[5] In addition to impacting the environment, animal agriculture poses a serious risk to human health. A startling 80% of antibiotics used in the United States go towards treating animals, resulting in the development of antibiotic resistant microorganisms, also known as superbugs. For years, food companies and farmers have administered antibiotics not only to sick animals, but also to healthy animals, to prevent illness. In September 2016, the United Nations announced the use of antibiotics in the food system as a crisis on par with Ebola and HIV.

[6] For at least these reasons, alternative dairy compositions produced without the use of mammalian milk have become increasingly popular. However, such compositions often fail to achieve the same organoleptic properties as their milk-derived counterparts. For example, some alternative dairy compositions on the market today are known to have an off-putting texture or taste, poor melt characteristics and/or lack of stretch. These compositions may also have reduced nutritional value compared to their milk-derived counterparts. For example, some "vegan" cheeses, typically made from oils and starch, may contain little to no protein.

[7] Accordingly, there is an urgent need to provide bovine milk and/or essential high-quality proteins from bovine milk in a more sustainable and humane manner, instead of solely relying on animal farming. Also, there is a need for selectively producing the specific milk proteins that confer nutritional and clinical benefits, and/or do not provoke allergic responses, and a need to prepare improved alternative dairy compositions which comparable nutritional value and similar organoleptic properties as their milk-derived counterparts.

SUMMARY

[8] Provided herein are recombinant fusion proteins comprising (i) a first milk protein, and (ii) a second milk protein. At least one of the first milk protein and the second milk protein may be, for example, α-S1 casein, α-S2 casein, β-casein, κ-casein, para-κ-casein, β-lactoglobulin, α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase, serum albumin, or an immunoglobulin. In some examples, at least one of the first milk protein and the second milk protein is β-lactoglobulin. In some examples, at least one of the first milk protein and the second milk protein is α-S1 casein, α-S2 casein, β-casein, κ-casein, or para-κ-casein. In some examples, i) the first milk protein is α-S1 casein, α-S2 casein, β-casein, κ-casein, or para-κ-casein; and ii) the second milk protein is α-S1 casein, α-S2 casein, β-casein, κ-casein, or para-κ-casein. In some

examples, at least one of the first milk protein and the second milk protein is κ-casein and comprises the sequence of SEQ ID NO: 4, or a sequence at least 90% identical thereto. In some examples, at least one of the first milk protein and the second milk protein is para-κ-casein and comprises the sequence of SEQ ID NO: 2, or a sequence at least 90% identical thereto. In some examples, at least one of the first milk protein and the second milk protein is β-casein and comprises the sequence of SEQ ID NO: 6, or a sequence at least 90% identical thereto. In some examples, at least one of the first milk protein and the second milk protein is α-S1 casein and comprises the sequence SEQ ID NO: 8, or a sequence at least 90% identical thereto. In some examples, at least one of the first milk protein and the second milk protein is α-S2 casein and comprises the sequence SEQ ID NO: 84, or a sequence at least 90% identical thereto. In some examples, the first milk protein and the second milk protein are different proteins. In some examples, the first milk protein and the second milk protein are the same proteins. In some examples, the fusion protein is plant-expressed. In some examples, the fusion protein is expressed in soybean plant. In some examples, the fusion protein comprises a protease cleavage site. In some examples, the protease cleavage site is a chymosin cleavage site.

[9] Also provided herein are nucleic acids encoding one or more of the recombinant fusion proteins of the disclosure, and expression vectors comprising the same. In some examples, the nucleic acids are codon-optimized for expression in a plant, such as a soybean.

[10] Additionally, provided herein are host cells comprising a nucleic acid or an expression vector of the disclosure; i.e., a nucleic acid or expression vector encoding a fusion protein. The host cells may be, for example, plant cells, bacterial cells, fungal cells, or mammalian cells. In some examples, the host cells are soybean cells.

[11] Also provided herein are plants stably transformed with a nucleic acid or an expression vector of the disclosure. In some examples, the fusion protein is expressed in the plant in an amount of 1% or higher per total protein weight of soluble protein extractable from the plant.

[12] Also provided herein are methods for making a fusion protein, the methods comprising: (a) transforming a host cell with a nucleic acid or an expression vector described herein; and (b) growing the transformed host cell under conditions wherein the fusion protein is expressed. In some examples, the method comprises co-expressing in the host cell a protein capable of forming a protein body, such as a prolamin selected from a gliadin, a hordein, a secalin, a zein, a

kafirin, or an avenin. In some examples, the method comprises expressing a kinase in the host cell. In some examples, expression of one or more proteases is knocked down or knocked out in the cell.

[13] Also provided herein are transgenic plants comprising a recombinant fusion protein, or a nucleic acid or expression vector comprising the same. In some examples, the transgenic plant is a soybean plant. In some examples, the fusion protein is expressed in the plant in an amount of 1% or higher per total protein weight of soluble protein extractable from the plant.

[14] Also provided herein are methods for stably expressing a recombinant fusion protein in a plant, the methods comprising: (i) transforming a plant with a plant transformation vector comprising an expression cassette comprising a nucleic acid molecule encoding the fusion protein; and (ii) growing the transformed plant under conditions wherein the recombinant fusion protein is expressed. In some examples, the fusion protein is expressed in an amount of 1% or higher per total protein weight of soluble protein extractable from the plant.

[15] Also provided herein are seed processing compositions comprising a fusion protein of the disclosure.

