(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% or more than 99% of microbes on the nuts or seeds.

In some embodiments, the non-dairy milk is centrifuged to remove insoluble solids. The non-dairy milk can have less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in the non-dairy milk before centrifugation. The non-dairy milk can have 99%, 95%, 90%, 80%, 70%, 60% or 50% of insoluble solids removed by centrifugation. Centrifugation of the non-dairy milk is described herein.

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 includes pasteurizing the non-dairy milk at 164°F -167°F for 10 to 20 seconds (e.g., 10, 12, 14, 16, 18, or 20) seconds. In sme embodiments, the microbial load in the non-dairy milk is reduced by exposure to UV light or high pressure pasteurization. A controlled chilling system can be used to rapidly bring non-dairy milk temperature down rapidly and store in a refrigerator at 36°F.

In some embodiments, the non-dairy milk is a non-dairy cream fraction.

In some embodiments, the non-dairy milk is an emulsion comprising one or more isolated and purified proteins and one or more isolated fats. In some embodiments the isolated and purified proteins are contained in a protein solution. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), Urea (0-2M) or any combination thereof. The solution can have a pH of 3 to 11. In some embodiments, the one or more isolated and purified proteins accounts for 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of said protein solution. In some embodiments, the one or more isolated and purified proteins accounts for 0.1-5%, 1-10%, 5-20%, 10-40%, 30-60%, 40-80%, 50-90%, 60-95%, or 70-100% of the protein content of said protein solution. In some embodiments, the total protein content of the protein solution is about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75%, 1%, 1.5%, 2%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, or more

than 20% weight/volume. In some embodiments, the total protein content of the protein solution is 0.1-5%, 1-10%, 5-20%, or more than 20% weight/volume. In some embodiments, the protein content of the protein solution contains 1-3 isolated and purified proteins, 2-5 isolated and purified proteins, 4-10 isolated and purified proteins, 5-20 isolated and purified proteins, or more than 20 isolated and purified proteins.

In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. In some embodiments, the non-dairy milk is isolated from a non-dairy source (e.g., a plant). Non-limiting examples of plant sources include grain crops such as, e.g., maize, oats, rice, wheat, barley, rye, triticale (a wheat rye hybrid), teff (*Eragrostis tef*); oilseed crops including cottonseed, sunflower seed, safflower seed, *Crambe*, *Camelina*, mustard, rapeseed (*Brassica napus*); leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, or cabbage; or green matter not ordinarily consumed by humans, including biomass crops such as switchgrass (*Panicum virgatum*), *Miscanthus*, *Arundo donax*, energy cane, *Sorghum*, or other grasses, alfalfa, corn stover, kelp or other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, or turnips; plants from the legume family, such as, e.g., clover, *Stylosanthes*, *Sesbania*, vetch (*Vicia*), *Arachis*, *Indigofera*, *Leucaena*, *Cyamopsis*, peas such as cowpeas, english peas, yellow peas, or green peas, or beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts (*Arachis hypogaea*); coconut; or *Acacia*. One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used in the present invention. In some embodiments, the plant source is not soybeans.

Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the cheese products. Accordingly, in some embodiments, the one or more isolated proteins comprise an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% of the total protein content of the source plant.

In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, or about 20-50% of the total weight of the dry matter of the source plant.

In particular embodiments, the one or more isolated proteins comprise an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo). RuBisCo is a particularly useful protein for cheese replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

In some embodiments, the one or more isolated proteins comprise an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins,

globulins, vicilins, convicilins, legumins, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, or oil body proteins such as oleosins, caloleosins, or steroleosins.

In some embodiments, the one or more isolated proteins comprise proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the cheese product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from moong bean, and 8S globulins from kidney bean.

In some embodiments, the one or more isolated and purified proteins is selected from the group consisting of ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), albumins, glycinins, conglycinins, globulins, legumins, vicilins, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, collagens, zein, kafirin, avenin, dehydrins, hydrophilins, late embyogenesis abundant proteins, natively unfolded proteins, 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, pea globulins, pea albumins, or any other protease described herein.

In some embodiments, the isolated and purified proteins are concentrated using any methods known in the art. The proteins may be concentrated 2-fold, five-fold, 10-fold, or up to 100 fold. The proteins may be concentrated to a final concentration of

0.001-1%, 0.05-2%, 0.1-5%, 1-10%, 2-15%, 4-20%, or more than 20%. Exemplary methods include, e.g., ultrafiltration (or tangential flow filtration), lyphilisation, spray drying, or thin film evaporation.

The fats used in preparing the emulsion can be from a variety of sources. In some embodiments, the sources are non-animal sources (e.g., oils obtained from plants, algae, fungi such as yeast or filamentous fungi, seaweed, bacteria, Archae), including genetically engineered bacteria, algae, archaea or fungi. The oils can be hydrogenated (e.g., a hydrogenated vegetable oil) or non-hydrogenated. Non-limiting examples of plant oils 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, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil; or margarine.

In some embodiments, the fat can be triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phospholipids such as phosphatidic acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl inositols, phosphatidyl ethanolamines, or phosphatidyl serines; sphingolipids such as sphingomyelins or ceramides; sterols such as stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol, or episterol; free fatty acids such as palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid (C18:2), eicosanoic acid (C22:0), arachidonic acid (C20:4), eicosapentanoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexanoic acid (C22:6), erucic acid (C22:1), conjugated linoleic acid, linolenic acid (C18:3), oleic acid (C18:1), elaidic acid (trans isomer of oleic acid), trans-vaccenic acid (C18:1 *trans* 11), or conjugated oleic acid; or esters of such fatty acids, including monoacylglyceride esters, diacylglyceride esters, and triacylglyceride esters of such fatty acids.

The fat can comprise phospholipids, sterols or lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids (e.g., see above), glycerol and polar groups. In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol

phosphates. In some embodiments, the lipids are, for example, sphingolipids, ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

In some embodiments, the fat are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflower seeds, safflower seeds, sesame seeds, rape seeds, almonds, macadamia nuts, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash, cashews, brazilnuts, chestnuts, hazelnuts, peanuts, pecans, walnuts, and pistachios. Methods for preparing a cream fraction are described herein.

The addition of controlled amounts of one or more fats can result in different cheese properties, including but not limited to firmness, water retention, oil leakage, melt-ability, stretching, color, and creaminess. The fats can be in the form of unsaturated oil, saturated oil, washed cream fraction, and/or unwashed cream fraction. The unsaturated oils can include, e.g., olive oil, palm oil, soybean oil, canola oil (rapeseed oil), pumpkin seed oil, corn oil, sunflower oil, safflower oil, avocado oil, other nut oils, peanut oil, grape seed oil, sesame oil, argan oil, and rice bran oil. The saturated oils can include, e.g., coconut, palm, cocoa, cottonseed, mango oil, etc. The cream fraction can be made from, by way of example only, sunflower seeds, safflower, sesame seeds, rape seeds, almonds, macadamia, and pistachios. Preparation and isolation of cream fractions are described herein.

In some embodiments, an emulsion is prepared by isolating and purifying one or more proteins, preparing a solution comprising the one or more isolated and purified proteins, admixing said solution with one or more fats, thereby creating said emulsion. The ratio of protein solution to fats can be about 1:10, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, or 10:1. The ratio of protein solution to fats can be about 10:1-1:2, 1:4-2:1, 1:1-4:1, or 2:1-10:1. The emulsion can be used as a non-dairy milk for the preparation of a non-dairy cheese. By way of example only, 0%- 50% fat can be added to a protein solution by weight/weight or weight/volume.

Method of isolating and mixing cream and skim fractions

In some embodiments, the non-dairy milk can be further separated into a cream fraction and a skim fraction. In some embodiments, defined amounts of cream fraction can be mixed with defined amounts of skim fraction to produce a non-dairy milk with a controlled fat profile. In one aspect, the invention provides a method for creating a non-dairy cheese replica with a controlled fat profile. In some embodiments, the method comprises isolating a cream and skim fraction from a non-dairy milk, and mixing a defined amount of said cream and optionally said skim fraction to produce a mixture with a controlled fat profile. In some embodiments, said isolating comprises separating a non-dairy milk into a cream and skim fraction. In some embodiments the cream fraction is enriched in fats relative to the skim fraction. The cream fraction may contain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more of the fat content of said non-dairy milk prior to separation. The cream fraction may comprise a fat content that is at greater than the fat content of a skim fraction. The cream fraction may comprise a fat content that is increased by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or greater than 100% as compared to the skim fraction. The cream fraction may comprise a fat content that is 0.2-fold greater, 0.5-fold greater, 0.75-fold greater, 1-fold greater, 1.2-fold greater, 1.3-fold greater, 1.4-fold greater., 1.5-fold greater, 2-fold greater, 3-fold greater, 4-fold greater, 5-fold greater, 7.5-fold greater, 10-fold greater, 15-fold greater, 20-fold greater, or more than 20-fold greater than the fat content of the skim fraction.

The cream and skim fractions can, for example, be separated by gravity or by centrifugation. Centrifugation generally refers to a process of separating components in a composition using centrifugal force. The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as g. The term “g” generally refers to the acceleration produced by gravity at the Earth's surface. In some embodiments, the cream and skim fractions are separated by centrifugation at 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10,000 RPM. In some embodiments, the cream and skim fraction are separated by centrifugation at about 500-2000, 1000-5000, 2000-7000, 4000-10,000, or greater than 10,000 RPM. In some embodiments, the cream and skim fractions are separated by centrifugation for about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50,

55, 60, or more than 60 minutes. In some embodiments, the cream and skim fractions are separated by centrifugation for 1-10, 5-30, 10-45, 30-60, or more than 60 minutes. In one embodiment, the cream and skim fractions are centrifuged in a JS-5.0 rotor at 5000 RPM for 30 mins. In some embodiments, the cream fraction and skim fraction are separated by centrifugal separation in a Flotwegg ac1500 or GEA ME55.

In some embodiments the cream fraction and the skim fraction are incompletely separated. The skim fraction and the cream fraction can be separated from the insoluble solids in a separation process. In some embodiments the skim fraction and the cream fraction are stored separately.

In some embodiments, a cream fraction can be used as a non-dairy cheese source.

In some embodiments, a protein solution can be admixed with a cream fraction to produce a non-dairy cheese source. Exemplary protein solutions are described herein.

In some embodiments, the method further comprises admixing the protein solution with a cream fraction isolated from a plant source. As used herein, the term "cream fraction" refers to an emulsion comprising fats, proteins and water that is enriched in fats as compared to an original emulsion (i.e., non-dairy milk). Exemplary cream fractions and methods of preparing the same are described herein. In some embodiments, a cream fraction is purified from a plant source, e.g., seeds, nuts, or legumes such as sunflowers, safflower, sesame seeds, rape seeds, almonds, macadamia, and pistachios. In some embodiments, the cream fraction is purified by blending seeds or nuts in water or a solution to create a slurry. Some embodiments includes blending seeds, nuts or legumes from 1 minute up to 30 minutes which could include a blending method by increasing the speed gradually to maximum speed over 4 minute, and blending at maximum speed for 1 minute. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), urea (0-2M) or any combination thereof. The solution can have a pH of 3 to 11. See, Example 11. The slurry can be centrifuged by any method known in the art or as described herein.

Centrifugation can result in a separation of liquid layers and an insoluble solid pellet. The top layer can be used as the cream fraction. The lower layer can be used as whey, and the pellet is removed. The cream fraction can then be used as is immediately

after centrifugation, or can be further washed with the solutions described above or heated in solution. Washing and heating removes the unwanted color and flavor molecules, or unwanted grainy particles to improve the mouth feel. In particular, washing with a high pH buffer (e.g., above pH 9) can remove bitter tasting compounds and improve mouth feel, washing with urea can remove storage proteins, washing below pH 9, followed by washing with a pH above pH 9 can remove unwanted color molecules, and/or washing with salts can decrease taste compounds. Heating increases the removal of grainy particles, color and flavor compounds. Heating can be from 0-24 hours, at 25°C to 80°C. Washing and heating can remove unwanted colors and flavor notes, and can remove unwanted grainy particles. In some embodiments, washing and heating improves mouthfeel. In some embodiments, the resulting creamy fraction comprises seed storage proteins. In some embodiments, the seed storage proteins are substantially removed from the resulting creamy fraction.

In some embodiments, a defined amount of a cream fraction and a defined amount of a skim fraction are mixed to produce a mixture. The cream and/or skim fractions can be pasteurized or not pasteurized. In some embodiments, the defined amounts are defined by a user to result in a mixture with a controlled fat profile. In some embodiments, the cream and skim fractions are mixed at a defined ratio to result in a mixture with a controlled fat profile. 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.

The mixture may then be used as a non-dairy cheese source to prepare a cheese replica. It is understood that any of the non-dairy cheese sources as described herein may be used individually, or in any combination thereof, when practicing the methods described herein.

Flavoring components/methods

In another aspect, the invention provides methods for flavoring cultured non-dairy products, including sour cream, crème fraiche, yogurt, or cheese replicas. In some embodiments, the method comprises comparing a flavor note profile of a test non-dairy product with one or more flavor additives and/or one or more individual microbial strains described herein to a flavor note profile of a control non-dairy product without the additives and/or individual microbial strain. The texture and flavor profile of the non-dairy product (e.g. cheese replica) can be ascertained by any method known in the art or described herein. Exemplary methods of ascertaining flavor and texture can be by a taste test, e.g., a blind taste test, or using gas chromatography–mass spectrometry (GCMS).

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 dairy cheese and a cheese replica. For example volatile chemicals can be detected from the head space around a dairy cheese or a cheese replica. 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 dairy cheese. In some embodiments the cheese replica has a GCMS profile identical to that of dairy cheese.

The flavor profile can be characterized by the presence and/or intensity of one or more flavor notes. Exemplary flavor notes include, but are not limited to butteriness, fruitiness, nuttiness, dairy, milky, chessy, fatty, fruity, pinnapple, waxy, buttery, tonka, dark fruit, citrus, sour, banana-like, sweet, bitter, musty, floral, goaty, sweaty, woody, earthly, mushroom, malty, spicy, pear, green, balsamic, pungent, oily, rose, fatty, butterscotch, orange, pine, carnation, melon, pineapple, vanilla, garlic, herbaceous, woody, cinnamon, rue, yogurt, peach, vanilla, hawthorn, and herbaceous. The flavor notes may be associated with the release of one or more volatile compounds. The flavor profile can be characterized by the absence or reduction in the intensity of one or more

flavor notes. Exemplary flavor notes include: planty, beany, soy, green, vegetable, nutty, dirty, and sour.

Exemplary volatile compounds include, e.g., gamma-nonanoic lactone, gamma-undecalactone, gamma-decalactone, delta- tetradecalactone, S-methyl thiopropionate, delta-tridecalactone, delta-tetradecalactone, δ-tetradecalactone, butyl butyryllactate, 2,3-hexandione, methyl hexanoate, butyrolactone, propanoic acid, 2-methyl propanoic acid, methyl isobutyl ketone, gamma octalactone, delta octalactone, gamma nonalactone , 5-hydroxy-4-octanone, 2-ethyl-1-hexanol, octane, ethanol, 2,3-butanedione, 2 heptanone, 1-butanol, acetoin, butanoic acid, nonanal, acetic acid, 1,3 butanediol, methyl-3-buten-l-ol, methanol, hexanol, dimethyl-benzene, ethyl-benzene, indole, limonene, toluene, acetophenone, pentan-2,3-dione, 2-pentanone, 2-heptanone, 2-nonanone, acetone, butanone, 2-methylpropionic acid, butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, 4-methylpentanoic acid, hexanoic acid, octanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, linolenic acid, propanol, butanol, pentanol, hexanol, heptanol, octanol, propan-2-ol, butan-2-ol, pentan-2-ol, hexan-2-ol, heptan-2-ol, nonan-2-ol, undecan-2-ol, octen-3-ol, octa-1,5-dien-3-ol, 3-methyl-2-cyclohexenol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol, 3-methylpentanol, phenylmethanol, 2-phenylethanol, 2-phenyl-ethan-2-ol, propan-2-one, butan-2-one, pentan-2-one, hexan-2-one, heptan-2-one, octan-2-one, nonan-2-one, decan-2-one, undecan-2-one, dodecan-2-one, tridecan-2-one, pentadeca-2-one, pentan-3-one, octan-3-one, 3-methylpentan-2-one, 4-methylpentan-2-one, methylhexan-2-one, hydroxypropan-2-one, hept-5-en-2-one, 4-methylpent-3-en-2-one, octen-3-one, octa-1,5-dien-3-one, nonen-2-one, undecen-2-one, methylfuryl ketone, phenylpropan-2-one, propiophenone, methyl butanoate, methyl hexanoate, methyl octanoate, methyl decanoate, methyl tetradecanoate, methyl hexadecanoate, methyl cinnamate, ethyl formate, ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl-3-methyl butanoate, propyl acetate, propyl butanoate, butyl formate, butyl acetate, amyl acetate, isoamyl formate, isoamyl acetate, isoamyl propanoate, isoamyl butanoate, diethyl phthalate, dimethyl phthalate, 2-phenylethyl acetate, 2-phenylethyl propanoate, 2-phenylethyl butanoate, 3-

methylthiopropanol, methanethiol, hydrogen sulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide, methylethyl disulfide, diethyl disulfide, 2,4-dithiapentane, methional, 3-methylthio-2,4-dithiapentane, 2,4,5-trithiahexane, 1,1-bis-methylmercaptodisulfide, methanethiol acetate, methyl thiopropanoate, methyl thiobenzoate, thiophen-2-aldehyde, methylindole, p-ethylphenol, p-cresol, acetaldehyde, butanal, 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, hexanal, heptanal, nonanal, 2-methylbuten-2-al, benzaldehyde, 3-methylheptyl acetate, 1-butanol, 1-butanol, 3-methyl, 1-heptanol, formic acid, 1-hexanol-2,ethyl, 1-octanol, 2-butanone, 2-hepten-1-ol , 2-hexanone, heptanal, 2-octen-1-ol, 1-octen-3-ol, 2-pentanone, 2,3-butanedione, 3-buten-1-ol, 5-Hepten-2-one, octane, ethanol, 2,3-butanedione, 2 heptanone, 1-butanol, butanoic acid, nonanal, acetic acid, 1,3 butanediol, methyl-3-buten phenylethyl alcohol, toluene, 1-pentanol, 3-octene-1-ol, 2 octene-1-ol, 2-undecanone, 1-octanol, Benzaldehyde, 1-heptanol, 2-heptanone, 4-methyl-2-nonanone, 2-methyl-2-nonanol, 1-hexanol, 2-methyl 2-propanol, Ethanol, 3 methyl 1-butanol, 1-hexanol, 2-methyl 2-nonanol, 2-nonanone, 2-heptanone, 4-methyl, 1-heptanol, 1-octanol, 2 octene-1-ol, 3-octene-1-ol, 1-octanol, 1-heptanol, 2-heptanone, 4-methyl-2-nonanone, 2-dodecanol, 2-dodecanone, 3-decene 1-ol acetate, benzyl alcohol, phenylethyl alcohol, 2-methoxy 4-vinylphenol, 3-decene 1-ol acetate, 2-dodecanone, 2-dodecanol, or 2-methoxy 4-vinylphenol.

In some embodiments, the improved flavors are due to the decreased levels of volatile flavor compounds, such as, e.g., benzaldehyde, 2-methyl-2-propanol, acetophenone, octane, ethanol, 2-pentanone, pentanal, 2 heptanone, 1-butanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-2-noonanol, 2-nonanone, 1-octanol, 2-undecanone, 2-octene-1-ol (Z), 1-octene-3-ol, acetophenone, 4-methyl-2 heptanone, nonanal, acetic acid, 3-methyl furan, 2-methyl furan, 1-hexanal, furan, 2-methyl-2-propanol, pyrazine, 1-heptanal, 2-ethyl furan, 2-pentyl furans, or 1,3 butanediol.

In some embodiments, the method further comprises preparing a cultured non-dairy product such as a cheese replica, yogurt, sour cream, or crème fraiche with a controlled flavor profile, by the controlled addition of defined combinations of flavor additives, described herein, to the non-dairy e source at any time point of the replica making process. Exemplary additives and specific combinations are described herein.

Flavor Generators

Control of flavor by addition of bacteria/microbes

Flavor compounds can be generated by microbes in the non-animal derived material used for producing many different non-dairy products described herein, including cheese replicas. The methods of flavoring generally include contacting a non-dairy milk or protein solution with one or more microbes, and preparing a cultured non-dairy product from the non-dairy milk. Microbes such as bacteria, yeast, or mold can be used to create a product with a desired flavor profile or be used as a component of the flavor in a product, as bacteria can create desirable flavors (e.g., buttery, creamy, dairy, or cheesy) in a neutral, planty, or beany product.

Exemplary non-dairy milks are described herein. Any of the non-dairy cheese milks or combinations thereof may be contacted with one or more microbes (e.g., a controlled amount of bacteria) to control the flavor of a resulting cultured non-dairy product such as a cheese replica. The microbes can be selected from bacteria, yeast, or molds. The bacteria can comprise mesophilic and/or thermophilic bacteria. The bacteria can comprise bacteria from a commercial starter. Exemplary commercial starters are described herein.

Flavor production in the replicas can be controlled by the use of one or more microbes e.g., one or more bacteria, yeast, or molds, including but not limited to Flavor production in the replicas can be controlled by the use of one or more microbes e.g., one or more bacteria, yeast, or molds, including but not limited to *Lactococcus* species such as *Lactococcus lactis lactis (LLL, used alone or as a component of commercial mix MA11), Lactococcus lactis cremoris (LLC, used alone or as a component of commercial mix MA11),* or *Lactococcus lactis biovar diacetylactis (LLBD, often used as commercial culture MD88*), a *Lactobacillus* species such as *Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei,* or *Lactobacillus rhamnosus,* a *Leuconostocaceae* species such as *Leuconostoc mesenteroides cremoris(LM),* a *Streptococcus* species such as *Streptococcus thermophiles (ST, often used as commercial culture TA61)* a *Pediococcus* species such as *Pediococcus pentosaceus,* a *Clostridium* species such as *Clostridium butyricum,* a

Staphylococcus species such as *Staphylococcus xylosus (SX),* a *Brevibacterium* species such as *Brevibacterium linens*, a *Propioniibacteria* species, a *Penicillium* species such as *Penicillium candidum*, *Penicillium camemberti*, or *Penicillium roqueforti*, a *Debaryomyces* species such as *Debaryomyces hansenii*, a *Geotrichum* species such as *Geotrichum candidum*, a *Corynebacteria* species, a *Verticillium* species such as *Verticillium lecanii,* a *Kluyveromyces* species such as *Kluyveromyces lactis,* a *Saccharomyces* species such as *Saccharomyces cerevisiae,* a *Candida* species such as *Candida jefer* or *Candida utilis,* a *Rhodosporidum* species such as *Rhodosporidum infirmominiatum,* a *Micrococcus* species., a *Halomonas* species., a *Psychrobacter* species. In some embodiments, lactic acid bacteria such as *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* or *Streptococcus* are used. In some embodiments, the bacteria do not comprise *Lactobaccilius acidophilus* strains. In some embodiments, a yeast such as *Saccharomyces cerevisiae*, *Kluveromyces lactis* and/or *Debaromyces hansenii* can be used. In some embodiments, a mold can be *Penicillium candidum*, *Penicillium camemberti*, *Penicillium roqueforti*, *Geotrichum candidum*, or a combination thereof.

In some embodiments, one or more of the follow microbes are used: *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus,* and *Brevibacterium linens*.

The one or more microbes can be cultured alone (e.g., bacteria, yeast, or mold alone), or in combination with two ore more microbes (e.g., two different bacteria, two different yeast, two different molds, a bacteria and a yeast, a bacteria and a mold, or a yeast and a mold). When two or more microbes are used, the microbes can be co-cultured or sequentially cultured, i.e., one microbe can be cultured for a length of time before adding another microbe. Particular good combinations for flavor generation in replicas are pre-culturing with SX, followed by either TA61 or MD88, or MD88 co-cultured with MA11.

The growth conditions of microbes also can control flavor generation in replicas. The temperature of microbes growth ranging from 4°C to 45°C can control the amount and type of flavor compounds produced in replicas. The amount of aeration by shaking

(e.g., 0 to 300 rpm) changes the flavor productions of many different bacteria in non-dairy media. Greater aeration during culturing by either SX, TA61, or MD88 generates more desired cheese and buttery compounds. Aeration also decreases the undesired flavor compounds. Desired cheese compounds such as 2-heptanone increase when SX, MD88, or TA61 are cultured with aeration. MD88's production of hexanoic methyl ester in cheese replicas is also modulated by aeration. An increase in aeration of SX during culturing in soymilk greatly increases 3-methyl and 2-methyl butanoic acid production and decreases the amounts of undesirable aroma compounds such as 2-ethyl furan or 2-pentyl furan in cheese replicas.

The amount of time the one or more microbes is cultured also can modulate the amount and types of flavor compounds. Culturing can range from 1 hour to multiple days. In some embodiments, one or more microbes and the non-dairy milk are incubated together for a length of time ranging from 1 min-60 minutes, 0.5-5 hours, 3-10 hours, 6-15 hours, 10-20 hours, or more than 20 hours. Most buttery compounds are created within the first 10 hours, while additional cheese compounds typically require 24-48 hours or more hours. Butyrolactone, a creamy, milky note compound is created in non-dairy media by MD88 and MA11 only after 20 hours of culturing in soymilk.

The one or more microbes also can be added at different inoculums, e.g., 10^2 - 10^9 cfu/mL or even greater. The phase of growth (i.e, stationary phase versus exponential phase) and the cell density of the bacterial culture affect the flavor compound profile of the medium. Higher inocula of a starter culture can protect the replica from unwanted microbial contamination (e.g., bacterial contamination). Therefore, an inoculum of 10^6 - 10^9 cfu/mL is usually used.

Flavor production by the one or more microbes also can be modulated by directing the metabolic pathways, e.g., by modulating their nitrogen source, carbon source, additional available nutrients, and growth conditions. Non-limiting examples of additives that can be used are shown in Table A. The additives can be added to the media at the same time as the bacteria or any time during the creation of the replica. When sequential culturing occurs additional additives can be added at the same time that the additional strains are inoculated.

TABLE A

Additives used to control flavor production by microbes in non-dairy replicas

$FeCl_2$	Asp	C8:0, Caprylic acid
$MgCl_2$	Cys	C10:0, Capric acid
$CaCl_2$	Glutamine	C12:0, lauric acid
$MnSO_4$	Glutamate	C14:0, myristic acid
$CoCl_2$	Gly	C16:0, palmitic acid
$CuSO_4$	His	C18:0, stearic acid
$ZnSO_4$	Ile	C16:1, palmitoleic acid
Adenine	Lue	Coconut Oil
Guanine	Lys	Castor Oil
Inosine	Met	Palm Oil
Uracil	Phe	Palm Fruit Oil
Xanthine	Pro	Jojoba Oil
Pyridoxamine	Ser	Sunflower Oil
Pyridoxine	The	Mango Butter
D-sorbitol	Trp	Ala
Citric Acid	Tyr	Arg
Lactic Acid	Val	Asn
α-ketoglutarate	Riboflavin	FAD
Pyruvic Acid	Thiamine	NAD
Ororic acid	Lipoic Acid	Biotin
oxalic acid	Nicotinic acid	Pantothenate
Ascorbic Acid	CoA hydrate	B12
Succinic acid	propanoic acid	Folic Acid
p-aminobenzoic acid	C4:0, butyric acid	
DL-Malate	C6:0, caproic acid	

The amount and type of sugar is a large driver of the type of flavors produced, including buttery compounds. In some embodiments, sugars are naturally present in the non-dairy milk. By way of example only, sucrose is present in a variety of nuts such as, e.g., almonds, which can be used to provide a non-dairy milk used for preparing the cheese replica. In some embodiments, a controlled amount of one or more sugars is added to the non-dairy milk or non-dairy cheese source.

In some embodiments, the sugar is a monosaccharide, including but not limited to glucose (dextrose), fructose (levulose), galactose, mannose, arabinose, xylose (D- or L-

xylose), and ribose, a disaccharide including but not limited to sucrose, lactose, melibiose, trehalose, cellobiose and maltose, a sugar alcohol such as arabitol, mannitol, dulcitol, or sorbitol, sugar acids such as galacturonate, glucuronate, or gluconate, oligosaccharides and polysaccharides such as glucans, starches such as corn starch, potato starch, pectins such as apple pectin or orange pectin, raffinose, stachyose and dextrans, a plant cell wall degradation product, β-galactosides, β-glucosides such as salicin, and/or sugar derivatives such as N-acetylglucosamine. In particular embodiments, the sugars are chosen from the group consisting of sucrose, maltose, glucose and fructose.

The relative growth of each isolated strain can be controlled by the addition of said one or more sugars. The addition of said one or more sugars can result in nondairy cheese replicas with significantly improved texture and flavor. A user, e.g., an individual or plurality of individuals practicing the invention, can select specific isolated strains and add controlled amounts of the selected strains to create a non-dairy cheese replica with a desired flavor and texture profile. A user can additionally select specific sugars and add controlled amounts of the selected sugars along with controlled amounts of specific isolated strains to create a non-dairy cheese replica with a desired flavor and texture profile. Table B provides non-limiting examples of bacteria culturing conditions for producing dairy flavor notes created in non-dairy cheese or other non-dairy products.

TABLE B

Genus	species	sub species	growth temperature	Aeration	sugar preference (based on generation time)
Lactococcus	*lactis*	*cremoris*	30C (25-40C)	yes	**glucose**=fructose >maltose >sucrose*
Lactococcus	*lactis*	*lactis*	30C (25-40C)	yes	**glucose**=fructose=sucrose >maltose*
Leuconostoc	*mesenteroides*	*cremoris*	30C (25-35C)*	yes	**glucose**=fructose=maltose >sucrose
Lactococcus	*lactis*	*biovar diacetylactis*	30C (25-40C)	yes	**glucose** >fructose=sucrose=maltose
Streptococcus	*thermophilus*		37C	no	**glucose** >maltose
Lactobacillus	*delbrueckii*	*lactis*	37C	no	**glucose** >maltose
Lactobacillus	*delbrueckii*	*bulgaricus*	37C	no	**glucose** >maltose
Lactobacillus	*helveticus*		37C	no	**glucose** >maltose
Lactobacillus	*plantarum*		37C	no	**glucose**=maltose
Lactobacillus	*casei*		37C	no	**glucose** >maltose
Lactobacillus	*rhamnosus*		37C	no	**glucose** >maltose
Staphylococcus	*xylosus*		30C	yes	**glucose** >maltose
Pediococcus	*pentosaceus*		30C		**maltose** >glucose
Clostridium	*butyricum*		37C	no	**glucose** >maltose

* Large variation in different strains of this subspecies

The effect of different sugars and microbes on flavor production also depends on the type and composition of the starting material (e.g., the starting material can include any non-animal derived material, including but not limited to soymilk, pea protein, moong protein, soy protein, coconut milk, yeast extract, protein hydrolysate, derived media, and synthetic media), and the amino acid composition of the proteins in the starting materials, the types of sugars and carbohydrates included, and the types of fats, triglycerides, and/or free fatty acids present. The amino acid composition of the protein can be broken down by enzymes, such as those created by the microbes and those added as part of the recipe, and the resulting amino acids or peptides can serve as precursors to particular flavor molecules. Synthetic media refers to using ammonium for the nitrogen source, and a defined sugar for the carbon source, with any other additives. The starting material can comprise isolated purified proteins, or crude plant extracts.

