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(54) **Title:** METHODS AND COMPOSITIONS FOR CONSUMABLES

(57) **Abstract:** Provided herein are methods and compositions for the production of cheese replicas. Generally the cheese replicas are produced by inducing the enzymatic curdling of non-dairy milks.



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## METHODS AND COMPOSITIONS FOR CONSUMABLES

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. application Ser. No. 61/507,096 filed July 12, 2011, which is incorporated herein in its entirety by reference.

### BACKGROUND OF THE INVENTION

[0002] Cheese making has relied on dairy milks as the major ingredient for more than 4000 years. Dairy cheese is made from curds formed from dairy milk. Dairy milks can readily be made to form curds suitable for making cheese by contacting the dairy milk with rennet (an aspartic protease which cleaves kappa-casein) at mildly acidic pH. Some cheeses, e.g., cream cheese, ricotta, cottage cheese and paneer, are made without rennet. In the absence of rennet, dairy cheese may be induced to curdle with acid (e.g., lemon juice, vinegar, etc.) or a combination of heat and acid. Acid coagulation can also occur naturally from starter culture fermentation. The strength of the curds depends on the type of coagulation. Most commercially produced cheeses use some type of rennet (animal, vegetable or microbial-derived) in their production.

[0003] The global dairy sector contributes an estimated 4 percent to the total global anthropogenic green house gas emissions. Producing 1 kg of cheddar cheese requires an average of 10,000 Liters of fresh water. Additionally, many individuals cannot digest and metabolize lactose. In these individuals enteric bacteria ferment the lactose, resulting in various abdominal symptoms, which can include abdominal pain, bloating, flatulence, diarrhea, nausea, and acid reflux. Additionally, the presence of lactose and its fermentation products raises the osmotic pressure of the colon contents. 3.4% of children in the U.S.A. are reported to have allergies to dairy milks. Many individuals choose to avoid milk for ethical or religious reasons.

[0004] Non-dairy milks, including plant-derived milks avoid many of the environmental, food sensitivity, ethical and religious problems associated with dairy milk and they can be made free of lactose, making the generation of dairy substitutes using the plant derived milks attractive. However, rennet is not an effective agent for inducing non-dairy proteins or emulsions, including plant-derived milks, including almond milk, chestnut milk, pecan milk, hazelnut milk, cashew milk, pine nut milk, and walnut milk, to curdle. Consequently, traditional cheese making techniques have not been successfully used to produce non-dairy cheese replicas.

[0005] Flavor and aroma in dairy cheese results in part from the degradation of lactose, proteins and fats, carried out by ripening agents, which include: bacteria and enzymes in the milk, bacterial cultures added during the cheese-making process, rennet, other proteases, lipases, added molds

and/or yeasts and bacteria and fungi that opportunistically colonize the cheese during ripening and aging.

[0006] Cheese replicas made principally of non-dairy ingredients are commercially available. Most of these cheese replicas include some dairy ingredients, for example, casein. Some commercially available cheese replicas contain no animal products. These include fermented cheese replicas made from nut milks from which insoluble carbohydrates have not been effectively removed, and made without using a protein crosslinking agent and several products in which a starch is a principal ingredient or containing agar, carrageenan and tofu to provide the desired texture. Most tasters consider none of the currently available cheese replicas to adequately replicate the taste, aroma and mouthfeel of dairy cheeses.

[0007] Complex carbohydrates in currently available cheese replicas made from nut milks have unfavorable effects on the texture, resulting in a product with a grainy mouthfeel and lacking the creaminess of dairy cheeses.

[0008] Starches that comprise the major gelling agent in many currently available cheese replicas lead to a relatively high carbohydrate content, which may be undesirable to consumers, for example those wishing to limit carbohydrate intake.

[0009] Because of these deficiencies, there is currently no cheese replica that is acceptable to most consumers as an alternative to traditional dairy cheeses.

[0010] Thus, it is clear that there is a great need in the art for an improved method and system for producing non-dairy cheese replicas while avoiding the shortcomings and drawbacks of the cheese replicas that have previously been available to consumers.

## SUMMARY OF THE INVENTION

[0011] The invention relates to methods and compositions for non-dairy milk and cheese products, including without limitation, plant-derived milk and cheese products, as an alternative to dairy products for human consumption.

[0012] The invention provides a method for producing a non-dairy cheese by preparing an emulsion comprising proteins and fats from plants or other non-animal sources, inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins, and producing a cheese-replica from the gel. In one embodiment, the emulsion contains less than 10% animal products. In one embodiment, the emulsion contains less than 8%, 7%, 6%, 5%, or 3% animal products. In one embodiment the emulsion contains no animal products. In one embodiment the inducing step of said method comprises adding an enzyme. In one embodiment of said method the enzyme used is transglutaminase. In one embodiment the enzyme used is Factor XIII (fibrin-stabilizing factor). In

one embodiment of said method the enzyme used is Keratinocyte transglutaminase (TGM1). In one embodiment the enzyme used is Tissue transglutaminase (TGM2). In one embodiment of said method the enzyme used is Epidermal transglutaminase (TGM3). In one embodiment the enzyme used is Prostate transglutaminase (TGM4). In one embodiment the enzyme used is TGM X (TGM5). In one embodiment of said method the enzyme used is TGM Y (TGM6). In one embodiment of said method the enzyme used is TGM Z (TGM7). In one embodiment, the enzyme is a transglutaminase from *Streptovorticillium mobaraense*. In one embodiment, the enzyme is similar or identical to a transglutaminase from *Streptovorticillium mobaraense*. In one embodiment, the enzyme is a lysyl oxidase. In one embodiment the emulsion is non-dairy milk. In one embodiment at least one of the plant sources are nuts. In one embodiment at least one of the plant sources are legumes. In one embodiment at least one of the plant sources are seeds. In one embodiment at least one of the plant sources are leaves. In one embodiment at least one of the plant sources are fruit from the family Fabaceae. In one embodiment at least one of the non-animal sources is a species of bacteria. In one embodiment at least one of the non-animal sources is a species of archaea. In one embodiment at least one of the non-animal sources is a species of fungus. In one embodiment at least one of the non-animal sources is a species of algae. In one embodiment the emulsion is a non-dairy milk. In one embodiment the nuts are one or more of following: almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts. One embodiment of said method has an additional step of adding a sugar to the emulsion. In one embodiment the sugar added to the emulsion is a monosaccharide. In one embodiment the sugar added to the emulsion is a disaccharide. In one embodiment said method has an additional step of inoculating with lactic acid bacteria. In the embodiment of inoculating with lactic acid bacteria the invention provides an addition step of allowing growth of the bacterial cultures. In one embodiment the method can include an additional step of adding one or more of the following *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* spp., *Lactobacillus* spp., *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*, *Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus* spp., or *Propionibacteria*. In one embodiment the method can include an additional step of adding one or more of the following *halophilic* bacterium or *archaea*. In one embodiment the invention provides for the use of thermal denaturing and cross-linking. In one embodiment the invention provides thermal denaturing used without the addition of any enzymes. One embodiment of said method has an additional step of cutting the gel. One embodiment of said method has an additional step of draining and shaping the cut gel. One embodiment of said method

has an additional step of adding a flavoring component. In one embodiment the flavoring component is one or more species of bacteria. In one embodiment the flavoring component is lactic acid bacteria. In one embodiment the flavoring component is a mold. In one embodiment the flavoring component is yeast. In one embodiment the method includes concentrated proteins prior to preparing the emulsion. In one embodiment the concentrated proteins are each essentially homogeneous proteins. In one embodiment the method includes the additional step of concentrating proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of concentrating proteins and fats from one or more non-animal sources. In one embodiment the method includes the additional step of isolating the proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of isolating the proteins and fats from one or more non-animal sources. In one embodiment the method includes the additional step of purifying the proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of purifying the proteins and fats from one or more non-animal sources. In one embodiment the invention provides for the concentrated proteins to come from the same essentially homogeneous plant-derived protein. In one embodiment the invention provides for the concentrated proteins to come from the same essentially homogeneous non-animal-derived protein. In one embodiment the invention provides for the concentrated proteins to come from separate plant species. In one embodiment the invention provides for the concentrated proteins to come from separate non-animal species.

**[0013]** In addition, the invention provides steps for preparation to undertake the claimed methods of producing a non-animal based cheese including (a) obtaining nuts or seeds, and (b) surface sterilizing the nuts or seeds. In one embodiment the method uses nuts or seeds as the plant sources. In one embodiment the surface sterilization step is blanching procedure. In one embodiment the preparation method can also include a step for washing the nuts or seeds. In one embodiment the method of preparation can also include the step of decompounding the nuts or seeds. In one embodiment the step of decompounding the nuts or seeds is a blending procedure. In one embodiment the method of preparation can also include centrifugation. In one embodiment the method of preparation can also includes a centrifugation procedure that results in the removal of at least 85% of the suspended solids. In one embodiment the method of preparation can also includes a centrifugation procedure that results in the removal of at least 75%, 65%, 55%, or 45% of the suspended solids.

**[0014]** The invention provides a method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyze the formation of crosslinks between proteins from the nuts or seeds.

[0015] The invention provides a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

[0016] In one embodiment the invention provides a composition comprising centrifuged non-dairy milk wherein at least 85% of the insoluble solids have been removed relative to the non-dairy milk prior to centrifugation.

[0017] In one embodiment the invention provides a composition comprising a non-dairy milk with less than 30% polysaccharides. In one embodiment the non-dairy milk composition has less than 10% polysaccharides. In one embodiment the non-dairy milk composition less than 1% polysaccharides. In one embodiment the non-dairy milk composition is entirely composed of ingredients derived from non-animal sources. In one embodiment the non-dairy milk composition is at least comprised of 20% protein isolated from a single plant species.

[0018] In one embodiment the invention provides for a composition comprised of non-dairy milk and a cross-linking enzyme. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Factor XIII (fibrin-stabilizing factor). In one embodiment of the non-dairy milk and a cross-linking enzyme composition, the enzyme is a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, the enzyme is similar or identical to a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Keratinocyte transglutaminase (TGM1). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Tissue transglutaminase (TGM2). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Epidermal transglutaminase (TGM3). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Prostate transglutaminase (TGM4). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM X (TGM5). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM Y (TGM6). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM Z (TGM7). In one embodiment the invention provides for a composition comprised of non-dairy milk and a cross-linking enzyme. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is lysyl oxidase. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents.

[0019] In one embodiment of the non-dairy milk and a cross-linking enzyme composition, at least 85% of the insoluble solids have been removed by centrifugation. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, proteins from non-animal sources comprise at least 50% of the total mass of the composition. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the starch content is less than 1% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the starch content is less than 5% by mass. One embodiment of the non-dairy milk and a cross-linking enzyme composition has less than 1% insoluble carbohydrate by mass. One embodiment of the non-dairy milk and a cross-linking enzyme composition has less than 5% insoluble carbohydrate by mass. One embodiment of the non-dairy milk and a cross-linking enzyme composition has less than 5% starch and less than 1% insoluble carbohydrate by mass. One embodiment of the non-dairy milk and a cross-linking enzyme composition has less than 5% starch and less than 5% insoluble carbohydrate by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition has a polysaccharide content less than 1% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition has a polysaccharide content less than 5% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 1% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 5% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 10% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, at least 80% of the protein content comprises a single monomeric or multimeric protein.

[0020] In some embodiments the invention provides for a composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes. In one embodiment of the composition of covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes composition, the proteins are not from soy. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes, less than 5% of the proteins are from soy. In one embodiment the composition comprises less than 20% insoluble solids. In one embodiment of the covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes composition also contains oils or fats isolated from non-dairy sources. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the crosslinking enzyme of the composition is a transglutaminase. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the crosslinking enzyme of the composition is a lysyl oxidase. In one embodiment of the composition comprising covalently

crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the cross-linking enzyme is Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7). In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, the enzyme is a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, the enzyme is similar or identical to a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, protein crosslinks are formed between lysine side chains of respective protein constituents.

[0021] In some embodiments the invention provides for a cheese replica composition comprised of a gelled emulsion of non-dairy proteins and fats. In one embodiment the cheese replica composition has between 10% and 40% proteins from non-dairy sources and between 0% and 65% fats from non-dairy sources. In one embodiment the cheese replica composition further comprises a crosslinking enzyme selected from the group consisting of Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) or TGM Z (TGM7). In one embodiment of the cheese replica composition, the enzyme is a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the cheese replica composition, the enzyme is similar or identical to a transglutaminase from *Streptovercillium mobaraense*. In one embodiment of the cheese replica composition, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents. In one embodiment of the cheese replica composition, protein crosslinks are formed between lysine side chains of respective protein constituents. In some embodiment of the cheese replica composition, formation of protein crosslinks is catalyzed by a lysyl oxidase.

[0022] In one embodiment the cheese replica composition further comprises cheese-making microbes. In one embodiment the cheese replica composition has one or more cheese-making microbes from the group consisting of *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* sps., *Lactobacillus* sps., *Lactococcus*, *Staphylococcus*,



*Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus* sps., or *Propionibacteria*.

