
pJAG-OST-LAA	Ampicillin (bacteria), G418 (yeast) (159)	P_AOX1 (153)	SP_OST (155)	α-lactalbumin (157)	TT_AOX1 (158)				
pLH37	Zeocin (151)	P_AOX1 (129)	SP_MFαT (132)	β-lactoglobulin (143)	TT_AOX1 (149)				
pLH0044	Zeocin (151)	P_GAP1 (130)	SP_MFαT (132)	β-lactoglobulin (143)	TT_AOX1 (149)				
pLH0045	Zeocin (151)	P_PGK1 (131)	SP_MFalphaT (132)	β-lactoglobulin (143)	TT_AOX1 (149)				

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pLH46	Zeocin (151)	P_GAP1 (130)	SP_β_casein (135)	β-casein (144)	TT_CYC1 (150)	P_PGK1 (131)	SP_αS1_casein (137)	αS1-casein (147)	TT_AOX1 (149)
pLH47	Kanamycin (bacteria), G418 (yeast) (152)	P_GAP1 (130)	SP_αS2_casein (133)	αS2-casein (145)	TT_CYC1 (150)	P_PGK1 (131)	SP_κ_casein (138)	κ-casein (148)	TT_AOX1 (149)
pLH48	Zeocin (151)	P_GAP1 (130)	SP_OST (134)	β-casein (144)	TT_CYC1 (150)	P_PGK1 (131)	SP_OST (134)	αS1-casein (147)	TT_AOX1 (149)
pLH49	Kanamycin (bacteria), G418 (yeast) (152)	P_GAP1 (130)	SP_OST (136)	αS2-casein (145)	TT_CYC1 (150)	P_PGK1 (131)	SP_OST (134)	κ-casein (148)	TT_AOX1 (149)
pLH50	Zeocin (151)	P_GAP1 (130)	SP_OST (136)	β-casein (144)	TT_CYC1 (150)	P_PGK1 (131)	SP_αS1_casein (137)	αS1-casein (147)	TT_AOX1 (149)
pLH51	Zeocin (151)	P_GAP1 (130)	SP_β_casein (135)	β-casein (144)	TT_CYC1 (150)	P_PGK1 (131)	SP_OST (134)	αS1-casein (147)	TT_AOX1 (149)
pLH52	Kanamycin (bacteria), G418 (yeast) (152)	P_GAP1 (130)	SP_αS2_casein (133)	αS2-casein K113E (146)	TT_CYC1 (150)	P_PGK1 (131)	SP κ_casein (138)	κ-casein (148)	TT_AOX1 (149)
pLH53	Kanamycin (bacteria), G418 (yeast) (152)	P_GAP1 (130)	SP_OST (136)	αS2-casein K113E (146)	TT_CYC1 (150)	P_PGK1 (131)	SP_OST (134)	κ-casein (148)	TT_AOX1 (149)
pLH54	Kanamycin (bacteri	P_GAP1 (130)	SP_OST (136)	αS2-casein (145)	TT_CYC1 (150)	P_PGK1 (131)	SP_κ_casein (138)	κ-casein	TT_AOX1 (149)

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	a), G418 (yeast) (152)							(148)	
pLH55	Kanamycin (bacteria), G418 (yeast) (152)	P_GAP1 (130)	SP_αS2_casein (133)	αS2-casein (145)	TT_CYC1 (150)	P_PGK1 (131)	SP_OST1 (134)	κ-casein (148)	TT_AOX1 (149)

[1]SEQ ID NO: 159 (Synthetic)

ATGGGTAAGGAAAAGACTCACGTTTCCAGACCAAGATTGAACTCTAACATGGACGCTGACTTGTA
CGGTTACAAGTGGGCTAGAGACAACGTTGGTCAATCTGGTGCTACTATTTACAGATTGTACGGTA
AGCCAGACGCTCCAGAGTTGTTCTTGAAGCACGGTAAGGGTTCTGTTGCTAACGACGTTACTGAC
GAGATGGTTAGATTGAACTGGTTGACTGAGTTCATGCCATTGCCAACTATTAAGCACTTCATTAG
AACTCCAGACGACGCTTGGTTGTTGACTACTGCTATTCCAGGTAAGACTGCTTTCCAAGTTTTGG
AGGAGTACCCAGACTCTGGTGAGAACATTGTTGACGCTTTGGCTGTTTTCTTGAGAAGATTGCAC
TCTATTCCAGTTTGTAACTGTCCATTCAACTCTGACAGAGTTTTCAGATTGGCTCAAGCTCAATC
CAGAATGAACAACGGTTTGGTTGACGCTTCTGACTTCGACGACGAGAGAAACGGTTGGCCAGTTG
AGCAAGTTTGGAAGGAGATGCACAAGTTGTTGCCATTCTCTCCAGACTCTGTTGTTACTCACGGT
GACTTCTCTTTGGACAACTTGATTTTCGACGAGGGTAAGTTGATTGGTTGTATTGACGTTGGTAG
AGTTGGTATTGCTGACAGATACCAAGACTTGGCTATTTTGTGGAACTGTTTGGGTGAGTTCTCTC
CATCTTTGCAAAAGAGATTGTTCCAAAAGTACGGTATTGACAACCCAGACATGAACAAGTTGCAA
TTCCACTTGATGTTGGACGAGTTCTTCTAA

These plasmids were then integrated into wildtype *P. pastoris* for expression. The production of the proteins was detected by SDS-PAGE, ELISA, and Western blot.

Alpha-Lactalbumin

Strain Construction

Three plasmids were created, placing the expression of bovine alpha-lactalbumin (bvLAA) under the control of the methanol-induced promoter P_{AOX1}, with either the native LAA signal peptide (pJAG-nat-LAA), the full length alpha mating factor signal peptide (pJAG-aMF-LAA), or the OST1 signal peptide (pJAG-OST-LAA).

Prior to transformation, 20 μg each plasmid was linearized by digestion with the restriction enzyme SacI. The digested plasmids were then concentrated by ethanol precipitation, and resuspended in 10 μl distilled water.

Competent *Pichia pastoris* cells were prepared as follows: A culture of *P. pastoris* was grown to log phase (OD600 ~1.0) in YPD media (10 g/L yeast extract, 20

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g/L peptone, 20 g/L dextrose). A 1.5 mL aliquot was harvested by centrifugation, then resuspended in 1 mL of a 1:1 mixture of YPD+20 mM HEPES (pH 8):1M lithium acetate. After adding 10 µL 1 M dithiothreitol, the cells were incubated for 15 min at 30°C in a shaker at 300 rpm. The cells were pelleted by centrifugation and washed three times in 1 mL ice cold 1 M sorbitol. After the final wash, the cells were resuspended in 50 µL 1 M sorbitol.

The cells were combined with the linearized plasmid DNA in a chilled 2 mm electroporation cuvette, and subjected to a 1.5 kV pulse (25 µF, 200 Ω). The cells were transferred to a culture tube with 200 µL cold 1:1 YPD:1 M sorbitol, and allowed to recover for 2 hours at 30°C (300 rpm). Finally, the cells were plated onto YPD agar plates containing zeocin and grown for two days at 30°C.

Protein Expression

Colonies were picked from the agar plates and grown in 750 µL BMD1% (0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml biotin, 1.1% glucose) at 30°C, 300 rpm. After 48 hours, 900 µL of culture was used to inoculate 750 µL BMM2 (0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml Biotin, 1% methanol). After 24 hours, 150 µL BMM10 (BMM10: 0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml Biotin, 5% methanol), and samples were harvested for analysis after one additional day.

Analysis

Protein expression was analyzed in samples of culture that were centrifuged to remove the cell mass. The clarified supernatant was then evaluated by SDS-PAGE, ELISA, and western blot.

To visualize total protein via SDS-PAGE, cell-free supernatant was treated with SDS-PAGE sample buffer, boiled, and run on a 10% polyacrylamide gel. The gel was stained with SYPRO Ruby stain (Life Technologies). The resulting gel shows that secretion of α-lactalbumin occurs using the OST1 or the native lactalbumin signal peptide (Figure 4).