[16] Also provided herein are food compositions comprising a fusion protein of the disclosure. In some examples, the food composition is selected from the group consisting of cheese and processed cheese products, yogurt and fermented dairy products, directly acidified counterparts of fermented dairy products, cottage cheese dressing, frozen dairy products, frozen desserts, desserts, baked goods, toppings, icings, fillings, low-fat spreads, dairy-based dry mixes, soups, sauces, salad dressing, geriatric nutrition, creams and creamers, analog dairy products, follow-up formula, baby formula, infant formula, milk, dairy beverages, acid dairy drinks, smoothies, milk tea, butter, margarine, butter alternatives, growing up milks, low-lactose products and beverages, medical and clinical nutrition products, protein/nutrition bar applications, sports beverages, confections, meat products, analog meat products, meal replacement beverages, weight management food and beverages, cultured buttermilk, sour cream, yogurt, skyr, leben, lassi, kefir, powder containing a milk protein, and low-lactose products. In some examples, the food composition comprises a total amount of casein protein; wherein about 32% to 100% by weight of the total amount of casein protein in the food composition is beta-casein. In some examples, the food composition is a cheese composition. In some examples, the cheese composition has the ability to stretch to at

least 3 cm in length without breaking, as determined by heating a 100 gram mass of the composition at a temperature of 225°C for 4 minutes and cooling to about 90°C and pulling with a fork placed beneath the mass.

[17] Also provided herein is method of making a food composition, comprising combining a fusion protein disclosed herein into a food composition.

[18] Also provided herein is an alternative dairy food composition comprising i) a recombinant fusion protein described herein; and ii) at least one lipid. In some examples, the recombinant fusion protein confers on the alternative dairy food composition one or more characteristics of a dairy food product selected from the group consisting of: taste, aroma, appearance, handling, mouthfeel, density, structure, texture, elasticity, springiness, coagulation, binding, leavening, aeration, foaming, creaminess and emulsification. In some examples, the alternative dairy food composition does not comprise any other milk proteins. In some examples, the alternative dairy food composition comprises calcium at a concentration of about 0.01 to about 2% by weight. In some examples, the alternative dairy food composition comprises a total amount of casein protein; wherein about 32% to 100% by weight of the total amount of casein protein in the food composition is beta-casein. In some examples, the alternative diary food composition has a pH of about 5.2 to about 5.9. In some examples, the alternative dairy food composition is selected from the group consisting of cheese and processed cheese products, yogurt and fermented dairy products, directly acidified counterparts of fermented dairy products, cottage cheese dressing, frozen dairy products, frozen desserts, desserts, baked goods, toppings, icings, fillings, low-fat spreads, dairy-based dry mixes, soups, sauces, salad dressing, geriatric nutrition, creams and creamers, analog dairy products, follow-up formula, baby formula, infant formula, milk, dairy beverages, acid dairy drinks, smoothies, milk tea, butter, margarine, butter alternatives, growing up milks, low-lactose products and beverages, medical and clinical nutrition products, protein/nutrition bar applications, sports beverages, confections, meat products, analog meat products, meal replacement beverages, weight management food and beverages, cultured buttermilk, sour cream, yogurt, skyr, leben, lassi, kefir, powder containing a milk protein, and low-lactose products. In some examples, the alternative diary food composition is a cheese composition.

[18a] In one aspect, the present disclosure refers to an alternative dairy food composition comprising: A) a plant-expressed recombinant fusion protein comprising: (i) a first casein milk protein; and (ii) a second casein milk protein and/or a zein protein; and B) at least one lipid.

[19] Also provided herein are solid phase, protein-stabilized emulsions comprising a fusion protein described herein, wherein the emulsions have the ability to stretch to at least 3 cm in length without breaking, as determined by heating a 100 gram mass of the emulsion to a temperature of about 225°C for 4 minutes and cooling to about 90°C and pulling with a fork placed beneath the mass.

[20] Also provided herein are colloidal suspensions comprising a fusion protein described herein, wherein the colloidal suspension has at least one, at least two, or at least three characteristics that are substantially similar to bovine milk selected from taste, appearance, mouthfeel, structure, texture, density, elasticity, springiness, coagulation, binding, leavening, aeration, foaming, creaminess, and emulsification.

[21] These and other examples are described in detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[22] The accompanying figures, which are incorporated herein and form a part of the specification, illustrate some, but not the only or exclusive, example embodiments and/or features. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than limiting.

[23] **FIGs. 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, 1O,** and **1P** show expression cassettes having different combinations of fusions between sequences encoding structured and intrinsically unstructured proteins (not to scale). Coding regions and regulatory sequences are indicated as blocks (not to scale). As used in the figures, "L" refers to linker; "Sig" refers to a signal sequence that directs foreign proteins to protein storage vacuoles, "5' UTR" refers to the 5' untranslated region, and "KDEL" refers to an endoplasmic reticulum retention signal.

[24] **FIGs. 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, 2M, 2N, 2O,** and **2P** show expression cassettes having different combinations of fusions between sequences encoding a first protein and a second protein (not to scale), wherein the first and/or second protein is a milk protein (not shown). Coding regions and regulatory sequences are indicated as blocks (not to scale). As used in the figures, "L" refers to linker; "Sig" refers to a signal sequence that directs foreign proteins to protein storage vacuoles, "5' UTR" refers to the 5' untranslated region, and "KDEL" refers to an endoplasmic reticulum retention signal.