[0023] In some embodiments the invention provides for a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human. In some embodiments the invention provides for a cheese replica composition, wherein human subjects cannot distinguish the cheese replica from a dairy cheese. In one embodiment of the cheese replica at least 20% of the protein content comprises a single monomeric or multimeric protein. In one embodiment the cheese replica composition is made without the addition of starches or rennet or any other extrinsic proteases other than those contributed by microbial cultures. In one embodiment the cheese replica composition has a pH less than 5.5 where the acidification was accomplished solely by microbial fermentation. In one embodiment the cheese replica composition has a pH less than 5 where the acidification was accomplished solely by microbial fermentation. In one embodiment the cheese replica composition has a pH less than 6 where the acidification was accomplished solely by microbial fermentation. In one embodiment the cheese replica composition contains no animal products. In one embodiment the cheese replica has less than 5% by mass insoluble carbohydrates. In one embodiment the cheese replica composition contains no rennet, vinegar, or lemon juice.

[0024] In some embodiments the invention provides for a cheese replica composition made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and /or aroma and /or texture of the cheese replica.

[0025] In some embodiments the invention provides for a cheese replica composition made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and /or aroma and/or texture of the cheese replica.

[0026] In some embodiments the invention provides for a soft fresh cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, transglutaminase, water, and salt. In one embodiment the soft fresh cheese replica composition includes the addition of vinegar. In one embodiment the soft fresh cheese replica composition includes the addition of microbial coagulant. In one embodiment the soft fresh cheese replica composition includes the addition of vinegar and microbial coagulant.

[0027] In some embodiments the invention provides for a salted cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, transglutaminase, and water. In one embodiment the salted cheese replica composition includes the addition of vinegar. In one embodiment the salted cheese replica composition includes a microbial coagulant. In one embodiment the salted cheese replica composition includes vinegar and a microbial coagulant.

[0028] In some embodiments the invention provides for a soft ripened cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, flora danica, *Geotrichum candidum*, *Penicillium candidum*, *Debaromyces hansenii*, transglutaminase, water, and salt. In one embodiment the soft ripened cheese replica composition includes vinegar. In one embodiment the soft ripened cheese replica composition includes a microbial coagulant. In one embodiment the soft ripened cheese replica composition includes vinegar and microbial coagulant.

[0029] In some embodiments the invention provides for a goat cheese replica composition composed of pasteurized almond milk, macadamia nut milk, mesophilic starter culture, transglutaminase, water, and salt. In one embodiment the goat cheese replica composition includes vinegar. In one embodiment the goat cheese replica composition includes a microbial coagulant. In one embodiment the goat cheese replica composition includes vinegar and a microbial coagulant.

[0030] In some embodiments the invention provides a method for making a cheese replica comprising removing from a non-dairy milk a proportion of insoluble solids. In some embodiments the invention provides a method for making a cheese replica comprising removing from a plant-based milk a proportion of insoluble solids. In some embodiments the invention provides a method comprising centrifuging a non-dairy milk to sediment a proportion of insoluble solids. In some embodiments about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% of the insoluble solids are removed.

[0031] In some embodiments the isolated or enriched proteins of the methods and compositions of the invention comprise one or more of: ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0032] In some embodiments the invention provides a composition comprising (A) covalently crosslinked plant-derived proteins from a set comprising ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases,

glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin; (B) a crosslinking enzyme, and (C) cheese microbes.

[0033] In some embodiments the invention provides a composition comprising (A) covalently crosslinked plant-derived proteins from a set comprising ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin.

### INCORPORATION BY REFERENCE

[0034] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0036] **Figure 1** shows freshly cut curds prepared by the method used to produce the soft fresh cheese replicas and salted cheese replicas described in the examples.

[0037] **Figure 2** shows drained curds prepared by the method used to produce the soft fresh cheese replicas and salted cheese replicas described in the examples.

[0038] **Figure 3** shows soft fresh cheese replicas made from non-dairy milk.

[0039] **Figure 4** shows Annatto-colored cheese replicas made from non-dairy milk.

[0040] **Figure 5** shows salted cheese replica made from non-dairy milk.

[0041] **Figure 6** shows salted cheese replica with paprika and fennel pollen made from non-dairy milk.

[0042] **Figure 7** shows waxed cheese replica made from non-dairy milk.

[0043] **Figure 8** shows soft ripened cheese replica made from non-dairy milk

[0044] **Figure 9** shows goat cheese replicas made from non-dairy milk (A) with black pepper (B) with chives.

[0045] **Figure 10** shows cheese replica gels made from partially purified plant proteins and vegetable oil, cultured with mesophilic culture MA11 (Danisco): (A) depicts cultured cheese replicas made from 2% Pea albumin fraction, 3% vegetable oil, heated and crosslinked with transglutaminase; (B) depicts cultured cheese replicas made from 2% Moong bean 8S globulin fraction, 3% vegetable oil, crosslinked with transglutaminase, without prior heating; (C) depicts cultured cheese replicas made from 7.5% Pea globulin fraction, 3% vegetable oil, heated and crosslinked with transglutaminase; (D) depicts cultured cheese replicas made from 7.5% Moong bean 8S globulin fraction, 3% vegetable oil, crosslinked with transglutaminase, without prior heating of proteins.

## DETAILED DESCRIPTION OF THE INVENTION

[0046] The present invention discloses methods and compositions based upon the realization that transglutaminase efficiently crosslinks the proteins in many non-dairy milks to produce a soft, moist, elastic gel (a “curdling” of non-dairy milks). This process allows the separation of the crosslinked proteins and associated fats from the “whey.” The crosslinked proteins can hold a fat emulsion, and have the essential physical characteristics needed for pressing, culturing and ripening a cheese replica derived from non-dairy milk. In various embodiments the current invention includes cheese replicas principally, entirely or partially composed of ingredients derived from non-animal sources. In additional embodiments the present invention includes methods for making cheese replicas from non-animal sources. In various embodiments these results are achieved by replicating the curdling process of cheese making in non-dairy milks using enzymes.

[0047] The term a cheese “substitute” or “replica” can be any non-dairy product that serves a role as food or in food that is commonly served by traditional dairy cheese. A cheese “substitute” or “replica” can be a product that shares visual, olfactory, textural or taste characteristics of cheese such that an ordinary human observer of the product is induced to think of traditional dairy cheese.

[0048] A purified protein can be a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 3 or more, or a factor of 5 or more, or a factor of 10 or more relative to the source material from which the specified protein was said to be purified.

[0049] The term “homogeneous” can mean a single protein component comprises more than 90% by mass of the total protein constituents of a preparation.

[0050] The term resemble can mean one composition having characteristics recognizably similar to another composition by an ordinary human observer.

[0051] The term “indistinguishable” can mean that an ordinary human observer would not be able to differentiate two compositions based on one or more characteristics. It is possible that two compositions are indistinguishable based on one characteristic but not based on another, for example two compositions can have indistinguishable taste while having colors that are different.

Indistinguishable can also mean that the product provides an equivalent function as or performs an equivalent role as the product for which it is substituting.

## **I. Non-dairy milks**

[0052] Cheese replicas may be made using non-dairy milk prepared from nuts or plant seeds. For example almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts can provide non-dairy milk that, in various embodiments of the invention, is used to produce cheese replicas. Nuts can include so called “true nuts” as well as a wide variety of dried seeds from plants. Any large, oily kernel found within a shell and used in food may be regarded as a nut. Plant seeds can include a wide variety of embryonic plants enclosed in a seed coat. Plant seeds can include for example legumes, cereals, and gymnosperms. Non-dairy products or compositions include products or compositions where the constituent proteins, fats and/or small molecules can be isolated from or secreted by plants, bacteria, viruses, archaea, fungi, algae, or they can be made synthetically by chemical synthesis or *in vitro*. Non-dairy products are generally not derived from cows, goats, buffalo, sheep, horses, camels, or other mammals. In some embodiments non-dairy products do not contain dairy proteins. In some embodiments non-dairy products do not contain dairy fats. In some embodiments non-dairy products do not contain enzymes derived from an animal.

[0053] The nuts or seeds of the present invention can be raw. In some embodiments the nuts or seeds used to produce non-dairy milk are all raw. Alternatively some or all of the nuts or seeds used in the production of a non-dairy milk can be processed. The processed nuts or seed can be roasted, dry roasted, toasted, or baked.

**[0054]** Milk, or non-dairy milk, can mean an emulsion comprising proteins and fats or a solution or suspension of proteins, sometimes further comprising other solutes that might include carbohydrates, salts and other small molecules that contribute to flavor, texture, emulsion stability, protein solubility or suspension stability, or its ability to support growth of microbial cultures used in making cheese replicas, yogurt replicas, or other replicas of cultured dairy products.

**[0055]** The non-dairy milk can be made by a method comprising preparing the nuts or plant seeds with processing steps such as sterilizing, blanching, shocking, decompounding, centrifugation, or washing.

**[0056]** The non-dairy milks can be produced by decompounding the nuts or plant seeds, for example by grinding or blending or milling the nuts in a solution comprising water. In various embodiments alternative methods for decompounding the nuts or dried seeds include crushing, tumbling, crumbling, atomizing, shaving, pulverizing, grinding, milling, water eroding (for example with a water jet), or finely chopping the nuts or plant seeds. In some embodiments the decompounding step takes place in a blender. In some embodiments the decompounding is in a continuous flow grinder. In some embodiments the decompounding is in a continuous flow mill. The decompounding can be followed by a sorting, filtering, screening, or separation step. In some embodiments the decompounded nuts or seeds can be stored prior to the formation of a non-dairy milk. In some embodiments an aqueous solution is added before, during, or after the decompounding.

**[0057]** The nuts or seeds used in some embodiments of the invention to make non-dairy milks may have contaminants on the surface which would make a non-dairy milk unsafe or unpalatable. Accordingly the nuts or seeds can be washed prior to use. The nuts or seeds can also be sterilized to remove, reduce, or kill any contaminants on the surface of the nuts or seeds. A sterilization step can be an irradiation step, a heat step (e.g. steam sterilization, flaming, or dry heat), or a chemical sterilization (e.g. exposure to ozone). In some embodiments the sterilization step kills more than 95% of microbes on the nuts or seeds. In some embodiments the sterilization step kills more than 99% of microbes on the nuts or seeds. Blanching is a process wherein the food is exposed to hot water (e.g. boiling), removed after a brief timed interval, and finally cooled by exposure to cold water (e.g. iced or cold running water). When nuts, such as almonds or pistachios, are blanched, the skin of the nut (botanically the seed coat surrounding the embryo) softens and can be easily removed later. Accordingly, in some embodiments the invention provides for non-dairy milk compositions with a reduced percentage of components found in the skin of the nut or seed. For example the composition can have 50%, 40%, 30%, 20%, 10% or less than 5% of the seed coat remaining after the preparation process. In some embodiments the invention provides for methods for making a non-dairy milk comprising removing the seed coat.

[0058] In one embodiment the blanching procedure is as follows: Place nuts into 212°F heated water and blanch for 30 seconds. Drain nuts. Immediately move drained nuts into cold water. In some embodiments the temperature of the water is altered by 5%, 10% or 20%. In some embodiments the cold water temperature is about 0°C, 5°C, 10°C, or 20°C. In some embodiments the blanching takes about 10, 20, 30, 40, 50 seconds or about 1, 2, or 5 minutes.

[0059] In some embodiments the nuts or seeds can be hydrated, for example by immersion in water for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. In some embodiments the hydration step lasts multiple days. In some embodiments the water contains other components such as salts or preservatives. In some embodiments the water is maintained at a constant cool temperature. In some embodiments the hydration step can occur prior to the decompounding step.

[0060] In some embodiments the nuts or seeds can be dried, for example by exposure to a low humidity and/or heated environment for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. In some embodiments the drying step lasts multiple days. The drying step can occur prior to the decompounding step or the drying step can occur after a decompounding step.

[0061] In one embodiment the decompounding step is a blending procedure as follows: Place nuts in a clean and sanitized blender. Add clean fresh water to the blender. Turn on the blender and gradually increase speed up to maximum, blending for 5 minutes. Accumulate blended slurry in clean and sanitized ice cold container. Immediately begin rapid cooling of slurry by stirring slurry. Once the slurry has cooled to 50°F, move slurry to refrigerator to continue cooling to below 41°F. Allow slurry to rest at 36°F overnight or up to 12 hours at 36°F. In some embodiments the blending times and speeds are altered by up to 100%. In some embodiments the temperatures are altered up to 20°.

[0062] Insoluble solids may be present in milks prepared by decompounding (e.g. grinding or blending) nuts or seeds. One surprising observation of the present invention is that these insoluble materials can hinder the formation of curds from the non-dairy milks. Insoluble solids can also result in curds or cheese replicas having a texture or mouthfeel perceived as grainy or pasty in comparison to the smoother, creamier texture of dairy cheese. Accordingly provided herein are methods for removing these insoluble materials and compositions of non-dairy milks and cheese replicas with reduced amounts of the insoluble materials. Compositions of the invention can have a mouthfeel or texture indicative of the absence of insoluble solids. For example a cheese replica can have a mouthfeel indicative of the absence of insoluble solids. The insoluble materials can comprise carbohydrates.