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To measure protein titers via ELISA, 25 µL of each sample were placed in a half-area 96 well microtiter plate, and allowed to bind overnight at 4°C. After removing the samples, the binding surface was blocked by filling each well with 1% (w/v) bovine serum albumin (BSA) dissolved in Tris Buffered Saline (50 mM Tris, pH 7.6, 150 mM NaCl) and incubating for 1 hour at room temperature. The samples were then incubated for 1.5 hr in primary antibody that was diluted in 1% BSA/TBS + 0.1% (v/v) Tween-20. Following three washes in TBS + Tween, the samples were incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (TMB Single Solution, Life Technologies) was added, and the absorbance at 650 nm was measured. The resulting data show that α-lactalbumin was secreted using the native α-lactalbumin signal peptide or the OST1 signal peptide (Figure 5).

To analyze samples via Western blot, one volume of sample was combined with an equal volume of SDS-PAGE sample buffer and run on a 10% polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane, which was blocked by treating with 1% BSA/TBS for 1 hr. After incubating for 1.5 hr with primary antibody diluted in 1% BSA/TBS+Tween, the blot was washed three times in TBS+Tween. The blot was then incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (1-Step Ultra TMB Blotting Solution, Thermo Fisher) was added. After staining was completed, the blot was washed in distilled water.

Beta-Lactoglobulin

Strain Constructions

Three plasmids were assembled, placing the expression of bovine beta-lactoglobulin (bvLGB) under the control of either a methanol-induced promoter (P_{AOX1} in pLH37) or one of two constitutive promoters (P_{GAP} in pLH44, or P_{PGK} in pLH45).

Prior to transformation, 20 µg pLH37 was linearized by digestion with the restriction enzyme SacI. The same amounts of pLH44 and pLH45 were linearized with

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the enzyme ApaLI. The digested plasmids were then concentrated by ethanol precipitation, and resuspended in 10 µl distilled water.

Competent *Pichia pastoris* cells were prepared as follows: A culture of *P. pastoris* was grown to log phase (OD600 ~1.0) in YPD media (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose). A 1.5 mL aliquot was harvested by centrifugation, then resuspended in 1 mL of a 1:1 mixture of YPD+20 mM HEPES (pH 8):1M lithium acetate. After adding 10 µL 1 M dithiothreitol, the cells were incubated for 15 min at 30°C in a shaker at 300 rpm. The cells were pelleted by centrifugation and washed three times in 1 mL ice cold 1 M sorbitol. After the final wash, the cells were resuspended in 50 µL 1 M sorbitol.

The cells were combined with the linearized plasmid DNA in a chilled 2 mm electroporation cuvette, and subjected to a 1.5 kV pulse (25 µF, 200 Ω). The cells were transferred to a culture tube with 200 µL cold 1:1 YPD:1 M sorbitol, and allowed to recover for 2 hours at 30°C (300 rpm). Finally, the cells were plated onto YPD agar plates containing zeocin and grown for two days at 30°C.

Protein Expression

To evaluate expression in clones transformed with the plasmid containing a methanol-inducible promoter (pLH37), individual clones were grown in 750 µL BMD1% (0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml biotin, 1.1% glucose) at 30°C, 300 rpm. After 48 hours, 900 µL of culture was used to inoculate 750 µL BMM2 (0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml Biotin, 1% methanol). After 24 hours, 150 µL BMM10 (BMM10: 0.2M Potassium Phosphate buffer, 13.4 g/l Yeast Nitrogen Base, 0.4 mg/ml Biotin, 5% methanol), and samples were harvested for analysis after one additional day.

To evaluate expression in clones transformed with a plasmid supporting constitutive expression (pLH44 or pLH45), individual clones were grown overnight in PG media (20 g/L peptone, 2% glycerol) at 30°C with shaking at 300 rpm. The cultures were diluted 1:10 in minimal sulfate media:

Glucose 20 g/L
Calcium Chloride (CaCl2) 1 g/L

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Sodium phosphate (Na2PO4) 24 g/L
Potassium sulfate (K2SO4) 18.2 g/L
Magnesium sulfate (MgSO4-7H2O) 14.9 g/L
Ammonium sulfate (NH4)2SO4 9 g/L
EDTA (Ethylenediaminetetraacetic acid) 65.25 mg/L
FeSO4-7H2O (Iron Sulfate heptahydrate) 12.18 g/L
ZnSO4-7H2O (Zinc sulfate heptahydrate) 25.0125 g/L
CaCl2-2H2O (Calcium chloride dihydrate) 12.615 g/L
CuSO4-5H2O (Copper sulfate pentahydrate) 2.175 g/L
NaMoO4-2H2O (Sodium molybdate dihydrate) 2.088 g/L
CoCl2-6H2O (Cobalt chloride hexahydrate) 2.0445 g/L
MnCl2-4H2O (Manganese chloride tetrahydrate) 1.392 g/L
Biotin 0.2175 g/L

After 48 hours, samples were harvested for analysis.

Analysis

Protein expression was analyzed in samples of culture that were centrifuged to remove the cell mass. The clarified supernatant was then evaluated by ELISA and Western blot.

To measure protein titers via ELISA, 25 µL of each sample were placed in a half-area 96 well microtiter plate, and allowed to bind overnight at 4°C. After removing the samples, the binding surface was blocked by filling each well with 1% (w/v) bovine serum albumin (BSA) dissolved in Tris Buffered Saline (50 mM Tris, pH 7.6, 150 mM NaCl) and incubating for 1 hour at room temperature. The samples were then incubated for 1.5 hr in primary antibody that was diluted in 1% BSA/TBS + 0.1% (v/v) Tween-20. Following three washes in TBS + Tween, the samples were incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (TMB Single Solution, Life Technologies) was added, and the absorbance at 650 nm was measured. The resulting data show the secretion of β-lactoglobulin (Figure 6).

To analyze samples via western blot, one volume of sample was combined with an equal volume of SDS-PAGE sample buffer and run on a 10% polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane, which was blocked by treating with 1% BSA/TBS for 1 hr. After incubating for 1.5 hr with primary antibody

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diluted in 1% BSA/TBS+Tween, the blot was washed three times in TBS+Tween. The blot was then incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (1-Step Ultra TMB Blotting Solution, Thermo Fisher) was added. After staining was completed, the blot was washed in distilled water. The resulting Western blot shows that β-lactoglobulin was secreted from the recombinant yeast (Figure 7).

Bovine Caseins

Dual expression plasmids were built, to support expression of α-S1-casein with β-casein in one plasmid, and α-S2-casein with kappa-casein in another plasmid. These pairings were chosen because the molar ratio of α-S1:α-S2:β:κ in fluid milk is approximately 5.5 : 1.5 : 4.0 : 1.5; it is therefore desirable to have a similar number of copies of α-S1-casein and beta-casein, and a similar number of copies of α-S2-casein and kappa-casein.

Beta-casein and α-S2-casein were placed under the control of the constitutive PGAP promoter in their respective plasmids, while α-S1-casein and κ-casein were placed under the control of the constitutive PPGK promoter.

In order to direct the proteins into the secretory pathway, the proteins were expressed with either their native signal peptide (pLH46 and pLH47), or the OST1 signal peptide (pLH48 and pLH49). In addition, plasmids were made in which one protein was expressed with its native signal peptide, and the other protein with the OST1 signal peptide:

pLH0050	OST1-beta, native-α-S1
pLH0051	native-β, OST1-α-S1
pLH0054	OST1-α-S2, native-κ
pLH0055	native-α-S2, OST1-κ

To generate strains expressing all four casein proteins, yeast cells were first transformed with the plasmid encoding beta-casein and α-S1-casein. Prior to transformation, 20 μg of each plasmid was linearized with the enzyme ApaLI. The

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digested plasmids were then concentrated by ethanol precipitation, and resuspended in 10 µl distilled water.

Competent *Pichia pastoris* cells were prepared as follows: A culture of P. pastoris was grown to log phase (OD600 ~1.0) in YPD media (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose). A 1.5 mL aliquot was harvested by centrifugation, then resuspended in 1 mL of a 1:1 mixture of YPD+20 mM HEPES (pH 8):1M lithium acetate. After adding 10 µL 1 M dithiothreitol, the cells were incubated for 15 min at 30°C in a shaker at 300 rpm. The cells were pelleted by centrifugation and washed three times in 1 mL ice cold 1 M sorbitol. After the final wash, the cells were resuspended in 50 µL 1 M sorbitol.