Acetoin and diacetyl ("buttery" compounds) are created by MD88 (LLBD) in the greatest abundance in a yeast extract medium with maltose added. On the other hand MD88 creates more of these buttery compounds in soymilk with glucose added. Acetoin and diacetyl are created in even higher amounts by MD88 and TA61 (ST) with the addition of citrate or pyruvate. Acetoin/diacetyl and 2,3-hexandione concentrations all increase in response to increased citrate concentration in cheese replicas made with strain MD88.

The addition of amino acids (see Table A) can directly control the production of particular flavor compounds in non-dairy replicas. The creation of these flavor compounds contributes to the overall flavor profile of the replica. Methionine can be added to cheese replica or media to produce methional by SX or dimethyl trisulfide by *Brevibacterium*. Methional and dimethyl trisulfide are two sulfur compounds that are found in many dairy cheeses, and contribute to the aged character of cheddar. Leucine added to soymilk, yeast extract media, or pea proteins significantly increase the 3-methyl butanoic acid production by SX bacteria, adding cheesy notes. Adding multiple compounds can further control flavor production by bacteria, e.g. alpha-ketoglutarate with leucine increases the 3-methyl butanoic acid production by SX bacteria. 3-methyl butanoic acid has a cheesy aroma and taste, it is similar to butanoic acid, which is a key flavor in American and cheddar cheeses. Organic acids can also control flavor production

by bacteria in non-dairy replicas. Oxalate added to yeast extract media and cultured by *Brevibacterium* creates cheese compounds butanoic acid, 3-methyl butanoic acid, and 2,6-nonadienal, and the flavors generated were described as "aged cheese". The creation of buttery compounds can be controlled by the addition of citrate, xanthine, and pyruvate, when added to cultures of TA61. Adding citric acid, pyruvate, riboflavin, and copper can affect the cheese and buttery flavor production by MD88. Other important aroma compounds like butanoic acid derivatives and 2-heptanone are produced when isoleucine, proline, alanine, malate, inosine, Fe^{2+}, Mg^{2+}, serine, and thiamine are added into replicas. Additionally, $ZnSO_4$, citric acid, lactic acid, pyruvic acid, succinic acid, malic acid, aspartic acid, lysine, tyrosine, valine, and jojoba oil allow for the creation of desired cheese flavor compounds by TA61 in replicas. The affect of each additive depends on the composition of the other stating materials.

Other additives can be added before bacteria culturing or after culturing. These include but are not limited to fruit extracts (0.0001% -0.2% wt/vol), fruit puree (peach, pineapple strawberry, mango, papaya, plum, etc) (0.001% -2% wt/vol), vegetable puree (potato, yam, onion, garlic, or broccoli) (0.001% -2% wt/vol), molasses (0.001% -2% wt/vol), yeast extracts (0.001% -2% wt/vol), protein hydrolysates (0.01% -10% wt/vol), red or white miso (0.01% -2% wt/vol), coconut milk (0.5%-60%), malt extracts (0.01% -2% wt/vol), or coconut cream (0.5%-60%), and combinations thereof. Malt extracts and molasses can add cheese flavor molecules like 3-methyl butanal, 2-methyl butanal, 2-heptanone, butanoic acid, or butyrolactone. Replicas with the addition of peach extract or puree were described by trained flavor scientists as having more cream flavor. Replicas with the addition of papaya were described as more cheesy by trained flavor scientist and had an increase in 3-methyl butanoic acid by GCMS. Red miso added to MD88 cultures, and white miso added to TA61 cultures resulted in improved flavor complexity and a perceived decrease in astringency by a trained flavor scientist.

Yeast extract can control bacteria growth and flavor generation, and contribute different starting flavors. There are many vitamins in yeast extracts that improve the growth of TA61 in replicas. All yeast extracts are not the same. TA61 growth was greater with Flavor House Flavor Spark, and BioSpringer Yeast Extract 2020, compared to other yeast extracts. BioSpringer Yeast Extract 2020 supports good growth and decent

flavor development for MD88 and TA61. Yeast extract can be added between 0.01% -2% wt/vol of media to improve growth and flavor production by the bacteria. Yeast extract can be added at 0.02%-0.1% improve growth and flavor production by the bacteria. Yeast extracts themselves can also provide certain flavors to the product including, brothy, whey, nutty, savory, roasted, malty, caramel, cooked-milk, light-sulfurous, and slight cheese-like. Yeast extracts also supports the production of flavor compounds by TA61, MA88, and SX that lead to a more cheesy and complexly flavored replicas.

In some embodiments, the one or more microbes, the non-dairy cheese source, and the one or more optional components that can be used to alter flavor (e.g., sugars, fats, carbohydrates, vitamins, organic acids, nucleotides, or food products) are incubated together for a sufficient period of time to achieve a desired pH. The pH can range from pH 3-5, 4-6, or 4.3-5.7. The desired pH can be pH 6 or lower, pH 5 or lower, or pH 4 or lower. Culturing the material by bacteria in some cases decreases the pH to 6.5, 6, 5.5, 5, 4.5, 4, or 3.5, while in other cases, flavors are generated with no change in pH. Culturing with *Lactococcus, Lactobacillus, Leuconostoc, Pediococcus* and/or *Streptococcus* typically results in a decrease in pH with most starting material, while culturing with *Staphylococcus, Brevibacterium,* and/or *Clostridium* typically has little or no effect on the pH.

In some embodiments, the method further comprises solidifying said non-dairy cheese source. Methods of solidifying are described herein.

In some embodiments, the method comprises isolating a plurality of microbial strains from a heterogeneous population, e.g., a commercial starter or a probiotic (e.g., Rejuvelac). In some embodiments, the isolated microbial strains are each characterized according to defined criteria. The defined criteria may include, for example, growth rates in non-dairy cheese sources comprising different sugars added therein. The defined criteria may include the contribution of each isolated strain to a flavor palette by characterizing flavor notes from a cheese replica with the isolated strain added therein as compared to a control cheese replica without the isolated strain added therein. The isolated microbial strains may be characterized by genetic sequencing, and/or determining sequences unique to each of the isolated strains.

In some embodiments, the method further comprises preparing a cheese replica with a controlled flavor profile, by the controlled addition of specific combinations of isolated strains to provide a desired array of flavor notes to the cheese replica.

In another aspect, the invention provides a library of isolated microbial strains. In some embodiments, the isolated strains in the library are selected to provide a palette of flavor profiles to non-dairy cheese. In some embodiments, the isolated microbial strains are bacterial strains. In some embodiments, the isolated strains are isolated from a commercial starter culture, e.g., a bacterial culture that is commercially available. In some embodiments, the commercial starter culture is a mesophilic bacterial culture. In some embodiments, the commercial starter culture is a thermophilic bacterial culture. Exemplary commercial starters include, e.g., MA11, MA14, MA19, LM57, MA4002, MM100, TA061, LH100, MD88, and Flora Danica.

Strains can be isolated by plating commercial mixes on selective or non-selective growth media, e.g., Reddy's selective agar, LB agar. In some embodiments, single bacterial cells of an individual strain will grow into discrete colonies on said growth media. In some embodiments, individual colonies can be screened by PCR. In some embodiments, PCR can involve the use of universal primers containing sequences that are common to all strains of a microbial species, or can involve the use of primers comprising sequences that are unique to particular subspecies of the microbial population. PCR products can be sequenced and the sequences compared to known sequences, e.g., in Gen Bank, and also to one another. pH profile, sugar fermentation, phenotype on Reddy's selective agar and more extensive sequencing can be carried out to help further identify and characterize individual strains.

In some embodiments, the bacterial strains are selected to provide to provide an array of flavors and textures, or other defined characteristics, to a non-dairy milk cheese. In dairy milks, LLL strains typically grow more quickly and rapidly acidify the milk, while LLC strains grow more slowly and provide more flavor. In dairy milks, both LLBD and LM contribute additional flavor compounds, notably diacetyl, which gives a buttery taste to the cheese. One or more isolated bacterial strains, e.g., LLL, may be selected to acidify a non-dairy milk quickly, e.g., a drop in pH within one hour, or an overall drop in pH from 6.3 to 4.3 in less than 15 hours. One or more isolated bacterial

strains, e.g., LLC, may be selected for a slower growth profile with greater production of flavor compounds and a less dramatic lowering of pH, e.g., from 6.3 to only 5.4 in 15 hours. Such a slowed growth rate may serve to, e.g., provide a cheesemaker greater control over the flavoring process and more tightly regulate the resulting taste profile of the non-dairy cheese replica. One or more isolated bacterial strains may be used to provide different flavor profiles to a non-dairy cheese. The different flavor profiles may be characterized by the release of specific volatile chemicals from the replica. For example, some bacterial strains, e.g., LLBD and LM, may be used to produce diacetyl when contacted with a non-dairy milk or protein solution. The diacetyl may produce a buttery flavor in the resulting non-dairy cheese.

It was found that single use direct vat cultures, such as MA11 and Flora Danica (FD), provide a fairly uniform but limited characteristic flavor and texture profile when used to make soft fresh (SF usingMA11) and soft ripened (SR using MA11 & FD) cheeses. To allow a much greater array of flavor and texture capability, individual bacterial strains were isolated and characterized from commercial preparations, e.g., Flora Danica and MA11. These isolated strains can be combined in new and varied combinations and in various proportions to create a much greater range of flavor and texture possibilities.

In general, the dominant sugar in dairy milk is lactose, which is not present in non-dairy milks. When controlled amounts of bacteria, e.g., controlled amounts of one or more isolated isolated strains of the invention are incorporated in non dairy cheese replicas that comprise one or more sugars, specific combinations of sugars and bacterial strains can alter the taste and texture profiles of the resulting non-dairy cheese replicas in unexpected ways. Such specific combinations can be used to prepare cheese replicas with a controlled flavor profile, e.g., to prepare cheese replicas that accurately mimic the flavor of specific dairy cheeses such as, by way of non-limiting example only, process cheese, swiss cheese, string cheese, ricotta, provolone, parmesan, muenster, mozzarella, jack, manchego, blue, fontina, feta, edam, double Gloucester, camembert, cheddar, brie, asiago and Havarti.

The desired flavor and texture profile can be selected to mimic the flavor and texture of a specific dairy cheese. By way of example only, by selecting and adding a

controlled amount of one or more isolated bacterial strains of the invention and optionally selecting and adding a controlled amount of one or more sugars, a user can create a non-dairy cheese replica that mimics the flavor and texture profiles of e.g., process cheese, swiss cheese, string cheese, ricotta, provolone, parmesan, muenster, mozzarella, jack, manchego, blue, fontina, feta, edam, double Gloucester, camembert, cheddar, brie, asiago and Havarti.

The texture and flavor profile of the non-dairy cheese can be ascertained by any method known in the art or described herein.

To generate dairy-like flavor in replicas or other non-dairy products, or a flavoring solution/paste to add to non-dairy products, particular stains can be used to generate cheesy, buttery, creamy, milky or other desired flavor compounds. See Table C for non-limiting examples of dairy flavor compounds that can be produced. Table C also provides examples of how to create the indicated flavor compound in non-dairy replicas.

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TABLE C

Compounds	Smell type	Bacteria to create	Additives, starting material
2-methyl butanal	cocoa, coffee, nutty	TA61	Calcium ions, proline in yeast extract media
3-methyl butanal	chocolate, peachy, fatty	SX with MD88	Pea Vicilin with coconut milk and yeast extract
acetoin	sweet buttery, creamy, dairy milky, fatty	MD88 or TA61	soy milk (plus pyruvate)
		MD88	yeast extract media plus maltose
		MD88 or TA61	xanthine, citrate, pyruvate in yeast extract media
diacetyl	buttery, creamy, milky	MD88 or TA61	xanthine, citrate, pyruvate yeast extract media
		TA61 or MD88	soy milk (plus pyruvate)
2,3-hexanedione	Creamy, buttery, fruity, caramellic	MD88	citrate with <50 mM glucose in yeast extract media
Hexanoic acid methyl ester	fruity pineapple	MD88	yeast extract media
2-methyl butanoic acid	Fruity, acidic with a dairy buttery and cheesey nuance	SX	Valine + >20 mM glucose
3-methyl butanoic acid	Cheesey, dairy, creamy, fermented, sweet, waxy	SX	Leucine + >20 mM glucose (alpha-ketoglutarate gives a further increase)
		Brevibacterium	Oxalate + yeast extract media
butyrolactone	Milky, creamy with fruity peach-like afternotes	MD88 or TA61	>20 hours culturing soymilk

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methional	musty tomato, potato, mold ripened cheeses	MD88	Methionine added to soymilk, Pea vicilin or yeast extract
dimethyl trisulfide	sulfurous, savory	brevibacterium	Methionine
butanoic acid	cheesy, dairy, creamy, sharp	*Clostridium Butyricum*	starches, corn steep liquor cultured in anaerobic
		Brevibacterium	Oxalate + yeast extract media
		SX	Pea Vicilin + coconut milk + yeast extract
2-heptanone	cheese, fruity, coconut	MD88 or TA61	soymilk no added sugar with shaking
2-undecanone	waxy, fruity with creamy cheese like notes	MD88	Methionine or Leucine added to yeast extract media
2-nonanone	green, herbal, cheesy, fresh	MA11	$CuSO_4$ or Alanine in yeast extract media
propanoic acid	acidic and dairy-like	*Brevibacterium*	Oxalate + yeast extract media
2-methyl propanoic acid	rancid butter	*Brevibacterium*	Oxalate + yeast extract media
		SX	Valine + yeast extract media
hexanoic acid	sour fatty sweat cheese	SX	> 5 mM glucose in yeast extract media with coconut oil
octanoic acid	fatty waxy rancid oily vegetable cheesy	SX	> 5 mM glucose in yeast extract media with coconut oil
ethyl butanoate	Fruity, sweet		
2-butanone	acetone-like, fruity, butterscotch	TA61	Ile, thiamine, Magnesium ions, or ascorbic acid in yeast extract media

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Acetic acid	sour	SX	< glucose in all systems
decanoic acid	rancid sour fatty goaty	SX followed by MD88 or TA61	Pea vicilins +coconut milk (with yeast extract)
Methyl Isobutyl Ketone	herbal, fruity and dairy nuances	SX followed by MD88	Pea vicilins +coconut milk (with yeast extract)
gamma-octalactone	sweet ,coconut, waxy, creamy, tonka, dairy, fatty	SX+TA61	Pea vicilins +coconut milk (with yeast extract)
delta-octalactone	Sweet, fatty, coconut, tonka, tropical dairy	SX+TA61	Pea vicilins +coconut milk (with yeast extract)
gamma-nonalactone	coconut, creamy, waxy, fatty milky	SX + MD88	Pea vicilins +coconut milk (with yeast extract)
5-Hydroxy-4-octanone	buttery	SX followed by TA61	soymilk
Ethyl octanoate	sweet, waxy, fruity and pineapple with creamy, and fatty	SX + MD88	yeast extract media with coconut milk
2-ethyl-1-Hexanol	sweet, fruity, fatty	TA61	Vitamin B12, Riboflavin, Thiamine, CoenzymeA, or Ketoglutarate in yeast extract

Case No.: IPR2023-00206
U.S. Patent No. 10,039,306

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It will be appreciated that many different types of bacteria can make buttery compounds. MD88 and TA61 are both very good at producing buttery compounds in non-dairy media and cheese replicas. They both can create the buttery compounds 2,3-butandione, acetoin, and 2,3-hexanedione in soymilk, purified pea proteins, purified soy proteins, purified moong proteins, and yeast extract medias. Additionally, LLC, LLL, and SX also can create buttery compounds in replicas. Additionally, greater refinement of flavor can come from controlling each concentration of the butter compound; in soymilk MD88 produces more acetoin while TA61 produces more diacetyl.

In some embodiments, buttery notes can be enhanced by selecting particular strains (e.g., LLBD, LM, LF2, LF5, and *Streptococcus thermophilus*) and/or selecting particular sugars to use (e.g., glucose, fructose, or sucrose). In some embodiments, the buttery notes are associated with increased levels of volatile flavor compounds. The volatile flavor compounds can be, e.g., acetoin, 2,3-butanedione, and butanoic acid. In some embodiments, the volatile flavor compounds can be, e.g., 2-heptanone, nonanal, butanol, 1-hexanol, 2-heptanone, 4-methyl, Ethyl acetate, or 2-nonanone.

In some embodiments, buttery notes can be decreased by selecting and adding a controlled amount of LM.

In some embodiments, said buttery flavor can be decreased by selecting one or more sugars from the group consisting of glucose, fructose, sucrose and maltose.

SX and *Brevibacterium* are particular good at generating cheesy compounds, including 3-methyl-butanoic acid, 2-methyl butanoic acid, and 2-methyl propanoic acid. SX and *Brevibacterium* can also be used to generate free fatty acids, including butanoic acid, propionic acid, dodecanoic acid, undecanoic acid, nonanoic acid, octanoic acid, and hexanoic acid, when cultured in the presence of fats. When SX is cultured in the presence of coconut oil, short and medium chain length free fatty acids are created. TA61 can also created additional types of cheese flavor compounds including 2-heptanoic and 2,4-heptadienal in non-dairy replicas. There are sulfur compounds including but not limited to dimethyl trisulfide and methional that are important in providing characteristic flavor to particular cheeses that can be generated in non-dairy medias by SX and *Brevibacterium*.

Other bacterial cultures, including LR, can drive floral flavor production and creamy notes can be created in replicas by LBB. In some embodiments, fruity notes are enhanced by selecting strains from the group consisting of LLBD, LM, LLL, LLC, LF2 , LF5, and *Streptococcus thermophilus* strains. In some embodiments, fruity notes are enhanced by selecting sugars from the group consisting of glucose, fructose, sucrose, and maltose.

In some embodiments, sour notes are enhanced by selecting strains from the group consisting of LLBD and LM strains. In some embodiments, sour notes are enhanced by selecting sugars from the group consisting of glucose, maltose and sucrose. In some embodiments, sour notes are associated with increased levels of volatile flavor compounds. The volatile flavor compounds can be, e.g., acetic acid, 2-methylbutanoic acid, hexanoic acid, propionic acid, and octanol.

In other embodiments, sour notes are enhanced by selecting strains from the group consisting of LLL, LLC, and LM. In particular embodiments, sour notes are enhanced by selecting sugars from the group consisting of glucose and sucrose. In some embodiments, the sour notes are characterized by increased levels of volatile flavor compounds. The volatile flavor compounds can be, e.g., nonanal or butanoic acid.

In some embodiments, floral notes can be enhanced by selecting strains from the group consisting of LLL, LLC and LM. In some embodiments, floral notes can be enhanced by selecting sugars from the group consisting of glucose, fructose, maltose and sucrose. In some embodiments, the floral notes are due to increased levels of volatile flavor compounds, e.g., nonanol.

In some embodiments, sweet notes are enhanced by selecting a LLBD strain. In particular embodiments, sweet notes are enhanced by addition of sugars. In some embodiments, sugars are added to a final concentration of 0-150 mM. In some embodiments, sugars are added to a final concentration of about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 mM. In some embodiments, the sugars are glucose, maltose, fructose or sucrose.

In some embodiments, the sweet flavor of non dairy cheeses comprising LLC, LLL, LM and LLBD can be enhanced by addition of sugars. The sugars can be, e.g., glucose, maltose, fructose, sucrose, or any combination thereof. In some embodiments,

the increased sweet flavor is due to increased levels of volatile flavor compounds. In some embodiments, the volatile flavor compound is butanoic acid.

In some embodiments, strains can be selected to enhance citrus flavor notes of non-dairy cheeses. In some embodiments, enhanced citrus flavor notes are due to increased levels of volatile flavor compounds, e.g., nonanal, limonene, and 1-octanol.

In some embodiments, strains can be selected to enhance mushroom flavor notes of non-dairy cheeses. In some embodiments, enhanced mushroom flavor notes are due to increased levels of volatile flavor compounds, e.g., 1-octene-3-ol, 1-hexanol, and 1-heptanol.

In some embodiments, the addition of bacteria decreases and or masks undesirable flavors including soy flavor, beany flavor, planty flavor, grassy flavor, and astringency. As described herein, the addition of SX decreased or masked the "soy" and "green" flavor and aroma of the replicas; the addition of leucine to the SX culture caused an even larger reduction in the "soy" notes. Growth of MD88 in replicas quickly decreases benzaldehyde, an off taste in soymilk. The soy (green, cereal) aroma present in soymilk and other plant derived material decreases as the coconut milk percentage increases in the cultured material. Characteristic undesirable aromas: Pentenols, Pentanol, 2-pentyl-furan, and 1-Hexanol, also decrease with an increasing coconut milk percentage. Samples were evaluated and compared by a 2-6 members of a trained sensory panel.

A limitation of currently available nut milk-based cheeses is the presence of undesirable nutty flavor in the cheese. The nutty flavor can detract from the taste profile of the cheese and make the cheese seem non dairy-like. Therefore, in one aspect, the invention provides a nut-milk based cheese replica with a reduced or undetectable nutty flavor, and methods of making the same. In some embodiments, the method comprises contacting a nut milk with a controlled amount of *Lactococcus* bacteria and a controlled amount of a sugar. In some embodiments, the nutty flavor is reduced by the selection of one or more isolated strains described herein. In some embodiments, the nutty flavor is reduced by the selection of one or more isolated strains selected from the group consisting of LLL, LLC, LM, and LLBD. In some embodiments, the nutty flavor is reduced by selecting sugars from the group consisting of glucose, fructose, sucrose and

maltose. In some embodiments, the reduced nutty flavor is associated with decreased levels of volatile flavor compounds. In some embodiments, said flavor compounds are benzaldehyde or 2-methyl-2-propanol.

The non-dairy cheese culture can be made from different sugar sources including but not limited to glucose, fructose, maltose, sucrose, and or galactose. The cultured material can be made into a solid like cheese or used as a liquid cultured material.

Replicas can also be flavored by adding artificial or natural flavors in either as a single compound or as complex product mixtures. Fatty acids (between 0.001%-0.5%) can be added to replicas, and these replicas are described by trained flavor scientists as being more cheesy with the addition of fatty acids. Compounds added to the replica cheese include but are not limited to 2,3 butanedione, acetoin, butanoic acid, 5,6 decenoic acid, y-heptalactone, y-hexalactone, y-octalactone, y-decalactone, y-nonanoiclactone, y-undecalactone, δ-decalactone, δ-dodecalactone, δ-nonanoic lactone, δ-octalactone, gamma-nonanoic lactone, gamma-undecalactone, gamma-decalactone,delta-tetradecalactone, S-methyl thiopropionate, delta-tridecalactone, delta-tetradecalactone, δ-tetradecalactone, butyl butyryllactate, isovaleric acid, 2-undecanone, valeric acid, 2-heptanone, 2-methyl butyraldehyde, 2-nonanone, 2-methyl butanoic acid, decanoic acid, methional, octanoic acid, 2-methyl butanal, 3-methyl butanal, ethyl-butanoate ester, hexanoic acid, and octanoic acid. Complex mixtures include but are not limited to coconut cream, yeast extract, molasses, fruit extracts, masking agents, cream flavor boosters, and miso. Flavor compounds can increase the cheesy, buttery, malty, creamy, coconut, milky, whey, and fruity taste. When present in cheese replicas these compounds increase the preferences of the cheese replica, and can be described by trained flavor scientists as more cheesy, more buttery, more creamy, more complex and with an increase in dairy notes has described by trained flavor scientists. The addition of flavor compounds can also decreases the off notes, including but not limited to planty, beany, nutty and sour notes in replicas. The concentrations of flavor compounds added to the replicas can be from 0.1 % to 0.000001% vol/wt of the final cheese replica. Complex flavor mixtures can be added from 10% to 0.01%. Flavor compounds can be added to cheese replicas made from bacterial cultured milks or other starting materials or acid coagulated milks or other starting materials. The flavor compounds added to the replicas

can be complementally to the flavors generated by the microbes. The flavor compounds can also be added to the non-dairy milk before coagulation, so the bacteria can use the flavor compound to generate additional compounds.

Control of Flavor and Texture Using Enzymes

One or more enzymes can be used alone or in combination any one the culturing methods and additives described to help modulate the flavor, texture, and/or melting profile, comprising contacting a non-dairy cheese source with one or more isolated and purified enzymes. The enzymes can be added before solidification, after solidification but before the whey is drained, or after whey is drained. Surprisingly, adding trace amounts of one or more isolated and purified enzymes (e.g., proteases, lipases, and/or amylases) greatly enhanced the texture, flavor, and/or meltability of the resulting non-dairy cheese replica, as determined by blind taste test or by the detection of volatile odorants by, e.g., GCMS. Using such enzymes also controls flavor production by microbial cultures (e.g., when soymilk is pre-treated with amylases, TA61 produces much more diacetyl).

In the nut milk cheeses or other non-dairy cheese replicas, the presence, type, amount, and the timing of addition of the protease can control the flavor profile as has determined by blinded taster testers and GCMS. By way of example only, plant based cheese replicas with proteases were judged as having a more liked flavor profile, a more complex flavor profile, a flavor profile that tasted more like dairy cheese, and in some cases indistinguishable to dairy cheese. In some cases plant based cheese replicas with lipases were judged as having more liked flavor profile, a more complex flavor profile, and a flavor profile that tastes more like dairy cheese. In some cases plant based cheese replicas with lipases and proteases were judged as having more liked flavor profile, a more complex flavor profile, and a flavor profile that tastes more like dairy cheese.

Particular proteases or combinations of proteases and lipases created distinct flavor profile that tasters described as particular types of dairy products. The addition of proteases also controls particular flavor notes, including but not limited to buttery, sweet, fruity, floral, nutty, and sour. In the cheese replicas the presence, the type, the amount, and the timing of addition of the protease can control buttery flavor as determined by

blinded taste testers and GCMS. The addition of proteases to non-dairy cheese replicas, particularly papain and aspartic protease, are significantly more buttery than the same non-dairy cheese without any proteases, as determined by blind taste testers. This is supported by GCMS data that shows the addition of proteases can greatly increase the production of compounds that create the buttery flavor in dairy cheeses, including diacetyl and acetoin. The contribution of specific added proteases and/or lipases to a flavor note profile of a non-dairy cheese replica can be ascertained using any of the methods described herein, e.g., taste test and/or GCMS.