**[0063]** In some embodiments the methods for producing cheese replicas includes a step for the removal of solids from non-dairy milks prior to curdling. For example, in some embodiments, the non-dairy milks are centrifuged to remove the insoluble solids. In some embodiments the present invention provides for a non-dairy cheese replica with less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in the non-dairy milk before removal of the insoluble solids. In some embodiments the present invention provides for a non-dairy milk extract with less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in non-dairy milk. In some embodiments the present invention provides for a non-dairy milk with 99%, 95%, 90%, 80%, 70%, 60% or 50% of insoluble solids found in non-dairy milk removed.

**[0064]** In one embodiment the centrifugation procedure is as follows: Pour decomposed nut or seed slurry into container. Using a centrifuge with the JS-5.0 rotor, spin at about 5000 RPM for 30 mins. In some embodiments the spin is at about 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10,000 RPM for about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 minutes. In some embodiments the centrifugation speed or time is altered, for example by 20%.

**[0065]** In one embodiment the centrifugation procedure is as follows: Pour decomposed nut or seed slurry at a flow rate of 4-20 gallons per minute, for example, at a flow rate of 8 gallons per minute, into decanter centrifuge (such as the Flottweg S4 sedicanter), spinning at a bowl speed of between 3000 and 5000 RPM. Collect the liquid outflow for further use as a non-dairy milk, and set aside the ejected solids.

**[0066]** Other methods for removal of solids from non-dairy milks include, but are not limited to, straining, filtering, allowing to settle, skimming, or using coagulants and flocculants (including cations, polymer flocculants, or polyelectrolytes such as pectin, carrageenan, alginates, or carboxymethyl cellulose) to agglomerate particles for removal.

**[0067]** Centrifugation step can result in a "cream layer" and a "skim layer". The cream layer is an emulsion comprising fats, proteins and water. The skim layer is a solution comprising proteins in water,. In some embodiments, the cream layer and skim layer are separated by centrifugal separation. In some embodiments, the cream layer and skim layer are separated by centrifugal separation in a Flotwegg ac1500 or GEA ME55. In some embodiments the cream layer and the skim layer are incompletely separated. The skim layer and the cream layer can be separated from the insoluble solids in a separation process. In some embodiments the skim layer and the cream layer are stored separately. The non-dairy milk can comprise the skim layer. The non-dairy milk can comprise the cream layer. Typically the skim layer and the cream layer are combined to form the non-dairy milk. The non-dairy milk can be up to 100% cream layer. In some embodiments the ratio



of cream layer to skim layer in the non-dairy milk is about 100:1, 90:1, 80:1, 70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, or 1:60. In some embodiments the methods described herein comprise measuring the amount of skim layer and cream layer being added to a non-dairy milk.

**[0068]** In one embodiment the separation procedure is as follows: Remove cream layer from centrifuged bucket and place in new container that is buried in an ice bath. Move the liquid layer (skim layer) from the bucket into a container that is ice cold. Keep skim and cream ice cold at all times.

**[0069]** In another embodiment the separation procedure is as follows: Flow the non-dairy milk, from which insoluble solids have been substantially removed by passage through a decanter centrifuge, into a separator centrifuge, for example, a Flotwegg ac1500 or GEA ME55. In some embodiments, the cream layer and skim layer are separated by centrifugal separation in the separator centrifuge. In some embodiments the separated skim and cream layers are kept refrigerated.

**[0070]** In some embodiments the non-dairy milk is pasteurized or sterilized. The pasteurization can be high-temperature, short-time (HTST), “extended shelf life” (ESL) treatment, or ultra-high temperature (UHT or ultra-heat-treated). In some embodiments the pasteurization procedure is as follows: Pasteurize blended non-dairy milk at 164°F -167°F for 16 seconds. Using controlled chilling system, bring non-dairy milk temperature down rapidly and store in a refrigerator at 36°F.

**[0071]** Cheese replicas may also be made using non-dairy milk prepared using proteins isolated, enriched, or purified from a plant, or microbial source or synthesized *in vitro*. For example ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) can isolated from a plant source and included in a non-dairy milk for the production of a cheese replica. For example 8S globulins can isolated from a Moong beans and included in a non-dairy milk for the production of a cheese replica. For example Pea globulins can isolated from Pea seeds and included in a non-dairy milk for the production of a cheese replica. For example Pea albumins can isolated from peas and included in a non-dairy milk for the production of a cheese replica. Isolated, enriched, or purified plant proteins can be combined with one or more oils or fats also isolated from plant sources, in a colloidal suspension, solution or emulsion to form the non-dairy milk for making a cheese replica. In some embodiments the isolated non-dairy proteins are combined with non-non-dairy oils or fats to form a non-dairy milk. In some embodiments multiple isolated, enriched or purified plant proteins are used to make a non-dairy milk. Without being bound by theory the non-dairy milks derived from isolated, enriched, or purified proteins may reduce the problems which can be caused by insoluble solids obtained in a slurry derived from a nut or seed. Without being bound by theory the non-dairy milks derived from isolated, enriched, or purified proteins may reduce the problems which can be caused by proteins

with undesirable properties, such as lipoxygenases or proteases. Additional non-limiting examples of proteins that may be isolated, enriched, or purified from a plant source for the production of a non-dairy milk for cheese replica production include: seed-storage proteins from any seed, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin. Non-limiting examples of oils that may be incorporated in a non-dairy milk include: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

**[0072]** In some instances the proteins used to make the non-dairy milk may be purified proteins. Accordingly, in some embodiments the total protein content of the cheese replica can, in some

embodiments, consist of greater than 25%, 50%, 75%, or 90%, of a purified protein, for example any single monomeric or multimeric protein.

[0073] Non-dairy milk compositions can also contain sugars or other fermentable carbon sources and other nutrients. Without being bound by theory these sugars or nutrients can favor growth of the cheese culture microbes or serve as substrates for production of lactic acid or other organic acids by the cheese culture microbes. For example, in some embodiments, the non-dairy milk comprises glucose. In other embodiments, the non-dairy milk comprises fructose. In other embodiments, the non-dairy milk comprises sucrose. In other embodiments, the non-dairy milk comprises high-fructose corn syrup. In other embodiments, the non-dairy milk comprises sugar-cane extract. In other embodiments, the non-dairy milk comprises a fruit juice. In other embodiments, the non-dairy milk comprises sugar-cane extract. In other embodiments, the non-dairy milk comprises agave syrup. In other embodiments, the non-dairy milk comprises molasses. Molasses can be cane molasses or beet molasses or made from other non-dairy sources. In other embodiments the non-dairy milk can comprises treacle, honey, refined sugars, or syrup (e.g high fructose corn syrup). In some embodiments fuel for glycolysis, or a component (or intermediate) of the glycolysis pathway, can be included in the compositions. Oligosaccharides can be a part of the non-dairy milk. Disaccharides (eg., maltose, sucrose) can be part of a non-dairy milk. Monosaccharides (e.g. fructose, glucose, or galactose) can be a part of the non-dairy milk. In some embodiments the sugars are added as an addition step. In some embodiments the sugars are not derived from the same organism as the proteins and fats in the composition.

[0074] In some embodiments a sugar in the composition of the invention is not treacle, honey, refined sugars, or syrup (e.g high fructose corn syrup). In some embodiments a sugar in the composition of the invention is not a monosaccharide (e.g. fructose, glucose, or galactose). In some embodiments a sugar in the composition of the invention is not a disaccharide. In some embodiments a sugar in the composition of the invention is not an oligosaccharide.

[0075] Non-dairy milk can, in some embodiments, contain one or more organic acids, such as lactic acid, or acetic acid, for example to adjust the pH and/or produce the characteristic sour taste of cheese. These organic acids may be used in addition to or as an alternative to microbial cultures. Accordingly, in some embodiments the non-dairy milk comprises an organic acid. The organic acid can be one or more of: lactic acid, acetic acid, citric acid, malonic acid, malic acid, propionic acid.

[0076] In some instances the compositions of the invention do not contain any animal products. In some embodiments the compositions of the invention use no components that are derived from animals. In some embodiments the compositions of the invention contain no fats from animals. In some embodiments the compositions of the invention contain no proteins from animals. In some

embodiments the compositions of the invention contain no enzymes from animals. In some embodiments the compositions of the invention contain no dairy products.

[0077] In one embodiment the invention provides a composition free of or substantially free of one or more of the following: dairy products, animal products, agar, carrageenan, or tofu.

[0078] In some embodiments the compositions have less than 20%, less than 15%, less than 10%, less than 5%, less than 1% or less than 0.5% starch content. In some embodiments no refined starch (e.g. cornstarch, tapioca, wheat, or potato starch) is added to the compositions of the invention.

[0079] In some embodiments the non-dairy milk contains no artificial flavoring or coloring.

[0080] In some instances the non-dairy milk does contain some animal products. For example, in some embodiments, non-dairy milk comprises enzymes obtained from animals. In some embodiments, the non-dairy milk contains dairy products. In some embodiments animal fats are included in the non-dairy milk. In some embodiments the non-dairy milk can comprise enzymes (e.g. proteases and lipases), and/or microbes (e.g. lactic bacteria, yeast and mold). Without being bound by theory these enzymes and or microbes can be added to non-dairy milk formulations to produce desirable flavor and aroma compounds.

[0081] Enzymes, including proteases and lipases, and microbes, including lactic bacteria, yeast and mold, can be added to non-dairy milk formulations to produce desirable flavor and aroma compounds.

[0082] In some embodiments the invention provides a method comprising isolating or purifying a single non-dairy protein, mixing the isolated non-dairy protein with a non-dairy fat source, and adding a microorganism selected from the group consisting of *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* sps., *Lactobacillus* sps., *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*, *Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus* sps., or *Propionibacteria*. In some embodiments at least 1 kg of the plant protein is isolated or purified from a single non-dairy source. In some embodiments the fat is isolated from another non-dairy source.

[0083] In some embodiments the invention provides for a composition comprising an isolated or purified non-dairy protein and a polynucleotide identifiable as derived from one or more of *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* sps., *Lactobacillus* sps., *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*,

*Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus* spp., or *Propionibacteria*. In some embodiments the cheese replicas comprise *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* spp., *Lactobacillus* spp., *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*, *Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus* spp., or *Propionibacteria*. In some embodiments compositions of the invention comprise *Penicillium*, *Geotrichum*, *Saccharomyces*, *Kluyveromyces*, or *Debaryomyces*.

## II. “Curdling” the non-dairy milk

[0084] Generally the cheese-replicas are made by causing cross linking or denaturation of proteins in non-dairy milks. These processes replicate the curdling process of traditional dairy cheese making.

[0085] Crosslinking is typically induced using an enzyme that creates covalent crosslinks between polypeptide chains. In some embodiments cross-linking enzymes are used in a cross-linking step to curdle the non-dairy milk. In various embodiments, transglutaminase is the cross-linking enzyme used to induce the curdling of non-dairy milk replicas. In some embodiments the crosslinking enzyme used to induce curdling in non-dairy milk replicas is a lysyl oxidase. In one embodiment, the invention provides for a method of inducing the curdling of a non-dairy milk comprising obtaining a non-dairy milk and adding a cross-linking enzyme to the non-dairy milk. In some embodiments the cross-linking enzyme is transglutaminase. In some embodiments the cross-linking enzyme is not derived from an animal source. In some embodiments the curdling process does not use rennet.

[0086] In some embodiments between 0.1 and 20 units (U) of transglutaminase is added per 1 mL of non-dairy milk. In some embodiments about 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 10, 15, or 20 U transglutaminase is added per 1 mL of non-dairy milk. In some embodiments the transglutaminase is followed by heated incubation, for example in a 100°F water bath. The heated incubation can be at a temperature optimized for the enzyme function. In some embodiments the temperature is about 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120 or 125 °F.

[0087] Transglutaminases are a family of enzymes that catalyze the formation of a covalent bond between a free amine and the gamma-carboxyl group of glutamine thereby linking proteins together. For example, transglutaminases catalyze crosslinking of e.g., lysine in a protein or peptide and the gamma-carboxamide group of a protein- or peptide- glutamine residue. The covalent bonds formed by transglutaminase exhibit high resistance to proteolytic degradation.

[0088] Many types of transglutaminase can be used in various embodiments of the invention. Acceptable transglutaminases include, but are not limited to, *Streptovorticillium mobaraense* transglutaminase, an enzyme is similar or identical to a transglutaminase from *Streptovorticillium mobaraense*, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase. Accordingly, in some embodiments an enzyme selected from the group consisting of a transglutaminase is used to crosslink a non-dairy milk protein. In some embodiments the transglutaminase is *Streptovorticillium mobaraense* transglutaminase, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase. In some embodiments the present invention provides for a composition comprising a non-dairy milk and *Streptovorticillium mobaraense* transglutaminase, an enzyme is similar or identical to a transglutaminase from *Streptovorticillium mobaraense*, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) and/or TGM Z (TGM7). In some embodiments the enzyme used for cross-linking is not Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase.

[0089] In some embodiments the invention provides for a composition comprising a non-dairy milk, wherein the composition is free of Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) and/or TGM Z (TGM7).