The cells were combined with the linearized plasmid DNA in a chilled 2 mm electroporation cuvette, and subjected to a 1.5 kV pulse (25 µF, 200 Ω). The cells were transferred to a culture tube with 200 µL cold 1:1 YPD:1 M sorbitol, and allowed to recover for 2 hours at 30°C (300 rpm). Finally, the cells were plated onto PG agar (20 g/L peptone, 2% (v/v) glycerol, 2% agar) plates containing zeocin and grown for two days at 30°C.

Six clones from the beta+alphaS1 plates were then grown in culture, and made competent for DNA uptake using the procedure described above. They were then transformed with the linearized alphaS2+kappa plasmids, and grown for two days at 30°C on PG plates containing G418.

Expression

To evaluate the production of bovine casein proteins, five clones expressing casein and a wildtype yeast negative control were grown overnight in PG media (20 g/L peptone, 2% glycerol) at 30°C with shaking at 300 rpm. All five of the casein-expressing clones expressed alphaS2- and κ-casein with the respective native casein signal peptides. Clones sLH115, 116, 117, and 118 expressed β-casein and α-S1-casein with the respective native signal peptides; clone sLH122 expressed beta-casein and α-S1-casein with the OST1 signal peptide. The cultures were diluted 1:10 in minimal sulfate media:

Glucose 20 g/L
Calcium Chloride (CaCl2) 1 g/L

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Sodium phosphate (Na2PO4) 24 g/L
Potassium sulfate (K2SO4) 18.2 g/L
Magnesium sulfate (MgSO4-7H2O) 14.9 g/L
Ammonium sulfate (NH4)2SO4 9 g/L
EDTA (Ethylenediaminetetraacetic acid) 65.25 mg/L
FeSO4-7H2O (Iron Sulfate heptahydrate) 12.18 g/L
ZnSO4-7H2O (Zinc sulfate heptahydrate) 25.0125 g/L
CaCl2-2H2O (Calcium chloride dihydrate) 12.615 g/L
CuSO4-5H2O (Copper sulfate pentahydrate) 2.175 g/L
NaMoO4-2H2O (Sodium molybdate dihydrate) 2.088 g/L
CoCl2-6H2O (Cobalt chloride hexahydrate) 2.0445 g/L
MnCl2-4H2O (Manganese chloride tetrahydrate) 1.392 g/L
Biotin 0.2175 g/L

After 48 hours, samples were harvested for analysis.

Analysis

Protein expression was analyzed in samples of culture that were centrifuged to remove the cell mass. The clarified supernatant was then evaluated by ELISA and western blot.

To measure protein titers via ELISA, 25 µL of each sample were placed in a half-area 96 well microtiter plate, and allowed to bind overnight at 4°C. After removing the samples, the binding surface was blocked by filling each well with 1% (w/v) bovine serum albumin (BSA) dissolved in Tris Buffered Saline (50 mM Tris, pH 7.6, 150 mM NaCl) and incubating for 1 hour at room temperature. The samples were then incubated for 1.5 hr in primary antibody that was diluted in 1% BSA/TBS + 0.1% (v/v) Tween-20. Following three washes in TBS + Tween, the samples were incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (TMB Single Solution, Life Technologies) was added, and the absorbance at 650 nm was measured. The ELISA data show that the different yeast strains can secrete α-S1 casein and β-casein into the culture medium (Figure 8).

To analyze samples via western blot, one volume of sample was combined with an equal volume of SDS-PAGE sample buffer and run on a 10% polyacrylamide gel.

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The proteins were transferred to a nitrocellulose membrane, which was blocked by treating with 1% BSA/TBS for 1 hr. After incubating for 1.5 hr with primary antibody diluted in 1% BSA/TBS+Tween, the blot was washed three times in TBS+Tween. The blot was then incubated with secondary antibody conjugated with horseradish peroxidase (HRP) for an additional hour. After three final washes in TBS + Tween, a chromogenic substrate (1-Step Ultra TMB Blotting Solution, Thermo Fisher) was added. After staining was completed, the blot was washed in distilled water.

The data in this Example show that the different expression vectors described herein can be used to generate transgenic yeast strains that secrete the different milk proteins.

Example 7. Method of Making a Composition

An exemplary composition described herein was generated using the specific method described below. A schematic diagram of this method is shown in Figure 9.

To prepare the milk product, laboratory equipment such as mixers, stirring plates, and sonicators are employed. For large scale production, standard fluid milk processing equipment should be used.

As Figure 9 shows, there are three main components to this method of making a composition. These steps include:

A. Preparation of the protein solution
B. Preparation of the oil mixture
C. Reconstitution of the milk solids

In step A, powdered micellar casein protein and whey protein are combined and blended (step 1) and subsequently mixed with deionized (DI) water (step 2) to obtain the protein solution **1**. Typically, this contains 2.8% powered micellar casein, 0.7% powered whey protein, and 85.5% water in this solution. The mixing vessel is covered to prevent evaporation of water. This mixing is performed by mixers, stirring plates, or a sonicator in a sufficient period of time (approximately 30 minutes). This mixing time ensures all

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proteins are dispersed in the water. The mixing speed has been optimized as medium which provides enough force to disperse the proteins and avoids the entrapment of air in the solution. The water content can be adjusted according to the usage of other ingredients.

In step 3, separate solutions of $CaCl_2$, KH_2PO_4, and Na_3 citrate in water are the mineral sources utilized to prepare similar mineral profile as native bovine milk. In a typical instance, $CaCl_2$ solution concentration is 0.1 g/mL, KH_2PO_4 is 0.27 g/mL, and Na_3citrate solution is 0.21 g/ml Na_3citrate. The water used to prepare KH_2PO_4 with Na_3citrate solution is usually warm to make sure the complete dissolution of KH_2PO_4. During the mixing of protein solution **1**, 0.015% $CaCl_2$ is added slowly (step 4). The volume of $CaCl_2$ solution used is adjusted according to the weight percent of $CaCl_2$ needed. The mixing continues for approximately 30 minutes to allow the complete interaction between proteins and Ca^{2+} ions. Subsequently, 0.27% KH_2PO_4 and 0.21% Na_3citrate are divided to 5 portions and each portion is added slowly into the mixing solution at an interval time of 5 to 10 minutes (step 5). 0.085% $CaCl_2$ is divided to 4 portions and each portion is added slowly into the mixing solution at an interval time of 5-10 minutes (step 6). The mixing continues for at least 30 minutes, preferably 1-2 hours, to obtain the protein solution 2.

In the process B, low speed mixing is sufficient to achieve the homogeneous mixing of different oil ingredients. The percent of each component used below for preparing the oil mixture 1 is based on the total oil mixture 1 weight. Initially, 65% sunflower oil, 29% coconut oil, and 2% tributyrin are mixed together form the oil base (step 7). The sunflower oil and coconut oil is deodorized to prevent an unwanted aroma. The combination of sunflower oil, coconut oil, and tributyrin can mimic a similar fatty acid profile as the native milk. The oil base ingredient and its content can be adjusted according to different needs (different types of products). The aroma mixture is prepared by mixing different the aroma components in the sunflower oil (step 8). The compounds used to mimic the aroma contain, but are not limited to ethyl butyrate, δ-decalactone, 2-furyl methyl ketone, 2,3-pentanedione, γ-undecalactone, δ-undecalactone, acetoin, furfuryl alcohol, furfural, 2-methylfurfural, and 2-methylpyrazine. Their contents can be adjusted by different applications and preference. 2.5% mono- and di-glycerides, 0.6%

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free fatty acids, 0.5% phospholipids, and 0.4% aroma mixture are added to prepare the oil mixture **1** with mixing (step 9). In a typical instance, free fatty acids contain 0.15% butyric acid and 0.45% hexanoic acid. Soy lecithin is used as the phospholipid source. Soy lecithin is readily available and is inexpensive. A β-carotene solution is prepared in sunflower oil at a concentration of 0.5 mg/g (step 10). 4% of oil mixture 1 and 0.06% the β-carotene solution are mixed together to obtain the oil mixture 2 (step 11). The usage of β-carotene is adjusted to achieve different color levels of the milk. The usage of oil mixture 1 can also be adjusted according to different milk product applications.