In some embodiments, the enzyme is aspartic protease. In some embodiments, the enzyme is papain. In some embodiments, the enzyme is not rennet. In some embodiments, the enzyme is a protease or peptidase. In general, a protease or peptidase is an enzyme that conducts proteolysis, that is, catalyzes the hydrolysis of peptide bonds that link amino acids together into peptide chains. The protease can be a serine protease, a threonine protease, an asparagine protease, a mixed protease, a cysteine protease, an aspartate protease, or a metalloprotease. The protease can be an exopeptidase, e.g., an aminopeptidease or carboxypeptidase, or the protease can be an endopeptidase, e.g., a trypsin, a chymotrypsin, pepsin, papain, cathepsin G, or elastase. The protease can be any protease selected from the group consisting of pepsin A, nepenthesin, walleye dermal sarcoma virus retropepsin, Ty3 transposon peptidase, Gypsy transposon peptidase, Osvaldo retrotransposon peptidase, retrotransposon peptidase, cauliflower mosaic virus-type peptidase, bacilliform virus peptidase, thermopsin, signal peptidase II, spumapepsin, Copia transposon peptidase, Ty1 transposon peptidase, presenilin 1, impas 1 peptidase, type 4 prepilin peptidase 1, preflagellin peptidase, gpr peptidase, omptin, DNA-damage inducible protein 1, HybD peptidase, PerP peptidase, skin SASPase, sporulation factor SpoIIGA, papain, bleomycin hydrolase, calpain-2, poliovirus-type picornain 3C, enterovirus picornain 2A, foot-and-mouth disease virus picornain 3C, cowpea mosaic comovirus-type picornain 3C, hepatitis A virus-type picornain 3C, parechovirus picornain 3C, rice tungro spherical virus-type peptidase, nuclear-inclusion-a peptidase, adenain, potato virus Y-type helper component peptidase, chestnut blight fungus virus p29 peptidase, chestnut blight fungus virus p48 peptidase, sindbis virus-type nsP2 peptidase, streptopain, clostripain, ubiquitinyl hydrolase-L1, legumain, caspase-1,

metacaspase Yca1, pyroglutamyl-peptidase I, murine hepatitis coronavirus papain-like peptidase 1, murine hepatitis coronavirus papain-like peptidase 2, hepatitis C virus peptidase 2, ubiquitin-specific peptidase 14, tymovirus peptidase, carlavirus peptidase, rabbit hemorrhagic disease virus 3C-like peptidase, gingipain R, gamma-glutamyl hydrolase, rubella virus peptidase, foot-and-mouth disease virus L-peptidase, porcine transmissible gastroenteritis virus-type main peptidase, porcine reproductive and respiratory syndrome arterivirus-type cysteine peptidase alpha, equine arteritis virus-type cysteine peptidase, equine arteritis virus Nsp2-type cysteine peptidase, beet necrotic yellow vein furovirus-type papain-like peptidase, calicivirin, bacteriocin-processing peptidase, dipeptidyl-peptidase VI, beet yellows virus-type papain-like peptidase, amidophosphoribosyltransferase precursor, acyl-coenzyme A:6-aminopenicillanic acid acyl-transferase precursor, hedgehog protein, staphopain A, Ulp1 peptidase, separase, D-alanyl-glycyl peptidase, pestivirus Npro peptidase, autophagin-1, YopJ protein, PfpI peptidase, vaccinia virus I7L processing peptidase, YopT peptidase, HopN1 peptidase, penicillin V acylase precursor, sortase A, sortase B, gill-associated virus 3C-like peptidase, African swine fever virus processing peptidase, Cezanne deubiquitinylating peptidase, otubain-1, IdeS peptidase, CylD peptidase, dipeptidase A, AvrRpt2 peptidase, pseudomurein endoisopeptidase Pei, pestivirus NS2 peptidase, AgrB peptidase, viral tegument protein deubiquitinylating peptidase, UfSP1 peptidase, ElaD peptidase, RTX self-cleaving toxin, L,D-transpeptidase, gamma-glutamylcysteine dipeptidyltranspeptidase, prtH peptidase, OTLD1 deubiquitinylating enzyme, OTU1 peptidase, ataxin-3, nairovirus deubiquitinylating peptidase, acid ceramidase precursor, LapG peptidase, lysosomal 66.3 kDa protein, McjB peptidase, DeSI-1 peptidase, USPL1 peptidase, scytalidoglutamic peptidase, pre-neck appendage protein, aminopeptidase N, angiotensin-converting enzyme peptidase unit 1, thimet oligopeptidase, oligopeptidase F, thermolysin, mycolysin, immune inhibitor A peptidase, snapalysin, leishmanolysin, bacterial collagenase V, bacterial collagenase H, matrix metallopeptidase-1, serralysin, fragilysin, gametolysin, astacin, adamalysin, neprilysin, carboxypeptidase A1, carboxypeptidase E, gamma-D-glutamyl--meso-diaminopimelate peptidase I, cytosolic carboxypeptidase 6, zinc D-Ala-D-Ala carboxypeptidase, vanY D-Ala-D-Ala carboxypeptidase, Ply118 L-Ala-D-Glu peptidase, vanX D-Ala-D-Ala dipeptidase,

pitrilysin, mitochondrial processing peptidase beta-subunit, eupitrilysin, leucyl aminopeptidase, aminopeptidase I, membrane dipeptidase, glutamate carboxypeptidase, peptidase T, Xaa-His dipeptidase, carboxypeptidase Ss1, carnosine dipeptidase II, O-sialoglycoprotein peptidase, beta-lytic metallopeptidase, lysostaphin, methionyl aminopeptidase 1, aminopeptidase P, IgA1-specific metallopeptidase, tentoxilysin, aminopeptidase S, glutamate carboxypeptidase II, IAP aminopeptidase, aminopeptidase Ap1, aminopeptidase T, hyicolysin, carboxypeptidase Taq, anthrax lethal factor, deuterolysin, fungalysin, isoaspartyl dipeptidase, FtsH peptidase, glutamyl aminopeptidase, cytophagalysin, pappalysin-1, pox virus metallopeptidase, Ste24 peptidase, HtpX peptidase, Oma1 peptidase, dipeptidyl-peptidase III, S2P peptidase, sporulation factor SpoIVFB, archaelysin, D-aminopeptidase DppA, BlaR1 peptidase, prtB g.p., enhancin, glycyl aminopeptidase, IgA peptidase, StcE peptidase, PSMD14 peptidase, JAMM-like protein, AMSH deubiquitinating peptidase, peptidyl-Asp metallopeptidase, camelysin, murein endopeptidase, imelysin, Atp23 peptidase, tryptophanyl aminopeptidase 7-DMATS-type peptidase, ImmA peptidase, prenyl peptidase 2, Wss1 peptidase, microcystinase MlrC, PrsW peptidase, mpriBi peptidase, NleC peptidase, PghP gamma-polyglutamate hydrolase, chloride channel accessory protein 3, IMPa peptidase, MtfA peptidase, NleD peptidase, TYPE ENZYME, nodavirus peptide lyase, tetravirus coat protein, Tsh-associated self-cleaving domain, picobirnavirus self-cleaving protein, YscU protein, reovirus type 1 coat protein, poliovirus capsid VP0-type self-cleaving protein, intein-containing V-type proton ATPase catalytic subunit A, intein-containing replicative DNA helicase precursor, intein-containing chloroplast ATP-dependent peptide lyase, DmpA aminopeptidase, chymotrypsin A, glutamyl peptidase I, DegP peptidase, lysyl peptidase, streptogrisin A, astrovirus serine peptidase, togavirin, IgA1-specific serine peptidase, flavivirin, subtilisin Carlsberg, kexin, prolyl oligopeptidase, dipeptidyl-peptidase IV, acylaminoacyl-peptidase, glutamyl endopeptidase C, carboxypeptidase Y, D-Ala-D-Ala carboxypeptidase A, D-Ala-D-Ala carboxypeptidase B, D-Ala-D-Ala peptidase C, peptidase Clp, Xaa-Pro dipeptidyl-peptidase, Lon-A peptidase, cytomegalovirus assemblin, repressor LexA, signal peptidase I, signalase 21 kDa component, TraF peptidase, lysosomal Pro-Xaa carboxypeptidase, hepacivirin, potyvirus P1 peptidase, pestivirus NS3 polyprotein peptidase, equine arteritis

virus serine peptidase, prolyl aminopeptidase, PS-10 peptidase, sobemovirus peptidase, luteovirus peptidase, C-terminal processing peptidase-1, tricorn core peptidase, penicillin G acylase precursor, dipeptidyl-peptidase 7, HetR peptidase, signal peptide peptidase A, protein C, archaean signal peptide peptidase 1, infectious pancreatic necrosis birnavirus Vp4 peptidase, dipeptidase E, sedolisin, rhomboid-1, SpoIVB peptidase, nucleoporin 145, lactoferrin, influenza A PA peptidase, EGF-like module containing mucin-like hormone receptor-like 2, Ssy5 peptidase, picornain-like cysteine peptidase, murein tetrapeptidase LD-carboxypeptidase, PIDD auto-processing protein unit 1, Tellina virus 1 VP4 peptidase, MUC1 self-cleaving mucin, dystroglycan, gpO peptidase, {Escherichia coli} phage K1F endosialidase CIMCD self-cleaving protein, White bream virus serine peptidase, prohead peptidase gp21, prohead peptidase, CARD8 self-cleaving protein, prohead peptidase gp175, destabilase, archaean proteasome, beta component, HslV component of HslUV peptidase, glycosylasparaginase precursor, gamma-glutamyltransferase 1, ornithine acetyltransferase precursor, polycystin-1, collagenase, protein P5 murein endopeptidase, Lit peptidase, homomultimeric peptidase, yabG protein, microcin-processing peptidase 1, AIDA-I self-cleaving autotransporter protein, and Dop isopeptidase.

In specific embodiments, the protease is papain, bromelain, AO protease, figin, rennet, protease type XXI from *Streptomyces griseus*, a protease from *Bacillus licheniformis*, a protease from *Aspergillus oryzae*, a protease from *Bacillus amyloliquefaciens*, a protease from *Aspergillus saitoi*, a thermolysin from *Bacillus thermoproteolyticus rokko*, Subtilisin A, protease type X, or a fungal protease type XIII.

In some embodiments, the enzyme is a lipase. The lipase can be any enzyme that catalyzes the hydrolysis of lipids. The lipase can break down fats and release fatty acids. The release of fatty acids can modulate the aroma, flavor profile, and texture profile of the resulting non-dairy cheese replica. The lipase may be derived from an animal source, or may be derived from a non-animal source. The animal source can be, e.g., a calf, a kid (goat), or a lamb. The non-animal source can be a plant source, or can be a bacterial source, a yeast source, or fungus. For example, the source can be from a *Lactococcus* species, a *Pseudomonas* species, an *Aspergillius* species, a *Penicillium* species such as *Penicillum roqueforti, Rhizopus,* a *Lactobacillus* species, a *Malassezia globusa* species, a

Mucor miehei species, or a *Candida* species, e.g., *Candida rugosa*. The non-animal source can be a genetically modified organism that expresses a lipase.

The lipase can be a bile salt dependent lipase, a pancreatic lipase, a lysosomal lipase, a hepatic lipase, a lipoprotein lipase, a hormone-sensitive lipase, a gastric lipase, an endothelial lipase, a pancreatic lipase-related protein 2, a phospholipase, a pregastric esterase, or a pancreatic lipase related protein. Exemplary lipases are described in U.S. Patent Nos. 3973042, 7622290, 7666618, 8012732, 7931925, 7407786, 7052879, and WIPO Patent Application No. WO/2004/113543, all of which are hereby incorporated by reference. Other exemplary lipases include, e.g., AK, Lipase G, Lipase PS, Lipase A, PC lipase and WG lipase.

The lipase can be a commercially available lipase. Exemplary lipases that are commercially available include Italase, often used to make mild-flavored cheeses such as, e.g., mozzarella, asiago, feta, provolone, blue cheese, and queso fresco, and Capilase, which is often used to make sharp-flavored cheese such as, e.g., provolone, romano, and parmesan cheese.

The added enzyme can account for 0.00001-0.005%, 0.001-0.01%, 0.01-0.1%, 0.05-1%, 0.1-2%, or 0.5-5% of the non-dairy cheese source by weight or volume. Preferably, the added enzyme can account for 0.00001-0.1% of the non-dairy cheese source by weight or volume.

In some embodiments, the protease is papain. In some embodiments, 0.001-0.01% of papain is added to the non-dairy cheese source. In particular embodiments, the non-dairy cheese source is a protein solution that comprises purified moong protein. In more particular embodiments, the protein solution with added protease is solidified by a heat/cool method. In some embodiments, addition of papain improves the softness and creaminess of the resulting non-dairy cheese replica.

In some embodiments, addition of one or more proteases improves texture by improving creaminess while maintaining stability of the cheese replica shape, e.g., the cheese replica remains firm enough to dice. In some embodiments, a non-dairy cheese replica prepared with added protease or lipase rates as significantly better in a blind taste test than a comparable non-dairy cheese replica made without protease or lipase. Proteases in some cases significantly improve the texture of the cheese due to an increase

in creaminess of the cheese, as judged by blind taste testers. In some cases the proteases decrease the firmness of the cheeses, as determined by texture analyses. In some cases the proteases increase creaminess without decreasing the firmness of the cheese. In some cases the non-dairy cheese replicas comprise one or more added lipases but no added protease, while in other embodiments they replicas comprise one or more added lipases and one or more added proteases.

One or more enzymes can be contacted with the non-dairy cheese source before solidifying or after solidifying (before the whey is drained, or after the whey is drained). In some embodiments, the flavor and/or texture profile is altered depending on whether the enzyme(s) are added before or after solidifying. In some embodiments, the enzyme(s) are added during the solidification process. In some embodiments, addition of the enzyme(s) during the solidification process results in a non-dairy cheese replica with a softer texture than a comparable non-dairy cheese replica prepared by adding the enzyme(s) at another step in the cheese-making process. In some embodiments, the enzyme(s) are added after solidification and after the whey is removed from the curd. In some embodiments, the non-dairy cheese source is a nut milk contacted with a controlled amount of bacteria, and one or lipases and/or proteases are added after solidifying but before the whey is drained. By way of example only, adding the enzyme(s) after the crosslinking step, while the curd is forming in a soft fresh cheese replica made with almond and macadamia milk with 0.47% transglutaminase, and 0.03% MA11 cultures resulted in a softer texture as compared to addition of the enzyme(s) before curdling has begun or after whey is drained from the curd. By way of other example only, addition of 0.004% rennet or 0.02% papain to the non-dairy cheese source before the non-dairy cheese source is curdled to a gel or after the whey is drained from the curd results in a firmer texture than when enzymes were added as the non-dairy milk is curdled.

In some embodiments, the addition of one or more proteases and/or one or more lipases can be used to enhance flavor notes in the resulting non-dairy cheese replica. The timing of adding the protease(s) and or lipase(s) at any step of the cheesemaking process (e.g., before solidification, after solidification but before whey is drained, or after whey is drained) can be adjusted to enhance desired flavor notes of the resulting cheese replica. Any of the flavor notes described herein can be enhanced by selecting individual

protease(s) and/or lipase(s) and by controlling the timing of adding the protease(s) and/or lipase(s). The enhanced flavor notes can be due to the increased release of specific volatile compounds as described herein. In some cases the flavor profile of the non-dairy cheeses changed without changes in the texture of the cheese.

By way of example only, cheese replicas created with 0.02% papain added after solidifying (either before or after the whey was drained) was significantly rated as more buttery than cheese replicas created without proteases or cheese replicas created when the proteases were added before solidifying. By contrast, aspartic protease added before solidifying, produced the most butter flavor, but in all cases the amount of butter flavor was greater than the control without proteases. See Example 2. The addition of proteases can greatly increase the production of compounds that create the buttery flavor in dairy cheeses, including diacetyl and acetoin, as ascertained by GCMS.

In some embodiments, the protein solution is contacted with a controlled amount of microbes as described herein. In some embodiments, the protein solution is admixed with a non-dairy milk, or a cream fraction, or a skim fraction, or a mixture comprising an isolated cream and skim fraction. Exemplary non-dairy milks, cream fractions, skim fractions, and methods of making are described herein. In some embodiments, the protein solution is contacted with one or more enzymes. In some embodiments, the one or more enzymes comprise a protease and/or lipase. Exemplary proteases and lipases are described herein.

In another aspect, the invention provides a non-dairy cheese replica and methods of preparing the same, comprising isolating a cream fraction, and solidifying the cream fraction. In some embodiments, a cheese replica may be made from about 0.1.%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, more than 90%, or 100% cream fraction. In some embodiments, a cheese replica may be made from about 0.1-1%, 0.5-5%, 2-10%, 5-15%, or 10-20%, 15-30%, 20-50%, 30-60%, 50-80%, 60-90%, or 80-100% cream fraction.

The method may further comprise adding controlled amounts of one or more fats to the non-dairy cheese source to create an emulsion.

By way of example only, some non-dairy cheese replicas were prepared by adding 0% - 50% fat to the non-dairy cheese source to create the emulsion, then solidifying the emulsion by protein denaturation, e.g., by heating. In some embodiments, the controlled amounts of one or more fats are added before solidifying, or after solidifying. In some embodiments, the controlled amount of one or more fats are added after solidifying and after draining the whey. By way of other example only, some cheese replicas made from protein denaturation have 0% to 50% fat added after solidification by denaturation, or 0% to 50% fat added after draining the whey. After the formation of a gel either protein denaturation or crosslinked, whey can be drained to increase the total fat in the cheese replica, further draining and aging the cheese can reduce the moisture content to increase the total fat of the cheese replicas.

In some embodiments, the addition of 5-20% unsaturated fats to enzyme crosslinked gels increased the firmness of the gel.

In some embodiments, addition of saturated fats from 5%-50% increased the firmness of the cheese replicas.

In some embodiments, cheese replicas made with cream fraction have an improved texture profile characterized by increased firmness significantly enhanced creaminess, as compared to cheese replicas made without any fat or with unsaturated or saturated oil added. The use of fat decreases the moisture content of the cheese.

In some embodiments, the type of fat added to the cheese replicas also determines the fat retention in the heat cool cheeses and crosslinked cheeses. The cheese replicas can be made with controlled fat retention through aging and heating by using different fat types and different forms of fats. The amount of fat retention was also dependent of the types of proteins in the cheese gels, for example pea-globulins has greater fat retention than the same amount of soy protein.

In some embodiments, cheese replicas made with the addition of fats, can also modulate the melt-ability of the cheese. The addition of fat can cause a greater change in viscosity when the non-dairy cheese is heated.

Modulating taste/texture/melting profile by adding salts or divalent cations

In dairy cheese, the interaction of calcium ions with casein molecules control the physical properties of the cheese. However, in non-dairy cheeses, no casein is present, so the impact of adding divalent cations to non-dairy cheeses and the impact of adding melting salts are unknown. It was found that the physical properties of the cheese replica can be further controlled by adding mono or divalent cations, e.g., Fe^{2+}, Mg^{2+}, Cu^{2+}, or Ca^{2+} (e.g., $CaCl_2$, $CaSO_4$), adding melting salts such as, e.g., sodium citrate, trisoidum phosphate, sodium hexameta phosphate, or disodium phosphate, or any combination thereof. The cation can be added to the non-dairy cheese source at any stage of the cheesemaking process, such as, e.g., before solidifying, after solidifying, or after draining the whey. Cations can be added at the same time as melting salts or at different times.

In heat/cool gels, divalent cations, e.g., $CaCl_2$, and/or $CaSO_4$ at 0.01% to 5% concentration can be added to a non-dairy cheese source. Exemplary non-dairy cheese sources include, e.g., non-dairy cream fractions, purified proteins, non-dairy milks, e.g., nut milks, protein fat emulsions, or any combination thereof. The resulting cheese replica exhibited improved meltability upon heating as compared to a comparable cheese replica made without the addition of divalent cations and/or melting salts. The improved meltability was characterized by reduced granulation, increased viscosity and increased expansion of the surface area upon heating. For example, a heat cool cheese replica made from 6% soy (7S and 11S), 0.03% MA11, and 1% glucose, did not melt. However, the addition of 1% melting salts (sodium citrate, trisodium phosphate, sodium hexameta phosphate, and/or disodium phosphate) after draining the whey, caused the resulting cheese replica to melt. For the above example, the addition of sodium hexamet phosphate had the greatest affect. In addition, even greater melting can be seen with the addition of divalent cation like $CaCl_2$ added with the emulsion before heat cooling.

Impact of divalent cations on creaminess

Adding divalent cations also can improve the texture profile of a cheese replica. For example, adding 1 mM $CaSO_4$ to cheese replicas prepared from a protein solution of pea globulins before crosslinking with 0.5%-2% transglutaminase improved creaminess as compared to a comparable cheese replica made without addition of $CaSO_4$. This effect

of $CaSO_4$ on creaminess was also observed with nut milk based cheeses and cheeses made from a RuBisCo protein solution.

Melting salts added to non-dairy cheeses also can modulate the firmness of the resulting cheese replica. Melting salts added before or after solidifying by heat cooling increases the firmness of the resulting cheese replica. In some cases, adding melting salts can cause a cheese replica that has been melted to become solid at room temperature. Melting salts added to non-dairy cheese replicas therefore can be used to improve the melting profile of a firmer cheese replica. For example, a protein denaturation cheese replica made with 4% moong protein, 30% palm oil, 1% glucose, and 0.03% MA11 resulted in a soft gel at room temperature that becomes liquid upon heating. When 3% sodium citrate is added to this cheese and heated, the cheese shows an increase in viscosity change, indicating an increase in melting and upon cooling forms a firmer cheese at room temperature.

It is understood that the invention provides methods of controlling the flavor profile, texture profile, melting profile, and/or stretching profile of a non-dairy cheese replica by selecting a specific combination of the following compositions and methods as described herein: non-dairy cheese source, method of solidifying, addition of one or more microbes, addition of one or more proteases and or lipases, addition of one or more fats, addition of one or more melting salts and/or cations, and varying the timing of adding microbes, fats, melting salts, proteases, lipases, or cations at specific stages of the cheesemaking process. Specific embodiments and examples are described herein.

Methods of solidifying

In another aspect, the invention provides cheese replicas and methods of making the same. In some embodiments, the method comprises solidifying a non-dairy cheese source (e.g., a non-dairy milk) (e.g., forming a gel). In some embodiments, the non-dairy milk is capable of retaining a shape after said solidifying. There are many ways in which the non-dairy cheese source can be solidified, including using enzymes, heat denature, forming cold gels, forming coacervate, liquid separation, acids, change in ionic strength, high pressure processing, solvents, chaotropic agents, or disulfide bond reducers as described in this section.

Enzymes (or chemicals) can be used to crosslink non-animal (e.g., plant based) proteins or non-dairy cream fractions, with or without emulsified fats or oils, sugars, and cultures. The resulting crosslinked cheese-replicas can have bacteria cultures added or not, and the timing of addition can be either before or after the crosslinking step. In some embodiments, solidifying involves a process of cross-linking components (e.g., polypeptides, also referred to as proteins herein) in the non-dairy cheese source. In some embodiments, cross-linking comprises contacting the non-dairy cheese source with a cross-linking enzyme, thereby creating crosslinks between polypeptide chains. The crosslinking enzyme can be a transglutaminase, tyrosinase, lipoxygenase, protein disulfide reductase, protein disulfide isomerase, sulfhydryl oxidase, peroxidase, hexose oxidase, lysyl oxidase, or amine oxidase.

In some embodiments, the cross-linking enzyme is a transglutaminase. 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, transgluaminases 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 can exhibit high resistance to proteolytic degradation.

Many types of transglutaminase can be used in various embodiments of the invention. Acceptable transglutaminases for crosslinking include, but are not limited to, *Streptoverticillium mobaraense* transglutaminase, an enzyme that is similar to a transglutaminase from *Streptoverticillium 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 a lysyl oxidase.

The timing of adding the cultures, the type of cultures, and amount of cultures change the pH of the emulsion, and therefore the activity of transglutaminase and the final texture of the cheese. In addition, changing the pH of the solution with the addition

of acid or base, and overall buffering capacity of the emulsion alters the crosslinking ability and the final texture of the cheese-replica.

In some embodiments the present invention provides for a composition comprising a non-dairy milk and a *Streptoverticillium mobaraense* transglutaminase, an enzyme is similar to a transglutaminase from *Streptoverticillium 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.

Transglutaminases can be produced by *Streptoverticillium mobaraense* fermentation in commercial quantities or extracted from animal tissues. Additionally, a transglutaminase (TGM) of the present invention may be isolated from bacteria or fungi, expressed in bacteria or fungi from a synthetic or cloned gene. In some particular embodiments, a transglutaminase is obtained from a commercial source, for example in the form of Activa™ from Ajinmoto Food Ingredients LLC.

In some embodiments, a transglutaminase is added at an amount between 0.0000001-0.001%, 0.0001-0.1%, 0.001-0.05%, 0.1-2%, 0.5-4%, or greater than 4% by weight/volume. In some embodiments, a transglutaminase is added at amounts greater than 0.1% and up to 10%.

In some embodiments, cross-linking by a transglutaminase can be done at temperatures ranging from 10-30°C, 20-60°C, 30-70°C, or 50-100°C. Transglutaminase cross-linking can occur for 10 minutes-24 hours.

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 of transglutaminase is added per 1 mL of non-dairy milk. In some embodiments after the transglutaminase is added, a heated incubation occurs, 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. In some embodiments, enzymatic cross-linking does not comprise contacting the non-dairy cheese source with glutaminase and transglutaminase. Transglutaminase crosslinking has been done at room temperature, and up to 65°C, for 10 minutes to 24 hours.

In some embodiments, solidifying comprises inducing protein denaturation. In some embodiments, denaturation is induced by heating the mixture, followed by cooling the mixture. In some embodiments, denaturation is induced by heating the mixture to a temperature between 30-35, 32-40, 37-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-95, 90-100 °C, or above 100°C. In some embodiments, denaturation is induced by heating the mixture for about 10-20, 15-30, 25-40, 30-50, 40-70 seconds or about 1-3, 2-5, 3-8, or 5-20 minutes. In some embodiments, the mixture is allowed to cool after heating. For example, proteins (e.g, purified or fractionated plant proteins such as from peas, moong, soy, RuBisCO, etc), preferably at concentrations >1%, can be homogenized with oils (such as canola oil, sunflower oil, palm oil or oil bodies from seeds such as sunflower) at 0.1-60% concentration. The emulsion can be subjected to a heat-cool cycle wherein it is heated to a temperature of 45-100°C for 5-60 minutes and then cooled to less than 30°C (e.g., 20-25°C). The resulting gel can be incubated at a temperature ≤30C, preferably for 2-16 hours and then drained through cheesecloth. The drained curds are ready to be shaped and aged or processed further by heating or pressing.

In some embodiments, solidifying comprising forming a coacervate using one or more plant proteins. Coacervation is the process during which a homogeneous solution of charged polymers undergoes a phase separation to result in a polymer-rich dense phase (the 'coacervate') and a solvent-rich phase (supernatant). Protein-polysaccharide coacervates have been used in the development of biomaterials. See, for example, Boral and Bohidar (2010) *Journal of Physical Chemistry B*. Vol 114 (37): 12027-35; and Liu *et al.*, (2010) *Journal of Agricultural and Food Chemistry*, Vol 58:552-556. Formation of such coacervates is driven by associative interactions between oppositely charged polymers. However, as described herein, coacervates can be formed using proteins (e.g.,

plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof). In general, a coacervate can be formed by acidifying a low ionic strength solution (e.g., a buffered solution at or below100 mM sodium chloride) comprising one or more isolated and purified plant proteins such as pea legumins or vicilins (e.g., a vicilin fraction comprising convicilins), a combination of both vicilins and legumins, or unfractionated pea proteins to a pH of 3.5 to 5.5. (e.g., pH 4 to 5). Under these conditions, the proteins separate out of solution and the mixture can be centrifuged to cleanly separate out the coacervate. This coacervate, unlike a precipitate, is a viscous material that can be stretched by pulling and that melts on heating. The process can be carried out in the presence of oils (up to 40%, e.g., palm or other oil), to form a creamy material. By varying the composition of the solution (ratio of vicilin: legumin, type and amount of oil used) the properties, such as melting and rheology, of the coacervate can be tuned as desired. Further, emulsifying salts such as disodium phosphate or trisodium pyrophosphate can be included in the initial mixture prior to acidification to improve flow characteristics of the coacervate (make less viscous, likely due to increased water retention in the coacervate). The resulting coacervate may be used as-is or in cheese replicas or processed further by cross-linking of proteins, or subjected to a heat-cool cycle or high pressure processing to obtain a firmer cheese replica. and used, for example, to prepare cheese replicas with stretching properties, or to prepare firm cheese replicas.

In some embodiments, solidifying comprises forming a cold set gel to avoid denaturation or the breakdown of any heat-labile components (e.g., volatile flavor molecules such as diacetyl can be lost from the food matrix upon heating; lactic acid bacterial cultures commonly used to coagulate milks and/or impart flavor as cheeses age will not survive the heating step). Hence, processes that can induce gelation without heating of such labile components are important in the development of cheeses. See, Ju and Kilara A. (1998) *J. Food Science*, Vol 63(2): 288-292; and Maltais *et al.*, (2005) *J. Food Science*, Vol 70 (1): C67-C73) for general methodologies for forming cold set gels. In general, cold set gels are formed by first heat denaturing a protein solution below its minimum gelling concentration (dependent on pH and type of protein, typically <8% (w/v) at pH 6-9 for globular plant proteins such as pea proteins). The protein solution can

heated to a temperature above the denaturation temperature of the protein under conditions where it does not precipitate out of solution (0-500 mM sodium chloride, pH 6-9). The solution can be cooled back to room temperature or below, and any heat-labile components (including fats at 0-50% (v/v), flavor compounds, enzymes, and bacterial cultures) that need to be incorporated into the gel can be added just prior to the addition of salt. Gelation can be induced by increasing the ionic strength using, for example, calcium chloride or sodium chloride (e.g., 5-100 mM), and incubating at or below room temperature to allow for gel formation (typically minutes-hours). The concentration of the salt needed to induce gel formation is dependent on the nature of the protein, its concentration, and the pH and ionic strength of the solution. The resulting gel is a soft material, with a yogurt-like texture, that can be used as a cheese replica as-is or processed further to obtain other cheese replicas.

Additional denaturation procedures are possible in additional embodiments of the invention. Acids, change in ionic strength, high pressure processing, solvents, chaotropic agents, or disulfide bond reducers can be used to denature the proteins in the non-dairy cheese source. In one embodiment urea is added to the non-dairy cheese source to form curds.

In some embodiments, solidifying results in the formation of solid curds and whey (resulting liquid that remains after curd is formed). In some embodiments, the curds are separated from the whey.

In some embodiments, solidifying comprises a combination of two or more methods. For example, solidifying can include crosslinking proteins and denaturation by heating followed by cooling. For example, a cold set gel can be crosslinked with transglutaminase to yield firmer gels or combined with other proteins such as soy, pea-legumins, pea-albumins, crude protein fraction from chick peas and lentils or materials (for example, fats or pea protein coacervates) to increase firmness and/or meltability.

In some embodiments, the non-dairy cheese source can be subjected to a shearing force during said solidifying. Said shear force can be used to cause protein components in said non-dairy cheese source to align, forming anisotropic fibers. Said formation of anisotropic fibers can be useful in creating a stretch cheese.

Formation of gels by crosslinking proteins

In some embodiments, the cheese replica comprises a cross-linking enzyme. In some embodiments, the cross-linking enzyme is used to solidify the non-dairy cheese source into a gel equivalent to a cheese curd. See the above section re solidifying gels.

For example, a method is provided for preparing a non-dairy cheese replica, comprising isolating and purifying a soy protein (7S and 11S), moong, pea-globulin, pea albumins, pea vicilins, pea legumins, late embryogenesis abundant proteins such as dehydrins, lentil proteins, chickpea proteins, oleosins, RuBisCo, prolamins (including but not limited to pea, corn and sorghum), proteins with or without fat emulsions, and non-dairy cream fractions create crosslinked gels that are cheese-replicas, or any combination thereof, preparing a protein solution comprising any combination of said isolated and purified proteins, and cross-linking the proteins in the solution using transglutaminase. In some embodiments, the soy proteins are used as isolated fractions, such as the 2S globulins, the 7S globulins or the 11S globulins. In some embodiments only the 7S globulins are used as the soy protein.

For example, cross-linked non-dairy cheese replicas can be created using a protein solution comprising soy protein alone ranging from 0.65% or higher weight/volume, using pea-globulin alone, moong bean protein alone, pea-prolamin, or RuBisCo protein alone ranging from 1% or any higher weight/volume.

As another example, cross-linked non-dairy cheese replicas can be created using a protein solution comprising more than one isolated and purified protein. For example, cross-linked non-dairy cheese replicas can be made using soy (7S and 11S) plus peas-globulin (pea-G), or soy (2S, 7S and 11S) plus RuBisCo. In some embodiments, the ratio of soy (2S, 7S and 11S) /pea-globulin or soy (2S, 7S and 11S) /RuBisCo can be about 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1. Cross-linked non-dairy cheese replicas can be made with a total protein amounts greater than 1%, at all possible ratios of the two proteins, including but not limited to 1% soy: 4% pea-G, 2% soy: 4% pea-G, 2% soy:6% pea-G, 4% soy:2% pea-G. Cross-linked non-dairy cheese- replicas can be made with soy (2S, 7S and 11S) plus RuBisCo with a total protein amounts greater than 1%, at all possible ratios of the two proteins. In some embodiments, the ratio of moong globulins/soy (7S and 11S) can be about 1:10, 1:9. 1:8. 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1,

2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1. Cross-linked non-dairy cheese- replicas can be made with moong globulins plus soy (2S, 7S and 11S) with a total protein amounts greater than 1%, at all possible ratios of the two proteins, including but not limited to 4% moong:1% soy, 4% moong: 2% soy, 2% moong: 4% soy, 6% moong: 1% soy, 6% moong: 2% soy, 8% moong:1% soy, 8% moong: 2% soy, and 10% moong:1% soy. Cross-linked non-dairy cheese replicas can be made with moong protein plus peas-globulin. In some embodiments, the ratio of moong protein/pea-G can be about 1:10, 1:9. 1:8. 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1. In some embodiments, the non-dairy cheese replica can be made with a total protein amounts greater than 1%, at all possible ratios of the two proteins, including but not limited to 2% moong: 4% pea, 4% moong: 2% pea, 4% moong: 4% pea, 6% moong: 2% pea. Cross-linked non-dairy cheese- replicas can be made with any combination of plant based proteins, including total isolated protein, and fractioned protein with a total protein concentration of 1% or greater. Other examples of proteins used to made cheese replicas including prolamins, late embryonic abundant proteins that can be used alone or in combination of the above proteins.