[0090] Transglutaminases can be produced by *Streptovorticillium mobaraense* fermentation in commercial quantities or extracted from animal tissues. Additionally, the transglutaminase (TGM) of the present invention may be isolated from bacteria or fungi, expressed in bacteria or fungi from a synthetic or cloned gene, Factor XIII, keratinocyte transglutaminase, tissue transglutaminase, epidermal transglutaminase, prostate transglutaminase, TGM X, TGM Y, TGM Z, or another member of the transglutaminase family. In some particular embodiments transglutaminase is

obtained from commercial sources, for example in the form of Activa™ from Ajinomoto Food Ingredients LLC.

[0091] In some embodiments the compositions of the invention have a detectable amount of a nucleic acid from the genome of a cell that encoded and produced an enzyme, for example a transglutaminase. For example a cheese replica can have a detectable amount of *Streptovorticillium mobaraense* DNA. This detectable amount can, for example, be a small amount of DNA that has carried over to the cheese replica from the production of transglutaminase by *Streptovorticillium mobaraense*.

[0092] In some embodiments the cross-linking of proteins in non-dairy milk is induced by transglutaminase. In some embodiments the cross-linking of proteins in non-dairy milk is induced by a lysyl oxidase. Various non-dairy milks have been crosslinked with transglutaminase. In some embodiments the invention provides a composition comprising a non-dairy milk and transglutaminase. In some embodiments the invention provides a composition comprising a curdled non-dairy milk and transglutaminase. In some embodiments the invention provides a cheese replica containing transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of degraded transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of improperly folded transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of transglutaminase by-product. In some embodiment the invention provides a cheese replica with a detectable peptide which is identifiable as portion of a transglutaminase used in the manufacturing process for making the cheese replica. In some embodiment the invention provides a cheese replica with one or more detectable peptides containing a crosslink between a lysine and glutamine residue. For example the crosslinked glutamine and lysine residues may be detectable by mass spectroscopy. In some embodiment the invention provides a cheese replica with one or more detectable peptides containing a crosslink between two lysine residues. For example the crosslinked lysine residues may be detectable by mass spectroscopy.

[0093] Denaturation can be used instead of, or in addition to, the cross-linking enzyme for curdling the non-dairy milk. For example, heat denaturation of a non-dairy milk followed by cooling of the mixture can result in a curd-like gel as, for example, described in example 7. Accordingly, the present invention provides for non-dairy milk curds comprising denatured non-dairy proteins. The present invention also provides for methods for making a non-dairy milk curd by heating a non-dairy milk to a temperature of about between 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 90-95, 95-100 °C for about 10, 20, 30, 40, 50, 60 seconds or about 1, 2, 3, 4, 5, 6,

7, 8, 9, or 10 minutes followed by cooling. In some embodiments non-dairy milks are curdled using both enzymatic cross-linking with transglutaminase and heat denaturation.

[0094] Additional denaturization procedures are possible in additional embodiments of the invention. Acids, solvents, chaotropic agents, or disulfide bond reducers can be used to denature the proteins in the non-dairy milk. In one embodiment urea is added to the non-dairy milk to form curds.

### **III. Cheese replica production from the curdled non-dairy milk**

[0095] Cheese replicas can be produced from the curdled non-dairy milk. Accordingly, the invention provides methods for producing cheese replicas from curdled non-dairy milk, as well as cheese replica compositions.

[0096] To create the cheese replicas one might use elements of the traditional cheese making process. Cheese replicas can be produced using one or more steps including cutting, draining, forming, pressing, waxing, aging, scalding, smoking, salting or ripening.

[0097] The curd and whey may be separated in a traditional way. For example, the cheese may be scalded to create a replica of traditional cheddar or other hard cheese, or, the whey and curd might be separated through a cloth drain.

[0098] The cheese replicas may be ripened in a way similar to traditional cheese. For example, surface mould may be allowed to grow to create a rind. In order to create a rind or color, the process may introduce certain bacteria to the cheese replicas in the ripening process. By way of example only, *Brevibacterium linens* can be introduced to produce an orange color and pungent aroma to the cheese replica.

[0099] In some embodiments, the cheese replica can be a salted or salt-washed cheese replica. The salting process can preserve the cheese replica and/or add flavor. In some embodiments the salt is chosen based on the geographic location of where the salt is originally obtained. In some embodiments salt is added to the cut curds. In some embodiments salt is contacted with the outside surface of the cheese replica, for example by rubbing. In some embodiments the cheese replica is contacted with or soaked in brine.

[00100] In some embodiments, the cheese replica can be a soft fresh cheese produced by procedures exemplified by example 3.

[00101] In some embodiments, the cheese replica can be a salted or salt-washed cheese produced by procedures exemplified by example 4.

[00102] In some embodiments, the cheese replica can be a soft ripened or molded-rind cheese produced by procedures exemplified by example 5.



[00103] In some embodiments, the cheese replica can be a goat cheese replica produced by procedures exemplified by example 6.

[00104] In some embodiments, the cheese replica can have edible materials added (e.g. herbs, pepper, spices) on its surface to enhance flavor or add to the visual appeal of the product, as exemplified by examples 4 and 6. In some embodiments the edible materials are embedded in the cheese replica.

[00105] In some embodiment the cheese replica can be shaped. For example the cheese replica can be shaped in a basket or a mold. In some embodiments the cheese replica is pressed, for example with a weight. The pressing can help expel any additional liquid from the cheese replica.

[00106] In some embodiments the production of the cheese replica includes a waxing step. In one embodiment the waxing procedure is as follows: Cut food-grade paraffin wax into ½-inch pieces. Place in double boiler and heat wax to 210°F. Place cheese replicas in standard freezer for fifteen minutes to reduce temperature of cheese replicas to 33°F. Using 3-grams of melted wax per piece, brush wax onto cheese replicas one side at a time. Placed waxed cheese replicas onto clean waxed paper on aging racks. Age waxed cheese replicas in aging room at 36°F with 75% humidity, for example for six months. In some embodiments the aging room is between 33-70°F. In some embodiments the humidity of the aging room is altered to aid in rind formation. In some embodiments the waxed cheese is stored for years, for example for 2 years or more.

[00107] In some embodiments the production of the cheese replica includes a smoking step. In some embodiments the cheese replica is cold smoked. In some embodiments the cheese replica is smoked at the curd stage or prior to the curd stage. In some embodiments the cheese replica is smoked after the cheese replica is formed. In some embodiment the smoking procedure is as follows: Soak wood chips for six hours. Drain chips of all water and place in smoking unit. Ignite smoker and as soon as chips have fully ignited, snuff out flames to create smoke-filled unit. Place cheese replicas on racks in smoker for five minutes per side. Remove from smoker and place on cooling racks. Place cheese replicas in cooling room for 24 hours, at 36°F. In various embodiments smoking times and cooling times and temperatures will be adjusted according to the particular cheese replica and particular desired taste profile.

[00108] In some instances the cheese replica is exposed to or injected with mold or yeast. In some embodiments the cheese replica is exposed to a particular bacterial strain or strains.

[00109] When the cheese replica is made without any dairy ingredients, the production process requires no dairy farming. Accordingly, the process for making cheese replicates is suitable for locations where keeping animals or storing milk is difficult or costly. In some embodiments the cheese replicas are made in non-dairy producing regions. This will reduce the need for shipping the

products. So in some embodiments the cheese replica is a product that is made in the same locality as its eventual consumption. For example the cheese replica is made within 10, 20, 50, or 100 miles of its point of sale. The cheese replica production process may also be useful in remote areas where dairy farming is difficult or impossible. For example the cheese replica production process could occur on an island, aboard an oil platform, or aboard a space station.

[00110] The cheese replicas may further contain microbes useful for cheese making. So in some embodiments the cheese replicas may be made by a process involving culturing with any microbes used for cheese making. The cheese replicates may be made by a process involving fermentation. In any process, the proteins may be crosslinked by treatment with an enzyme, which may be from the family of transglutaminase. The cheese replicas may be created from curds formed by a treatment of a protein-containing liquid with a transglutaminase, or other enzymes that promote gelling, or they may be prepared by a process involving heat denaturation of one or more of the protein components in the solution, suspension or emulsion to induce formation of a gel.

[00111] In some embodiments the cheese replicas comprise *Penicillium*, *Geotrichum*, *Saccharomyces*, *Kluyveromyces*, or *Debaryomyces*.

#### IV. Non-dairy cheese replicas

[00112] The cheese replicas can be made for consumption by either humans or other animals or both. In some instances the cheese replicas are used to feed domesticated animals. For example, a cheese replica of the present invention can be fed to a dog. In one particular embodiment, the cheese replica contains a veterinary medication. In another embodiment the cheese replica comprises a treat for a domestic animal. In some embodiments the domestic animal treat is shaped like traditional a wedge of cheese. In some embodiments the animal treat is artificially colored to resemble dairy cheese, e.g. cheddar cheese.

[00113] The cheese replicas include products similar to conventional cheese, yogurt and other dairy products. Cheese replica can be made from a liquid extract of nuts or other fruits or seeds. The current invention also includes cheese replicas made from proteins and fats isolated and enriched or purified from one or more plant. The current invention also includes cheese replicas made from proteins and fats isolated and enriched or purified from one or more non-dairy source.

[00114] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides.

[00115] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides because of centrifugal separation.

[00116] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides because of the use of purified proteins.

[00117] The cheese replicas may be suitable for consumption by humans or animals who are unable to eat certain animal products, such as a person who is lactose-intolerant or allergic to dairy. Accordingly, in some embodiments the cheese-replica does not contain lactose. The cheese replicas might contain enough protein or other nutrients to be nutritionally equivalent to traditional cheese or other animal products.

[00118] The cheese replicas may contain less fat, less saturated fat or less cholesterol than traditional cheese or animal products, and may be suitable for a healthier diet. So in some embodiments provided herein is a method for distributing cheese replicas comprising the steps of informing a lactose intolerant person of the cheese replica, receiving an order for a cheese replica, and making the cheese replica available to the lactose intolerant person.

[00119] In some embodiments the lactose intolerant person is informed through advertising. In some embodiments the lactose intolerant person is informed by their personal physician. In some embodiments the order for the cheese replica is sent by the lactose intolerant person. In some embodiments the lactose intolerant person receives the cheese replica via a distributor that places the order. In some embodiments the informing and receiving steps take place on a network, for instance a computer network and are enacted with specialized computer software stored on a computer readable media. In some embodiments the method further comprises producing a cheese replica of the present invention and supplying it to lactose intolerant person.

[00120] The cheese replicas are, in some embodiments, designed to replicate the experience of eating cheese. The look, texture, and taste of the cheese replicas can be such that it is similar or indistinguishable from cheese. The invention provides for methods for determining whether a animal or human can distinguish the cheese replicas from cheese. In some embodiments a property of the compositions of the invention is that an animal, for example a human, will identify the composition as cheese. In some embodiments the human identifies the composition of the invention as equivalent to cheese. In some embodiments one or more properties of cheese are equivalent according to an ordinary human's perception. Such properties include the properties that can be tested listed below. In some embodiments an ordinary human identifies a cheese replica of the present invention as more cheese like than cheese substitutes found in the art.

[00121] One method to determine whether the cheese replica is comparable to cheese is to a) define the properties of cheese and b) determine whether the cheese replicas has similar properties. Properties of cheese that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of cheese or a

cheese replica that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. The properties of the cheese replica can be described using terms such as “soft,” “firm” or “hard” describe hardness; “crumbly,” “crunchy,” “brittle,” “chewy,” “tender,” “tough,” “short,” “mealy,” “pasty,” or “gummy,” to describe cohesiveness; “thin” or “viscous” or “spreadable” to describe viscosity; “plastic” or “elastic” to describe elasticity; “sticky,” “tacky” or “gooey” to describe adhesiveness; “gritty,” “grainy” or “coarse” or “heterogeneous” to describe particle shape and size; “fibrous,” “cellular” or “crystalline” to describe particle shape and orientation, “dry,” “moist,” “wet,” or “watery” to describe moisture content; or “oily” or “greasy” to describe fat content. In one embodiment a group of people can be asked to rate a certain cheese, for instance cheddar, according to properties which describe the cheese. These ratings can be used as an indication of the properties of the cheese. The cheese replicas of the present invention can then be compared to the properties of traditional dairy cheese to determine how similar the cheese replica is to the dairy cheese. In some instances the properties of the cheese replicas are then altered to make the cheese replica more similar to the cheese. In some embodiments, the cheese replica has a property or properties that are rated similar to cheese according to human evaluation. In some embodiments the cheese replica is indistinguishable from real cheese to a human. In some embodiments the cheese replica is distinguishable by some properties from real cheese to a human.

**[00122]** In some embodiments the cheese replica is compared to real cheese based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. In some embodiments the cheese replica is a product that causes similar olfactometer readings to those of a particular target cheese. In some embodiments the cheese replica is a product that causes nearly similar, but slightly different olfactometer readings to those of a particular target cheese.

**[00123]** Gas chromatography–mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a cheese replica. For example volatile chemicals can be isolated from the head space around cheese. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around cheese is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the

properties of the cheese replicas. The GCMS could be used to refine the cheese replica. In some embodiments the cheese replica has a GCMS profile similar to that of cheese. In some embodiments the cheese replica has a GCMS profile identical to that of cheese.