In the process C, oil mixture 2 is added slowly to protein solution 2 and mixed thoroughly to prepare product mixture 1 (step 12). The mixing can be performed by mixers or sonicators. In a typical instance, oil mixture 2 and protein solution 2 are mixed under medium to high speed to ensure sure the oil is uniformly dispersed in the aqueous solution. Subsequently, sonication is applied to break down the oil globules into smaller size, which leads to an increase of their stability in the solution. It is necessary to prevent the entrapment of air bubbles in the solution during mixing. A mixing time of least 20 minutes is utilized to stir the oil mixture 2 into the aqueous solution and allow the thorough dispersion. A 4% maltose solution is added into product mixture 1 and was mixed continuously for an additional 30 minutes to yield product mixture 2 (step 13). The sweetness can be adjusted by the sugar content according to different applications. The source of the sugar can also be adjusted according to requests. Extra DI water may be required to make up the final total weight to 100%.

No intensive homogenization, pasteurization, and sterilization is included in this process. However, it will be necessary to apply these steps to prepare the product mixture in the process C for a scale-up production.

Equipment Used

Mixer: IKA-Labortechnik RW16 Basic, speed level (4-6)
Tip sonicator: Qsonica Model CL-188, Amplitude 70%
Water bath sonicator: Bransonic Model 1510R-MT

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Example 8. Example Formulations

Example formulations compositions that have a similar taste and texture profile as whole milk, cream, high protein milk, fat-free milk, and sugar-free milk are provided in Tables 11-15 below.

As can be appreciated in the art, the compositions listed in Tables 11-15 are made by making the necessary modifications to the process described in Example 7.

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Table 11. Composition like Whole Milk

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Table 12. Composition like Cream

Theoretical Cream (40% Milk fat) Formulation

Total Sample Weight 100 g

Protein Component 3 g	**Wt%**	**Amount in Section**	**Weight Percent in 100 g Sample**
Micellular Casein	80%	2.4 g	2.40%
Whey Protein	20%	0.6 g	0.60%
Fat 40 g			
Sunflower Oil	65%	26 g	26.0%
Coconut Oil	29%	11.6 g	11.6%
Tributyrin	2%	0.8 g	0.8%
Mono and Di Glycerides	2.50%	1 g	1.0%
Free fatty acids (butyric and hexanoic acid)	0.60%	0.24 g	0.24%
Phospholipids	0.50%	0.2 g	0.2%
Aroma Compounds 0.4 %	0.40%	0.16 g	0.16%
Minerals 0.54 g			
Calcium		0.1005g	0.1005%
Phosphorus		0.09 g	0.090%
Potassium		0.078 g	0.078%
Sodium		0.0545 g	0.0545%
Citrate		0.1493 g	0.1493%
Chloride		0.064 g	0.064%
Sugar 4 g			
Maltose		4 g	4%
Water		52.46 g	52.46%

Aroma Compounds List

δ–Decalactone
Ethyl butyrate
2-furyl methyl ketone
2,3-pentanedione
γ–Undecalactone
δ–Undecalactone

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Table 13. Composition like Protein Rich Milk

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Table 14. Composition like Fat-Free Milk

Total Sample Weight 100 g

Protein Component 3 g	**Wt%**	**Amount in Section**	**Weight Percent in 100 g Sample**
Micellular Casein	80%	2.4 g	2.40%
Whey Protein	20%	0.6 g	0.60%
Minerals 0.54 g			
Calcium		0.1005g	0.1005%
Phosphorus		0.09 g	0.090%
Potassium		0.078 g	0.078%
Sodium		0.0545 g	0.0545%
Citrate		0.1493 g	0.1493%
Chloride		0.064 g	0.064%
Sugar 4 g			
Maltose		4 g	4%
Water		92.46 g	92.46%
Aroma Compounds List			
δ–Decalactone			
Ethyl butyrate			
2-furyl methyl ketone			
2,3-pentanedione			
γ–Undecalactone			
δ–Undecalactone			

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Table 15. Composition like Sugar Free Milk

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Example 9. Exemplary Composition

An exemplary composition made by the presently described methods is shown in Figure 10. The composition in Figure 10 has a similar look (color), viscosity, foaming property, flavor, and nutritional value as a mammal-produced milk. The composition shown in Figure 10 comprises mammal-derived proteins.

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What is claimed is:

1. A substitute dairy food composition, wherein:

(a) the substitute dairy food composition comprises one or more identified recombinant milk proteins;

(b) the substitute dairy food composition is free of milk proteins other than the one or more identified recombinant milk proteins;

(c) the one or more identified recombinant milk proteins are:

i. a recombinant β-lactoglobulin protein and a recombinant β-lactalbumin protein,

ii. a recombinant β-lactoglobulin protein and a recombinant κ-casein protein,

iii. a recombinant β-lactoglobulin protein and a recombinant β-casein protein,

iv. a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, a recombinant β-casein protein, a recombinant α-S1-casein protein, a recombinant α-S2-casein protein, and a recombinant κ-casein protein,

v. a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, and a recombinant β-casein protein,

vi. a recombinant β-casein protein and a recombinant α-S1-casein protein,

vii. a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, and a recombinant κ-casein protein,

viii. a recombinant β-casein, a recombinant α-S1-casein protein, and a recombinant α-S2-casein protein,

ix. a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148,

x. a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant β-lactoglobulin protein,

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xi. a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant α-lactalbumin protein, or

xii. a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant β-lactoglobulin protein, and a recombinant α-lactalbumin protein; and

(d) the one or more identified recombinant milk proteins confer on the substitute dairy food composition one or more characteristics of a dairy product selected from the group consisting of: taste, flavor, aroma, appearance, mouthfeel, density, structure, texture, elasticity, springiness, coagulation, binding, leavening, aeration, foaming, creaminess, and emulsification.

2. The substitute dairy food composition of claim 1, wherein the one or more identified recombinant milk proteins are free of milk impurities.

3. The substitute dairy food composition of claim 1 or 2, wherein at least one of the one or more identified recombinant milk proteins comprises an amino acid sequence that is at least 80% identical to an amino acid sequence of a cow milk protein, sheep milk protein, horse milk protein, or goat milk protein.

4. The substitute dairy food composition of claim 1 or 2, wherein at least one of the one or more identified recombinant milk proteins comprises an amino acid sequence that is at least 90% identical to an amino acid sequence of a cow milk protein, sheep milk protein, horse milk protein, or goat milk protein.

5. The substitute dairy food composition of claim 1 or 2, wherein at least one of the one or more identified recombinant milk proteins comprises an amino acid sequence that is at least 95% identical to an amino acid sequence of a cow milk protein, sheep milk protein, horse milk protein, or goat milk protein.

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6. The substitute dairy food composition of any one of claims 1 to 5, wherein at least one of the one or more identified recombinant milk proteins is produced by a fungal cell or a bacterial cell.

7. The substitute dairy food composition of claim 6, wherein the fungal cell is *Aspergillus* or *Trichoderma.*

8. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein and a recombinant α-lactalbumin protein.

9. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein and a recombinant κ-casein protein.

10. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein and a recombinant β-casein protein.

11. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, a recombinant β-casein protein, a recombinant α-S1-casein protein, a recombinant α-S2-casein protein, and a recombinant κ-casein protein.

12. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, and a recombinant β-casein protein.

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13. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-casein protein and a recombinant α-S1-casein protein.

14. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-lactoglobulin protein, a recombinant α-lactalbumin protein, and a recombinant κ-casein protein.

15. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant β-casein, a recombinant α-S1-casein protein, and a recombinant α-S2-casein protein.

16. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148.

17. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant β-lactoglobulin protein.

18. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant milk protein that is encoded by a nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant α-lactalbumin protein.

19. The substitute dairy food composition of any one of claims 1 to 7, wherein the one or more identified recombinant milk proteins are a recombinant milk protein that is encoded by a

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nucleic acid having a substantial homology to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 148, and a recombinant β-lactoglobulin protein, and a recombinant α-lactalbumin protein.