In some embodiments, the cheese replica is made using cream fraction as a non-dairy cheese source, cross-linked by enzymatic cross-linking or by denaturation. For example, cross-linked non-dairy cheese- replicas have been made with cream fraction alone from 5% to 100%. Cheese replicas made from crosslinking the cream fraction can be modulated by the purification method of the cream fraction. For example, sunflower cream fraction purified with a high pH wash, crosslinks very well, forming firm curd that is white. In other cases, purification of the sunflower cream fraction from a urea wash, does not crosslink well, and more crosslinking enzyme or more cream fraction is required for curd formation. In even another case, sunflower cream fraction washed only at pH 7, can result in a green/brown colored cheese.

In some embodiments, the cheese replica is made using a non-dairy cheese source that comprises a cream fraction and a protein solution comprising one or more isolated and purified proteins. Cross-linked non-dairy cheese-replicas can be made with purified proteins and cream fraction. Cross-linked non-dairy cheese-replicas can be made with soy plus sunflower cream fraction, with amounts including but not limited to 0.6% soy

(7S and 11S): 20% cream, 2% soy (7S and 11S): 20% cream, 4% soy(7S and 11S), 20% cream, 2% soy(7S and 11S): 10% cream, 4% soy(7S and 11S): 10% cream, and 4% soy(7S and 11S): 30% cream. Cross-linked non-dairy cheese- replicas can be made with pea globulins plus cream, with amounts including but not limited to 4% pea globulins: 20% cream, 7% pea globulins: 20% cream, 7% pea globulins: 10% cream, 4% pea globulins: 10% cream, 10% pea globulins: 20% cream. Cross-linked non-dairy cheese-replicas can be made with moong plus sunflower cream, with amounts including but not limited to 4% moong: 20% cream, 6% moong: 20% cream, 8% moong: 20% cream, 8% moong: 10% cream. Cross-linked non-dairy cheese- replicas can be made with multiple purified proteins plus cream fraction, for example 2.5% pea globulins: 3% soy(7S and 11S): 20% sunflower cream, 2% pea globulins: 4% soy (7S and 11S): 20% sunflower cream, 6% pea: 2% soy (7S and 11S): 10% sunflower cream fraction. Cheese-replicas made with purified proteins plus the addition of cream fraction can have an improved creamy texture as compared to cheese-replicas made with purified proteins alone or made with the cream fraction alone. For example, a cheese-replica made with with 4-8% pea globulins plus 10-20% cream fraction, cross-linked by transglutaminase, results in a firm but creamy non-dairy cheese replica as compared to a comparable non-dairy cheese replica created with only purified proteins or the cream fraction alone, both of which result in a more granular cheese replica. Cheese-replicas made with purified proteins plus the addition of cream fraction can also hold oil well through aging. Such replicas can exhibit minimal to no oil leakage in aging studies lasting a week or more. By contrast, cheese replicas prepared from crosslinked washed cream fraction alone are firm and hold a cheese form, are able to be diced, but may exhibit oil leakage with aging. Surprisingly, it was found that addition of one or more isolated and purified proteins, even trace amounts of isolated and purified proteins lower than 1%, significantly minimizes oil leakage from the resulting cheese replica, as compared to a cheese replica made using cream fraction alone. Addition of 0.65% wt/volume or more of isolated and purified proteins to the cheese replica can significantly minimize oil leakage as compared to a replica that does not comprise isolated and purified proteins.

The invention also provides methods of creating a non-dairy cheese replica with improved melting profile. Some currently available non-dairy cheeses exhibit poor

melting capabilities. Instead of exhibiting smooth melting, the non-dairy cheeses often curdle during heating and are unable to melt smoothly. In some embodiments, the method comprises solidifying a non-dairy cheese source by denaturation. By way of example only, the property of meltability can be imparted to cheese replicas prepared by mixing protein solutions mixed with one or more fats into an emulsion, then solidifying the emulsion by denaturation. Exemplary methods of denaturation are described herein. In some embodiments, a protein solutions, preferably at concentrations >5% protein, is homogenzied with one or more oils at a concentration between 0.1-60%. The resulting emulsion can be subjected to a heat-cool cycle wherein it is heated to a temperature of 45-100C for 5-60 minutes and then cooled back. The gel can be incubated at a temperature <=25C, preferably for 2-16 hours and then optionally drained through cheesecloth. In some embodiments, the drained curds are ready to be shaped and aged or processed further by heating or pressing.

As described herein, unfractionated pea globulins do not form reversible gels between pH 4-9 and NaCl concentrations 0-1M. However, once fractionated (to >90% purity as judged by gel electrophoresis) into vicilin (+ convicilin) and legumin, these proteins can form reversible gels under certain conditions. Once purified (to >90% purity as determined by gel electrophoresis), the 7S and 11S proteins from peas could be used to obtain meltable gels under certain pH and NaCl conditions. Purified pea-7S proteins could be induced to form a meltable gel at pH7 , 100mM NaCl. On the other hand, purified pea-11S proteins formed meltable gels between pH 7-9 when NaCl concentration was 300mM.

In some cases, cheese-replicas comprising proteins from plants sources other than soybeans (such as moong bean globulins, pea proteins etc) can be induced to form meltable gels by using them in combination with soy (7S and 11S). The mixture, preferably at total protein concentrations of >5% (comprising > 0.2% soy proteins) can then be emulsified with various oils or oil bodies and then taken through the heat-cool cycle described above (e.g., heating to 95°C and cooling back to 25°C) to form meltable gels. Melting salts are useful for helping form the meltable gel, as well as retaining meltability for specific protein+ oil mixture (depending on protein, not oil).

In some embodiments, the gels can be inoculated with microbial cultures to improve flavor, texture and/or appearance of the cheese-replica. Microbes, such as bacterial cultures (such as, e.g., *Lactococci, Lactobacilli, Streptococci*, and *Propionii* bacteria) or molds (such as *Penicillium,*or *Geotrichum*) can be added at 0-1% concentration to the non-dairy cheese source. The microbes can be combined with 0.5-3% sugars (such as glucose, fructose, maltose, sucrose etc) at any point during the cheese-making process. In some embodiments, the microbes and optionally sugars are added to the protein-fat emulsion during the cool-down phase of the heat-cool cycle, preferably when the mixture is at or below 40C. The mixture can be held at this temperature for 1hr and then cooled further before draining and shaping.

The meltability of the curds formed by denaturation, e.g., by a heat/cool cycle can also be modulated by adjusting the pH and/or ionic strength of the protein mixture. For example, protein solutions, preferably at concentrations >5%, can be induced to form meltable gels by heat-cool cycling (heating to 45-100C and then cooling down to <=30C) by maintaining pH of the solution between 3-9 and an ionic strength between 0-0.5M sodium chloride.

The appearance of curds formed by heat-cool method can also be modulated by pH and ionic strength of the solution. By way of example only, a solution of moong 8S globulins comprising 200 mM sodium chloride and kept at pH 8-9 form opaque curds greyish in color. However, when the ionic strength of the solution is lowered (50mM sodium chloride), the appearance of the gel changes to a translucent white-light grey.

In some embodiments, various combinations of the heat-cool method and crosslinking (TG) are used to modulate the appearance, texture and meltability of the cheese-replicas. For example, a solution of 6% soy (7S and 11S) can be subject to the heat-cool method to obtain curds that readily and reversibly melt. In addition, if transglutaminase (0.1%) is added to the curd at during the cool down phase, preferably at 40C, the resulting curds are more granular in appearance and melt more controllably.

Stretching:

In some embodiments, the cheese replica is made to form stretchy strings upon heating ("stretchy cheese replica"). A stretchy cheese replica can be made, for example,

by using mixtures that comprise isolated and purified plant proteins such as, e.g., pea, moong and soy globulins, albumins, prolamins (zeins, pea prolamins) and late embryonic stage abundant proteins (LEAs). Sources of these proteins include, by way of example only, cereal grains including, e.g., barley, wheat, legumes such as, e.g., moong, plants from the *Arabidopsis* genus, and *S. cereviseae*. These proteins can be used at varying concentrations, preferably >5%, in solution. The protein solution can be mixed with 0-60% fat to create an emulsion. In some embodiments, the protein solution comprises isolated and purified prolamins. In some embodiments, the prolamins are isolated from peas. The addition of these prolamins increases stretching.

The emulsion can be solidified by crosslinking or by heat-cooling or a combination of both crosslinking and heat-cooling. In some cases, 0-2% melting salts are used to improve meltability of the gels. In some cases, 0-1% microbial cultures could be added to improve texture and flavor of the cheese-replicas.

The stretchy cheese replica can be prepared using a number of non-dairy cheese sources, including but not limited to non-dairy cream fractions, purified proteins, non-dairy milk, e.g., nut milks, protein solutions comprising one or more isolated and purified proteins, emulsions of isolated proteins and isolated fats, or any combination thereof. In some embodiments, the non-dairy cheese source is a non-dairy milk isolated from plant sources with isolated and purified pea prolamin proteins, zein (purified corn prolamins) added therein. In some embodiments, the pea prolamins are added to achieve a concentration of 0.1-10%, or 0.5-8%. The addition of pea prolamins or zein to the non-dairy milk can improve stretchability of the resulting cheese replica, as compared to a cheese replica made with just non-dairy milk.

In some embodiments, stretching in cheese replicas can be improved by incorporating oacervates. Coacervates may be used as-is to form stretchable cheeses, or combined with other proteins with/without use of crosslinking enzymes such as transglutaminase to form cheese replicas with stretching properties.

Stretching also can be increased by adding starches, including but not limited to xanthan gum, carrageenan, cassia gum, konjac gum, methylcellulose and hydroxypropyl methylcellulose, hydroxypropyl, alginates, guar, locust bean gum, pectin and gum arabic. The gum may be added to achieve a final concentration of 0.01-4%, or 0.05-2%.

In some embodiments, the gum is xanthan gum. Additing xanthan gum to the non-dairy cheese source can increase the stickiness of the curd after solidifying, which thus can increase the stretchability of the resulting cheese replica. Xanthan gum added from 0.05% to 2% to plant based emulsions including but not limited to non-dairy cream fractions, purified proteins, nut milks, protein fat emulsions, or a mixture of these components, increased the stickiness of the curd, allowing for the cheese to stretch. The addition of xanthan gum can increase stretching of an otherwise non-stretching cheese replica, or can increase the stretching of a stretchy cheese replica.

Hard Cheeses

In another aspect, the invention provides a method of preparing a firm cheese replica that mimics the texture, flavor, and firmness of a hard cheese, such as parmesan or Cheddar. In some embodiments, a non-dairy milk is inoculated with thermophilic bacteria cultures before solidifying into a gel. The temperature may be increased during the solidification to allow the curd to release more whey. The curd can be cut into smaller pieces, e.g., 1/2 inch squares. The smaller pieces can be allowed to acidify. The smaller pieces can be allowed to solidify for 10 minutes while still suspended in their own whey. After acidification, the curd can be broken into smaller pieces, e.g., pea-sized pieces by, for example, whisking. The temperature of the whey/coagulum can be increased by 2 degrees every five minutes until a desired curd temperature is reached. The desired internal temperature can be 50-200°F. The curd can be stirred, e.g., every 10 minutes to prevent reaggregating. The temperature can be raised to 125-130°F. The curds can be separated and drained to form a hard cheese replica. The hard cheese replica can optionally be aged.

The cheese replicas may be ripened in a way similar to traditional cheese. For example, surface mold 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.

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. In some embodiments the edible materials are embedded in the cheese replica.

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.

In some embodiments, 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.

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.

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.

EXAMPLES

EXAMPLE 1: The use of enzymes to improve non-dairy cheese replica texture

Soft ripened (SR) cheese replicas were created with proteases and lipases added at different time points and evaluated for the ability of the proteases and lipases to modulate the texture of the replicas. Eighteen cheese replicas were made with pasteurized almond and macadamia nut milk (see Example 20). The nut milk was cultured with 0.03% MA11, 0.15% Flora Danica, 0.0045% *Geotrichum candidum*, 0.009% *Penicillium candidum*, and 0.007% *Debaryomyces hansenii* cultures, then crosslinked with 0.5% transglutaminase (ACTIVA TI, from Ajinomoto). Five different combinations of a protease and/or a lipase were examined, along with three controls without protease or lipase as follows: A = papain 0.02%, B= Fromase™ (an aspartic protease from *Rizomucor* with a similar specificity to chymosin from rennet), 0.004%, C= papain 0.01% + Fromase™ 0.002%, D= papain 0.02% + Lipase G 0.001%, E = papain 0.01%, PC lipase 0.0001% and F= control. The proteases and lipases were added at these three times: (i) with the cultures, (ii) after crosslinking had started (after TG hour), and (iii) after draining the whey.

The cheese replicas were evaluated by blind taste testers and by texture analysis (see FIG. 1 and FIG. 2.). The tasters rated the cheese replicas from 1-5 as follows: 1: too soft, 2: creamy yet stays intact (e.g., wedge stays intact), 3: slightly too firm, 4: much too firm and 5: rubbery, and breakable. FIG. 1 depicts the average texture scores of the above cheese replicas as determined by taste testers. As shown in FIG. 1, the cheese replicas with added proteases (samples A-E, referred to as 1-5 in FIG. 1) were mostly rated as "creamy and wedge stays intact" and were significantly creamier and softer than cheese replicas without any proteases. The control replicas with no proteases were mostly rated as "much too firm." The data indicate the addition of proteases can increase the creaminess of the cheese replica.

The cheese replicas also were tested for firmness using a texture analyzer (texture Technologies XT plus). FIG. 2 depicts firmness of each of the soft ripened cheese

replicas with the addition of proteases as determined by the texture analyzes. As shown in FIG. 2, the cheese replicas with proteases had a much softer texture than the cheese replicas without proteases. The data indicates the addition of proteases can decrease the firmness of the cheese replica. The data from the texture analyzer supports the data collected by taste testers.

EXAMPLE 2: The use of enzymes to improve non-dairy cheese replica flavor (to create flavor that is indistinguishable from a comparable dairy cheese).

Soft fresh (SF) cheese replicas (Example 21) created with proteases and lipases added at different times during the creation of the cheese replicas were evaluated for the ability of the proteases and lipases to modulate the flavor of the non-dairy cheese replicas. Eighteen cheese replicas were made with pasteurized almond and macadamia nut milk as detailed in Example 1. Five different combinations of protease and/or lipase mixtures were examined, along with three controls without protease or lipase as follows: A = Fromase™ 0.004%, B = Papain 0.02% + Lipase G 0.001%, C = control (no protease or lipase), D = Papain 0.02%, E = Papain 0.01% + Fromase™ 0.002%, and F = Papain 0.01% + PC lipase 0.0001%. The proteases and lipases were added at these three times: with the cultures, after crosslinking had started, and after draining the whey.

Blind taste testers and GCMS were used to evaluate the cheese replicas for a variety of flavors. FIG. 3A depicts the sum of the preference scores from 12 blind taste testers. Each test tester rated the cheese replica from 1-3, 1 being the favorite, 2 being all the rest, and 3 being the least favorite. Figure 3B depicts the sum of the flavor scores from 12 blind taste testers. Each taste tester rated the cheese replicas from 1-5, 1 being the best flavor (sweet, fermented, fresh, little sharp, salt), 5 being the worst flavor (off flavors: nutty, plastic, metallic).

The tasters also rated the replicas for the following: how much they liked the flavor, their overall preferred cheese replica, the amount of acidity and the amount of buttery flavor. The rating was from 1-5, with 1 being the most liked for flavor or the overall favorite, or 1 being least amount for acidity or buttery flavor.

Overall preference: The least preferred cheese replicas were the three controls, which had no protease and lipases added; these were less liked than all of the cheese

replicas that contained proteases and/or lipases and were significantly ($p<0.05$, two-tailed T-test) less liked than the cheese replicas that contained either papain 0.02%, or papain 0.01% and Fromase™ 0.002% . The most preferred cheese replica had papain at 0.02% added after draining the whey.

Flavor preference: The cheese replicas least liked for flavor were the three controls, which had no added proteases or lipases. These were less liked than all of the cheese replicas that contained proteases and/or lipases, and were significantly ($p<0.05$, two-tailed T-test) less liked than the cheese replicas that contained papain 0.02%, and the cheese replicas with papain 0.01% and Fromase™ 0.002%. The most preferred cheese replicas contained either papain at 0.02%, papain at 0.01% or papain 0.01% plus 0.002% Fromase™, added after draining the whey.

Buttery flavor: FIG. 4 depicts the butteriness score for the cheese replica as determined by taste testers. Each test tester rated the cheese replicas from 1-5, 1 being the least buttery (no butter taste), 3 (good amount of butter taste), and 5 (too much butter taste).The least buttery cheese replicas were the three controls, which had no added proteases or lipases. These were less buttery than the cheese replicas that contained proteases and/or lipases, and were significantly ($p<0.05$, two-tailed T-test) less buttery than the cheese replicas that contained papain 0.02% and papain 0.01% and Fromase™ 0.002%. The most buttery cheese replicas were created by adding either papain at 0.02%, papain 0.01% or 0.01% papain plus 0.002% Fromase™, after draining the whey.

Acidity: FIG. 5 depicts acidity scores of cheese replicas. Each test tester rated the cheese replicas from 1-5, 1 being the least acidity (no acidity), 3 (good amount of acidity), and 5 (too much acidity). The amount of acidity varied little among the samples.

The cheese replicas were also evaluated using GCMS. The volatile chemicals were isolated from the head space around the cheese replicas homogenized in 400 mM NaCl. These aroma chemicals were identified using GCMS, and the peaks further evaluated to identify the compounds in each cheese replica sample. This set of cheese replicas with and without proteases and lipases were compared for the chemical compounds in each sample to identify compounds created by the presence of protease and/ or lipases. The control cheese replicas without any proteases or lipases were found to have the least amount of two known buttery compounds: 2, 3, butanedione and acetoin.

The sample with papain at 0.02% added after draining the whey had the greatest amount of both 2, 3, butanedione and acetoin compared to all other samples. The amount of 2, 3, butanedione in this sample was 30 fold higher than its control with no added proteases or lipases, and acetoin was greater than 10-fold higher in the sample containing papain added after draining the whey, compared to the control. The GCMS results were consistent with the tasting results, which showed that adding papain at 0.02% after draining the whey resulted in the most buttery tasting cheese replica. Table 1 depicts the relative amounts of buttery compounds identified by GCMS that vary among the samples with different proteases and lipases, and that also vary depending on when in the cheese-replica-making process the proteases and lipases are added. The amount of each flavor component is shown using a qualitative scale. Where there is no symbol, the molecule was present below detection levels. The number of + signs designates the relative amount of the target molecule detected in the experiment, where ++++ is more than +++ and significantly more than +.

TABLE 1

	Timing of adding proteases and lipases		
	with cultures	after TG	after draining
2, 3, Butanedione			
A = FromaseTM 0.004% (samples: 1, 7, 13)	+	+++	++
B = Papain 0.02% + Lipase G 0.001% (samples: 2, 8, 14)	+++	+	+
C = control (no proteases or lipases), (samples: 3, 9, 15)			
D = Papain 0.02%, (samples: 4, 10, 16)	+++	++	+++++
E = Papain 0.01% + FromaseTM 0.002% (samples: 5, 11, 17)	+++	+++	+++
F = Papain 0.01% + PC lipase 0.0001% (samples: 6, 12, 18)	++++	+++	++++
Acetoin			
A = FromaseTM 0.004% (samples: 1, 7, 13)	+	++++	++
B = Papain 0.02% + Lipase G 0.001% (samples 2, 8, 14)	++	+	+
C = control (no proteases or lipase), (samples			

3, 9, 15)			
D = Papain 0.02%, (samples: 4, 10, 16)	++	++	+++++
E = Papain 0.01% + FromaseTM 0.002% (samples: 5, 11, 17)	++	+++	+++
F = Papain 0.01% + PC lipase 0.0001% (samples: 6, 12, 18)	+++	++	++++

EXAMPLE 3 - Selection of desired lactic acid bacteria

Twenty-one (21) individual bacterial strains were isolated from the commercial products MA11, MA14, MA19 and Flora Danica. These mixed direct vat cultures were plated on non-selective media and screened by PCR with primers (Table 3) that were designed specifically to distinguish among the various strains (Table 2, where LLL refers to *Lactococcus lactis lactis*, LLC refers to *Lactococcus lactis cremoris*, LM refers to *Leuconostoc mesenteroides*, and LLBD refers to *Lactococcus lactis biovar diacetylactis*. Table 2 depicts the individual isolated strains from the starter cultures. The first column identifies the source of the isolated strains, and the first row depicts the bacterial subspecies classification of the individual strains. In some cases it was exploited that certain strains, e.g., LLC, grew poorly on certain media (e.g., LB+ glucose) and the smallest colonies were selected for PCR analysis.

Table 2

List of isolated bacterial strains.

Source	LLL	LLC	LM	LLBD	Other
MA11	LF5 LF7	LF2			
MA14	LF24LF27 LF40	LF43			
MA19	LF28	LF33			
Flora Danica	LF16 LF50 LF17	LF15 LF54LF55	LF21LF53	LF13 LF14 LF49	LF51 undefined species of lactococcus

Table 3 depicts the primers used for sequence analysis of the isolated strains. Primer sets 1-3 (primer 1 pair, SEQ ID NOs: 1-2; primer 2 pair, SEQ ID NOs: 3-4;

primer pair 3, SEQ ID NOs. 5-6):were used in preliminary identification of LLL, LLC and LLBD strains. Primer pair 4 (SEQ ID NOs. 5 and 6) was used to identify LM strains.

TABLE 3

List of primers.

Primer	Forward primer (5'→3')	Reverse primer (5' → 3')
Primer 1	TATGAAAGGAACTTATCTTAAAGTT	ATTTTCAATCTCCATTTTTTAGAGT
Primer 2	ATTCTTGATTTCAAAAAACCTGATT	AAATTGATTGAAGTCGGTCAAAAGT
Primer 3	CAAAGTTCTTTGACATTATGTTG	CTAATGATGATTTAGATATGATGAC
Primer 4	CCAGACTCCTACGGGAGGCAGC	CTTGTGCGGGCCCCCGTCAATTC

Strains were further identified by sequencing the PCR products and by carrying out more extensive whole genome sequence analysis. Phenotypic analysis included: growth on Reddy's selective medium, pH profile in nut media, sugar fermentation, GCMS, and the tasting of curd and cheese replicas produced with individual strains. Cheese replica tastings were blinded to the tasters and carried out multiple times. The tasting results were evaluated for statistical significance.

The effect of selected strains on pH of filtered nut media was also tested. Nut media was incubated with LF2, LF5, or a 1:1 ratio of LF2 and LF5 strains for at least 17 hours, and the pH sampled at different time points. FIG. 6 depicts the effect of LF2 and LF5 strains on pH of filtered nut medium. LF2 (an LLC isolated from MA11) lowered the pH in filtered nut medium from 6.25 to 5.35 at T 17h, whereas LF5 (an LLL isolated from the same commercial mix) lowered the pH to 4.23 in the same time period.

EXAMPLE 4 - The use of specific lactic acid bacteria to produce desired flavors

The individual strains identified in Example 3 were used to make SF cheese replicas (see Example 21). Combinations of LF2, LF5 and LF7 (including 1/3 each) and combinations of LF2 and LF5 (including 1/2 each) were liked as well or better than MA11 in blind curd and cheese replica tastings.

A 50:50 mixture of LF2:LF5 was tested over a 4 x range of bacterial cell concentration at inoculation, from 1.5 x 10^8 cfu/ml – 3.8 x 10^7 cfu/ml per strain, and

found to result in the same final pH (4.3) as MA11 (at an inoculum concentration of 3 x 10^8 cfu/ml). Tasters found no significant flavor difference among samples in this inoculum range.

In blind taste tests, SR cheese replicas (see Example 20) made with isolated strains of LLL, LLC, LLBD and LM (e.g., a mixture of LF2, LF5, LF21 and LF14) were as well liked as or preferred to samples made with Flora Danica (FD).

SF cheese replicas (Example 21) also were prepared using individual strains to better characterize the flavor and texture profiles contributed by each strain. The results of the taste test/texture preference tests are depicted in Table 4.

TABLE 4

Texture and flavor of cheese replicas made with various starter cultures and isolated bacterial strains

Strain	Average Texture Score	Predominant Flavors	Less Dominant Flavors
LLBD (MD88)	2	Buttery = nutty	Sour
LM (LM57)	1.11	Buttery = sour	Nutty = sweet > floral = woody
LF2 (LLC)	1.44	Sour > buttery > nutty	Bitter = sweet
LF5 (LLL)	1.67	Sour > buttery = nutty	sweet

Predominant = more than 50% of tasters scored the flavor as present; Less dominant = a third or more of the tasters scored the flavor as present. Texture was scored on a scale of 1 – 5, where 1 = softest, and 2 = ideal texture for a SF cheese replica.

Some of the observations in the tasting studies included:

Cheese replicas made with LM alone had a much softer texture than cheese replicas made with LLBD alone.

Cheese replicas made with LM alone had a predominantly sour flavor. Samples also were quite buttery, but not as much so as those made with LLBD alone. Other prominent flavors in cheese replicas made with LM alone included nutty, sweet, floral and woody.

Cheese replicas made with LLBD alone had a predominantly buttery taste. They also were sour, but less so than samples made with LM. LLBD samples also had a nuttier and sweeter flavor than cheese replicas made with LM. Other prominent LLBD flavors included fruity and floral.

Cheese replicas made with LLC may have more bitter and sour flavors than those made with LLL alone.

EXAMPLE 5: EFFECT OF ADDED SUGARS ON FLAVOR PRODUCTION IN SF CHEESE REPLICAS

SF cheese replicas were prepared using the standard SF recipe (see Example 21). Each coagulation was inoculated with either LM57 (LM, commercial product) or MD88 (LLBD, commercial product). Sugar (glucose, fructose, sucrose, or maltose) at 20 mM final concentration was added to each sample except for the no added sugar control. Nut milk formula is about 55 mM sucrose with negligible amounts of glucose, fructose, and maltose. SF cheese replicas were taste tested by 10 individuals blinded to the identity of the cheese replicas.

Table 5 depicts a listing of cheese replica samples and their respective bacterial/sugar experimental conditions.

TABLE 5

List of cheese replica samples and description.

Sample	Description
A	LM57 no added sugar
B	LM57 + 20 mM Glucose
C	LM57 + 20 mM Fructose
D	LM57 + 20 mM Sucrose
E	LM57 + 20 mM Maltose
F	MD88 no added sugar
G	MD88 + 20 mM Glucose
H	MD88 + 20 mM Fructose
I	MD88 + 20 mM Sucrose
J	MD88 + 20 mM Maltose

Tasters were asked to score one point for each flavor tasted from the following categories: buttery, nutty, sweet, sour, fruity, floral, bitter, earthy and nutty. For example, a cheese replica judged to be buttery by all ten tasters would receive a score of 10. Flavor scores for each sample are summarized in Tables 6A & 6B. Combined flavor scores for each strain, irrespective of added sugar, are shown in Table 6C.

TABLE 6A

Effect of added sugar on flavors produced by LM

Flavor	no added sugar	glucose	fructose	sucrose	maltose
buttery	8	7	4	6	4
nutty	4	2	3	4	2
sweet	4	1	1	2	4
sour	8	8	9	7	8
fruity	0	1	1	1	2
floral	3	1	1	2	2
bitter	1	1	0	1	0
earthy	2	2	0	1	0
woody	3	1	2	2	0

TABLE 6B

Effect of added sugar on flavors produced by LLBD

Flavor	no added sugar	glucose	fructose	sucrose	maltose
buttery	6	9	9	8	9
nutty	6	5	6	5	3
sweet	2	6	3	5	3
sour	4	4	4	3	4
fruity	2	2	1	1	2
floral	2	3	1	1	0
bitter	1	1	1	1	0
earthy	1	1	1	0	2
woody	0	0	0	1	1

TABLE 6C

Flavors produced by LM (LM57) and LLBD (MD88), irrespective of added sugars

Flavor	LM	LLBD
buttery	29	41
nutty	15	25
sweet	12	19
sour	40	19

fruity	5	8
floral	9	7
bitter	3	4
earthy	5	5
woody	8	2

The predominant flavors that the tasters detected were buttery, sour, nutty and sweet. A number of differences were observed between LM and LLBD samples. Both sample sets scored high for buttery flavor, but the presence of added sugar affected butteriness differently, uniformly increasing this flavor in LLBD samples but tending to decrease it in LM samples (FIG. 7, see Table 5 for a description of each sample). Samples cultured with LM were rated more sour than samples made with LLBD, and sourness was relatively unaffected by the presence of added sugar. FIG. 8 shows the combined buttery and sourness scores irrespective of added sugar.

Likewise differences were observed between the two strains for nuttiness and sweetness. Overall, samples made with LM are less nutty and less sweet than samples made with LLBD (see FIGs. 9 & 10).

Each cheese replica was subjected to analysis by GC-MS. The results are summarized in Table 7.

TABLE 7

Volatile flavor compound	**Sugar**	**LLBD**	**LM**
Octane	All conditions	-	-
Ethanol	+ Maltose	+++	++
Ethanol	No added sugars	++	+
2,3-Butanedione	All conditions	+	++
Acetoin	No sugar or +sucrose	+++	++
Acetoin	+ other sugars	+++	+
Butanoic acid	All conditions	+++	++
2-heptanone	No added sugars	-	-
2-heptanone	+ Glucose	+++	+
Nonanol	No added sugars	-	+
Nonanol	+ Glucose	-	++
Acetic Acid	+Sucrose or +maltose	++++	+++
Acetic Acid	No added sugars	-++++	++

Acetic Acid	+ Glucose	-++++	+
Acetic Acid	+ Frucose	-++++	+
1,3-Butanediol	All conditions	-+++	++

From these data, the following conclusions were reached:

Sourness: Cheese replicas made with LM alone have a predominantly sour flavor. Samples made with LLBD are also sour, but less so than those made with LM. Acetic acid was produced by both LLBD and LM: more was produced by LLBD than LM. Although sucrose and maltose increased acetic acid production by LM and glucose and fructose reduced it, sugar did not seem to affect the tasters' perception of sour flavor. It is likely that acetic acid is not the only sour flavor compound produced by these strains.

Butteriness: Both LM and LLBD samples have prominent buttery flavors, with LLBD overall more buttery than LM. There are three buttery flavor compounds produced by both strains: 2,3-butanedione, acetoin, and butanoic acid. LM produces more 2,3-butanedione, and LLBD produces more acetoin and butanoic acid. Glucose, fructose and maltose decrease the amount of acetoin produced by LM, and tasters likewise found samples made with fructose and maltose to be the least buttery. Overall added sugar appears to increase butteriness for LLBD samples.