[00124] The cheese replicas may be modified to fit into traditional cheese-types, including: fresh, bloomy, semi-soft, washed, firm, hard and blue. There may be additional processes to modify the cheese replicas and fit them into any cheese-type category. These categories also include different presentations of the cheese replicas, for example: sliced, shredded, block, with rind, without rind, wet or dry. The cheese replicas may be aged, or a method may be created to mimic the taste of traditionally-aged dairy cheese. The cheese replicas may be classified in taste the same way traditional cheese is. For example, they may be tart, tangy, smooth, creamy, buttery, fluffy, rich, earthy, pungent, aromatic, eggy, fruited, sharp, dry, caramelly, grainy, punchy or complex, among other descriptions. The invention includes methods to modify the cheese replicas to fit into any category or taste description.

[00125] The cheese replicas may be modified to have or not have a rind, may be coated in wax, and may have craters or veins typical to blue cheese. The cheese replicas may be spreadable, such as cream cheese. The cheese replicas can contain flavor additives, for example: truffle, mushrooms, nuts, herbs, chives, and other flavors.

[00126] The cheese replicas may be meltable, and have other properties of conventional cheese.

[00127] The cheese replicas may also be similar in consistency and taste to traditional yogurt or cottage cheese. The cheese replicas might be flavored with fruits, sweeteners, or other flavor additives. The cheese replicas might contain bacteria to aid with digestion or other health additives. The cheese replicas may be modified to have an appropriate or appealing consistency.

[00128] In various embodiments the cheese replicas may be used in all cooking and recipes as a substitute for traditional cheese. In some embodiments the cheese replicas may be used in cooking and recipes as a flavor substitute for traditional cheese. In some embodiments the cheese replicas may be used in cooking and recipes as a functional substitute for traditional cheese.

## EXAMPLES

[00129] **EXAMPLE 1**

[00130] THE EFFECTIVENESS OF TRANSGLUTAMINASE IN CURDLING VARIOUS NUT MILKS WAS COMPARED.

[00131] Cashews, almonds, macadamia nuts, brazil nuts were used to produce a slurry, by adding fresh water in a 3:1 weight ratio to the nuts, then blending in a Vitamix 4500 blender at high

speed for 2 minutes. Because we found in preliminary experiments that the insoluble solids in the milks at this stage (slurry) inhibited formation of optimal creamy curds and produced a cheese replica with grainy mouth feel the slurry was subjected to centrifugation at 10,000 G for 15 minutes at 4°C to remove insoluble solids. The liquid supernatant, consisted of two layers, a lighter, creamy, opaque layer and a slightly denser clear to translucent aqueous layer. Liquid supernatant layers were recovered and blended together and the pelleted solids were discarded. The blended supernatants are referred to as “nut milks” (a non-dairy milk). Each of the 4 resulting nut milks and each pair-wise combination at a 1:1 blend, were evaluated for curdling in the presence of transglutaminase (TG): 30 U TG was added to 15 mls of each nut milk or blend in a 20 ml glass vial, followed by incubation in a 100°F water bath. After 5 hours all but the pure cashew nut milk had formed sturdy curds, sufficient to retain their form when the glass vial was inverted. The cashew milk and cashew brazil nut milks formed soft curds. The macadamia and almond milks formed the most solid curds. Control samples without added transglutaminase remained liquid under these conditions.

**[00132]           EXAMPLE 2**

**[00133]           PREPARATION OF ALMOND AND MACADAMIA NUT MILKS**

**[00134]           Blanching (surface sterilization of the nuts):**

**[00135]           Note:** Almonds and macadamia nuts were blanched separately.

**[00136]           A 30 gallon tilt skillet filled to ½ -capacity with fresh water was heated to 212°F. 25 pounds of either almonds or macadamia nuts were dumped into the boiling water bath and left for 30 seconds. The nuts were recovered by draining into a colander then immediately immersed in fresh ice-cold water in a second tilt skillet to rapidly cool them. After rapid cooling the nuts were recovered by draining in a colander and spread on sheet trays to dry.**

**[00137]           Macadamia nuts proceeded directly to the blending process. Almonds were first hydrated as described below before blending.**

**[00138]           Hydration of Almonds:**

**[00139]           The blanched almonds were transferred to Cambro buckets and fresh water was added to a level two inches above the level of the nuts. The buckets were then placed at 36°F for up to 16 hours. After hydration, the nuts were recovered by draining into a colander, rinsed with fresh water, then proceeded to the blending step.**

**[00140]           Blending of nuts:**

**[00141]           Note:** Almonds and macadamia nuts were blended separately.

[00142] One pound batches of nuts were placed in a Vitamix blender (model Vitaprep 3), with one liter of fresh water and blended for 5 minutes, gradually increasing the blender speed to high. The blended slurries from each batch were collected in a stainless steel bain-marie sitting in a large container of ice and stirred with a frozen cooling stick to cool. Once the accumulated slurry cooled to 50°F, the bain-marie was placed in a refrigerator at 36°F for up to 12 hours.

[00143] Separation of skim and cream:

[00144] Sets of four 1800 ml aliquots of the blended slurry were centrifuged at 5000 RPM / 7480 G at 4°C for 30 minutes in a Beckman-Coulter Avanti J-HC Centrifuge with a JS-5.0 rotor, resulting in separation of the blend into 3 layers, a dense pellet of insoluble solids, a clear to translucent aqueous layer (which we refer to as the “skim”), and a lighter, creamy, opaque layer (which we refer to as the “cream”). The cream layer was carefully collected from each bucket using a spoon, and placed in a pan sitting in a container of ice. The viscous aqueous layer (skim) from each bucket was carefully poured into a ½-Cambro sitting in a container of ice. The accumulated cream and skim layers were kept at 36°F until the pasteurization step. Typical yields were about 0.77 lbs of cream and 0.95 lbs of skim per lb of macadamia nuts and about 0.29 lbs of cream and 1.62 lbs of skim per lb of almonds.

[00145] Blending

[00146] Almond skim and cream were combined in a ratio specified by the recipe for the specific cheese replica being produced (Table 1), and blended with an immersion blender. Macadamia skim and cream were combined in a ratio specified by the recipe for the specific cheese replica being produced (Table 1), and blended with an immersion blender. We hereafter refer to each blended mixture of skim and cream as “non-dairy milk.” The two non-dairy milks were then blended in a ratio specified by the recipe for the specific cheese replica being produced (Table 1).

[00147] Pasteurization:

[00148] The non-dairy milk blend was pasteurized at 167°F for 16 seconds after which the non-dairy milk temperature was rapidly reduced to 50°C in 12 seconds by a controlled chilling system. The non-dairy milk temperature was then further reduced by placing containers in an ice bath at 36°F. Pasteurized non-dairy milks were stored at 36°F. Non-dairy milks were discarded if the pH of the non-dairy milk dropped below pH 6.0 during storage, indicating spoilage.

**[00149]      EXAMPLE 3****[00150]      PRODUCTION OF A SOFT FRESH CHEESE REPLICA.**

**[00151]**      The ingredients and amounts needed for one batch (about one and a half pieces) of soft fresh cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

**[00152]**      Soft fresh cheese replica was prepared by the following procedure:  
non-dairy

**[00153]**      Bring pasteurized non-dairy milk formula (see Table 2) to 80°F in a water bath.

**[00154]**      Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in the starter culture with a spatula for two minutes. Hold at 80°F for one hour.

**[00155]**      Increase the water bath temperature to begin to bring the non-dairy milk temperature to 100°F.

**[00156]**      Optional step: Add microbial “rennet” and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

**[00157]**      Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

**[00158]**      Remove the container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

**[00159]**      Cut curd into ½ inch dice pieces. (**Figure 1**). Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.



[00160] Place curd in a mixing bowl. Add cheese salt (see Table 2). Gently whisk the curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

[00161] Place 13.75 ounces of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (**Figure 2**). Drain at room temperature for one hour without the follower in place. Then place the follower in the mold and add 600 grams of weight to the follower. Refrigerate at 36°F for 24 hours.

[00162] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for 1/2 hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00163] Remove cheese replica from the mold. Place on draining mat and return it to 36°F for 24 hours.

[00164] The soft fresh cheese replica (**Figure 3**) is now ready for packaging and shipment. Packaging process includes nitrogen flush and airtight heat-sealing.

[00165] **EXAMPLE 4**

[00166] **PRODUCTION OF A SALTED CHEESE REPLICA.**

[00167] The ingredients and amounts needed for one batch (about one and a half pieces) of salted cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00168] Bring pasteurized non-dairy milk formula (see Table 2) to 80°F in a water bath.

[00169] Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in starter culture with a spatula for full two minutes. Hold at 80°F for one hour.

[00170] Increase water bath temperature to begin to bring non-dairy milk temperature to 100°F.

[00171] Optional: Add microbial “rennet” and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

[00172] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00173] Remove container of non-dairy milk formula from the water bath and cover. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00174] Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.

[00175] Place curd in mixing bowl. Optional: For the classic presentation of a cheddar cheese replica (**Figure 3**), annatto— a natural vegetable dye, is added to create the yellow cheddar color. The percentage of annatto evenly distributed throughout the curd is 0.08%. Gently whisk curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

[00176] Place 16.3 ounces of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (see **Figure 1**). Drain at room temperature for one hour without the follower in place. Then place the follower in the mold and add 600 grams of weight to the follower. Refrigerate at 36°F for 24 hours.

[00177] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for ½ hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00178] Remove the cheese replica from the mold. Place the cheese replica on a draining mat and return it to 36°F for 24 hours.

[00179] Move the cheese replica into a drying room for seven days at 55°F, with 55% humidity, and minimal airflow. Turn the cheese replica and place on a fresh draining mat daily.

[00180] After the seven-day drying period, remove the draining mats and place the salted cheese replica (**Figures 4 and 5**) directly on an aging rack.

[00181] If the cheese replica is to be waxed or smoked, return the cheese replica to the drying room for two additional weeks at 55°F, with 55% humidity and minimal airflow. Then follow the procedure for waxing or smoking.

[00182] If the cheese replica is to be rolled in paprika and fennel pollen mixture, follow the steps for the paprika & fennel pollen process.

[00183] Paprika and fennel pollen process:

[00184] Brush the salted cheese replica with cold water using a pastry brush. Roll the cheese replica in 1.5 grams of a mixture of 10-parts organic paprika and 1-part fennel pollen. Place the coated cheese replica on an aging rack and return it to the drying room for two weeks at 55°F, with 55% humidity and minimal airflow, turning daily.

[00185] Following the drying period, move the cheese replica to 36°F for 24-hours. The paprika-fennel pollen cheese replica (**Figure 6**) is now ready for packaging and shipment.

[00186] Waxing Process:

[00187] Cut food-grade paraffin wax into ½-inch pieces and heat to 210°F in a double boiler.

[00188] Place the cheese replica in a freezer to reduce its temperature to 33°F. Using three grams of melted wax per piece of cheese replica, brush wax onto cheese replica one side at a time. Place waxed-side down onto waxed paper and then continue brushing remainder of the cheese replica including sides and top. Placed waxed cheese replica (**Figure 7**) onto clean waxed paper on an aging rack.

[00189] Age the waxed cheese replica in aging room at 36°F with 75% humidity for up to six months. The waxed cheese replica is now ready for packaging and shipment.

[00190] Smoking Process:

[00191] Soak equal parts apple and cherry wood chips for six hours. Drain chips of all water and place in a smoking unit. Ignite smoker and as soon as chips have fully ignited, snuff out flames to create a smoke-filled unit. Place salted cheese on rack into smoker for five minutes per side. Remove cheese from smoker and place on cooling rack.

[00192] Place the cheese at 36°F for 24 hours. Smoked cheese is now ready for vacuum aging or packaging and shipment.

**[00193]      EXAMPLE 5**

**[00194]      PRODUCTION OF A SOFT RIPENED CHEESE REPLICA.**

**[00195]**      The ingredients and amounts needed for one batch (about one and a half pieces) of soft ripened cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

**[00196]**      Bring pasteurized non-dairy milk formula (see Table 2) to 90°F in a water bath.

**[00197]**      Sprinkle lactose-free preparations of Florica Danica, Mesophilic Starter, Geotrichum Candidum, Penicillium Candidum, and Debaromyces hansenii (see Table 2) onto the milk formula. Optional: Add microbial “rennet.” (see Table 2). Let milk sit for 5 minutes. Gently fold in and top stir with a spatula for two minutes. Hold at 90°F for 90 minutes.

**[00198]**      Increase water bath temperature to start to bring non-dairy milk temperature up to 100°F.

**[00199]**      Optional: Add in distilled vinegar (see Table 2), folding gently with spatula. Hold 15 minutes.

**[00200]**      Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

**[00201]**      Remove container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

**[00202]**      Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.

**[00203]**      Place curd in mixing bowl. Add cheese salt. Gently whisk curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

**[00204]**      Place 17.64 ounces (500 grams) of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (**Figure 8**) on a draining mat. Drain at room temperature for one hour without the follower in place. Then place the follower in the mold just

touching the cheese replica. Do not add any additional weight to the follower. Refrigerate at 36°F for 24 hours.

[00205] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for ½ hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00206] Remove the cheese replica from the mold. Place on draining mat and return it to 36°F for 24 hours.

[00207] Transfer the cheese from 36°F to dry yeasting room for three days at 60°F, with 75% humidity.