20. The substitute dairy food composition of any one of claims 1 to 19, wherein the substitute dairy food composition is substitute ice cream , a powder composition, substitute cream, substitute milk, substitute cream cheese, substitute cottage cheese, a nutritional supplement composition, substitute yogurt, substitute cheese, substitute crème fraiche, substitute buttermilk, substitute butter, substitute frozen custard, or substitute curd.

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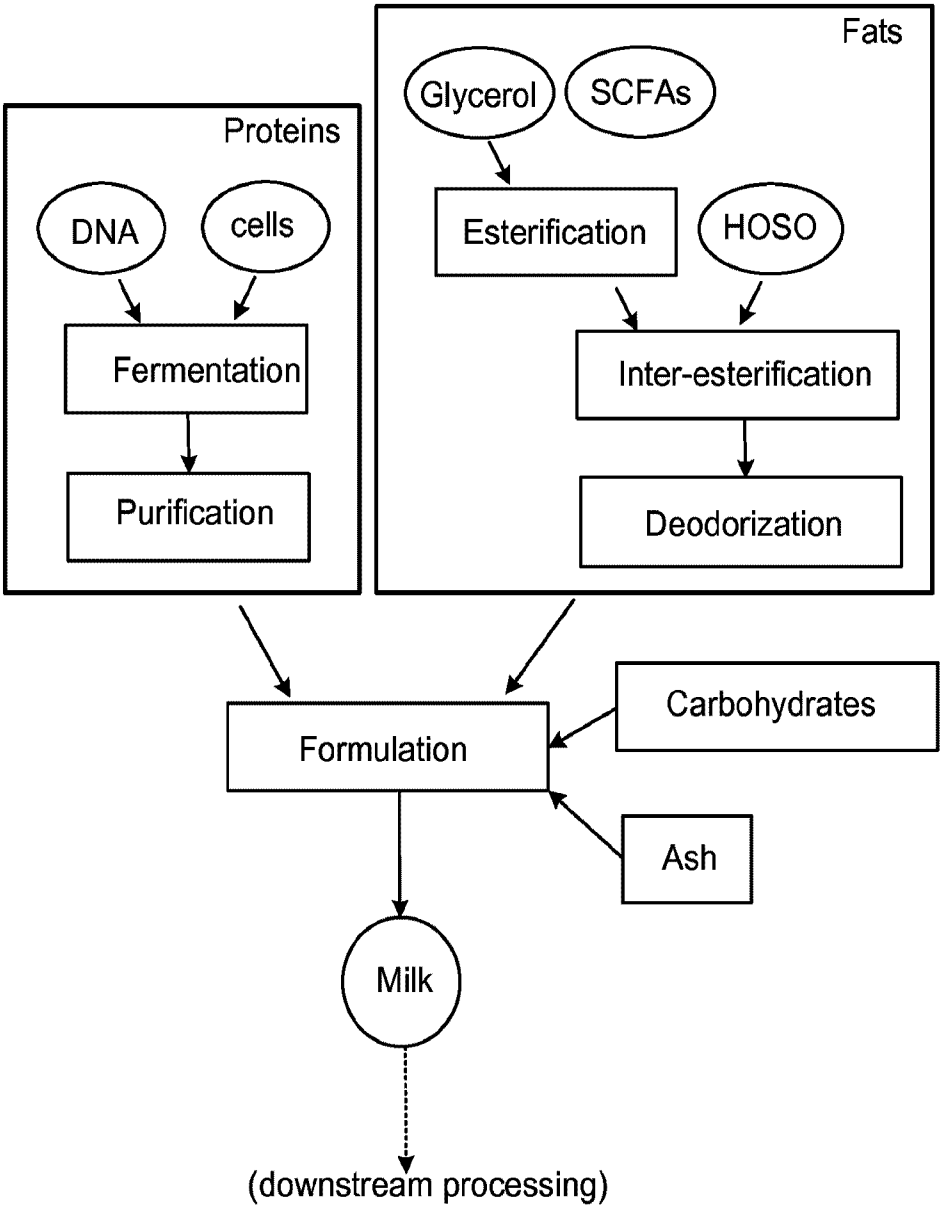

FIG. 1

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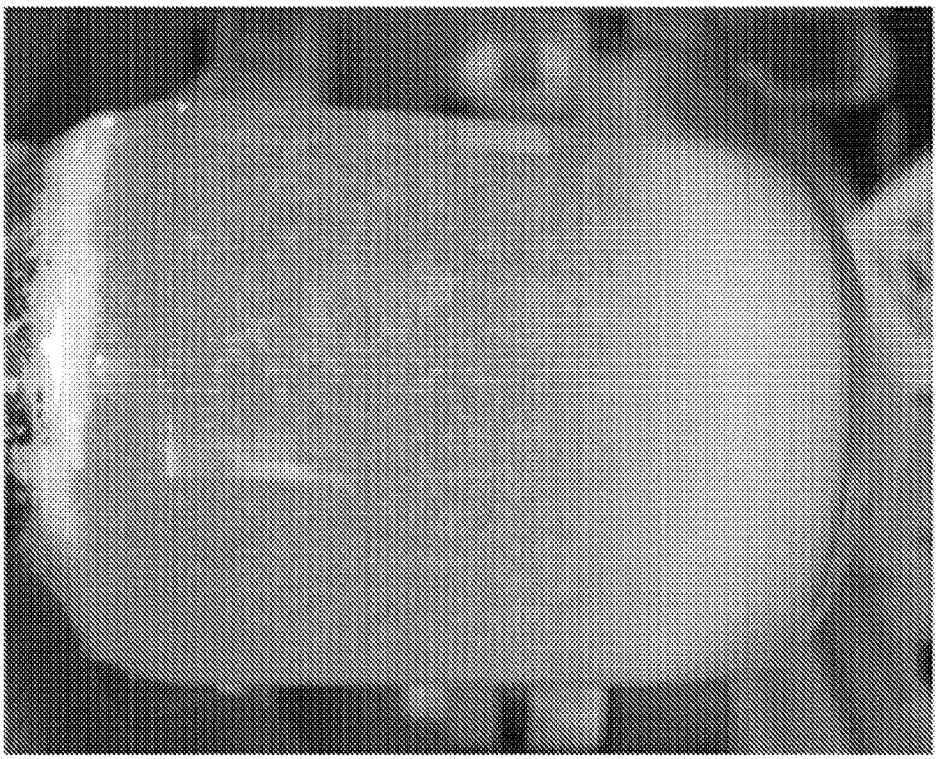