LLBD samples also have a nuttier and sweeter flavor than cheese replicas made with LM. Sweetness may increase with LLBD with added sugar.

EXAMPLE 7 - TITRATION OF NUT MILK CREAM AND SKIM FRACTIONS TO MAKE CHEESE REPLICAS

Typically, the nuts milks are prepared from a 55:45 mixture of almond milk to macadamia milk with the following recipe:

49.45 % almond skim

22.40% macadamia skim

5.12 % almond cream (which is ~59% fat) (see Example 1 of WO 2013/010037)

23.02 % macadamia cream (which is ~63% fat)

This recipe contains 28% cream (5.12 % almond cream plus 23.02 % mac cream). The percentage of fat in this formulation is approximately 17.5 g fat/ 100 g formula.

The ratio of almonds to macadamias was changed to 78:22 (23.6% almond skim, 7 % almond cream, 4.7% macadamia nut skim, 4% macadamia nut cream), maintaining 28% cream, and carried out blind tastings. SF cheese replicas made with this formulation were liked as much as the 45:55 mix, and were indistinguishable in flavor and texture.

EXAMPLE 8 CONTROL OF FAT RETENTION

Cheese replicas were made with different types and different amounts of fat and compared for their ability to retain fat. The cheese replicas were all made with a 4% solution of moong 8S protein homogenized with each of the following: the sunflower cream fraction from 5-40%, sunflower oil from 5-40%, or palm oil from 5-40%, each emulsion was heated to 95°C and then cooled back to 30°C. At 30°C, 0.03% MA11 and 1% glucose was added to both samples. The mixtures were then incubated at 25°C overnight, followed by the non-gelled liquid being drained through cheesecloth.

All the cheese replica gels were compared for their ability to retain fat at room temperature and upon heating up to 100°C. The non-dairy cheese replicas with 4% moong protein, and the sunflower cream fraction (all tested amounts from 5% to 40%) had no fat leakage at room temperature, and no fat leakage upon heating to 100°C. The cheese replica gels made with 4% moong protein, and 10% to 40% sunflower oil all had fat leakage at room temperature, and even greater oil leakage upon heating. Oil leakage was seen for cheese replica gels made with 4% moong protein, and 5% sunflower oil upon heading. The cheese replica gels made with 4% moong protein and 20% to 40% palm oil all had fat leakage at room temperature, and even greater oil leakage upon heating. Oil leakage was seen for cheese gels made with 4% moong protein, and 10% palm oil upon heating.

EXAMPLE 9: PURIFICATION OF DESIRED PROTEINS

All steps were carried out at 4˚C or room temperature. Centrifugation steps were at 8000 g for 20 mins, 4˚C or room temperature. The flour is suspended in a specific buffer, the suspension is centrifuged and the supernatant is microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10

kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system.

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 10 volumes of 50 mM K Phosphate buffer, pH 7.4, + 0.5 M NaCl. The suspensions were centrifuged and the supernatants microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

(i) Pea-albumins: Dry green or yellow pea flour was used as a source of pea albumins. The flour was suspended in 10 volumes of 50 mM sodium acetate buffer pH 5 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and pea seed debris by either centrifugation (8000 g, 20 minutes) or filtration through a 5 micron filter. Supernatant or filtrate, respectively, was collected. To this crude protein extract, solid ammonium sulfate was added to 50% wt/v 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% wt/v saturation. The solution was stirred for 1 hour, and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20˚C until further use. Protein was recovered from the pellet and prepared for use as described above, with the exception that final buffer can contain 0-500 mM sodium chloride.

In some embodiments, the flour was suspended in 10 volumes of 50 mM NaCl, pH 3.8 and stirred for 1 hour. Soluble protein was separated from un-extracted protein and pea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 kD molecular weight cutoff PES membrane.

(ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was suspended in 10 volumes of 50 mM potassium phosphate buffer pH 8 and 0.4M sodium chloride and stirred for 1hr. Soluble protein was separated from

pea seed debris by 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. Protein was recovered from the pellet and prepared for use as described above.

iii) Soybean 7S and 11S globulins: Globulins from soybean flour were isolated by first suspending lowfat/defatted soy flour in 4-15 volumes of 10 (or 20) mM potassium phosphate pH 7.4. The slurry was centrifuged at 8000 rcf for 20 mins or clarified by 5 micron filtration and the supernatant was collected. The crude protein extract contained both the 7S and 11S globulins. The solution then was 0.2 micron filtered and concentrated using a 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system or by passing over anion-exchange resin prior to use in experiments. The 11S globulins were separated from the 7S proteins by isoelectric precipitation. The pH of the crude protein extract was adjusted to 6.4 with dilute HCl, stirred for 30 min-1 hr and then centrifuged to collect the 11S precipitate and 7S proteins in the supernatant. The 11S fraction was resuspended with 10mM Potassium phosphate pH 7.4 and the protein fractions were microfiltered and concentrated prior to use.

Soybean proteins also can be extracted by suspending the defatted soy flour in 4-15 volumes (e.g., 5 volumes) of 20 mM sodium carbonate, pH 9 (or water, pH adjusted to 9 after addition of the flour) or 20 mM potassium phosphate buffer pH 7.4 and 100 mM sodium chloride to decrease off-flavors in the purified protein. The slurry is stirred for one hour and centrifuged at 8000 xg for 20 minutes. The extracted proteins are ultrafiltered and then processed as above or alternatively, the supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(iv) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first suspending the flour in 4 volumes of 50 mM 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. Protein was recovered from the pellet and prepared for use as described above.

Moong bean globulins also can be extracted by suspending the flour in 4 volumes of 20 mM sodium carbonate buffer, pH 9 (or water adjusted to pH 9 after addition of the moong flour) to reduce off-flavors in the purified protein fractions. The slurry is centrifuged (or filtered) to remove solids, ultrafiltered and then processed as described above.

(v) Late embryogenesis abundant proteins: Flour (including but not limited to moong bean and soy flour) was suspended in 20 mM Tris–HCl, pH 8.0, 10 mM NaCl, and stirred at room temperature for 1 hour then centrifuged. Acid (HCl or acetic acid) was added to the supernatant to a 5% concentration (v/v), stirred at room temperature then centrifuged. The supernatant was heated to 95°C for 15 minutes, and then centrifuged. The supernatant was precipitated by adding Trichoroacetic acid to 25%, centrifuged, then washed with acetone. Heating and acid wash steps can be carried out in the reverse direction as well.

(vi) Pea-Prolamins: Dry green pea flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged (7000g, 20min) and the supernatant collected. The ethanol in the supernatant was evaporated by heating the solution to 85°C and then cooling to room temperature. Ice-cold acetone was added (1: 4 v/v) to precipitate the proteins. The solution then was centrifuged (4000g, 20min), and protein recovered as the light-beige colored pellet.

(vii) Zein-Prolamins: Corn protein concentration or flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged. Ethanol in supernatant was evaporated with heat, and then the solution is centrifuged, and the protein recovered as the pellet.

(viii) RuBisCO was fractionated from alfalfa greens by first grinding leaves with 4 volumes of cold 50 mM potassium phosphate buffer pH 7.4 buffer (0.5M NaCl + 2mM DTT + 1mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1hr and then centrifuged. The pellet from this step

was discarded and additional ammonium sulfate was added to the supernatant to 50 % (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1hr. The pellet from this step contains RuBisCO, and was kept at -20°°C until used. Protein was recovered from the pellet and prepared for use as described above.

RuBisCO also can be purified by adjusting the crude lysate to 0.1M NaCl and applying to an anion exchange resin. The weakly bound protein contaminants are washed with 50 mM KPhosphate buffer pH 7.4 buffer + 0.1M NaCl. RuBisCO was then eluted with high ionic strength buffer (0.5M NaCl).

RuBisCO solutions were decolorized (pH 7-9) by passing over columns packed with activated carbon. The colorants bound to the column while Rubisco was isolated in the filtrate.

RuBisCO solutions were also alternatively decolorized by incubating the solution with FPX66 (Dow Chemicals) resin packed in a column (or batch mode). The slurry is incubated for 30mins and then the liquid is separated from the resin. The colorants bind to the resin and RuBisCO was collected in the column flow-through.

In some embodiments, RuBisCO was isolated from spinach leaves by first grinding the leaves with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

In some embodiments, RuBisCO was extracted from alfalfa or wheatgrass juice powder by mixing the powder with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xi) Oleosin. Sunflower oil bodies were purified from sunflower seeds. Sunflower seeds were blended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 1 mM EDTA at 1:3 wt/v. Oil-bodies were collected by centrifugation (5000g, 20min), and resuspended at 1:5 (wt/v) in 50 mM sodium chloride, 2M urea and stir for 30min, 4°C. 2M urea wash and centrifugation steps were repeated. Oil-bodies collected by centrifugation were resuspended in 100 mM sodium phosphate buffer pH 7.4, 50mM

sodium chloride. Centrifugation and washing steps were repeated once more, and the final washed oil-bodies fraction was obtained from a last centrifugation step. Oil-bodies were resuspended at 10% wt/w in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 2% wt/v vegetable oil fatty acid salts, homogenized at 5000 psi and incubated at 4°C for 12 hr. Solution was centrifuged (8000g, 30min), top layer removed and soluble fraction collected. SDS-PAGE analysis suggested that oleosins are a major protein present in the soluble fraction. Oleosin concentration was 2.8 mg/ml.

(ix) Pea total proteins: Dry green or yellow pea flour was used to extract total pea proteins. The flour was suspended in 10 volumes of 20mM potassium phosphate buffer pH 8 and 100 mM sodium chloride and stirred for 1hr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 kDa cutoff PES membrane.

(x) Pea vicilin and Pea legumin: Dry green or yellow pea flour was used to extract total pea proteins as described above. The crude pea mixture obtained thereof was fractionated into pea vicilin and pea legumin using ion-exchange chromatography. Material was loaded on Q Sepharose FastFlow resin and fractions were collected as salt concentration was varied from 100 mM to 500 mM NaCl. Pea vicilin was collected at 350 mM sodium chloride while pea legumin was collected at 460 mM sodium chloride. The collected fractions were concentrated using a 10 KDa cutoff PES membrane.

(xi) Amaranth flour dehydrins: Amaranth flour was suspended in 5 volumes of 0.5 M sodium chloride, pH 4.0 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 3 kDa cutoff PES membrane. Further enrichment of dehydrins from this fraction was obtained by boiling the concentrated protein material, spinning at 8000 g for 10 minutes and collecting the supernatant.

EXAMPLE 10: ADDITION OF BACTERIAL CULTURES TO MELTABLE GELS COMPRISING PURIFIED PROTEINS

Meltable gels were made with bacterial cultures by subjecting a mixture comprising 2% pea globulins and 4% soy proteins to homogenization. The emulsion was

heated to 95°C, held at 95°C for 15 minutes and then cooled back to room temperature, then 0.03% *Lactococcus lactis* culture and 1% glucose was added at 30°C during the cool-down phase. The gel was allowed to incubate at 25°C overnight and drained to obtain a gel that melts reversibly at 70°C.

EXAMPLE 11: PREPARATION OF CREAM FRACTION FOR USE IN CHEESE REPLICAS

A cream fraction was created by blending sunflower seeds into 5 times the weight to volume of a solution of 40 mM potassium phosphate pH 8, with 400 mM NaCl and 1 mM EDTA, then cooled to 20°C. The resulting slurry (slurry 1) was centrifuged. The top cream layer was removed from slurry 1 and blended in the same buffer, then heated for 1 hour at 40°C, the lower aqueous layer is the skim fraction. The resulting slurry (slurry 2) was cooled down then centrifuged; the cream layer was removed from slurry 2 and mixed with 5 times the weight to volume of 100 mM sodium carbonate pH 10 with 400 mM NaCl, then centrifuged to obtain slurry 3. The top layer from slurry 3 was then mixed with 5 times the weight to volume of water and centrifuged again. The resulting cream was very creamy white, and described by taste testers as has having no bitter tasting, a neutral taste, and having an excellent mouth feel.

EXAMPLE 12 - CREATION OF MELTING CHEESES REPLICAS WITH ISOLATED SOY PROTEINS

A 6% solution of soy protein (7S + 11S globulins) was homogenized with 20% sunflower oil and the emulsion was heated to 95°C and then cooled back to 25°C. The mixture was incubated at temperatures ≤25°C overnight and then drained through cheesecloth. The resulting gel melted reversibly, i.e., melts on heating and sets on re-cooling. The gel can be poured into molds for shaping.

A gel comprising 4% moong globulins was induced to melt upon heating if unfractionated soy protein at 0.6% was added to the mixture before the gel formed, i.e., before the heat-cool cycle (heating to 95°C, holding at 95°C for 15 minutes, and cooling back to 25°C) was applied.

A gel comprising 2% pea globulins was induced to melt upon heating if soy protein at 4% concentration was added to the mixture before forming the gel with a heat-cool cycle as described above.

EXAMPLE 13 - Meltable gels comprising isolated proteins without soy proteins

A soy-free meltable gel was made from moong 8S globulins at a concentration at 7% in a solution at pH 8 when the concentration of sodium chloride was maintained at 80 mM and the solution was subjected to a heat-cool cycle (heating to 95°C and cooling back to 25°C). The gel reversibly melted.

A solution of moong 8S globulins at pH 7.4 and 50 mM sodium chloride also formed a gel that reversibly melts after applying a heat-cool cycle (heating to 95°C and cooling back to 25°C).

EXAMPLE 14: NON-DAIRY MELTING CHEESE REPLICAS, BY ADDING MELTING SALTS AND CATIONS

In this example, a 4% solution of moong 8S protein was homogenized with 20% palm oil and the emulsion was heated to 95°C and then cooled back to 30°C, and 0.03% MA11, and 1% glucose was added. The mixture was incubated at 25°C overnight and then the gelled and non-gelled liquid (i.e., the whey) were separated by draining through cheesecloth. The resulting cheese replica gel was very soft at room temperature and did not melt upon heating as indicated by no change in viscosity reversibly. The addition of 3% sodium citrate after draining the whey caused the cheese replica gel to melt, with the cheese replica gel becoming liquid upon heating and increasing in firmness upon cooling to room temperature. The resulting cheese replica gel can be poured into molds for shaping.

In this example, two samples were compared, with and without $CaCl_2$; one sample was a 6% solution of soy protein (7S and 11S) mixed with 1 mM $CaCl_2$, and the other was a 6% solution of soy protein (7S and 11S) without $CaCl_2$. Both samples were heated to 95°C and then cooled back to 30°C. At 30°C, 0.03% MA11 and 1% glucose were added to both samples. The mixtures were then incubated at 25°C overnight, followed by

separation of the gelled material from the non-gelled liquid by draining the non-gelled liquid through cheesecloth. The resulting gels were solid at room temperature and did not melt upon heating, i.e., no change in viscosity. The addition of 1% melting salts (sodium citrate, trisodium phosphate, sodium hexameta phosphate, or disodium phosphate) added after draining the whey caused both cheeses to change viscosity upon heating to some extent, with both cheese replicas being firm at room temperature. The cheese replica gels with $CaCl_2$ with the melting salts had a large increase in viscosity. In particular, the addition of 1% salt hexametaphosphate to the $CaCl_2$ containing cheese replica gel, resulted in the gel becoming meltable as it became liquid upon heating. Upon cool down, all of the cheese replica gels were firm at room temperature.

EXAMPLE 15: NON-DAIRY, NON-SOY MELTING CHEESE REPLICA, BY THE ADDITION OF FAT

In this example, two samples were compared, with and without saturated fat. One sample was a 6% solution of rubisco mixed with 20% palm oil, and the other was a 6% solution of rubisco without palm oil. Both samples were heated to 95°C and then cooled back to 30°C. At 30°C, 0.03% MA11 and 1% glucose were added and the mixture was then incubated at 25°C overnight. The cheese replica gels were compared for firmness and meltablity. The resulting gel without palm oil was very soft and had no viscosity change upon heating. The sample with 6% rubisco and 20% palm oil was a gel at room temperature, and upon heating increased the viscosity to become liquid and then re-solidified once cooled again. The addition of saturated fat allowed for a non-melting gel to be made into a melting cheese replica. This also was observed when saturated fat was added to moong protein cheese replica gels.

EXAMPLE 16: CREATION OF NON-DAIRY STRETCHY CHEESE REPLICAS USING ISOLATED PROTEINS

In this example, two samples were compared, with and without prolamins purified from pea flour; one sample was a 4% solution of soy protein (7S and 11S), 2% pea-globulin, mixed with 2% pea-prolamins, and the other sample was a 6% solution of soy protein (7S and 11S) mixed with 2% pea-globulin; both samples were heated to 95°C and

then cooled back to 30°C. At 30°C, 0.03% MA11 and 1% glucose were added to both samples. The mixtures were then incubated at 25°C overnight, followed by separation of the gelled material from the non-gelled liquid by draining the non-gelled liquid through cheesecloth. The resulting cheese replica gels were both solid at room temperature, with the cheese replica gel containing prolamins being firmer. Both gels also melted upon heating, as shown with an increase in viscosity. There was no noticeable different in the viscosity of the samples upon heating, but there was a greater change in viscosity for the prolamin sample upon cooling as it was firmer at room temperature. Upon heating, the cheese replica with prolamins had an increase in the interactions between the molecules. As a portion of the cheese replica with prolamins was pulled away from the rest of the sample, it showed stretching, due to rest of the gel retaining its intermolecular interactions. These stretching properties were not seen in the sample without prolamins.

EXAMPLE 17: CREATION OF STRETCHY NON-DAIRY CHEESE REPLICAS USING POLYSACCHARIDES

Cheese replicas were compared with and without 0.5% xanthan gum. One sample was a 4% solution of soy protein (7S and 11S), 2% pea-globulin, mixed with 2% pea-prolamins, and 0.5% xanthan gum; another was a 4% solution of soy protein (7S and 11S), 2% pea-globulin, and mixed with 2% pea-prolamins; another was a 4% solution of soy protein (7S and 11S), 2% pea-globulin; the last sample was a 4% solution of soy protein (7S and 11S), 2% pea-globulin and 0.5% xanthan gum. All samples were heated to 95°C and then cooled back to 25°C. The mixtures were then incubated at 25°C overnight, then drained through cheesecloth to separate gelled material from non-gelled liquid. The resulting cheese replicas gels were all gelled at room temperature, with the gel containing prolamins and/or xanthan gum being firmer than the others. All the gels melted upon heating, as shown with an increase in viscosity. Upon heating, it was also shown that the two cheese replicas with xanthan gum had an increase in the interactions between the molecules, greater than the cheese replicas with no xanthan gum. As in Example 16, the cheese replicas with prolamins exhibited stretching properties.

EXAMPLE 18: CREATION OF CHEESE REPLICAS USING CROSSLINKED ISOLATED PROTEINS

A cheese replica was made from an emulsion of 4% pea-globulins, 20% sunflower cream fraction, and 1% glucose, by heating to 24°C, then adding MA11 at 0.03% and incubating for 1 hour. After the incubation, the heat was increased to 38°C, and 0.6% transglutaminase was added and the temperature held at 38°C for 1 hour. The mixture in the vat/beaker was held at room temperature for 12 hours to allow the mixture to coagulate. After 12 hours, the final curd had a pH of 4.2. The whey then was drained from the curd through butter muslin, and then the curd was whisked and spooned into mold forms and held at room temperature for 1 hour. After 24 hours at room temperature, the cheese replicate was removed from the mold form and stored at 4°C.

EXAMPLE 19 - METHOD OF MAKING HARD CHEESE REPLICAS

The non-dairy milk was inoculated with thermophilic cultures before formation of the gel. During formation of the gel, the temperature was slowly increased to allow the curd to release more whey. The curd was cut into 1/2 inch squares and allowed to acidify for 10 minutes still suspended in its own whey. After 10 minutes the curd was stirred with a large whisk breaking it up into pea sized pieces. At that time the internal temperature of the whey/coagulum was increased by 2 degrees every five minutes by heating in a waterbath until the desired internal temperature (130°F) was reached. The curd was stirred every 10 minutes to assure that it was not reaggregating. The curds were then separated and drained to form a hard cheese replica. The hard cheese replica was then aged.

EXAMPLE 20 - METHOD OF MAKING A SOFT RIPENED CHEESE REPLICA

The following is the standard recipe used throughout the examples to make soft ripened (SR) cheese replica. The standard pasteurized milk mixture that was used has 28% cream, made from 55% almonds and 45% Macadamia. The milk is heated to 90 ± 3° F, then Florica Danica, Mesophilic Starter (MA11, LF2, LF5, LF7, LF21, MD88 or other cultures), *Geotrichum candidum*, *Penicillium candidum*, and *Debaromyces hansenii*

cultures were allowed to hydrate on top of milk for 5 minutes before it is stirred into the milk. Proteases and/or lipases (dissolved in water) can be added at this step. The milk containing the cultures was held at 90 ± 3° F for 90 minutes. After an hour, the pH of the formula usually drops to 5.6 ± 0.2. After the hour, the circulator was adjusted to 110°F, and transglutaminase (dissolved in water right before use) was added. The formula was stirred until completely and evenly mixed into the milk and left unstirred for the rest of the coagulation. Once the formula reached 100°F, the circulator was turned off, and the formula was kept in the waterbath for 10 minutes. Proteases and/or lipases (dissolved in water) also can be added at this step. At that point, the vat/beaker was taken from the water and covered with plastic wrap to coagulate for 12 hours at room temperature.

After 12 hours, the final curd had a pH of 4.4 . The curd was cut into 1 inch squares, and allowed to sit for 5 minutes. The whey was then drained from the curd through butter muslin, usually resulting in 50% curd and 50% whey. Proteases and/or lipases (dissolved in water) also can be added to the curd at this step. The curd was whisked for 5 minutes, spooned into the micro-perforated mold forms to the desired weight, and then allowed to sit at room temperature for 1 hour. After an hour, a cover was placed on the curd on each mold form. The curd was allowed to drain for an additional 1 hour at room temperature in the mold form, and then the curd in the mold form was pressed for 24 hours at 36° F. After the pressing time, the cheese replicas were brined in their mold forms (time is dependent on the size of the piece, 6 oz cheese replica = 20 mins).

Cheese replicas still in their mold forms were fully immersed in preheated saturated brine at 50°F. After brining, the molds were placed on a draining rack and returned to 36°F for 24 hours. Each cheese replica then was removed from its mold and placed on a draining mat and returned to 36°F for 24 hour. After 24 hours, the cheese replica was transferred from 36°F to a dry yeasting room for three days at 60°F, with 75% humidity. After three days, the cheese replica was transferred from a draining mat to an aging mat. The mat was placed on an aging rack, and moved to a ripening room at 50°F, with 90% humidity and continuous airflow. Every two days, the cheese replica was turned and the mat replaced. After seven days, the cheese replica was transferred directly onto an aging rack, allowing maximum aeration for seven more days, or until mold

coverage was complete. After mold coverage was complete, the cheese replica was moved to 36°F for sixteen hours. The cheese replica was then wrapped in perforated paper. Cheese replicas can be tasted two weeks later.

EXAMPLE 21 - METHOD OF MAKING A SOFT FRESH CHEESE REPLICA

The following is the standard recipe used throughout the examples to make soft fresh (SF) cheese replicas. The standard pasteurized milk used to make SF cheese replica has 28% cream, made from 55% almonds and 45% Macadamia. The milk was heated to 83 ± 3° F, then MA11 or other bacterial cultures were added (cultures are allowed to hydrate on top of milk for 5 minutes before it is stirred into the milk). Proteases and/or lipases (dissolved in water) can be added at this step. The milk containing the cultures was held at 83 ± 3° F for 1 hour. After an hour, the pH of the formula usually drops to 5.6 ± 0.2. After the hour, the circulator was adjusted to 110°F, and transglutaminase (dissolved in water right before use) was added. The formula was stirred completely and evenly into the milk and left unstirred for the rest of the coagulation. Once the formula reached 100°F (it takes 50 ± 10 minutes to reach temperature), the circulator was turned off, and the formula was kept in the waterbath for 10 minutes. Proteases and/or lipases (dissolved in water) also can be added at this step. At that point the vat/beaker was removed from the waterbath and covered with plastic wrap and allowed to coagulate for 12 hours at room temperature. After 12 hours the final curd has a pH of 4.4 ± 0.1 (if normal formula). The curd was cut into 1-inch squares, and allowed to sit for 5 minutes. The whey was then drained from the curd through butter muslin, usually resulting in 50% curd and 50% whey. The proteases and/or lipases (dissolved in water) also can be added to the curd at this step. The curd was whisked for 5 minutes, spooned into the mold forms to the desired weight, and then let to sit at room temperature for 1 hour. After an hour, a cover was placed on the curd and 600g of weight on each mold form were added. The curd was allowed to drain for an additional 1 hour at room temperature in the mold form. The curd in the mold form was then pressed for 24 hours at 36° F. After the pressing time, the cheese replicas were brined in their mold forms (time is dependent on the size of the piece, 8 oz cheese replica = 10 mins). Cheese replicas in their mold forms were

placed back at 36° F with their cover but without the weight. After 24 hours, the cheese replicas were removed from their mold forms and placed at 36° F on draining mats. After another 24 hours, the cheese replicas were placed on a clean tray and the entire tray was wrapped with plastic wrap, and maintained at 36° F until tasting.

EXAMPLE 22: Preparation of a Blue Cheese Replica

Standard pasteurized nut milk has 28% cream, made from 55% almonds and 45% macadamia nuts. The milk is heated to 83 ± 3° F, then MA11 and *Penicillium roquefortii* are added (cultures are allowed to hydrate on top of milk for 5 minutes before it is stirred into the milk). The proteases and/or lipases (dissolved in water) can be added at this step. The milk containing the cultures is held at 83 ± 3° F for 1 hour. After an hour, the pH of the formula usually drops to 5.6 ± 0.2. After the hour, the circulator is adjusted to 110°F, and transglutaminase (dissolved in water right before use) is added. The formula is stirred completely and evenly into the milk and left unstirred for the rest of the coagulation. Once the formula reaches 100°F (it takes 50 ± 10 minutes to reach temperature), the circulator is turned off, and the formula stays in the waterbath for 10 minutes. The proteases and/or lipases (dissolved in water) can be added at this step. At that point the vat/beaker is taken from the water and covered with plastic wrap and allowed to coagulate for 12 hours at room temperature. After 12 hours the final curd has a pH of 4.4 ± 0.1 (if normal formula). The curd is cut into 1 inch squares, and allowed to sit for 5 minutes. The whey is then drained from the curd through butter muslin, usually resulting in 50% curd and 50% whey. Proteases and/or lipases (dissolved in water) also can be added to the curd at this step. The curd is whisked for 5 minutes, spooned into the mold forms to the desired weight, and then let to sit at room temperature for 1 hour. After an hour, the follower is placed on the curd and 600g of weight on each mold form are added. The curd is allowed to drain for an additional 1 hour at room temperature in the mold form. The curd in the mold form is then pressed for 36 hours at 36°F. After the pressing time, the cheese replicas are brined in their mold forms (time is dependent on the size of the piece, 8 oz cheese replica = 10 mins). Cheese replicas in their mold form are place back at 36°F with their topper but without weight. The cheese replica forms will be aged at 36°F for 2 days then the salt rub will be applied to the exterior of the cheese replicas

each day for a 3 day period. The cheese replicas are then matured for 20 days at 41°F. The cheese replica is then pierced with a small needle several times into the pressed cheese replica to allow the mold to innoculate in these holes and spread through out the interior surface of the cheese replica. This process is repeated on the 30th day of maturation. At that point, the cheese replica is to age for another 5 months at 40°F. The drying room will be kept at 46 to 53°F and the ripening room will be held at 35 to 50°F.

EXAMPLE 23: Preparation of a Blue Cheese Replica

A nut milk composed of 60% almond milk and 40 % macadamia milk, having a fat content of 14.2%, was pasteurized. The pH of the milk after pasteurization was 6.4. The milk was heated to 81°F and individual microbial cultures (LF2 (LLC), MD88 subculture (LLBD), LF5 (LLL), *Penicillium roquetforti* and *Debaryomyces hansenii*) were added at one time. After incubating at 81°F for 1 hour, the pH of the milk ranged from 5.9 to 6.0. The temperature of the milk was raised to 100°F, and a transglutaminase (ACTIVA TI, from Ajinomoto), hydrated in a 2 to 1 ratio water to enzyme and allowed to sit for 5 minutes, was added to the 100°F milk. The enzyme was stirred in gently and the milk was held for 1 hour at 100°F without stirring. The heat was turned off and the milk allowed to sit covered for 4 hours to form a strong coagulation. After the 4 hours, the pH of the milk was about 5.8. There was a strong resilient gel formed before cutting the curd into 1/2 inch squares and left to heal for 15 minutes in the whey with a pH of 5.9.

The curd was stirred with a large whisk to just break up the curd for about 60 seconds. The temperature of the curd then was increased 2 degrees every 5 minutes until about 120°F with stirring every 10 minutes to insure no further matting of the curd occurred. Once the target temperature was reached, the curd was separated and firm, and had a pH of about 5.6. The curd was put into a tightly woven linen draining bag and hung at room temperature for 8 hours, then placed in a sterilized stainless steel bowl and 1% Kosher salt was added and distributed evenly. After salting the curd, it was ladled into cylindrical molds on plastic draining mats, and allowed to drain for 12 hours, with flipping once every hour for 6 hours. At this point, the forms were salted lightly on all sides and put back into the molds, then put into a yeasting room at 42°F and 75% relative humidity (RH) for 5 days, turning the forms 2x per day. The forms then were moved to

the molding room at 51°F and 90% RH, and punctured multiple times with a stainless steel needle to increase oxygen flow to the center. The cheese replica was maintained under these conditions for 3 weeks to encourage yeast and mold growth to occur. After 3 weeks, the yeast and mold were washed off the exterior of the forms with a 5% salt solution and wrapped in perforated foil. The cheese replica was kept in a cold aging room at 38°F for a minimum of 30 days for cold aging to occur. After aging, the cheese replicas can be wrapped in new foil or plastic wrap to prohibit mold growth on the exterior.

Blue cheese replica crumbles were formed in a similar fashion, except that the curd was spread out on mats and put into a 51°F and 90% RH aging environment to allow the yeast and mold to grow and stabilize the natural microflora. The curd was then aged in the molding room for 3 weeks and packaged in air tight containers to cut off oxygen flow and stop the bluing process.