[00208] After three days, transfer cheese from a draining mat to an aging mat. Place the mat on an aging rack, and move to ripening room at 50°F, with 90% humidity and continuous airflow. Turn the cheese replica and replace mat daily.

[00209] After seven days, transfer the cheese replica directly onto an aging rack, allowing maximum aeration for seven more days, or until mold coverage is complete.

[00210] After the cheese replica has thoroughly molded (**Figure 8**), move it to 36 °F for sixteen hours. Wrap cheese in perforated paper and place in a wooden box for shipment.

[00211] **EXAMPLE 6**

[00212] **PRODUCTION OF A GOAT CHEESE REPLICA.**

[00213] The ingredients and amounts needed for one batch (about one and a half pieces) of goat cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00214] Bring pasteurized milk formula (see Table 2) to 80°F in a water bath.

[00215] Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in starter culture with a spatula for full two minutes. Hold at 80°F for one hour.

[00216] Increase water bath temperature to begin to bring non-dairy milk temperature to 100°F.

[00217] Optional: Add microbial “rennet” and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

[00218] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00219] Remove container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00220] Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a maximum of 24 hours until proper viscosity and density of curd is achieved. The drained curd should weigh about 50% of original formula weight.

[00221] Place curd in mixing bowl. Add cheese salt (see Table 2). Using Hobart mixer on high speed, mix and aerate completely.

[00222] Place sixteen ounces of curd (enough to yield eight ounces after completion of draining and brining) into a cylindrical goat cheese mold (**Figure 9**) and stand the mold upright on a draining mat. Refrigerate at 36°F for 24 hours allowing the cheese replica to drain.

[00223] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, horizontally into the preheated brine for ½ hour.

[00224] After brining, stand mold on draining mat placed at 36°F for 24 hours. Turn mold and change mat every twelve hours for two days.

[00225] Slide shaped goat cheese replica out of mold directly onto a bed of three grams of coarse ground pepper. Gently roll the cheese replica in pepper, covering evenly and completely. Place the cheese replica on waxed paper and return to 36°F for 48 hours.

[00226] The goat cheese replica (**Figure 10**) is now ready for packaging and shipment. Packaging includes Cryovac sealing process.

[00227] **EXAMPLE 7**

[00228] **CULTURED GELS MADE WITH EMULSIONS OF PURIFIED PLANT PROTEINS AND FATS.**

[00229] Procedure

**[00230]** Mixtures of purified or partially purified protein + glucose + oil were assembled and mixed with a vortex mixer to create an emulsion. Emulsions were either treated with transglutaminase (Ajinomoto Activa TI at a final concentration of 2% or 4%, as noted) or first heated to 85°C, rapidly cooled on ice and then treated with transglutaminase. Mesophilic starter culture (Danisco Choozit MA11) was added to a concentration of 0.01% (wt/vol) concomitantly with transglutaminase to allow cultures to ferment, acidify and impart flavors and aroma to the gels. Reactions were incubated overnight at 30°C before evaluating for gel formation and aroma.

**[00231]** Protein purification methods:

**[00232]** All steps were carried out at 4°C whenever possible. Centrifugation steps were at 8000 g for 20mins, 4°C. Once fractionated, all ammonium sulfate precipitate fractions of interest were stored at -20°C until further use. Prior to their use in experiments, the precipitates were resuspended in 50mM KPhosphate buffer pH 7.4 + 0.5M NaCl and dialyzed overnight against the same buffer to remove ammonium sulfate. The dialyzed solution was then centrifuged at 12000 g for 20min to remove precipitate and then used in experiments. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

**[00233]** (i) Pea-albumins: Dry flour of green peas was used as a source of pea albumins. The flour was resuspended in 10 volumes of 50mM sodium acetate buffer pH 5 and stirred for 1hr. The slurry was centrifuged at 8000 g for 20 minutes and the supernatant was collected. To this crude protein extract, solid ammonium sulfate was added to 50% saturation. The solution was stirred for 1 hour and then centrifuged. To the supernatant from this step, ammonium sulfate was added to bring to 90% saturation. After stirring for 1 hour, the solution was stirred and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20°C until further use.

**[00234]** (ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was resuspended in 10 volumes of 50mM KPhosphate buffer pH 8 + 0.4M NaCl and stirred for 1hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation in two steps at 50% and 80% saturation. The 80% pellet containing globulins of interest was stored at -20°C until further use.

**[00235]** (iii) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first resuspending the flour in 4 volumes of 50mM KPhosphate buffer pH 7 (+ 0.5M NaCl for lab scale purifications). After centrifugation, proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps at 50% and 90% saturation respectively. The precipitate from the 90% fraction contained the 8S globulins and was saved at -20°C until further use.

[00236] Gel formation was tested with the following protein fractions:

[00237] (i) Pea-albumins (90% ammonium sulfate fraction from green pea dry seed flour)

[00238] (ii) Pea-globulins (80% ammonium sulfate fraction from green pea dry seed flour)

[00239] (iii) Moong bean 8S globulin (90% ammonium sulfate fraction from moong flour)

[00240] The protein solutions were dialyzed into 50mM KPhosphate buffer pH 7-7.4 + 0.5M NaCl and used at concentrations between 8mg/ml and 75mg/ml (0.8-7.5%) as described below.

[00241] Canola oil was used to make emulsions. Stock solutions of transglutaminase (Ajinomoto) were prepared at 40% in 50mM KPhosphate pH 7. Mesophilic starter culture (MA011 from Danisco) was used as the cheese bacterial culture; the freeze-dried culture was resuspended at a 10% in 50mM KPhosphate buffer and used immediately in the experiments. Glucose (1%) was used as sugar source for growth of mesophilic cultures; stock solution was prepared at 40% in water.

[00242] Two conditions were used to form gels:

[00243] (i) 'Set 1': 2% protein + 3% oil + 4% transglutaminase; the rest water. Emulsify by vortex mixing.

[00244] (ii) 'Set 2': 7.5% protein + 3% oil + 2% transglutaminase; the rest water. Emulsify by vortex mixing.

[00245] Both gel compositions listed above were tested for gel formation catalyzed by transglutaminase, with and without heat denaturation of the protein component. All reactions (total volume of each, 1ml) were first assembled in 1.5 ml micro-centrifuge tubes with protein + oil+ sugar components. Emulsions were made by vortexing the samples for 30sec. Samples to be heat denatured were immediately placed in a water bath and heated at 85°C for 30minutes. These samples were subsequently rapidly cooled on ice for 20 minutes. Transglutaminase and starter culture were added to all samples and mixed. Samples that formed gels upon heat/cool step were vortexed to mix the enzyme and cultures in. All samples were removed from the reaction tubes and evaluated for gel consistency and aroma after overnight incubation at 30°C.

[00246] Aroma evaluation:

[00247] The following gels were tested for aroma (smell by 4 individuals):

[00248] Set 1: pea-albumin (heat/cool), moong (heat/cool), moong (no heat/cool),

[00249] Set 2: pea globulin (heat/cool), moong (no heat/cool)

[00250] All four individuals reported yogurt-like/fermented/sour aroma in the moong (set 1, no heat/cool) gels. They reported similar smells but to a lower degree in the moong (set2, no heat/cool) and pea globulin (set 2, heated) gel samples. For pea-albumins (set 1, heated) and moong (set 1,



heated), very faint or no aroma was reported. One of the four testers reported the aroma from moong (set 2, no heat/cool) - to be unpleasant.

[00251] Gel firmness evaluation: **(Figure 10)**

[00252] ‘Set 1’: all samples formed gels. In general these gels were weak and disintegrated easily. Pea albumins (heat/cooled) were slightly firmer in this regard.

[00253] ‘Set 2’: All samples with exception of pea-globulins (no heat/cool) formed gels. Gels were stronger compared to Set 1 and held their shape even after removal from the tubes. Gels formed without heat/cool.

**Table 1: MILK FORMULAS FOR SOFT FRESH, SALTED, SOFT RIPENED AND GOAT CHEESE REPLICAS**

Type of cheese replica	milk component	Amount (grams)
Soft fresh, salted or goat	<i>Almond milk (per 800 ml batch).</i>	
	Almond skim	725.0
	Almond Fat	75.0
	<i>Macadamia nut milk (per 660 ml batch)</i>	
	Macadamia nut skim	328.5
	Macadamia nut fat	337.5
Soft ripened	<i>Almond milk (per 800 ml batch).</i>	
	Almond skim	725.0
	Almond Fat	75.0
	<i>Macadamia nut milk (per 660 ml batch)</i>	
	Macadamia nut skim	291.0
	Macadamia nut fat	375.0

**Table 2: INGREDIENTS FOR ONE BATCH OF CHEESE REPLICA**

<b>Ingredients</b>	<b>Amount (grams)</b>
<i>Soft Fresh Cheese Replica:</i>	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set )	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
Cheese salt (internal)	2.5
<i>Salted Cheese Replica</i>	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
<i>Soft Ripened Cheese Replica</i>	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.3
Flora Danica	0.2
Geotrichum candidum	0.1
Penicillium candidum	0.1
Debaromyces hansenii	0.1
Transglutaminase (733 units in 15 mls of water)	15.0
Cheese salt (internal)	4.2
<i>Goat Cheese Replica</i>	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set )	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
Cheese salt (internal)	2.5
<i>Optional Ingredients</i>	
Distilled white vinegar	5.3
Microbial "rennet:"	0.2

**Notes:**

"Transglutaminase" here refers to Activa TI, 100U/g, from Ajinomoto

"Mesophilic starter culture" here refers to Danisco Choozit lyophilized MA 11

"Geotrichum candidum" here refers to Danisco Choozit GEO17 LYO 10D

"Debaromyces hansenii" here refers to Chr. Hansen Debaryomyces hansenii LAF3

"Flora Danica" here refers to Chr. Hansen "Flora Danica freeze-dried lactic culture for direct vat set"

"Penicillium candidum" here refers to "Penicillium candidum FD PC A1" from Chr. Hansen

Microbial "rennet" here refers to FROMASE 50, protease from Rhizomicrobium mucor

## CLAIMS

### WHAT IS CLAIMED IS:

1. A method comprising:
  - (a) preparing an emulsion comprising proteins and fats from plant sources
  - (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins, and
  - (c) producing a cheese-replica from the gel.
2. The method of claim 1, wherein the emulsion contains less than 10% animal products.
3. The method of claim 1, wherein the emulsion contains no animal products.
4. The method of claim 1, wherein the inducing step comprises adding an enzyme.
5. The method of claim 4, wherein the enzyme is transglutaminase.
6. The method of claim 5, wherein the transglutaminase is selected from the group consisting of: a transglutaminase from *Streptovercillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptovercillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), or TGM Z (TGM7).
7. The method of claim 1, wherein the emulsion is a non-dairy milk.
8. The method of claim 1, wherein at least one of the plant sources are nuts.
9. The method of claim 8, wherein the nuts are selected from one or more of following group: almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts.
10. The method claim 1, wherein the preparation includes the steps of
  - (a) obtaining nuts or seeds, and
  - (b) surface sterilizing the nuts or seeds,wherein the nuts or seeds comprise the plant sources.

11. The method of claim 10, wherein the surface sterilization is blanching.
12. The method of claim 8, wherein the method further comprises washing the nuts or seeds.
13. The method of claim 10, wherein the preparation further comprising decompounding the nuts or seeds.
14. The method of claim 13, wherein the decompounding is blending.
15. The method of claim 10, further comprising centrifuging the sample.
16. The method of claim 12, wherein the centrifuging removes at least 85% of the suspended solids.
17. The method of claim 1, further comprising adding a sugar to the emulsion.
18. The method of claim 17, wherein the sugar is a monosaccharide.
19. The method of claim 17, wherein the sugar is a disaccharide.
20. The method of claim 1, further comprising inoculating with lactic acid bacteria.
21. The method of claim 20, further comprising incubating to allow growth of the bacterial cultures.
22. The method of claim 1, further comprising cutting the gel.
23. The method of claim 22, further comprising draining and shaping the cut gel.
24. The method of claim 1, further comprising adding a flavoring component.
25. The method of claim 24, wherein the flavoring component is a lactic acid bacteria, mold or yeast.
26. The method of claim 1, further comprising adding one or more of *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensii*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* sps., *Lactobacillus*

*sps.*, *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*, *Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus sps.*, or *Propionibacteria*.

27. The method of claim 1 further comprising adding a halophilic bacterium or archaea.
28. The method of claim 1, wherein at least one of the plant sources is a legume.
29. The method of claim 1, wherein the proteins are concentrated prior to preparing the emulsion.
30. The method of claim 29, wherein the concentrated proteins are each essentially homogeneous proteins.
31. The method of claim 1, further comprising concentrating, isolating, or purifying the proteins and fats from one or more plant sources.
32. The method of claim 30, wherein the concentrated proteins are all the same essentially homogeneous plant-derived protein.
33. The method of claim of claim 30, wherein each concentrated protein is from a separate plant species.
34. A method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.
35. A method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.
36. The method of claim 1, wherein thermal denaturing is used in addition to enzymatic cross-linking.
37. The method of claim 1, wherein thermal denaturing is used without the addition of any enzymes.
38. A composition comprising a centrifuged non-dairy milk wherein at least 85% of the insoluble solids have been removed relative to the non-dairy milk prior to centrifugation.