FIG. 2A

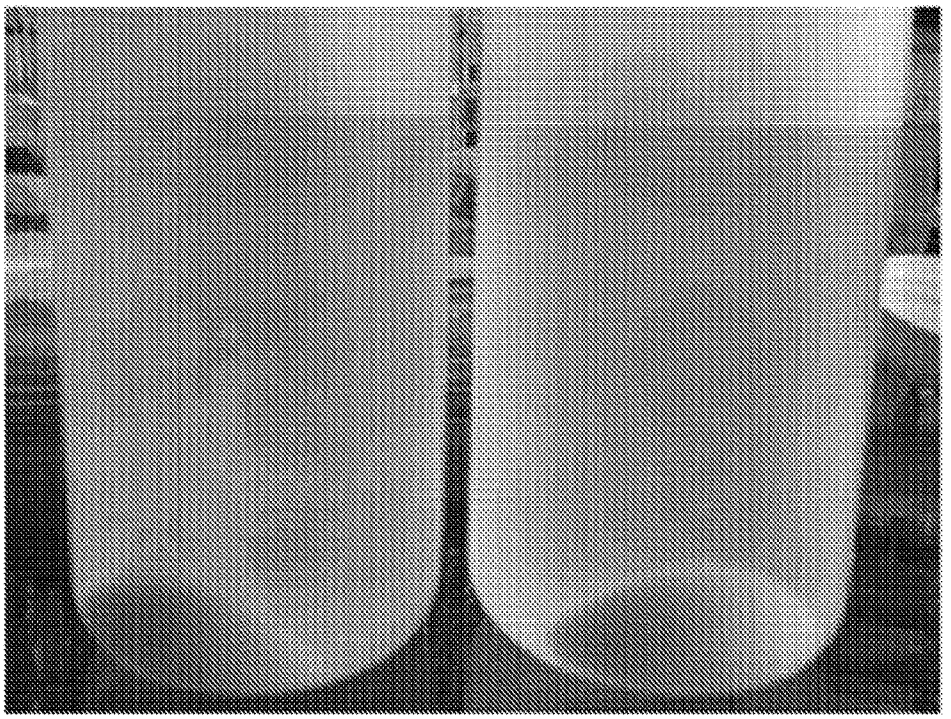

FIG. 2B

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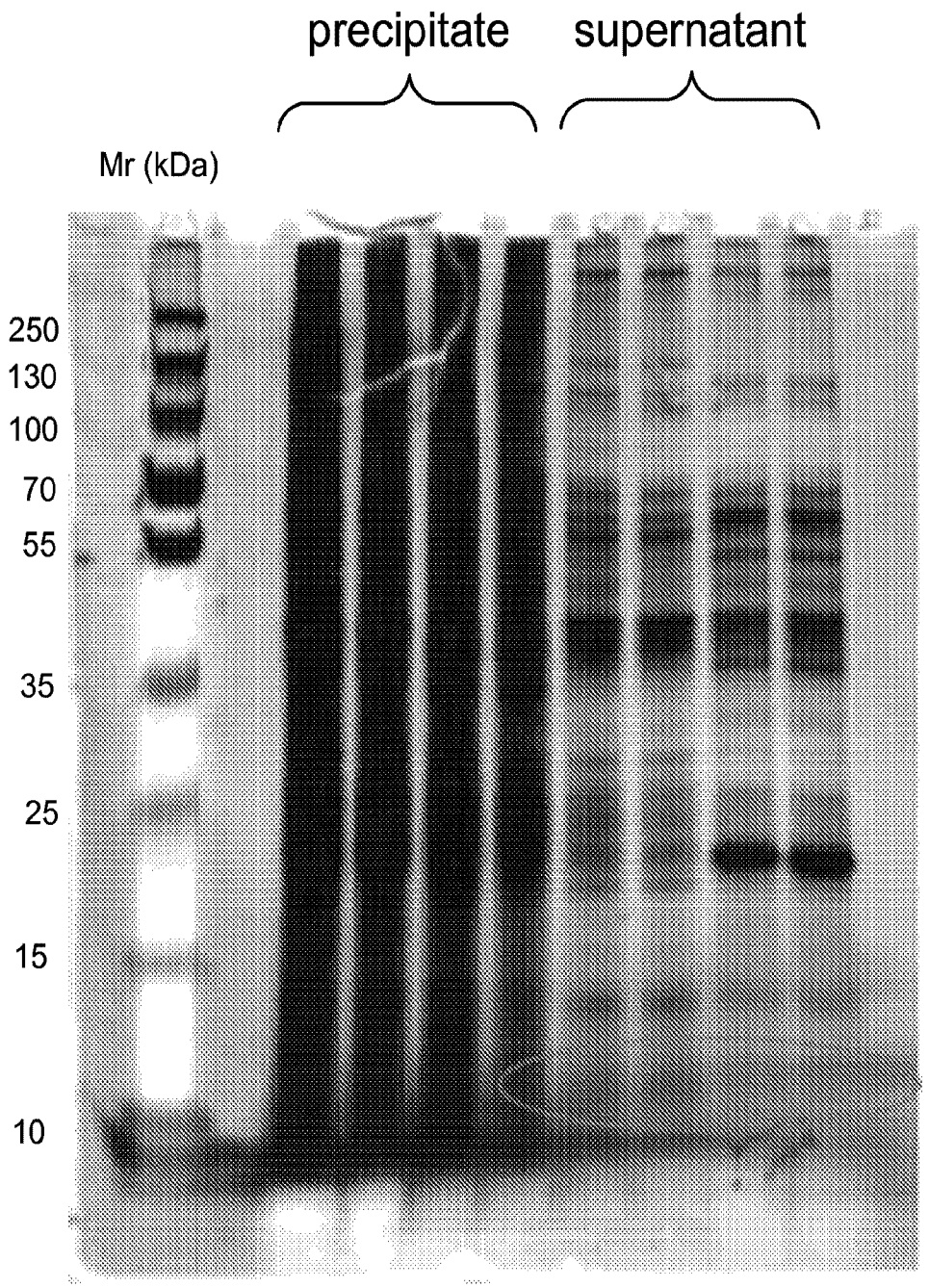

FIG. 3

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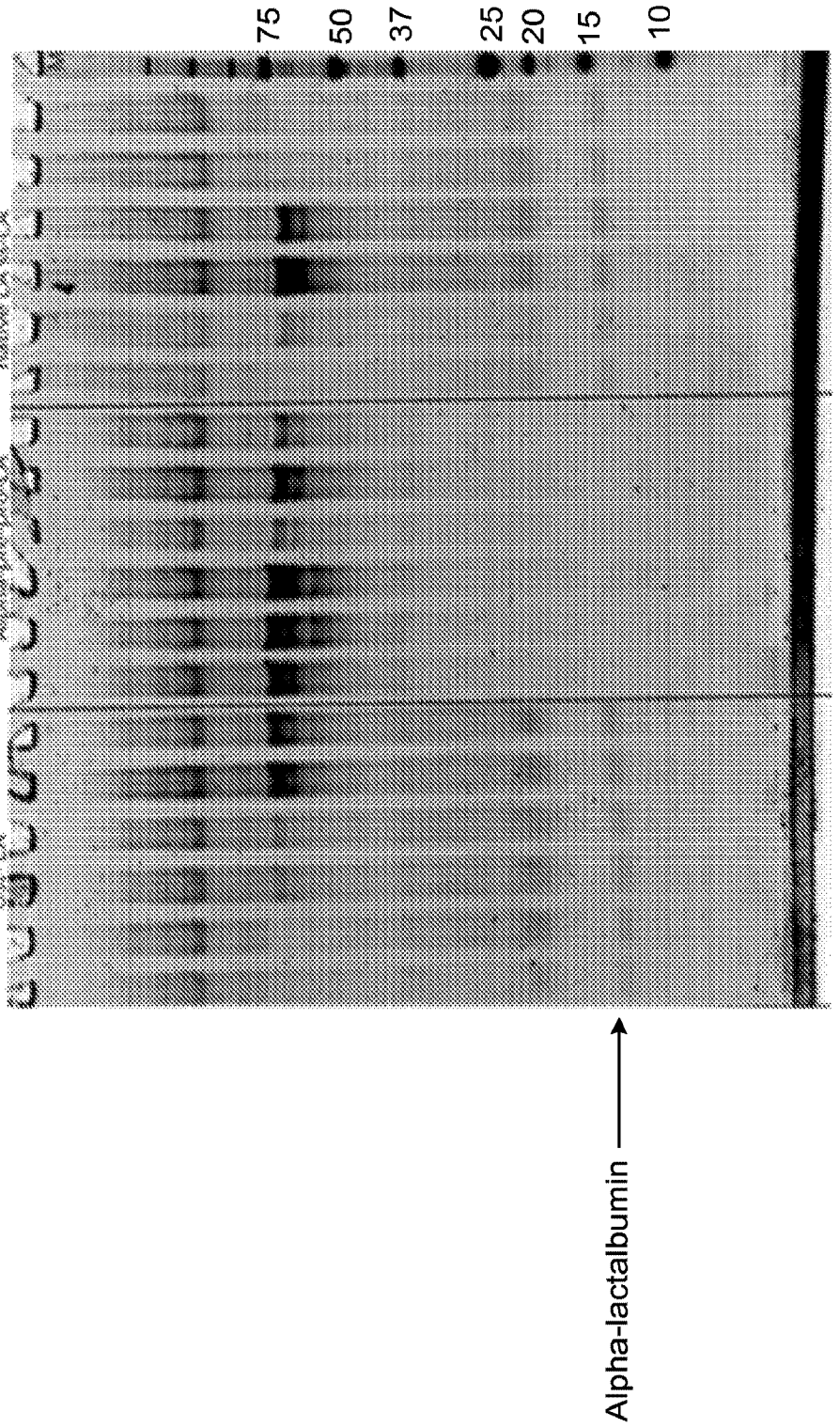