EXAMPLE 24: Preparation of a washed rind cheese replica

The milk (pasteurized milk has 28% cream, made from 55% almonds and 45% macadamia nuts) was heated to 83 ± 3° F, then MA11, yeast, Micrococci, *Coryneform* bacteria and *Geotrichum candidum* were added (cultures were allowed to hydrate on top of milk for 5 minutes before being stirred into the milk). The proteases or lipases (dissolved in water) can be added at this step. The milk containing the cultures was held at 83 ± 3° F for 1 hour. After an hour, the pH of the formula usually drops to 5.6 ± 0.2. After the hour the circulator was adjusted to 110°F, and transglutaminase (dissolved in water right before use) was added. The formula was stirred until completely and evenly mixed into the milk and left unstirred for the rest of the coagulation. Once the formula reached 100°F, the circulator was turned off, and the formula was left in the waterbath for 10 minutes. The proteases or lipases (dissolved in water) also can be added at this step. At that point the vat/beaker was taken from the water and covered with plastic wrap to coagulate for 12 hours at room temperature. After 12 hours the final curd has a pH of 4.4 ± 0.1 (if normal formula). The curd was cut into 1-inch squares, and let to sit for 5 minutes. The whey was then drained from the curd through butter muslin, usually resulting in 50% curd and 50% whey. The proteases and lipases (dissolved in water) also

can be added at this step to the curd. The curd was whisked for 5 minutes, spooned into the mold forms to the desired weight, and then allowed to sit at room temperature for 1 hour. After an hour, a cover was placed on the curd and 600g of weight on each mold form were added. The curd was allowed to drain for an additional 1 hour at room temperature in the mold form. The curd in the mold form was then pressed for 36 hours at 36°F. After the pressing time, the cheese replicas were brined in their mold forms (time is dependent on the size of the piece, 8 oz cheese replica = 10 min). Cheese replicas in their mold form were place backed at 36°F with their cover but without weight. The cheese replica forms were aged at 36°F for 2 days. The cheese replica forms were brined in a saturated brine and allowed to drain for 48 hours at 36°F. After the 48 hour period the cheese replicas were brushed with a solution of *Brevibacterium linens* and 5% salt solution for two weeks turning the forms every two days. After that time, the washing of the form was decreased. Once the *Brevibacterium linens* was visible on the rind, the washing was reduced to 2x per week. After that period, the rind was brushed with a solution of yeast and water to help dry out the rind with help from good fresh air movement as the cheese replicas age.

The drying room temperature for washed rind cheese replica was 57 to 64° F. and the ripening room temp was 52 to 57° F. The cheese replicas were wrapped in breathable paper and aged for an additional 60 days at 35° F or higher.

EXAMPLE 25 - USE OF CITRATE IN YEAST EXTRACT MEDIA TO DEVELOP "BUTTERY" FLAVORS

A 2-dimensional matrix of glucose concentrations vs. citrate concentrations was set up with MD88 (LLBD) to evaluate the relationships between citrate and glucose and the production of "buttery" compounds (e.g., acetoin and 2,3-butanedione). The yeast extract media (YEM) used was composed of 0.5% yeast extract (Flavor House, Inc, Item# X11020) and 20 mM Potassium Phosphate buffer, pH 7.0. This YEM was inoculated with 0.005% (w/v) MD88 lyophilysate (Danisco CHOOZIT, Item# MD088 LYO 50 DCU). Glucose and citrate stocks were added to 9 volumes of YEM with MD88 in such a way to create a 3x3 matrix of [Glucose] vs. [Citrate]. Glucose was added to this media at 200 mM, 50 mM, or 10 mM, and citrate was added as trisodium citrate hydrate

at 50 mM, 10 mM, or 2 mM. All media samples were incubated for 17 hours at 30°C with 200 rpm shaking.

Cultured samples were smelled by a trained flavor scientist and analyzed by GCMS. Table 8 provides the recorded aroma descriptions and the GCMS data for each sample after the 17-hour aerobic incubation. A "buttery" aroma could be smelled in all samples down to 2 mM Citrate and this aroma was stronger with higher glucose concentrations. All samples with 10 mM glucose had a very mild aroma and higher pH suggesting poorMD88 growth at this glucose concentration.

TABLE 8

Aromas and pH of each sample

[Glucose](mM)/[Citrate] (mM)	pH	Aroma
200/2	4	yeasty, light butter
200/10	4	buttery, pungent/sharp sour
200/50	4.5	buttery, strong sour
50/2	4	media, light butter
50/10	4	buttery, sour
50/50	7	buttery, sour, light almonds
10/2	5.5	bready, light butter
10/10	6.5	bready, yeasty
10/50	7.5	light media

GCMS was used to detect the different volatile compounds present in each sample using a solid phase micro-extraction (SPME) fiber containing polydimethylsiloxane (PDMS) to adsorb volatile compounds from the sample headspace. Five mL volumes of each sample in glass GCMS vials were sealed and the volatile compounds were extracted from the headspace for 12 mins at 50°C while samples were agitated at 500 rpm. The GCMS data for "buttery" aroma compounds (2,3-butandione, acetoin, and 2,3-hexanedione) support the aroma descriptions. All three compounds increased with increasing citrate concentration and glucose concentrations over about 25 mM. At 10 mM Glucose, MD88 did not grow well and therefore could not produce any aroma compounds. See, FIGs. 12-14.

EXAMPLE 26A: USE OF STAPHYLOCOCCUS XYLOSUS TO DEVELOP "CHEESY" FLAVORS IN YEAST EXTRACT MEDIA

Three different microbes were cultured in YEM with 5% Refined Coconut Oil at 4 different glucose concentrations to determine if microbial growth in a low carbon environment could promote lipid degradation to free fatty acids. The YEM was composed of 0.5% yeast extracts (Flavor House, Inc, Item# X11020) and 20 mM potassium phosphate buffer, pH 7.0 which was sterile filtered and then combined with warm coconut oil. The whole mixture was sonicated to create a homogenous emulsion. The emulsified media mix was distributed to glass vials and sets were spiked with different glucose concentrations: 20mM, 5mM, 1mM, or 0mM. Each glucose gradient set then was inoculated with 0.005% (w/v) MD88 (CHOOZIT, Item# MD088 LYO 50 DCU), 0.005% (w/v) TA61 (CHOOZIT, Item# TA 61 LYO 50 DCU), or $5x10^7$ cells/mL of *Staphylococcus xylosus* (SX) (in-house isolation). Samples inoculated with MD88 or SX were incubated at 30°C with 200 rpm of shaking for 19 hours. Sample inoculated with TA61 were incubated at 37°C with 150 rpm of shaking for 19 hours. Cultured samples were then smelled by two individuals, the pH measured, and analyzed by GCMS (SPME fiber sampling of headspace). Table 9 contains the recorded aroma descriptions for each sample after the 19-hour aerobic incubation. The samples inoculated with SX were the most interesting. At higher glucose concentrations the samples smelled fermented and fruity, while at lower glucose concentrations the samples smelled waxy and plastic suggesting the presence of free fatty acids. These observations were supported by the GCMS data which detected C6:0, C8:0, C9:0 & C11:0 free fatty acids in samples with 0 mM and 1 mM Glucose. See FIG. 15. At 20 mM Glucose, SX produced 2-methyl-butanoic acid and 3-methyl-butanoic acid, which are described in literature to have fermented, fruity, and cheesy aromas. See, FIG. 16.

TABLE 9

Aromas of each sample and the final pH of each sample

Media Condition (SX)	pH	Aroma Results 1	Aroma Results 2
20 mM Glucose	5.5	fruity, fermented,	sweaty(butyric acid-like), feety-

		brothy	apricot
5 mM Glucose	7	brothy, slight waxy	bready, potato
1 mM Glucose	5.5	cheesy, plastic/waxy	waxy (C10:0)
0 mM Glucose	4.5	plastic/waxy	lighter waxy, slight fruity noticed

EXAMPLE 26B: USE OF BREVIBACTERIUM TO DEVELOP "CHEESE" FLAVORS IN YEAST EXTRACT MEDIA

Brevibacterium was cultured in YEM with several different keto-acids to determine if *Brevibacterium* will synthesize free fatty acids from keto-acids. The YEM was composed of 0.5% yeast extract (Flavor House, Inc, Item# X11020), 20 mM Potassium Phosphate buffer, pH 7.0, and 50mM Glucose. Separate volumes were spiked with 10 mM of Pyruvic Acid (Sigma, Cat# 10736), 10mM trisodium citrate (QC Unlimited, LLC), or 10 mM Oxalix acid (Sigma, Item# 194131). All samples were inoculated with 0.02% (w/v) *Brevibacterium*/*Corynebacteriae* (CHOOZIT, Item# LR LYO 10D) and incubated at 30°C with 200 rpm of shaking for 22 hours. Cultured samples were then smelled, pH measured, and then analyzed by GCMS (SPME fiber sampling of headspace). Table 10 provides the recorded aroma descriptions by a trained flavor scientist for each sample after the 22-hour aerobic incubation. The sample with oxalic acid added was described and "goaty" and "waxy" which are characteristic odors of short chain free fatty acids. This observation was further supported by the GCMS data showing butanoic acid, propanoic acid, 3-methyl butanoic acid, and 2-methyl propanoic acid ("cheese" acids) only in the sample with oxalic acid.

TABLE 10

Aroma descriptions and measured pH values for *Brevibacterium* samples

Sample Name	Aroma	pH
Pyruvic Acid	brothy, rotten	6
Citrate	earthy, musty, mushroom	7
Oxalic Acid	brothy, mushroom, goaty, waxy (aged cheese-like)	4.5
None	fermented, brothy, malty	7

EXAMPLE 27: USE OF PYRUVIC ACID AND TA61 TO ENHANCE "BUTTERY" FLAVORS IN CULTURED SOYMILK

MD88 and TA61 were cultured in soy milk with varying concentrations of trisodium citrate and/or pyruvic acid to determine the affects on "buttery" aroma compound production. The soy milk (WestSoy, Unsweetened, Organic) was supplemented with 20% Coconut Milk (Sprouts, Premium), 50 mM Glucose, and 50 mM Sodium Chloride. Two separate volumes of soy milk media were inoculated with either 0.01% (w/v) MD88 (CHOOZIT, Item# MD088 LYO 50 DCU) or 0.01%(w/v) TA61 (CHOOZIT, Item# TA 61 LYO 50 DCU). Each set was divided into 12 volumes and spiked with: Na3-Citrate (QC Unlimited, LLC) at 20mM, 10mM, 5mM, or 2mM; Pyruvic Acid (Sigma, Cat# 10736) at 20mM, 10mM, 5mM, or 2mM; and Citrate and Pyruvate at 10mM & 10mM, 5mM & 5mM, 1mM & 1mM; one sample was spiked with neither. Samples inoculated with MD88 were incubated at 30°C for 24 hours, and samples inoculated with TA61 were incubated at 37°C for 24 hours. After incubation, the samples were smelled by a trained flavor scientist, tasted, the pH measured, and then analyzed by GCMS (SPME fiber sampling of headspace). Tables 11 and 12 contain the recorded aroma and flavor descriptions for each sample after the 24-hour incubation. Soymilk samples inoculated with TA61 had a stronger buttery aroma in comparison to samples inoculated with MD88. The most "buttery" TA61 samples were those spiked with only low concentrations of pyruvate or citrate; a higher pyruvate concentration resulted in a more "creamy" aroma. The addition of higher citrate concentrations also resulted in more sour and astringent tasting samples. The GCMS results from each sample set show that only the addition of pyruvate to soymilk cultured with TA61 increase the acetoin production. The addition of citrate to soymilk does not significantly enhance the production of buttery aroma compounds by MD88 or TA61 in soymilk. Similarly, the addition of pyruvate to soymilk has no effect on acetoin or 2,3-butanedione production by MD88.

TABLE 11

Measured pH, and aroma and flavor descriptions of each MD88 cultured sample.

Samples with MD88	Aroma	pH	Taste
No Addition	butter, coconut, cream, light green	4.5	sour, creamy, soymilk
2 mM Citrate	butter, sour, cream	4.5	sour, creamy, soymilk
5 mM Citrate	creamy, butter, light cereal	4.5	sour, creamy, soymilk
10 mM Citrate	creamy, wheat, light butter	4.5	sour, astringent, soymilk

			taste
20 mM Citrate	creamy, light butter, wheat	4.5	sour, astringent, citrus, soymilk
2 mM Pyruvate	buttery, sour, light green	4.5	sour, creamy, soymilk
5 mM Pyruvate	malty, wheat	4.5	sour, soymilk
10 mM Pyruvate	sour, light butter, wheat	4.5	sour, soymilk
20 mM Pyruvate	sour, creamy, wheat	4.5	sour, soymilk
1 mM Pyruvate, 1mM Citrate	butter, creamy, wheat	4.5	light sour, soymilk
5mM Pyruvate, 5mM Citrate	butter, cream	4.5	sour, soymilk
10mM Pyruvate, 10mM Citrate	sour, slight citrus, light butter, wheat	4.5	sour, soymilk

TABLE 12

Measured pH, and aroma and flavor descriptions of each TA61 cultured sample.

Samples with TA61	Aroma	pH	Taste
0 mM Citrate or Pyruvate	butter, soy	4	sour, cream, soymilk
2 mM Citrate	butter, soy	4	sour, soymilk
5 mM Citrate	butter, cream, wheat	4	sour, soymilk
10 mM Citrate	creamy, light butter, wheat	4	sour, citrus, soymilk
20 mM Citrate	cream, wheat	4	sour, citrus, soymilk
2 mM Pyruvate	butter, creamy	4	sour, soymilk
5 mM Pyruvate	buttery, cream, sweet, wheat	4	sour, soymilk
10 mM Pyruvate	creamy, light butter, wheat	4	sour, soymilk
20 mM Pyruvate	cream, sweet, wheat	4	sour, soymilk
1 mM Pyruvate, 1mM Citrate	buttery, creamy	4	sour, soymilk
5mM Pyruvate, 5mM Citrate	cream, butter, sweet	4	very sour, soymilk
10mM Pyruvate, 10mM Citrate	sweet, cream, wheat	4	very sour, citrus, soymilk

EXAMPLE 28A: USE OF *STAPHYLOCOCCUS XYLOSUS* TO DEVELOP "CHEESY" ACIDS IN SOYMILK

SX was cultured in soy milk with varying branched chain amino acids to determine affects of supplemental amino acids on 3-methyl-butanoic acid, 2-methyl

butanoic acid, and 2-methyl propanoic acid production by SX. The soy milk (WestSoy, Unsweetened, Organic) was supplemented with 20% Coconut Milk (Sprouts, Premium), 50 mM Glucose, and 50 mM Sodium Chloride. Leucine (Leu), Isolecuine (Ile), and Valine (Val) were spiked in at 10 mM into separate samples. An additional sample was spiked with all 3 amino acids at 6mM. A final sample included 0.5% yeast extract (Flavor House Inc, Item# 090656) as a source of branched chain amino acids. All samples were then inoculated with $1x10^8$ cells/mL of SX and incubated at 37°C for 24 hours. Cultured samples were analyzed by GCMS (SPME fiber sampling of headspace). FIGs. 20-22 depict the GCMS data for the 3-methyl-butanoic acid, 2-methyl butanoic acid, and 2-methyl propanoic acid detected in each sample after the 24-hour incubation. The addition of leucine increased 3-methyl butanoic acid production while the addition of isoleucine increased 2-methyl butanoic acid production. Similarly, addition of valine increased 2-methyl propanoic acid production.

EXAMPLE 28B: USE OF STAPHYLOCOCCUS XYLOSUS TO DEVELOP 3-METHYL BUTANOIC ACID IN SOYMILK

SX was cultured in soymilk with varying concentrations of leucine to determine affects of supplemental leucine on 3-methyl-butanoic acid production by SX. The soymilk (WestSoy, Unsweetened, Organic) was supplemented with 20% Coconut Milk (Sprouts, Premium), 50 mM Glucose, and 50 mM Sodium Chloride. Leucine was spiked in to separate samples at 30 mM, 20 mM, 10 mM, 5 mM, 2 mM, and 0 mM. Additionally, a sample with 10 mM leucine was spiked with an additional 10 mM of alpha-ketoglutaric acid (aKG). All samples were then inoculated with $1x10^8$ cells/mL of SX and incubated at 30°C for 24 hours. Cultured samples were smelled by a trained flavor scientist, tasted, the pH measured, and then analyzed by GCMS (SPME fiber sampling of headspace). Table 13 contains the recorded aroma and flavor descriptions for each sample after the 24-hour incubation. All samples spiked with leucine had a "dried apricot" aroma and a sweet taste for the lower leucine concentrations and a savory taste for the greater leucine concentrations. The GCMS supports the aroma descriptions and shows all samples with additional leucine have significantly higher 3-methyl butanoic acid. A change in GCMS signal was considered to be significant if was greater

than 3x the signal of a compared sample. In this case, the sample with 2 mM Leu was detected to have 28x the signal for 3-methybutanoic acid as the sample without Leu (0 mM).

TABLE 13

Measured pH, aroma descriptions, and flavor descriptions of each SX cultured sample

[Leucine] (mM)	Aroma	pH	Taste
0	old dried apricots, sour, slight musty	5.5	soymilk, slight astringent
2	dried apricots, caramel	5.5	slight sweet, yogurt-like, wheat
5	dried apricots, caramel	5.5	slight sweet, savory-note, wheat
10	dried apricots, malty	5.5	savory, dried apricots
20	dried apricots, malty	5.5	savory, light sickly-sweet, apricots
30	dried apricots, malty, slight octane	6	savory, wheat, salty, apricots, musty
10, plus 10mM aKG	dried apricots, malty, slight musty	5.5	savory, dried apricots

EXAMPLE 29: USE OF BREVIBACTERIUM AND METHIONINE TO PRODUCE DIMETHYL TRISULFIDE

Brevibacterium was cultured in soy milk and YEM with varying concentrations of methionine to determine affects of supplemental methionine on "cheesy" aroma production by *Brevibacterium*. The soymilk (SunOpta, SoyBase) was supplemented with 50 mM Glucose and the YEM was composed of 0.5% yeast extracts (Flavor House, Inc, Item# X11020), 20 mM potassium phosphate buffer, pH 7.0, and 50 mM glucose. Both media types were inoculated with 0.02% (w/v) *Brevibacterium*/*Corynebacteriae* (CHOOZIT, Item# LR LYO 10D) and incubated at 30°C with 200 rpm of shaking for 22 hours. Cultured samples were then smelled, pH measured, and then analyzed by GCMS (SPME fiber sampling of headspace). Table 15 contains the recorded aroma and flavor descriptions for each sample after the 22-hour incubation. All samples with methionine added had a "fermented" and "fishy" aroma. The GCMS data suggests that this strong aroma is from dimethyl trisulfide. Methionine can be deaminated to create methional which is a desired aged/cheddar cheese aroma compound. Additionally, *Brevibacterium*

is normal cultured on the outside of cheese and will produce methional during the aging process.

TABLE 15

Aroma descriptions and sample pH after 22-hour incubation of Brevibacterium in SoyBase and yeast extract media with varying Methionine concentrations.

Sample Name	Aroma	pH
SoyBase-10mM Met	fermented, thiol, potato	7
SoyBase-5mM Met	fermented, fishy, potato	7
SoyBase-1mM Met	fishy, fermented, strong fried potato	7
SoyBase	soy milk, mushroom, earthy	7
YEM-10mM Met	fermented, fishy, potato	7.5
YEM-5mM Met	fermented, fishy, potato	7
YEM-1mM Met	lighter fermented, fishy, potato	7
YEM	fermented, brothy, malty	7

EXAMPLE 30: PRODUCTION OF MICROBIAL CHEESE FLAVOR PRODUCTION IN PEA CHEESE REPLICAS

A 50 mg/mL solution of isolated pea vicilin (PV) protein (purified to greater than 80%) was heated to 90°C for 30 minutes to denature the proteins used as the base for cheese flavor development by SX, TA61, and MD88 cultures. The denatured PV was supplemented with 20% Coconut Milk (Aray-D), 20 mM Glucose, and 0.5% (w/v) yeast extract (BioSpringer, Item# 2020). Four different culturing procedures were followed: (i) SX culture followed by MD88 culture (SX/MD88), (ii) SX and MD88 co-culture (SX+MD88), (iii) SX culture followed by TA61 culture (SX/TA61), and (iv) SX and TA61 co-culture (SX+TA61). All samples were inoculated with $1x10^8$ cells/mL SX and specified samples were inoculated with 0.05%(v/w) MD88 (CHOOZIT, Item# MD088 LYO 50 DCU) or 0.05% (w/v) TA61 (CHOOZIT, Item# TA 61 LYO 50 DCU). For sequentially cultured samples, the SX was cultured at 30°C with 200 rpm of shaking for 24 hours. Then specified samples were inoculated with MD88 or TA61 and additional substrates (40mM Glucose, 10mM Na3-Citrate, 2mM Methionine, and 3mM $MgCl_2$).

All samples were incubated a second time with 200 rpm of shaking for 22 hours; MD88 inoculated samples were incubated at 30°C and TA61 inoculated samples were incubated at 37°C. Co-cultured samples contained SX, the additional substrates (40mM

Glucose, 10mM Na_3-citrate, 2mM methionine, and 3 mM $MgCl_2$), and MD88 or TA61. Samples with MD88 were incubated at 30C while samples with TA61 were incubated at 37°C, but all samples were shaken at 200 rpm for the 24-hour incubation. Once all samples were cultured, the samples were smelled and tasted by a trained flavor scientist, the pH was measured, and then samples analyzed by GCMS (SPME fiber sampling of headspace). The samples were created into cheese replicas by adding in 20 mM $CaCl_2$ to solidify the replicas. Table 16 contains the recorded aroma and flavor descriptions for all samples cultured with PV. Most samples smelled "buttery" and/or "creamy" and these descriptions are supported by the GCMS data showing the presence of acetoin and 2,3-butanedione. See, FIGs. 25 and 26. FIG. 27 shows the free fatty acids detected by GCMS in cultured PV with coconut milk.

TABLE 16

Aroma, flavor and pH descriptions of cultured pea vicilins with coconut milk.

Sample Name	Final pH	Aroma	Flavor
SX+MD88 A	5	buttery, slight burnt, light cream, coconut	burnt, buttery, slight astringent
SX+MD88 B	4.5	buttery, creamy, slight burnt	sour, buttery, slight grainy
SX+TA61 A	5	buttery, creamy, nutty	strong burnt, pea, sour
SX+TA61 B	4.5	buttery, creamy, pea protein	sour, strong savory, burnt
SX/MD88 A	5	sweet, floral, buttery, light burnt	strong savory, sour, burnt
SX/MD88 B	5	fruity, liquor	savory, burnt
SX/TA61 A	4.75	malty, caramel, cream	savory, burnt
SX/TA61 B	4.75	fruity, malty	fruity, savory, light burnt

EXAMPLE 31 - DIRECT CONTROL OF THE CREATION OF FLAVOR COMPOUNDS BY BACTERIA

To control flavor production of different aroma compounds produced by bacteria, MA11, MD88, TA61 were tested in a low odor media (LOM). Two controls were run, one with only LOM+MA11 and one with LOM only. A stock volume of LOM was made, sterile filtered, and stored at 4°C thorough the study. Bacteria concentration of 1x 10^8 cells/mL was added to a stock. This LOM with bacteria was aliquoted into the required

number of 10 mL GC vials. To each individual vial, the pre-determined amount of additive was added. All vials were covered tightly with tin foil, stored at 30°C for ~ 24 hours, and then 4°C for ~ 24 hours. After culturing, the vials were allowed to warm to room temperature as the final reaction pH was checked with pH strips. The pH of each vial was adjusted to 3-3.5 with 6M HCl. Each vial was smelled by a trained flavor scientist and the aroma was recorded. All vials were capped and run on the GCMS. Original data was analyzed using the ChromoTOF library then aligned for each additive type. Table 17 shows the LOM that was used for each of the different bacteria, TA61 was not able to grow in the other media. Table 18 shows the compounds created or eliminated by which bacteria and the additives that control its production.

TABLE 17

Low odor media to determine how additives control flavor generation by different bacteria

	TA61	MD88, MA11
yeast extracts	0.50%	0
Peptone (from Pea)	0.10%	0.10%
K-phos buffer, pH 7.15	50	100
MgCl2	2	2
FeCl2	2	0
Glucose	50	25
NaCl		20

TABLE 18

Aroma compounds production controlled by different bacteria with additives

Aroma	Compound	To increase the compound…	To decrease the compound…
herbal, sweet green	1-Hexanol	Mango+MD88	
	2- Nonanone	Ala + MA11, (CoA, FAD, Lipoic Acid, Thiamine)+MA11, $CuSO_4$+MA11	(Folic Acid, Citric Acid, Pyruvate) + MA11
acetone-like, fruity, butterscotch	2-Butanone	Ile + TA61, Thiamine +TA61, Ascorbic acid + TA61, Mg +TA61, Riboflavin + MA11, Ascorbic Acid + MA11, $FeCl_2$+MA11, Ascorbic Acid+MD88, C10:0-C16:1+MD88, Oils+MD88	Ca + TA61, C3:0 +MA11, C4:0 + MA11, C6:0 + MA11, C8:0+ MA11, amino acids +MD88, Citric acid+MD88, C3:0-C8:0+MD88
sweet, fruity, fatty	2-ethyl-1-Hexanol	NAD, FAD, B12, Folic, Nicotinic, Riboflavin, Thiamine, **CoenzymeA,** lipoic acid, α-Ketoglutarate with TA61	TA61+Amino Acids
cheese, fruity, coconut	2-Heptanone	Pro + TA61, Ser +TA61, Thiamine + TA61, Mg + TA61, NAD + MA11, ($CoCl_2$, $CuSO_4$)+MA11, Coconut Oil+MD88, Valine+MD88	Asp + TA61, Glu + TA61, Met + TA61, Co +TA61, Ala + MA11, citric acid + MA11, $CaCl_2$+MA11, $MNSO_4$+MD88, Nicotinic Acid+MD88
green, herbal, cheesy, fresh	2-Nonanone	Pro + TA61,Ser + TA61, Citric acid + TA61, Malic acid + TA61, Lipoic acid+ TA61, Valine+MD88	

fruity, woody, fermented	2-Pentanone	Amino Acid+MD88, Metals+MD88	Most Organic Acids+MD88
buttery	2,3-Butanedione	Ala + TA61, Phe + TA61, Pro + TA61, Pyridoxine + TA61, **Citric + TA61, Pyruvate + TA61, Xanthine + TA61**, Malate + TA61, Inosine + TA61, Zn^{2+} + TA61, Pyruvate + MA11, CoCl2+MA11, Citric Acid+MD88, Pyruvate+MD88, Riboflavin+MD88, C16:1+MD88	Ile + TA61, Lue + (Ala, Asn, Glu) + MA11, Nicotinic acid + MA11, C3:0 + MA11, C4:0, C6:0, C10:0, C12:0, C14:0, Most Oils and Fatty Acids
pungent, fruity, musty	Acetylaldehyde	Thr + TA61, Riboflavin, + TA61, Thiamine + TA61, Ascorbic acid+ TA61, Lipoic acid + TA61, Mg + TA61, Riboflavin + MA11, ($CuSO_4$, $FeCl_2$)+MA11, Thr+MD88, Ascorbic Acid+MD88	Ala + TA61, Asp + TA61, Glu + TA61, Mn + TA61, Ala + MA11, ($CaCl_2$, $CoCl_2$)+MA11
sour	Acetic acid	Ile +TA61, Biotin +TA61, p-aminobenzoic acid +TA61, Citric acid+TA61, Malic acid +TA61, Inosine +TA61, Pyruvate+TA61, MA11, (Riboflavin+CoA)+MA11, CuSo4+MA11, Citric Acid+MD88,Riboflavin+MD88	Asp+TA61, Glu+TA61, MA11 + Ala, NAD+MA11, $FeCl_2$+MA11
buttery	Acetoin	Ile+TA61, Biotin+TA61, p-aminobenzoic acid+TA61, Thiamine+TA61, **Citric acid +TA61, Inosine+TA61, Pyruvate+TA61, Malate+TA61,** Xanthine+TA61, Mg^{2+} +TA61, Zn^{2+} +TA61, Co +TA61, Citric Acid+MD88, Pyruvate + MA11,	Asp+TA61, Glu +TA61, (Ala, Asn, Glu) + MA11, Nicotinic acid+ MA11, C3:0, C4:0, C6:0, C10:0, C12:0, C14:0

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		$CoCl_2$+MA11Pyruvate+MD88, Riboflavin+MD88, C16:1+MD88, Citric Acid+MD88, Pyruvate+MD88, Riboflavin+MD88, C16:1+MD88	
	Acetone	Pro +TA61, Ascorbic acid +TA61, Citric acid +TA61, (Ala, Asn, Met, Pro) + MA11, (NAD, Riboflavin, CoA, FAD, Lipoic Acid, Nicotinic acid, thiamine, B12, Folic Acid, Biotin) + MA11, $CaCl_2$+MA11, Oxalic Acid+MD88, Ascorbic Acid+MD88, Met+MD88	Riboflavin+MD88, Citric Acid+MD88
bitter almonds, cherry	Benzaldehyde	Riboflavin+TA61, Ascorbic+TA61, Mn^{2+} +TA61, $MnSO_4$+MA11, (C16:0, C18:0)+MA11, Nicotinic Acid+MD88	Ala+TA61, Pro+TA61, p-aminobenzoic acid+TA61, Inosine+TA61, Malate+TA61, Xanthine+TA61, Ca^{2+} +TA61, Mg^{2+} +TA61, (Ala, Asn, Asp, Glu, Ile, Leu, Lys, Met, Pro, Tyr) + MA11, (FAD, NAD, Thiamine, riboflavin, ascorbic acid) +MA11, ($CaCl_2$, $CuSO_4$, $FeCl_2$)+MA11, (C10:0, C12:0)+MA11, $FeCl_2$+MD88, $CuSO_4$+MD88, Ascorbic Acid+MD88
Cocoa, green, malty	Butanal		TA61 in general

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coco, coffee, nutty	Butanal, 2-methyl-	Pro+TA61, Ca^{2+} +TA61, Ascorbic Acid+MD88	amino acid + MD88
chocolate, peachy, fatty	Butanal, 3-methyl-	Riboflavin +TA61, Ascorbic Acid+MD88, p-aminobenzoic acid+MD88	TA61 in general , amino acid + MD88, Nicotinic acid +MD88, Riboflavin+MD88, Thiamine+MD88
acidic, dirty, cheese nuance	Butanoic Acid and Derivatives	Ile +TA61, Ala+TA61, Pro+TA61, Inosine+TA61, Malic acid +TA61, Fe^{2+} +TA61, Mg^{2+} +TA61	Thiamine +TA61
	Ethanol	Ile, Biotin, p-aminobenzoic acid, Inosine, Malate, Mg^{2+} with TA61, MA11, (NAD, Riboflavin, CoA, FAD, Lipoic Acid, Nicotinic acid, thiamine, B12, Folic Acid, Biotin) + MA11, Oxalic Acid+MD88	
	Ethyl Acetate	Pro + MA11	
	Methyl Butanals	(CoA, Lipoic Acid, Riboflavin, Nicotinic Acid, B12, Folic Acid, Biotin) +MA11	(Ala, Asn, Met, Pro) + MA11, MA11 in general, Ca^{2+} +MA11, C3:0, C4:0, C6:0
spicy note, floral	2-methyl-Propanal	Pro+TA61, Riboflavin+TA61, Ascorbic+TA61, Mn^{2+} +TA61, Ascorbic Acid+MD88, $MnSO_4$+MD88	Ala+TA61, Asn+TA61, Pro+TA61, p-aminobenzoic acid+TA61, Citric+TA61, Malate+TA61, Ca^{2+} +TA61

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EXAMPLE 32 - CULTURING CONDITIONS FOR AMERICA CHEESE TYPE FLAVOR IN NON-DAIRY CHEESE REPLICA

To prepare a non-dairy replica with an American cheese type flavor, the ingredients in Table 19 can be cultured at 30°C for 24 hours in a closed container with headspace. The resulting material from this culture can be cultured with the ingredients in Table 20 at 37°C for 24 hours in a closed container with headspace.