39. A composition comprising a non-dairy milk with less than 30% polysaccharides.
40. The composition of claim 38, with less than 10% polysaccharides.
41. The composition of claim 38, with less than 1% polysaccharides.
42. The composition of claim 38, wherein the composition is entirely composed of ingredients derived from non-animal sources.
43. The composition of claim 38, wherein the composition is at least comprised of 20% protein isolated from a single plant species.
44. A composition comprising a non-dairy milk and a cross-linking enzyme.
45. The composition of claim 44, wherein the cross-linking enzyme is selected from the group consisting of: a transglutaminase from *Streptovorticillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptovorticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase.
46. The composition of claim 44, wherein at least 85% of the insoluble solids have been removed by centrifugation.
47. The composition of claim 44, wherein proteins from non-animal sources comprise at least 50% of the total mass of the composition.
48. The composition of claim 44, wherein the composition has a starch content less than 1% by mass.
49. The composition of claim 44, wherein the composition has a starch content less than 5% by mass.
50. The composition of claim 44, wherein the composition has less than 1% insoluble carbohydrate by mass.
51. The composition of claim 44, wherein the composition has less than 5% insoluble carbohydrate by mass.

- 52.** The composition of claim 43, wherein the composition has less than 5% starch and less than 1% insoluble carbohydrate by mass.
- 53.** The composition of claim 44, wherein the composition has less than 5% starch and less than 5% insoluble carbohydrate by mass.
- 54.** The composition of claim 44, wherein the composition has a polysaccharide content less than 1% by mass.
- 55.** The composition of claim 44, wherein the composition has a polysaccharide content less than 5% by mass.
- 56.** The composition of claim 44, wherein the composition has a carbohydrate content less than 1% by mass.
- 57.** The composition of claim 44, wherein the composition has a carbohydrate content less than 5% by mass.
- 58.** The composition of claim 44, wherein the composition has a carbohydrate content less than 10% by mass.
- 59.** The composition of claim 44, wherein the composition has at least 80% of the protein content from a single monomeric or multimeric protein.
- 60.** A composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.
- 61.** The composition of claim 60, wherein the plant derived proteins are not soy based.
- 62.** The composition of claim 60, wherein the water content of the composition is less than 5%.
- 63.** The composition of claim 60, with less than 20% insoluble solids.
- 64.** The composition of claim 60, further comprising oils or fats isolated from plant sources.
- 65.** The composition of claim 60, wherein the crosslinking enzyme is selected from the group consisting of: atransglutaminase from *Streptoverticillium mobaraense*, an enzyme is similar or

identical to a transglutaminase from *Streptovorticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) , TGM Z (TGM7), or lysyl oxidase.

66. The composition of claim 60, wherein the crosslinks are formed between glutamine and lysine side chains of respective protein constituents.

67. A cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

68. The composition of claim 67, comprising between 10% and 40% proteins from plant sources and between 0% and 65% fats from plant sources.

69. The composition of claim 67, further comprising a crosslinking enzyme selected from the group consisting of: a transglutaminase from *Streptovorticillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptovorticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase.

70. The composition of claim 67, further comprising cheese-making microbes.

71. The composition of claim 67, wherein the cheese-making microbes are selected from one or more of: *Penicillium camemberti*, *Geotrichum candidum*, *Penicillium roqueforti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Khuyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Debaryomyces hansensil*, *Rhodospiridium infirmominiatum*, *Candida jefer*, *Cornybacteria*, *Micrococcus* sps., *Lactobacillus* sps., *Lactococcus*, *Staphylococcus*, *Halomonas*, *Brevibacterium*, *Psychrobacter*, *Leuconostocaceae*, *Streptococcus thermophilus*, *Pediococcus* sps., or *Propionibacteria*.

72. The method of claim 67, further comprising adding a halophilic bacterium or achaea.

73. A cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

74. The cheese replica of claim 73, comprising protein, wherein at least 20% of the protein content comprises a single monomeric or multimeric protein.



**75.** The cheese replica of claim 73, made without the addition of starches or rennet or any other extrinsic proteases other than those contributed by microbial cultures.

**76.** The cheese replica of claim 73, with a pH less than 5.5 where the acidification was accomplished solely by microbial fermentation.

**77.** The cheese replica of claim 73, without animal products.

**78.** The cheese replica of claim 73, with less than 5% by mass insoluble carbohydrates.

**79.** The cheese replica of claim 73, made without rennet, vinegar, or lemon juice.

**80.** A cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and /or aroma and /or texture of the cheese replica.

**81.** A cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and /or aroma and/or texture of the cheese replica.

**82.** A soft fresh cheese replica comprising:

- (a) pasteurized almond milk,
- (b) pasteurized macadamia nut milk,
- (c) mesophilic starter culture,
- (d) transglutaminase,
- (e) water, and
- (f) cheese salt.

**83.** A salted cheese replica comprising

- (a) pasteurized almond milk,
- (b) pasteurized macadamia nut milk,
- (c) mesophilic starter culture,
- (d) transglutaminase, and
- (e) water.

**84.** A soft ripened cheese replica comprising:

- (a) pasteurized almond milk,
- (b) pasteurized macadamia nut milk,
- (c) mesophilic starter culture,

- (d) *flora danica*,
- (e) *Geotrichum candidum*,
- (f) *Penicillium candidum*,
- (g) *Debaromyces hansenii*
- (h) transglutaminase,
- (i) water, and
- (j) cheese salt.

**85.** A goat cheese replica comprising:

- (a) pasteurized almond milk
- (b) macadamia nut milk,
- (c) mesophilic starter culture,
- (d) transglutaminase,
- (e) water, and
- (f) cheese salt.

**86.** The composition of claims 82-85 further comprising distilled white vinegar.

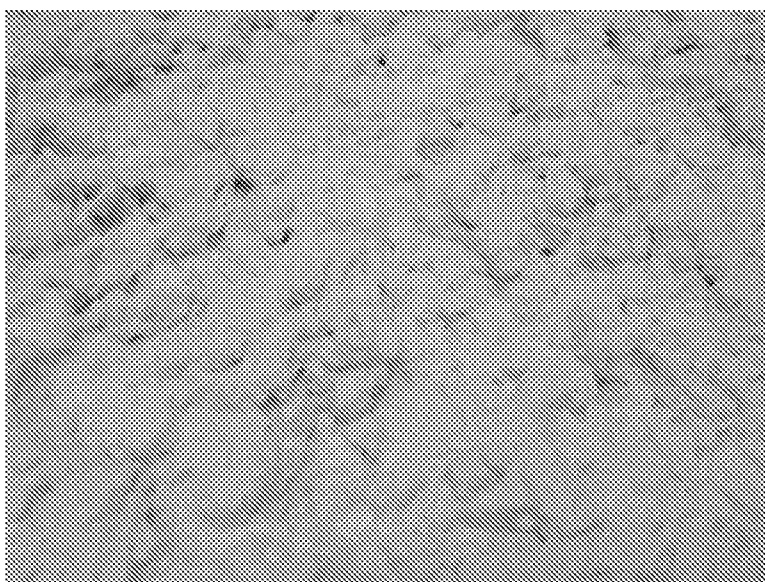
**87.** The composition of claims 82-85 further comprising microbial coagulant.

**88.** The composition of claims 82-85 further comprising white vinegar and microbial coagulant.

**89.** The method of claim 4, wherein the enzyme is lysyl oxidase.

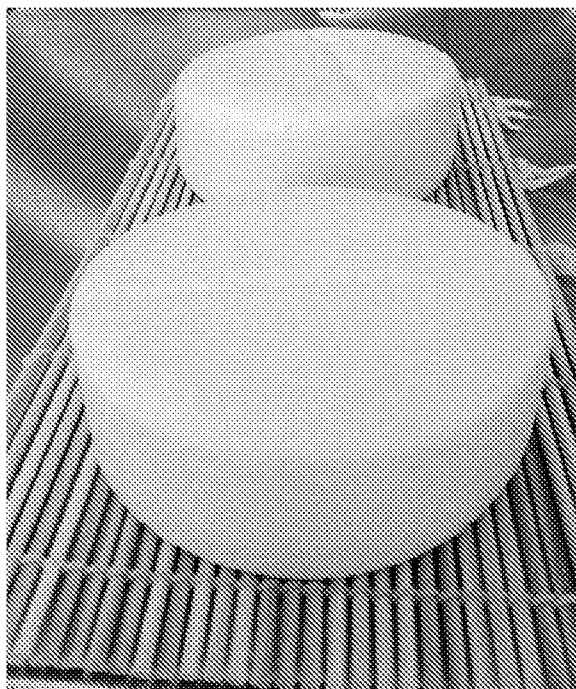


**Figure 1.**

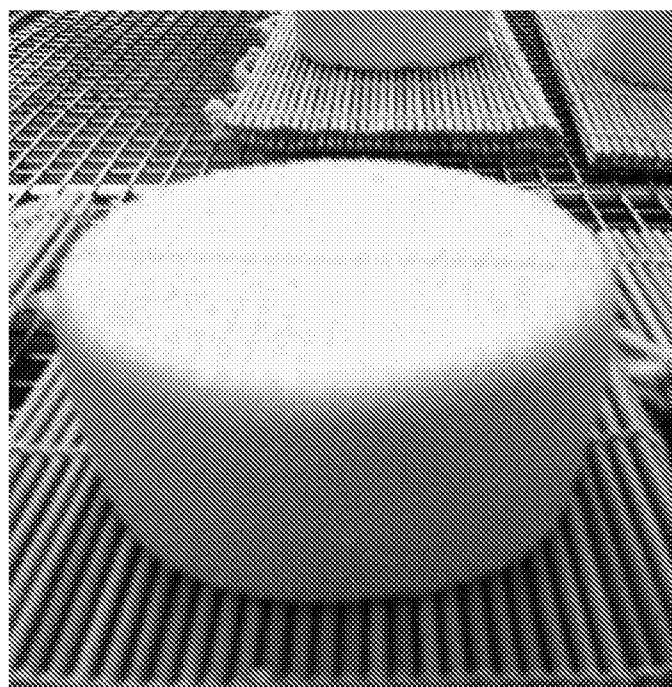


**Figure 2.**

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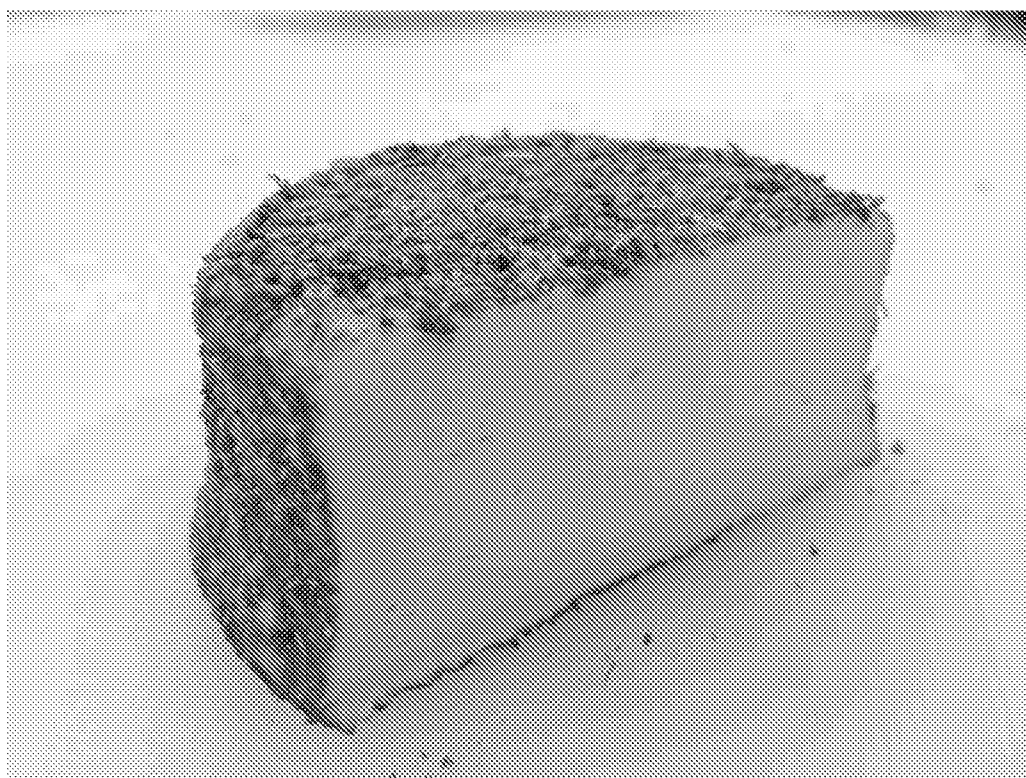
**Figure 3.**