FIG. 4

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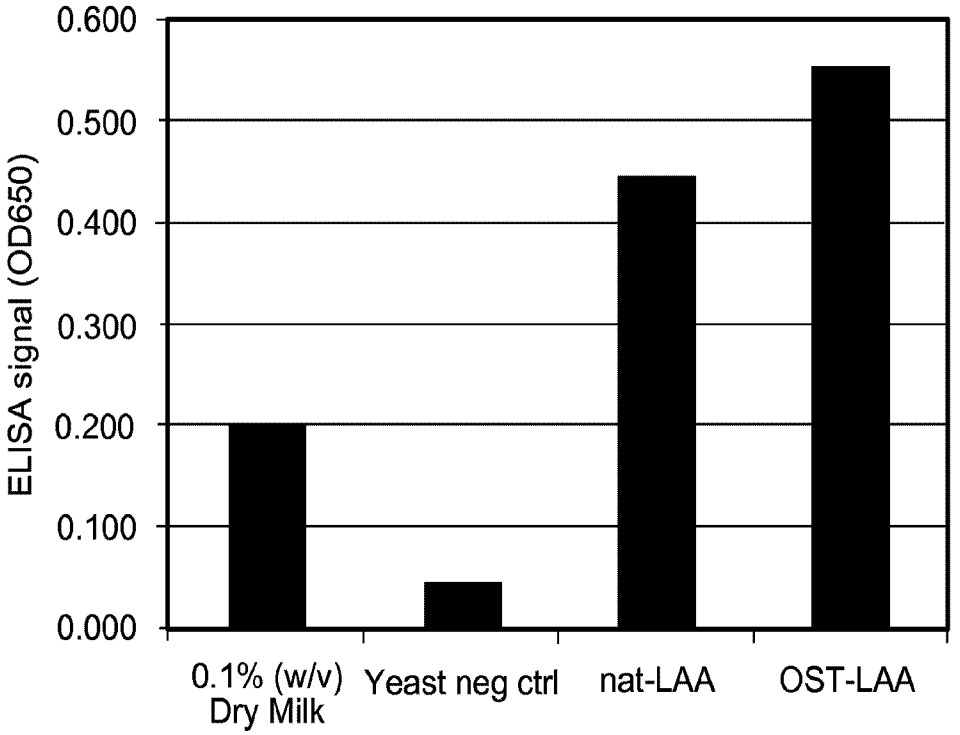

FIG. 5

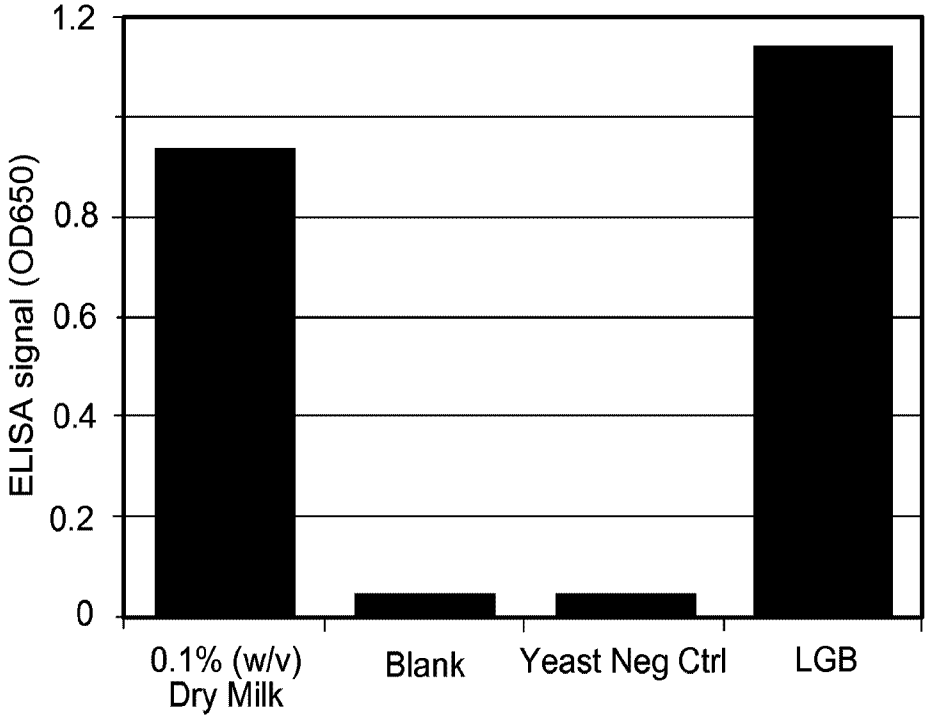

FIG. 6

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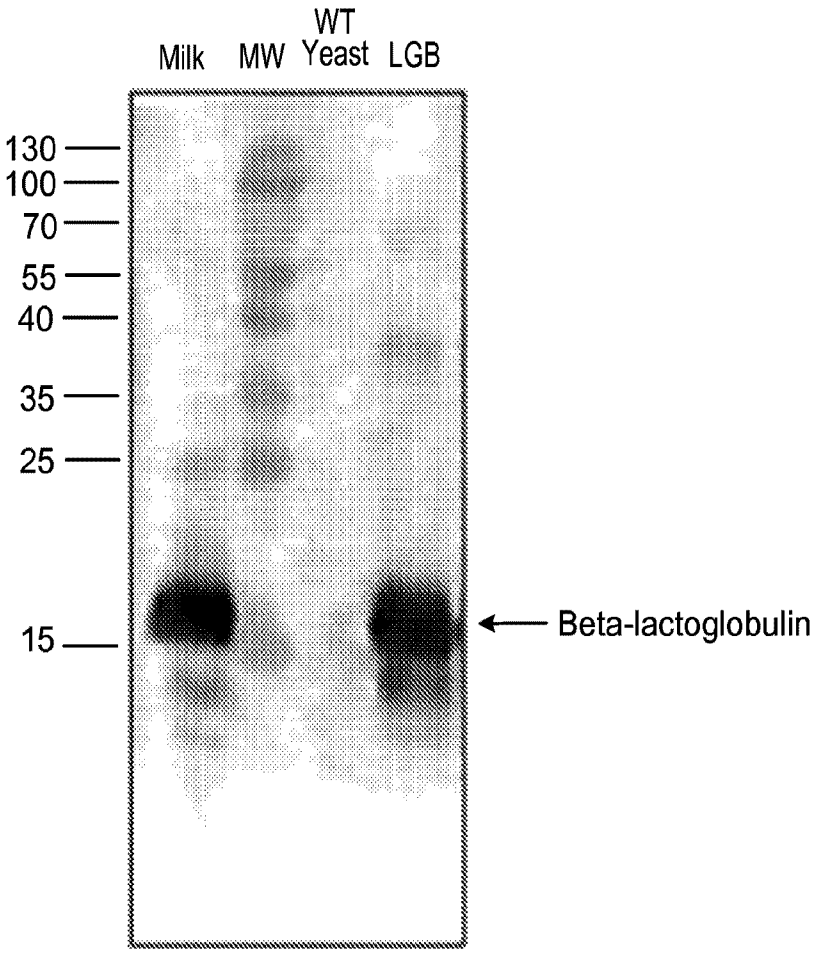