TABLE 19

Components	Concentrations
Soymilk or Pea Vicilin	80 %
Coconut	20 %
Leucine	2 mM
Yeast Extract 090656 or X11020	0.5 %
Glucose	50 mM
Isolated SX Culture	$1x10^8$ cells/mL

TABLE 20

Components	Concentrations
Material from '1st culture'	100 %
Methionine	5 mM
$MgCl_2$	5 mM
Pyruvate	15 mM
Glucose	50 mM
TA61	$1x10^8$ cells/mL

EXAMPLE 33A - CHEESE REPLICA CONTAINING COACERVATES MADE OF PLANT PROTEINS

A cheese replica was made by first preparing a 3% (w/v) solution of pea vicilins and pea legumins (vicilin:legumin ratio of 3:1 by weight, purified to >90%) in 20mM potassium phosphate pH 7.4 + 100mM sodium chloride. Melted palm oil (from Jedwards

International) was added to the solution to a final concentration of 5% (v/v) and mixed by vortexing. The emulsion was then acidified by addition of hydrochloric acid while stirring to a pH of 5. The resulting slurry was centrifuged and the liquid top layer was decanted off to obtain the coacervate. This material was creamy in texture at room temperature, solidified when on chilled ice, and melted when heated.

EXAMPLE 33B - CHEESE REPLICA CONTAINING COACERVATES THAT IS FURTHER PROCESSED BY CROSSLINKING PROTEINS

A cheese replica was prepared by first making a coacervate from a 3% (w/v) solution of pea proteins at 3:1 pea vicilin: legumin ratio and cocoa butter (from Jedwards International) using the method described in Example 33A. The coacervate was collected by centrifugation and further processed by enzymatically crosslinking its constitutent proteins using a transglutaminase (Ajinomoto) at a final concentration of 1% (w/v). The material was allowed to incubate with stirring overnight at 30°C. The resulting cheese replica was a firm, elastic material similar to aged cheeses such as cheddar at room temperature.

EXAMPLE 33C - CHEESE REPLICA CONTAINING COACERVATES THAT IS FURTHER PROCESSED BY A HEAT-COOL CYCLE

A cheese replica was prepared by first making coacervate from a 3% (w/v) solution of pea proteins at a 3:1 pea vicilin: legumin ratio and canola oil using the method described in Example 33A. The coacervate was collected and heated in a water bath to 70°C for 10 minutes in a closed container, then removed from the water bath to allow it to cool back to room temperature. The resulting material was a firm cheese replica resembling hard cheeses.

EXAMPLE 33D - USE OF COACERVATE TO MAKE MELTABLE CHEESE REPLICA SLICES USING HIGH PRESSURE PROCESSING

A cheese slice-replica was made by mixing 12 g soymilk curd (soymilk from Westsoy sequentially cultured with cheese cultures TA61 and MD88 (both TA61 and MD88 from Danisco) and drained to collect curd) with 12 g of crude soy protein mixture

(at a protein concentration of 14% w/v), 6.7 mL of canola oil (final concentration of 20% v/v) and 2.8 g freeze-dried pea legumin (final pea legumin concentration of 8% w/v). The mixture was acidified to pH 5 using lemon juice to produce a cheese sauce-like consistency at room temperature. The sample was sealed in a heat-sealable food-saver plastic bag and then subjected to high pressure processing (85k psi for 5 minutes in an Avure 2L Isostatic Food Press). The samples were removed from the bags and then evaluated for firmness and melting properties.

The high pressure processing caused the samples to solidify into a cheese slice-replica that melted when heated in an oven set to 350°F. Firmness of the replica was found to be comparable to Kraft American singles using compression test on a TA.XT2 texture analyzer (slices were cut into 1cm diameter disks and stacked to a height of ~5mm. Flat cylinder probe of diameter 25mm was used to compress the samples at 0.5mm/sec compression rate up to a distance of 2mm. The compression force was measured to be 19.2g for Kraft American singles and between 7.3g-12.2g for the replicas).

EXAMPLE 33E – MODULATING VISCOSITY OF THE COACERVATE BY VARYING THE PEA LEGUMIN: VICILIN RATIO

Coacervates were prepared as described in Example 33A using 1:1 or 1:3 pea vicilin: legumin mixtures at a total protein concentration of 10% (w/v) in 20 mM potassium phosphate buffer pH 7.4 + 100 mM sodium chloride, 10% canola oil (v/v) + 10% cocoa butter (v/v) (from Jedwards International). The mixtures were acidified to pH 5 using 1N hydrochloric acid and centrifuged at 5000 x g for 10 minutes to collect the coacervate. The coacervate sample from the 1:1 vicilin:legumin mixture was more creamy and sauce-like in appearance at room temperature and set when chilled on ice. In contrast, the sample prepared from the 1:3 vicilin:legumin ratio was more viscous and did not flow at room temperature.

EXAMPLE 33F – MODULATING VISCOSITY OF THE COACERVATE BY VARYING THE TYPE OF OIL

Coacervate were prepared as described in Example 33A using a 1:1 pea vicilin: legumin mixture at a total protein concentration of 10% (w/v) in 20 mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride. Fat, either canola oil or cocoa butter at 16% w/v, or 10% canola oil (v/v) + 10% cocoa butter (v/v) (all oils from Jedwards International), was added to the protein solution and the mixture was emulsified by sonication, then acidified to a pH of 5 using 1N hydrochloric acid. The coacervates (cheese replicas) were collected by centrifugation at 5000 x g for 10 minutes. The replica made using cocoa butter was viscous, did not flow easily at room temperature and solidified on ice. In contrast, the replica made using canola oil was less viscous and creamy sauce-like at room temperature and became less runny when cooled on ice. The replica made with a mixture of 8% canola oil + 8% cocoa butter was of intermediate viscosity at room temperature, but set when cooled on ice.

EXAMPLE 33E – MODULATING VISCOSITY OF THE COACERVATE USING EMULSIFYING SALTS

Coacervate samples were prepared by first making an emulsion of pea vicilin: legumin (3:1 ratio by weight, total protein concentration of 10%) and a mixture of canola and palm oils (10% each v/v, sourced from Jedwards International). Calcium chloride (Sigma) was added to a final concentration of 1 mM and trisodium pyrophosphate dodecahydrate (TSP12, from Prayon) was added to 1% (w/v). The mixture then was acidified using 1N hydrochloric acid and centrifuged at 5000 x g for 10 minutes to collect the coacervate. In a control experiment, coacervate was formed using the same protein and fat mixture but without addition of calcium chloride and emulsifying salts. Although both the coacervate with TSP12 and control sample were cheese sauce-like in nature at room temperature, the coacervate formed from the mixture containing TSP12 showed much lower viscosity and flowed more readily than the control.

EXAMPLE 34A – FORMING COLD GELS WITH FAT AND CHEESE STARTER CULTURES

A cheese replica was formed by first heating a solution of pea-vicilin (>90% purity as judged by gel electrophoresis) at a concentration of 6% (w/v)in 20 mM

potassium phosphate buffer pH 7.4 + 100mM sodium chloride at 95°C for 30 minutes. The solution was cooled back down to room temperature. Palm fruit oil (20% v/v, from Jedwards International), glucose (1% w/v), and a starter culture (*Lactococcus lactis lactis subsp. diacetylactis* from Danisco) were added to the solution at 0.02% (w/v) and mixed. Calcium chloride was added to a final concentration of 20 mM and the solution was incubated at 30°C for 24 hours to allow for growth of the Lactococcal culture. The resulting gel was a soft, yogurt-like material that was smooth in texture. The gel did not melt on heating.

EXAMPLE 34B – COLD GELS WITH CROSSLINKED PROTEINS

A cheese replica was made by heat denaturing a solution of pea-vicilin (>90% purity as judged by gel electrophoresis) at a concentration of 6% (w/v) in 20 mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride at 100°C for 30 minutes. The solution was cooled back down to room temperature and gelled by addition of palm oil (to 40% v/v from Jedwards International) and calcium chloride to 20 mM. The solutions were transferred to 4°C to obtain a soft, thick yogurt-like gel. The increased amount of fat resulted in a thicker gel. Soy protein (unfractionated) was added to a final concentration of 5% (w/v) and crosslinking was initiated by addition of a transglutaminase (from Ajinomoto) at 0.5% (w/v). The materials were stirred for 1 hr at room temperature and subsequently acidified to a pH of 5 (by addition of 1N hydrochloric acid) while mixing to produce cheese replicas with improved texture (increased firmness, resembling cottage cheese) and meltability (melts in oven set to 350°F).

EXAMPLE 34C – COLD GELS COMBINED WITH COACERVATE TO FORM MELTABLE CHEESE REPLICAS

A cheese-replica was prepared by combining a cold gel with coacervate as described below. The cold gel component was first prepared by heat denaturing a solution of pea-vicilin (>90% purity as judged by gel electrophoresis) at a concentration of 6% (w/v) in 20 mM potassium phosphate buffer pH 7.4 + 100 mM sodium chloride at 100°C for 30 minutes. The solution was cooled back down to room temperature and gelled by

adding palm oil (to 40% v/v from Jedwards International) and calcium chloride to 20 mM. The mixture was incubated for approximately 10 minutes at room temperature to allow the gel to form.

The coacervate component was formed from a mixture of 3:1 pea vicilin: legumin (total protein concentration of 11% w/v) and palm fruit oil (5% v/v). The mixture was sonicated to form an emulsion and acidified with 1N hydrochloric acid to pH 5. The mixture was then centrifuged at 5000 x g for 10 minutes to collect the coacervate (heavier phase at bottom).

The cold gel was mixed with the coacervate in a 2:1 proportion by weight to form a cheese-replica with a soft but thick curd-like consistency at room temperature. Upon heating in an oven at 350°F, the replica melted like cheese.

EXAMPLE 35 – FORMATION OF MELTABLE GELS WITH FRACTIONATED PROTEINS

Solutions of pea proteins at 7-9% protein concentrations (unfractionated), 50 mM NaCl, were adjusted to pH 3-9 using acid (HCl) or base (NaOH) as appropriate. The solutions were subjected to a heat-cool cycle by heating to 95°C in a water bath, holding at 95°C for 1hr and then turning off the heat and allowing the samples to slowly cool back to room temperature while in the water bath. The samples were then removed from the containers and the gels were evaluated for appearance and meltability. All samples showed formation of an opaque, white precipitate-like curd that did not melt upon heating.

To evaluate properties of the pea protein fractions comprising the globular proteins in peas, the proteins were fractionated via anion exchange chromatography. A mixture of crude pea proteins (0.5% w/v) in 20 mM potassium phosphate buffer pH 8 + 50mM NaCl was passed over Q-Sepharose (GE Life Sciences). The unbound protein fraction was collected (albumins) and the bound proteins were fractionated over a salt gradient 50-500mM NaCl. Proteins were observed to elute in 2 major peaks (peak 1 = vicilin + convicilin, peaks 2 = legumin), fractions were pooled and then frozen until use.

Pea protein fractions (vicilin + convicilin and legumin) were tested for formation of meltable gels by dialyzing proteins into buffered solutions pH 4-9 at NaCl

concentrations of 50, 100mM and 300mM (Buffers used at 20mM each: pH 4,5 sodium acetate, pH 6-8 potassium phosphate, pH 9 sodium carbonate). Solutions of 7% protein (w/v) at respective NaCl and pHs were subject to heat-gelation as described above. The samples were then removed from the tubes and evaluated for appearance and melting behavior. Observations are summarized in Table 21.

TABLE 21

Summary of types of gels comprising pea protein legumins and vicilins obtained under various pH and NaCl concentrations

Pea-vicilin + convicilin

	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
100mM NaCl	phase separation		some precipitate	translucent gel, meltable		
300mM NaCl	precipitate	opaque gel, no melt				

Pea-legumin

	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
100mM NaCl	phase separation		some precipitate	opaque gel, does not melt		
300mM NaCl	opaque gels, do not melt			translucent gel, meltable		

It was found that pea vicilins (+ convicilin) and legumins formed meltable gels at pH >7 but did so at different NaCl concentrations. This likely explains why a mixture of pea-legumins and vicilins (+ convicilin) did not form a meltable gel under any condition.

It was further explored how pure the legumin or vicilin fractions needed to be in order to obtain meltable gels. Fractionated pea proteins (legumin and vicilin (+ convicilin)) fractionated as described above were mixed in ratios legumin: vicilin 0:8, 1:7, 2:6, 3:5, 1:1, 5:3, 6:2, 7:1 and 8:0 (at total protein concentration of 8% w/v in 20 mM potassium phosphate buffer pH 7.4 at 158 mM and 300 mM NaCl concentrations. The samples were subject to heat-cool cycle by heating to 95°C in a water bath, holding at 95°C for 15 minutes and cooling to 30°C at the rate of 0.5C/min. The samples were then evaluated for melting properties. At 158 mM NaCl where the viclin (+ convicilin) fraction was expected to form a meltable gel, only samples at legumin:vicilin ratios of 2:6, 1:7 and 8:0 formed meltable gels. This suggests that vicilin (+convicilin) fraction needs to be at least 75% pure to form meltable gels. On the other hand, at 300mM NaCl where legumins are expected to form meltable gels, only samples at a legumin:vicilin

ratio of 7:1 and 8:0 formed meltable gels. This suggests that legumin fraction needs to be at least 87.5% pure to form meltable gels.

EXAMPLE 36 – USING MELTING SALTS FOR GELLED EMULSIONS OF PROTEINS

Solutions of pea legumin, vicilin (+ convicilin), soy proteins, and moong 8S proteins were prepared at 7% (w/v) in 20 mM potassium phosphate buffer pH 7.4 at 100 mM NaCl (except pea legumins at 300 mM NaCl). Melted palm oil (from Jedwards International) was added at 20% (v/v), calcium chloride (from Sigma) at 1 mM and a melting salt (disodium phosphate (DSP), trisodium pyrophosphate dodecahydrate (TSP12), sodium hexametaphosphate (SHMP) or trisodium citrate (TSC)) was added at 1% (w/v) and the mixtures were sonicated to form an emulsion. Each salt was tested for every protein based emulsion. No melting salt added to control samples. The emulsions were transferred to closed containers in a water bath and then subjected to a heat-cool cycle to induce gelation (heated to 95°C for 15 minutes, held at 95°C for 15 minutes and cooled to 30°C at 0.5-1C/min). The samples were then removed from the container and evaluated for meltability.

It was found that while protein solutions described here readily form meltable gels at pH 7.4 at 100 mM NaCl (300 mM NaCl for pea-legumin), the presence of fats appears to interfere with meltability of the gels, i.e., control samples containing protein + oil +/- calcium chloride did not form meltable gels. However, addition of melting salts allowed the protein gels to retain meltability as summarized in Table 23.

TABLE 23

Summary of specificity of melting salts for emulsions comprising different proteins. (✔) indicates heat-induced gel formed from emulsion was meltable, (x) indicates gel was not meltable; (*) indicates sample was heated to a maximum of 85C during the gelling procedure.

Protein fraction in emulsion	DSP	TSP	SHMP	TSC
pea-vicilin (+ convicilin)	✔	✔	✖	✖
pea-legumin	✖	✔	✔	✔
soy (unfractionated)	✔	✖	✖	✖
mung 8S globulin	✔ *	✖	✖	✖

EXAMPLE 37 – MAKING A NUT MILK RICOTTA

A pasteurized nut milk containing 12-14% fat and having a pH between 6.0 to 6.3 was used to prepare ricotta cheese replica. *Lactococcus lactis lactis* (0.02g/L) and *Lactococcus lactis cremoris* (0.02 g/L) were inoculated into the nut milk held at about 80°F, then stirred for 15 minutes. Hydrated transglutaminase (ACTIVA TI, from Ajinomoto) was added to the inoculated milk and was allowed to coagulate at temperatures between 12°C and 25°C for 10 to 14 hours. When the pH was below 4.6, the curd was cut into 1" cubes and drained and pressed for 24 hours at 41°F to obtain firm ricotta with a moisture range from 62 to 68%. The curd can be salted and whipped as desired.

1. A non-dairy cheese replica comprising a coacervate comprising one or more isolated and purified proteins from a non-animal source.

2. The non-dairy cheese replica of claim 1, wherein said one or more isolated and purified proteins are plant proteins.

3. The non-dairy cheese replica of claim 2, wherein said one or more isolated and purified plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins.

4. The non-dairy cheese replica of claim 3, wherein said pea proteins comprise pea vicilins and/or pea legumins.

5. A non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt.

6. The non-dairy cheese replica of any one of claims 5, wherein said non-dairy cheese replica comprises one or more heat-labile ingredients.

7. The non-dairy cheese replica of claim 6, wherein said heat-labile ingredients comprises one or more fats, microbes, volatile compounds, or enzymes.

8. The non-dairy cheese replica of any one of claims 1-7, further comprising one or more microbes selected from bacteria, molds, and yeast.

9. The non-dairy cheese replica of any one of claim 1-8, comprising one or more microbes selected from the group consisting of a *Penicillium* species, a *Debaryomyces* species, a *Geotrichum* species, a *Corynebacterium* species, a *Streptococcus* species, a *Verticillium* species., a *Kluyveromyces* species, a *Saccharomyces* species, a *Candida* species, a *Rhodosporidum* species, a *Cornybacteria* species, *a Micrococcus* species, a *Lactobacillus* species, a *Lactococcus* species, a *Staphylococcus* species, a *Halomonas* species, a *Brevibacterium* species, a *Psychrobacter* species, a *Leuconostocaceae* species, a *Pediococcus* species, a *Propionibacterium* species, and a lactic acid bacterium.

10. The non-dairy cheese replica of any one of claims 1-9, wherein said one or more microbes are selected from the group consisting of *Lactococcus lactis lactis (*LLL*), Leuconostoc mesenteroides cremoris (LM), Lactococcus lactis cremoris* (LLC), *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis,*

Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus (SX), *Lactococcus lactis biovar diacetylactis* (LLBD), *Penicillium roqueforti*, *Penicillium candidum*, *Penicillium camemberti, Penicillium nalgiovensis Debaryomyces hansenii, Geotrichum candidum*, *Streptococcus thermophiles* (TA61), *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Rhodosporidum infirmominiatum* and *Brevibacterium linens.*

11. A non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids, said cheese replica comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus,* and *Brevibacterium linens*

12. A non-dairy cheese replica comprising a solidified non-dairy milk, nut milk, and one or microbes selected from the group consisting of *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus,* and *Brevibacterium linens,* wherein at least 85% of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk.

13. A non-dairy cheese replica comprising an isolated and solidified non-dairy cream fraction and one or microbes selected from the group consisting of *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus,* and *Brevibacterium linens*.

14. The non-dairy cheese replica of any one of claims 11-13, said replica further comprising one of more microbes selected from the group consisting of *Penicillium roqueforti*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium candidum*, *Corynebacteria*, *Streptococcus thermophiles, Penicillium camemberti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces*

cerevisiae, Candida utilis, Rhodosporidum infirmominiatum, Cornybacteria, a Micrococcus species, a *Lactobacillus species,* a *Lactococcus species, Lactococcus lactis lactis (*LLL*), Leuconostoc mesenteroides cremoris (LM), Lactococcus lactis cremoris* (LLC)*,* a *Staphylococcus species,* a *Halomonas species,* a *Brevibacterium sps,* a *Psychrobacter sps,* a *Leuconostocaceae sps,* a *Pediococcus species, Leuconostoc mesenteroides, Lactococcus lactis biovar. diacetylactis* (LLBD) or a *Propionibacterium* species

15. The non-dairy cheese replica of any one of claims 9-14, said replica comprising two of LLL, LLC, and LLBD or comprising SX and TA61.

16. The non-dairy cheese replica of claim 15, said replica comprising LLC and LLL.

17. The non-dairy cheese replica of claim 15, said replica comprising LLL and LLBD or LLL, LLC, and LLBD.

18. The non-dairy cheese replica of claim 17, said replica further comprising *Penicillium roqueforti* and *Debaryomyces hansenii*.

19. The non-dairy cheese replica of any one of claims 5-18, wherein said one or more non-animal based proteins are plant proteins.

20. The non-dairy cheese replica of claim 19, wherein said plant proteins are selected from the group consisting of a seed storage protein or an oil body protein.

21. The non-dairy cheese replica of claim 20, wherein said seed storage protein is an albumin, glycinin, conglycinin, globulin, legumin, vicilin, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin, proteinoplast, secalin, triticeae gluten, or zein.

22. The non-dairy cheese replica of claim 20, wherein said oil body protein is an oleosin, a caloleosin, or a steroleosin.

23. The non-dairy cheese replica of claim 19, wherein said plant protein is selected from the group consisting of ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation

factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), collagens, kafirin, avenin, dehydrins, hydrophilins, and natively unfolded proteins.

24. The non-dairy cheese replica of any one of claims 1-23, said replica further comprising one or more sugars.

25. The non-dairy cheese replica of claim 24, wherein said one or more sugars are selected from the group consisting of sucrose, glucose, fructose, and maltose.

26. The non-dairy cheese replica of any one of claims 1-25, said replica further comprising one or more purified enzymes.

27. The non-dairy cheese replica of claim 26, wherein said one or more isolated enzymes is a lipase, a protease, and/or an amylase.

28. The non-dairy cheese replica of any one of claims 1-27, said replica further comprising a melting salt.

29. The non-dairy cheese replica of claim 26, wherein said melting salt is sodium citrate, trisodium pyrophosphate, sodium hexametaphosphate, disodium phosphate, or any combination thereof.

30. The non-dairy cheese replica of any one of claims 1-27, further comprising a divalent cation

31. The non-dairy cheese replica of claim 28, wherein said divalent cation is Fe^{2+}, Mg^{2+}, Cu^{2+},or Ca^{2+}.

32. The non-dairy cheese replica of any one of claims 1-31, further comprising an isolated amino acid or other additive selected from the group consisting of a food product, a yeast extract, miso, molasses, a nucleobase, an organic acid, a vitamin, a fruit extract, coconut milk, and a malt extract.

33. The non-dairy cheese replica of claim 32, wherein said isolated amino acid is methionine, leucine, isoleucine, valine, proline, or alanine.

34. The non-dairy cheese replica of any one of claims 1-33, wherein said non-dairy cheese replica comprises one or more plant-derived lipids, one or more oils derived from an algae, fungus, or bacterium, or one or more free fatty acids.

35. The non-dairy cheese replica of claim 34, wherein said one or more plant-derived lipids comprises corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil,

sesame oil, cottonseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, palm fruit oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil.

36. The non-dairy cheese replica of claim 35, wherein said one or more plant-derived lipids are canola oil, cocoa butter, and/or coconut oil.

37. The non-dairy cheese replica of any one of claims 1-36 further comprising a cross-linking enzyme.

38. The non-dairy cheese replica of claim 37, wherein said cross-linking enzyme is a transglutaminase or a lysyl oxidase.

39. The non-dairy cheese replica of any one of claims 1-38, wherein said cheese replica has one or more of: a) increased creamy, milky, buttery, fruity, cheesy, free fatty acids, sulfury, fatty, sour, floral, or mushroom flavor or aroma notes; 2) reduced nutty, planty, beany, soy, green, vegetable, dirty, or sour flavor or aroma notes; 3) an increased creamy texture; 4) an improved melting characteristic; and 5) an increased stretching ability, relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

40. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of acetoin, diacetyl, 2,3-hexandione, or 5-hydroxy-4-octanone, or a decrease in one or more of benzaldehyde, 1-hexanol, 1-hexanal, furan, benzaldehyde or 2-methyl-2-propanol, pyrazine, or heptanal relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

41. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in methional and/or dimethyl trisulfide relative to a corresponding cheese replica lacking said one or more microbes, sugar, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

42. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of butanoic acid, propanoic acid, hexanoic

acid, octanoic acid, or decanoic acid relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

43. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of 2-heptanone, 2-undecanone, 2-nonanone, 2-butanone, 2-methyl propanoic acid, 2-methyl butanoic acid, or 3-methyl butanoic acid relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

44. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of ethyl butanoate or methyl hexanoate relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

45. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has (i) an increase in ethyl octanoate and/or 2-ethyl-1-hexanol or (ii) an increase in 2-methyl butanal and/or 3-methyl butanal relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

46. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in acetic acid relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

47. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of gamma-octalactone, delta-octalactone, gamma-nonalactone, butyrolactone, or methyl isobutyl ketone relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids, yeast extract, plant-derived lipids, or combinations thereof.

48. The non-dairy cheese replica of any one of claims 9-39, wherein said cheese replica has an increase in one or more of nonanol or 1-octen-3-ol relative to a

corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids, yeast extract, plant-derived lipids, or combinations thereof.

49. A method of making a non-dairy cheese replica, said method comprising solidifying a mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated fats, said mixture comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus,* and *Brevibacterium linens*.

50. The method of claim 49, wherein solidifying comprises cross-linking said proteins using a transglutaminase or a lysyl oxidase.

51. The method of claim 49, wherein solidifying comprises subjecting said mixture to a heat / cool cycle.

52. The method of claim 49, wherein solidifying comprises forming a cold set gel.

53. The method of claim 49, wherein solidifying comprises forming a coacervate comprising one or more isolated and purified proteins from a non-animal source.

54. The method of any one of claims 49-53, said method further comprising adding one or more isolated enzymes to said mixture before or after solidifying.

55. The method of claim 54, wherein said one or more isolated enzymes is a lipase, protease, and/or an amylase.

56. The method of any one of claims 49-55, said method further comprising adding one or more sugars to said mixture.

57. The method of claim 56, wherein said one or more sugars are selected from the group consisting of sucrose, glucose, fructose, and maltose.

58. The method of any one of claims 49-57, said method further comprising adding a melting salt to said mixture.

59. The method of claim 58, wherein said melting salt is sodium citrate, trisodium pyrophosphate, sodium hexametaphosphate, disodium phosphate, or any combination thereof.

60. The method of any one of claims 49-59, further comprising adding a divalent cation to said mixture.

61. The method of claim 60, wherein said divalent cation is Fe^{2+}, Mg^{2+}, Cu^{2+}, or Ca2+.

62. The method of any one of claims 49-61, further comprising adding an isolated amino acid to said mixture, a yeast extract, or a food product to said mixture.

63. The method of claim 62, wherein said isolated amino acid is methionine, leucine, proline, or alanine.

64. The method of any one of claims 49-63, wherein said one or more isolated fats comprises corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, palm fruit oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil, an algal oil, or an oil derived from bacteria, oil derived from fungi, or rice bran oil.

65. The method of claim 64, wherein said one or more fats are canola oil and/or cocoa butter.

66. The method of any one of claims 49-65, said method comprises further aerating said mixture.

67. The method of any one of claims 49-66, wherein said method comprises incubating said mixture with one microbe for a period of time and then adding a second microbe to said mixture.

68. A method for making a cold set gel comprising:

a) denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution;

b) optionally adding any heat-labile components to said solution of denatured protein;

c) gelling said solution of denatured protein between 4°C and 25°C by increasing the ionic strength; and

d) optionally subjecting said cold set gel of c) to high pressure processing.

69. The method of claim 68, wherein said plant proteins are pea proteins, chickpea proteins, seed storage proteins, proteins from a legume, lentil proteins, or Lupine proteins.

70. The method of claim 69, wherein said pea proteins comprise pea vicilins and/or pea legumins.

71. The method of any one of claims 68-70, wherein said heat labile components comprise one or more microbes.

72. The method of any one of claims 68-71, wherein gelling is induced using 5 to 100 mM sodium or calcium chloride.

73. A method of making a coacervate, said method comprising

a) acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; and

b) isolating said coacervate from said solution; and

c) optionally subjecting said coacervate to high pressure processing.

74. The method of claim 73, wherein said isolated and purified proteins are plant proteins.

75. The method of claim 74, wherein said plant proteins are seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins.

76. The method of claim 75, wherein said pea proteins comprise pea vicilins and/or pea legumins.

77. The method of claim 76, wherein said pea vicilins comprises convicilins.

78. The method of any one of claims 73-77, wherein said acidifying step is done in the presence of a plant based oil.

79. The method of any one of claims 73-78, wherein said pH is between 4 and 5.

80. A method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases.

81. The method of claim 80, wherein said ligand is bound to a solid substrate.

82. A method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with activated carbon then removing the activated carbon from said composition.

83. A method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a lipoxygenase inhibitor and/or an antioxidant.

84. The method of any one of claims 80-83, wherein said composition is a food composition.

85. The method of claim 84, wherein said composition is a cheese replica.

86. A method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated.

87. The method of claim 86, further comprising adding one or more sugars, divalent cations, isolated enzymes, isolated amino acids or other additives, plant-derived lipids, algal oils, or oil derived from bacteria, oil derived from fungi, or free fatty acids to said mixture during said culturing step.

88. The method of claim 86 or claim 87, further comprising solidifying said microbe containing mixture.

89. The method of any one of claims 86-88, wherein said product is a cheese replica.

90. The method of any one of claims 86-88, wherein said product is yogurt or sour cream, crème fraiche, or kefir.

91. A non-dairy cheese replica comprising a coacervate comprising one or more isolated proteins from a non-animal source.

92. The non-dairy cheese replica of claim 91, wherein said one or more isolated proteins are plant proteins.

93. The non-dairy cheese replica of claim 92, wherein said one or more isolated plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins.

94. A non-dairy cheese replica comprising (i) a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids or (ii) a solidified non-dairy milk, nut milk, and one or microbes; wherein said non-dairy cheese replica has a) an increased creamy texture; b) an improved melting characteristic; or an increased stretching ability.