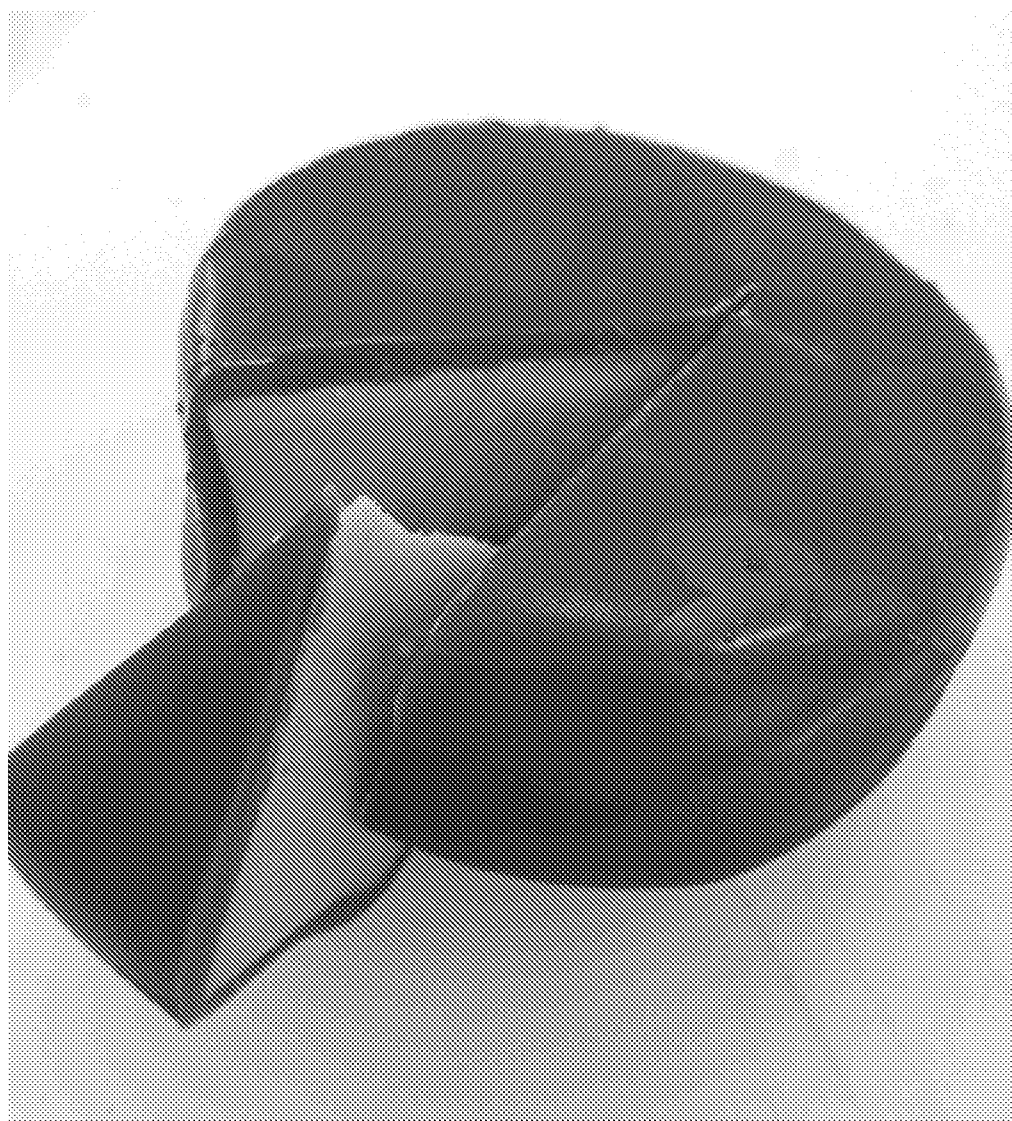
**Figure 4.**



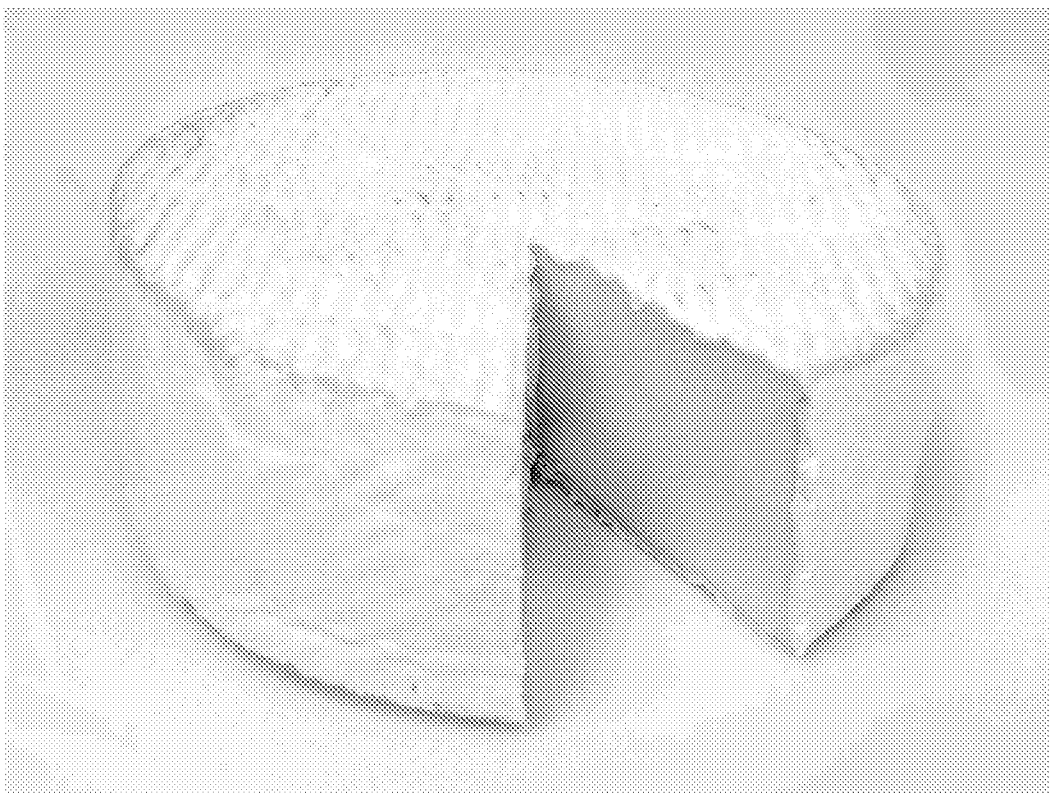
**Figure 5.**



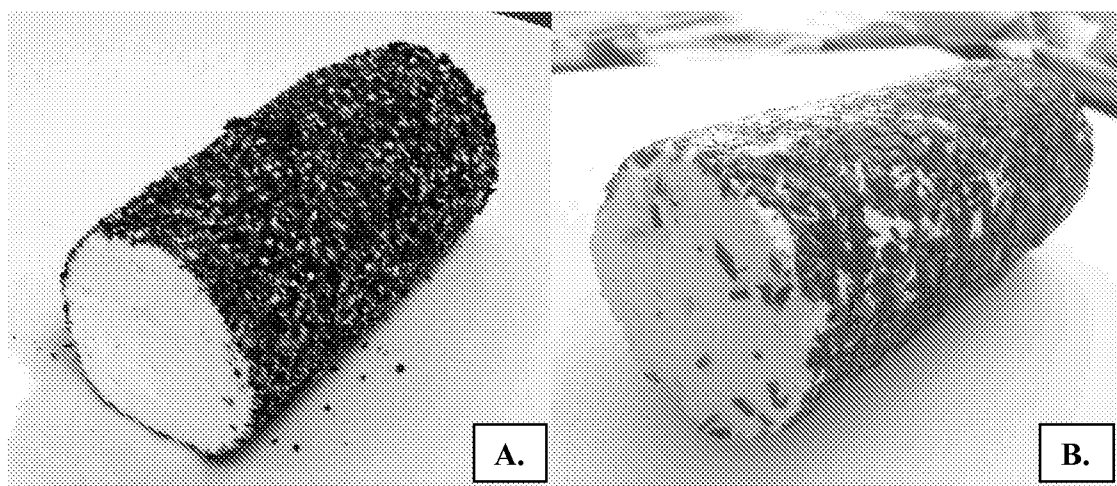
**Figure 6.**



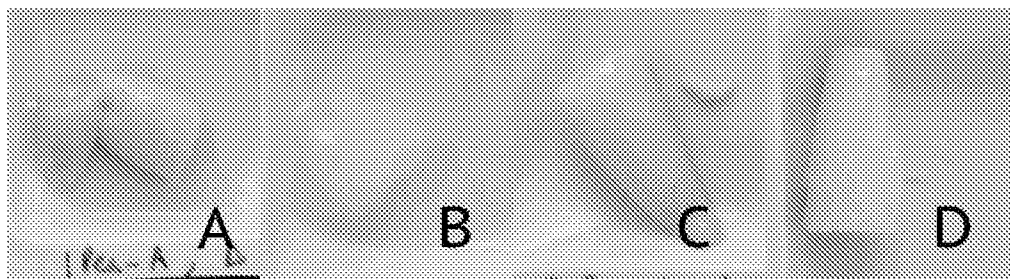
**Figure 7.**



**Figure 8.**



**Figure 9.**



**Figure 10.**



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46552

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)

USPC - 426/573, 426/602, 426/632

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC - 426/573, 426/602, 426/632

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 426/573, 426/602, 426/632; 435/128

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Lyrical Foods, Casino, Voccola, Varadan, transglutaminase, cheese, replica, imitation, plant, nut, soy, milk, extract, lysyl oxidase,

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,055,310 A (Nonaka et al.) 18 October 1991 (18.10.1991), especially col 1, ln 35-36; col 2, ln 9-59; col 4, ln 15-46;	1-33 and 89
Y	US 4,678,676 A (Ishizuka et al.) 07 July 1987 (07.07.1987), especially col 2, ln 50 to col 3, ln 2; col 3, ln 45-56;	1-33 and 89
Y	US 2008/0299254 A1 (Kim et al.) 04 December 2008 (04.12.2008), especially para [0013]	20-21 and 25-26
Y	US 2011/0064847 A1 (McCready et al.) 17 March 2011 (17.03.2011), especially para [0004], [0026]	8-16 and 33
Y	US 5,807,601 A (CARPENTER et al.) 15 September 1998 (15.09.1998) abstract; col 2, ln 1-5	8-16 and 33
Y	US 2003/0224476 A1 (CHOU) 4 December 2003 (04.12.2003) abstract; para [0003]; [0008]; [0042]; [0050]; [0176]	89
Y	US 2008/0292749 A1 (GOODWINS et al.) 27 November 2008 (27.11.2008) para [0021]; [0029]; [0034]	27

☐ Further documents are listed in the continuation of Box C.


\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

31 October 2012 (31.10.2012)

Date of mailing of the international search report

19 NOV 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46552

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-33 and 89, directed to a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources; (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins; and (c) producing a cheese-replica from the gel.

Group II: claims 34, 38, 40-43 and 52, directed to a method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-33 and 89

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46552

### Continuation of Box III: Lack of Unity of Invention

Group III: claims 35-37, directed to a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

Group IV: claim 39, directed to a composition comprising a non-dairy milk with less than 30% polysaccharides

Group V: claims 44-51 and 53-59 directed to a composition comprising a non-dairy milk and a cross-linking enzyme.

Group VI: claims 60-66, and 82-85, directed to a composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

Group VII: claims 67-72, directed to a cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

Group VIII: claims 73-79, directed to a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

Group IX: claim 80, directed to a cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and/or aroma and /or texture of the cheese replica.

Group X: claim 81, directed to a cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and/or aroma and/or texture of the cheese replica.

The inventions listed as Groups I - X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-X are indicated above in the Group descriptions.

There is no common technical element shared by all of the above groups. Groups I and VII-X are related to a cheese replica. Groups II-V are related to a non-dairy milk, which, in Groups II and III may be a nut milk. Groups I-III, V, and VI share the common technical element of a crosslinking enzyme, which, in Groups I-III and VI may be a transglutaminase. These common technical elements do not represent an improvement over the combined prior art of US 5,055,310 A to Nonaka et al. (hereinafter "Nonaka") and US 2005/0220937 A1 to Ito et al. (hereinafter "Ito"), as follows: Nonaka teaches a shelf-stable soybean curd product which is prepared by reacting a non-dairy milk (soy milk) with a solidifying agent and a transglutaminase (abstract); wherein the milk is produced by separating (filtering) plant solids from the liquid (col 9, ln 24-35). Although Nonaka does not specifically recite wherein the milk may also be a nut milk, it would have been obvious to a person of ordinary skill in the art to substitute a nut milk in place of the soy milk, as both were plant milk products in common use at the time of the invention. Further, Ito teaches producing a cheese-like food product (para [0058], [0071], [0072]) by using a transglutaminase (para [0059]) to enable crosslinking (shaping) of a soybean paste foodstuff (para [0059]), wherein the foodstuff comprises a non-dairy milk product (soy milk; para [0071]). It would have been obvious to a person skilled in the art to combine the teaching of Nonaka regarding the formation of a solid non-dairy milk product directly from the liquid with the teaching of Ito regarding the formation of a cheese replica made from plant material in order to make a cheese replica directly from the non-dairy milk without undue experimentation. A person of ordinary skill in the art would have desired to form a non-dairy cheese replica in a manner similar to dairy cheese making, while avoiding the use of allergenic dairy products to provide a cheese replica to users unable to consume dairy products.

Therefore, the inventions of Groups I-X lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.