FIG. 7

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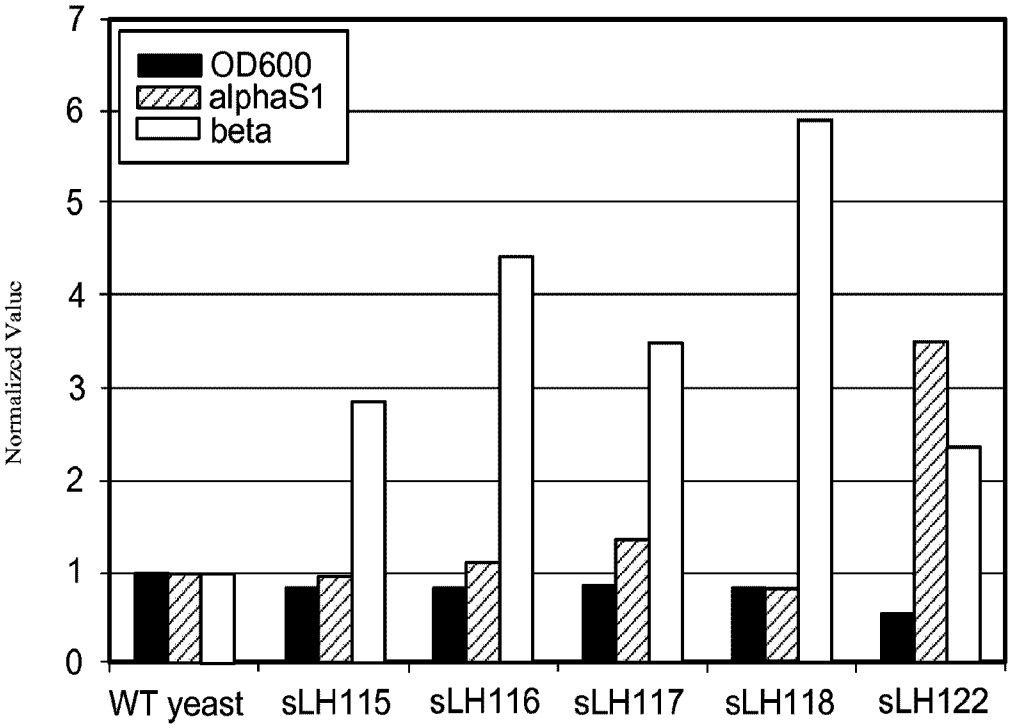

FIG. 8

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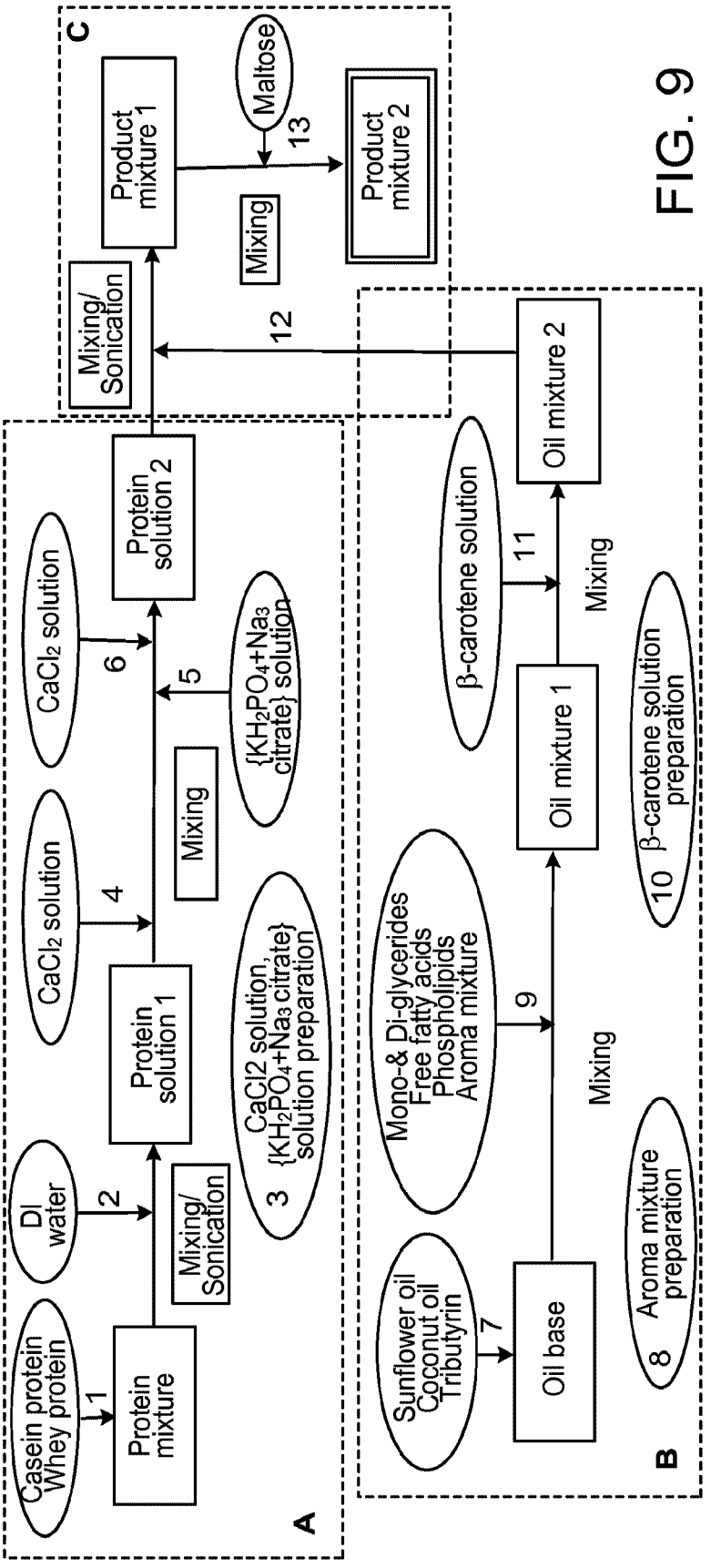
A
Casein protein
Whey protein
1
Protein mixture
DI water
2
Mixing/ Sonication
3
CaCl2 solution, {KH2PO4+Na3 citrate} solution preparation
Protein solution 1
CaCl2 solution
4
Mixing
{KH2PO4+Na3 citrate} solution
5
CaCl2 solution
6
Protein solution 2
B
Sunflower oil
Coconut oil
Tributyrin
7
Oil base
8
Aroma mixture preparation
Mono-& Di-glycerides
Free fatty acids
Phospholipids
Aroma mixture
9
Mixing
Oil mixture 1
10
β-carotene solution preparation
β-carotene solution
11
Mixing
Oil mixture 2
C
Mixing/ Sonication
12
Mixing
Product mixture 1
Maltose
13
Product mixture 2

FIG. 9

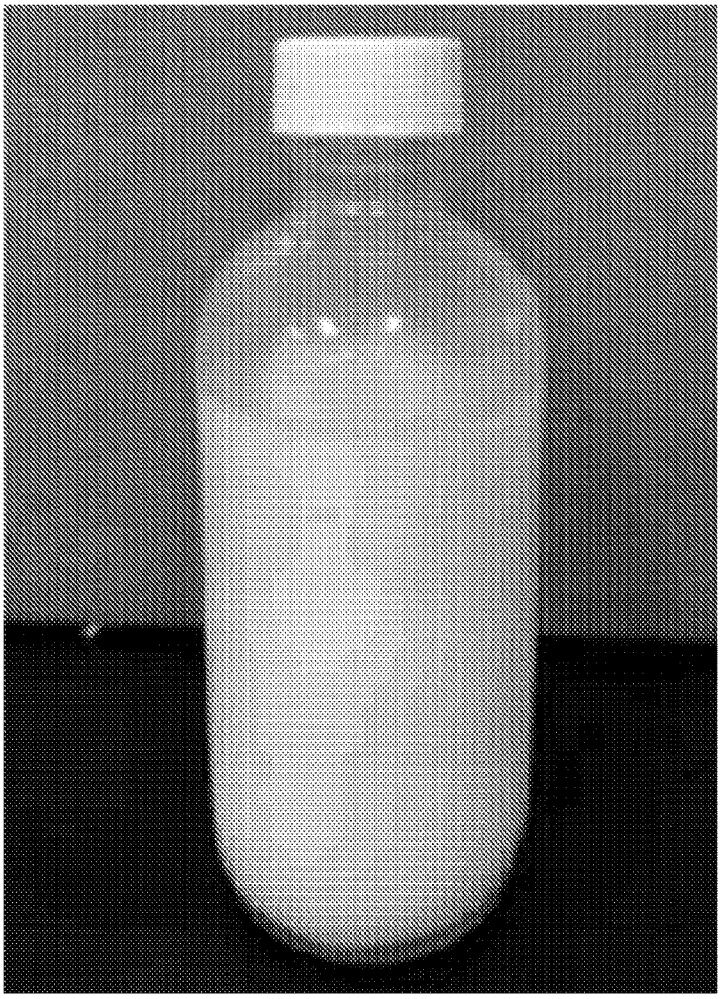

FIG. 10

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