95. A method of making a non-dairy cheese replica, said method comprising solidifying a mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated fats using high pressure processing, said mixture comprising one or more microbes comprising one or more microbes selected from the group consisting of a *Penicillium* species, a *Debaryomyces* species, a *Geotrichum* species, a *Corynebacterium* species, a *Streptococcus* species, a *Verticillium* species., a *Kluyveromyces* species, a *Saccharomyces* species, a *Candida* species, a *Rhodosporidum* species, a *Cornybacteria* species, *a Micrococcus* species, a *Lactobacillus* species, a *Lactococcus* species, a *Staphylococcus* species, a *Halomonas* species, a *Brevibacterium* species, a *Psychrobacter* species, a *Leuconostocaceae* species, a *Pediococcus* species, a *Propionibacterium* species, and a lactic acid bacterium. .

96. A ricotta cheese replica comprising a solidified nut milk, *Lactococcus lactis Lactis*, and *Lactococcus lactis cremoris*.

97. The ricotta cheese of claim 96, further comprising a transglutaminase.

98. The ricotta cheese of claim 96 or 97, wherein said nut milk comprises a mixture of almond milk and macadamia nut milk.

99. A blue cheese replica comprising a solidified nut milk, *Lactococcus lactis cremoris*, *Lactococcus lactis diacetylactis*, *Lactococcus lactis lactis*; *Penicillium roquetforte*, and *Debaryomyces hansenii*.

100. The blue cheese of claim 99, further comprising a transglutaminase.

101. The blue cheese of claim 99 or 100, wherein said nut milk comprises a mixture of almond milk and macadamia nut milk.

102. A method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising:

a) obtaining a starter culture comprising a heterogenous population of microbial strains;

b) isolating one or more individual microbial strains from said heterogenous population; and

c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica.

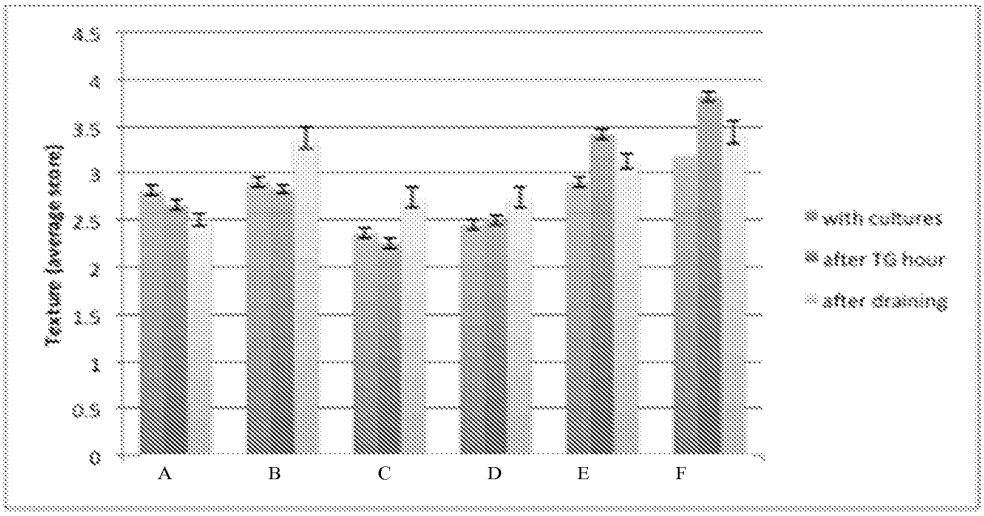

Texture (average score)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
A
B
C
D
E
F
with cultures
after 16 hour
after draining

FIG. 1

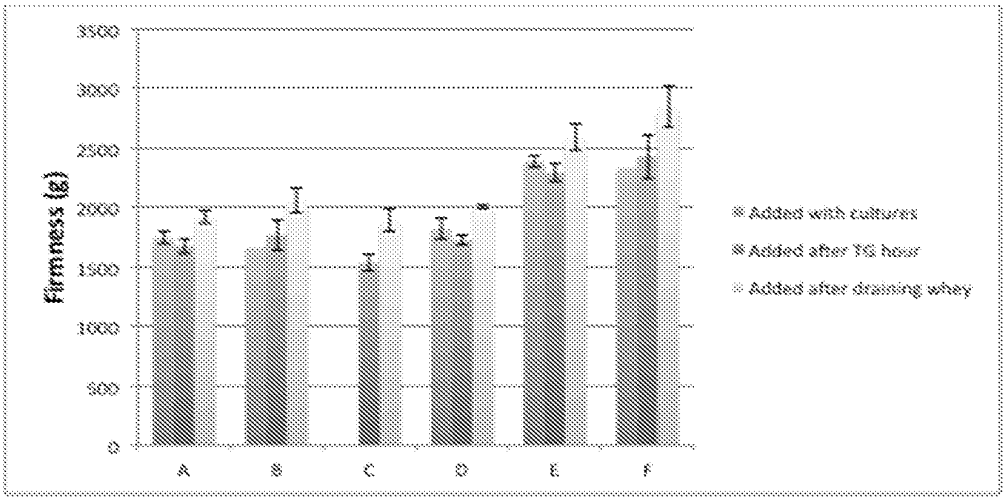

firmness (g)
3500
3000
2500
2000
1500
1000
500
0
A
B
C
D
E
F
Added with cultures
Added after TG hour
Added after draining whey

FIG. 2

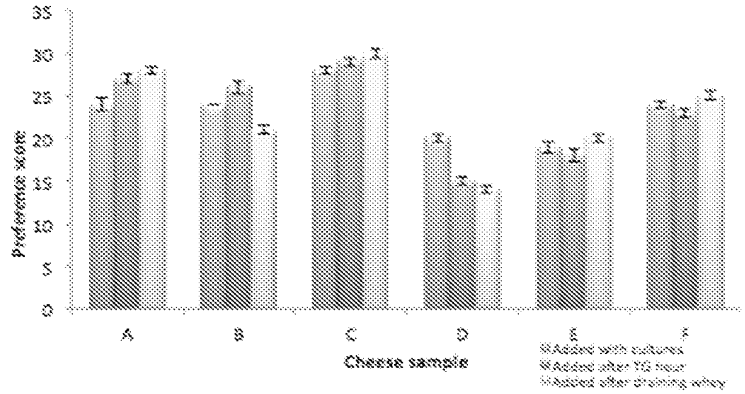

Preference score
Cheese sample
A
B
C
D
E
F

FIG. 3A

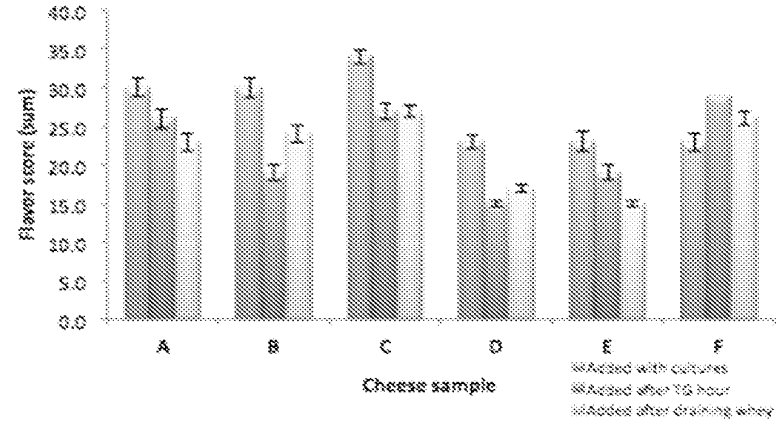

Flavor score (sum)
Cheese sample
A
B
C
D
E
F

FIG 3B.

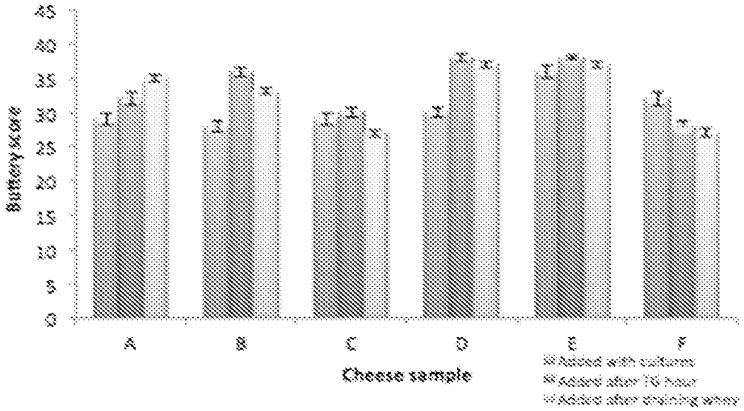


FIG. 4.

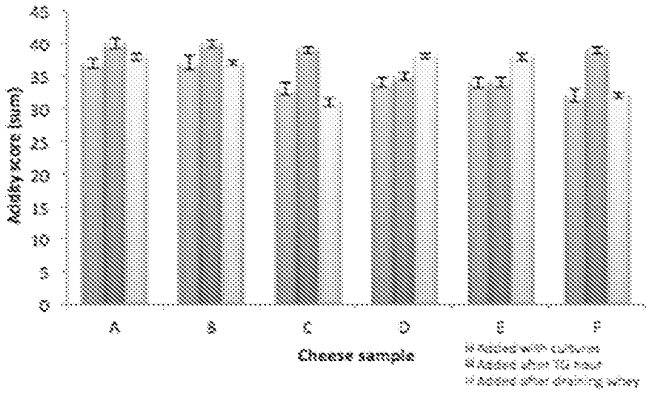


FIG. 5

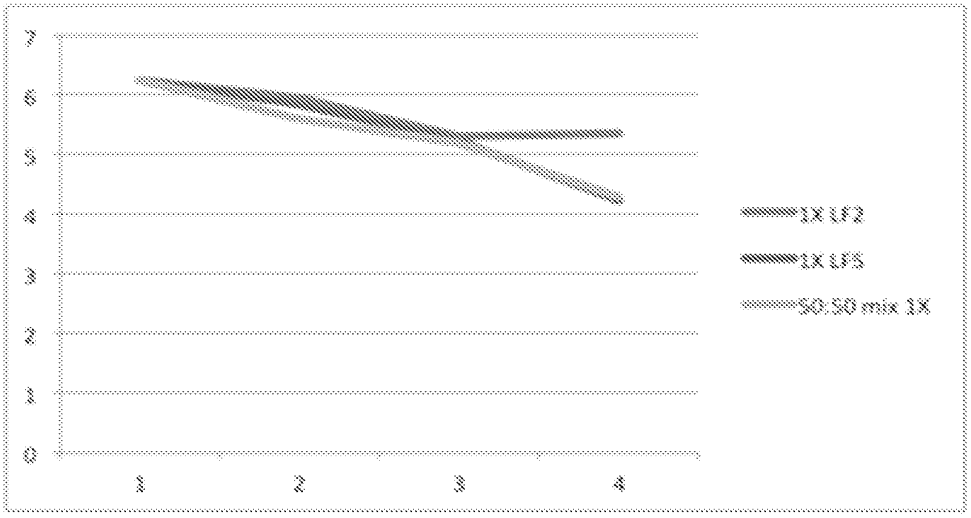
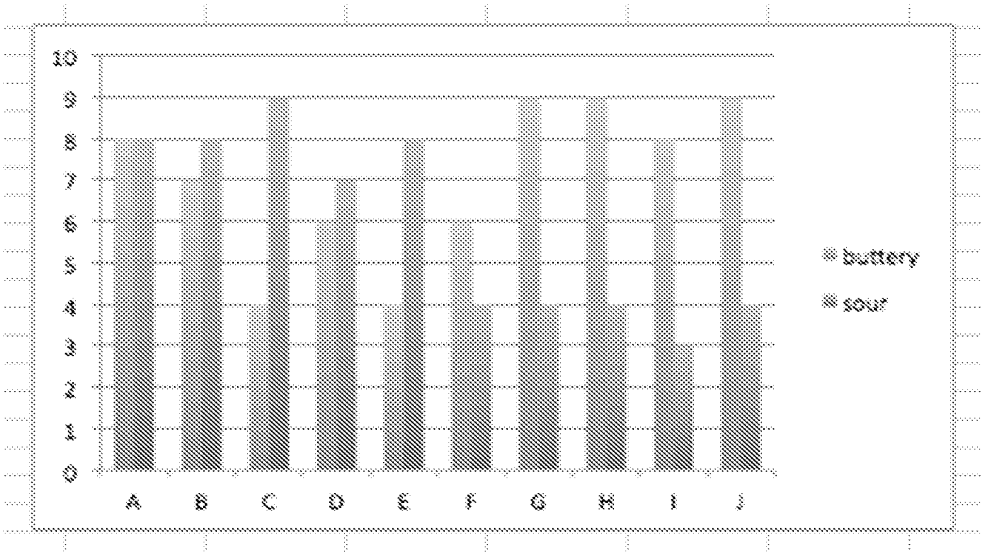


FIG. 6



LM LLBD

FIG 7

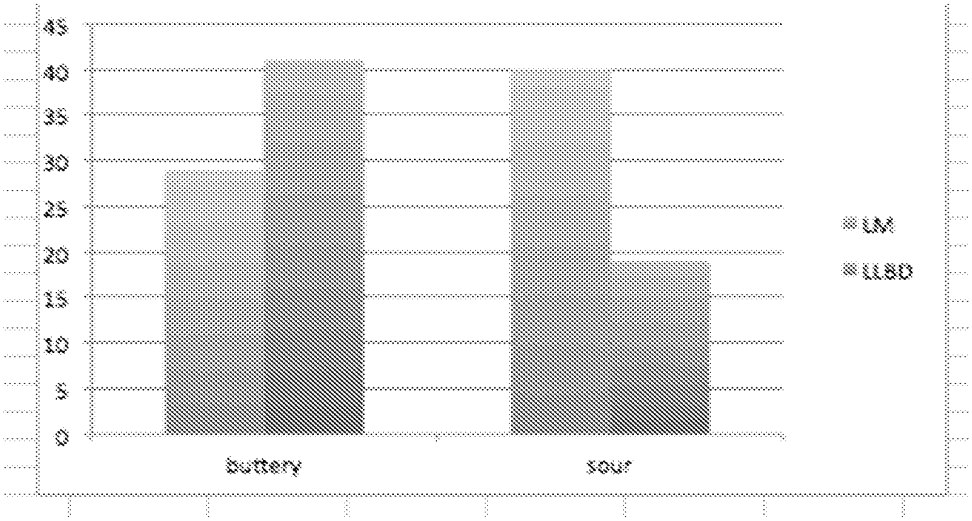


FIG. 8

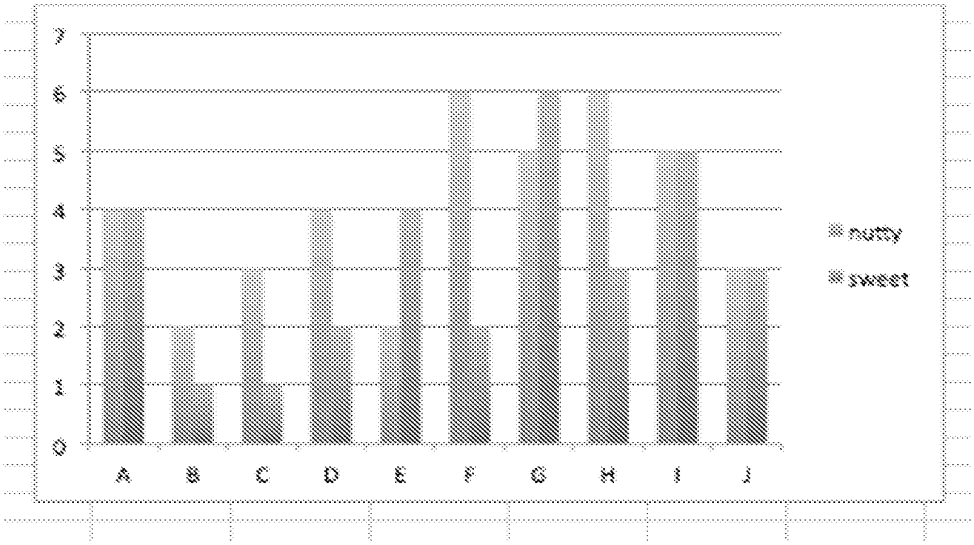


FIG. 9



FIG. 10

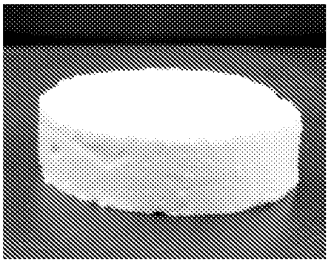


FIG. 11

FIGURE 12

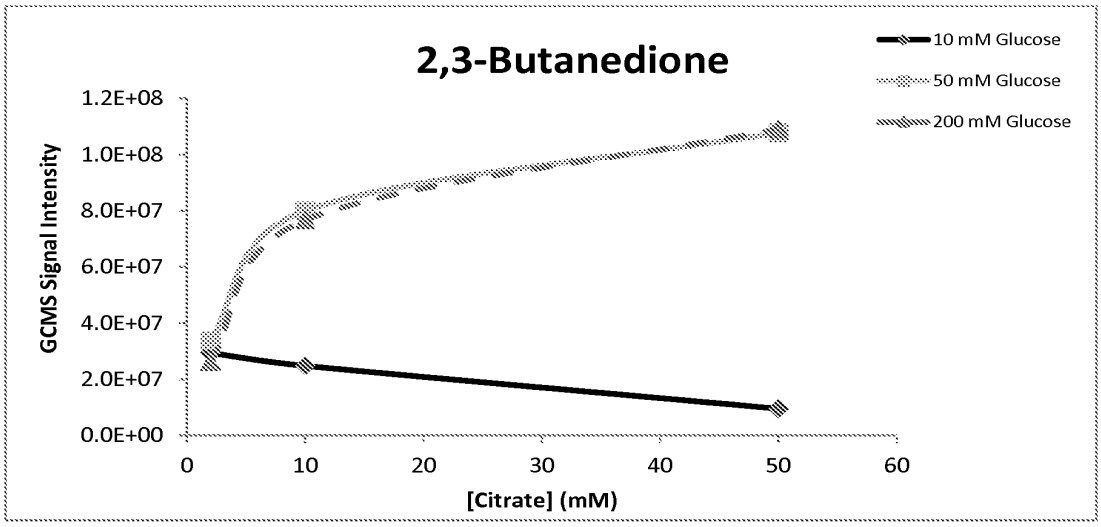

2,3-Butanedione
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 13

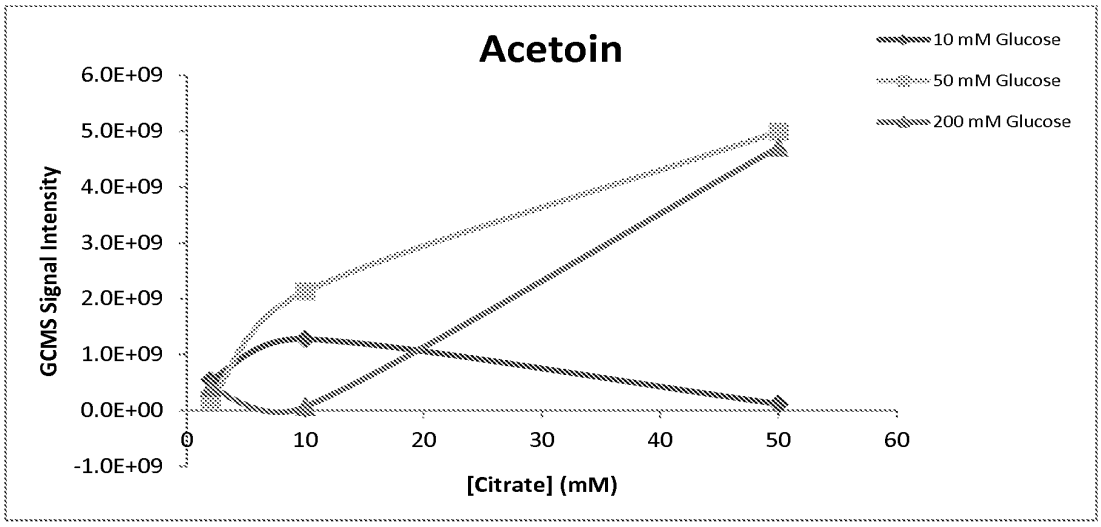

Acetoin
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
6.0E+09
5.0E+09
4.0E+09
3.0E+09
2.0E+09
1.0E+09
0.0E+00
-1.0E+09
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 14

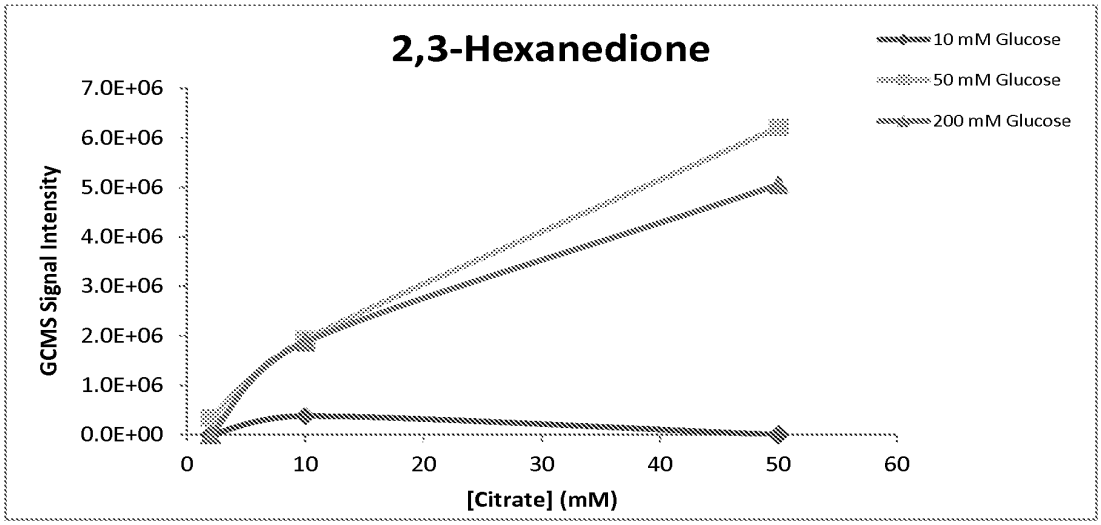

2,3-Hexanedione
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 15

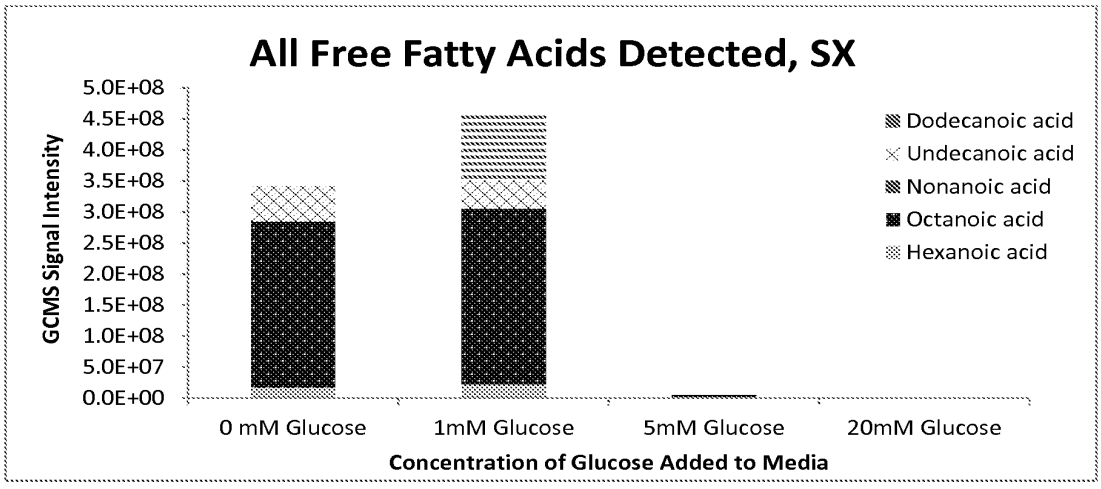

All Free Fatty Acids Detected, SX
GCMS Signal Intensity
5.0E+08
4.5E+08
4.0E+08
3.5E+08
3.0E+08
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
0 mM Glucose
1mM Glucose
5mM Glucose
20mM Glucose
Concentration of Glucose Added to Media
Dodecanoic acid
Undecanoic acid
Nonanoic acid
Octanoic acid
Hexanoic acid

FIGURE 16

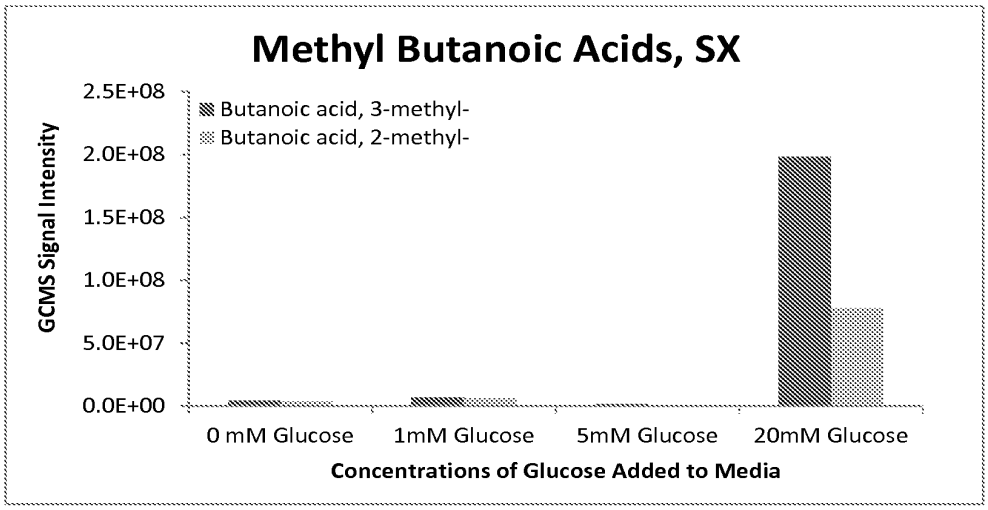

Methyl Butanoic Acids, SX
GCMS Signal Intensity
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
Butanoic acid, 3-methyl-
Butanoic acid, 2-methyl-
0 mM Glucose
1mM Glucose
5mM Glucose
20mM Glucose
Concentrations of Glucose Added to Media

FIGURE 17

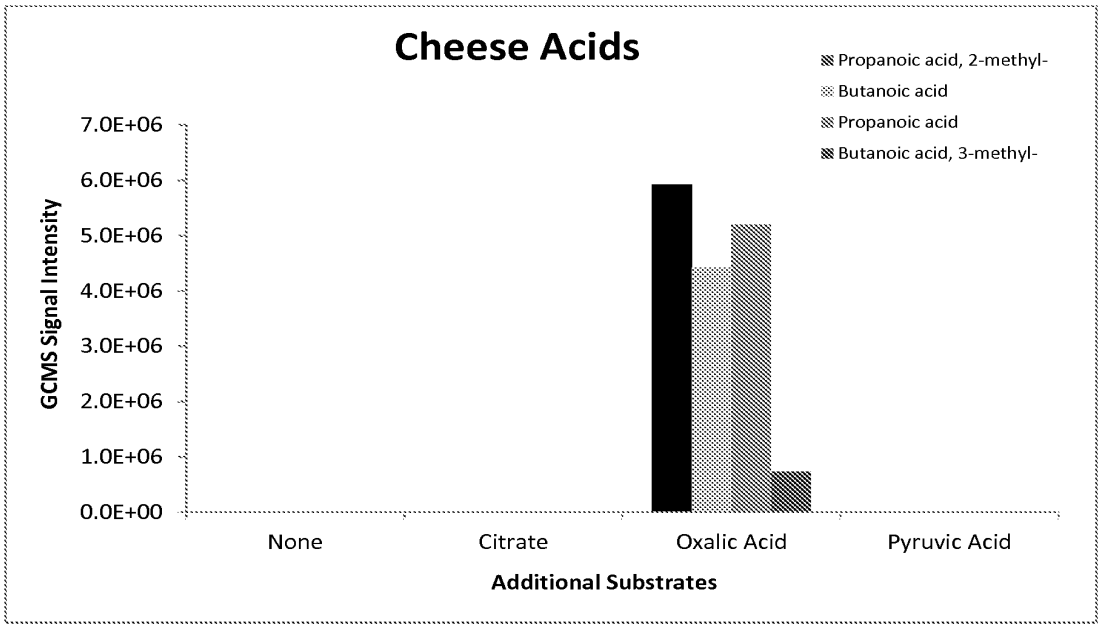

Cheese Acids
Propanoic acid, 2-methyl-
Butanoic acid
Propanoic acid
Butanoic acid, 3-methyl-
GCMS Signal Intensity
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
None
Citrate
Oxalic Acid
Pyruvic Acid
Additional Substrates

FIGURE 18

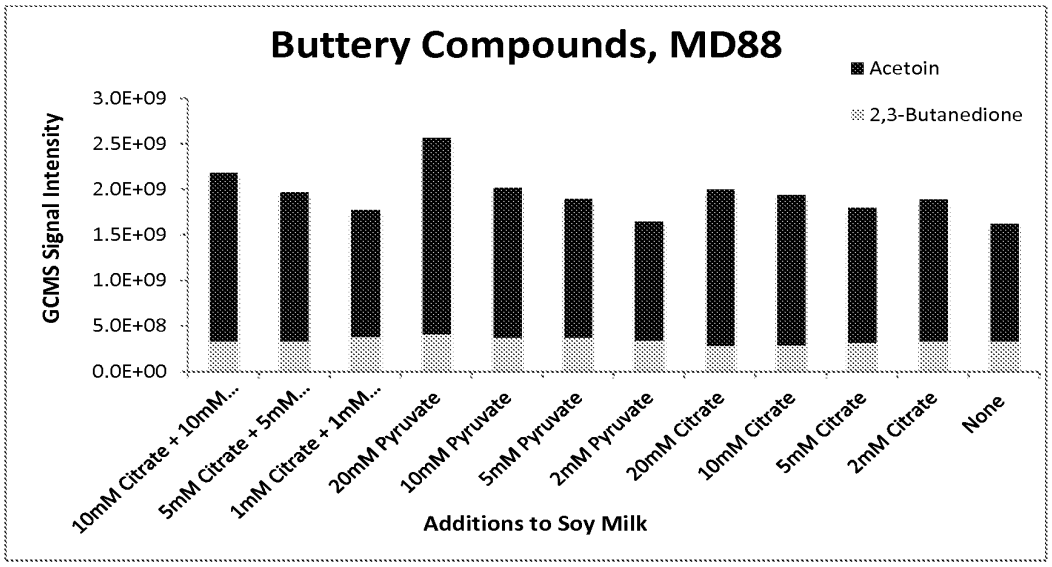


FIGURE 19

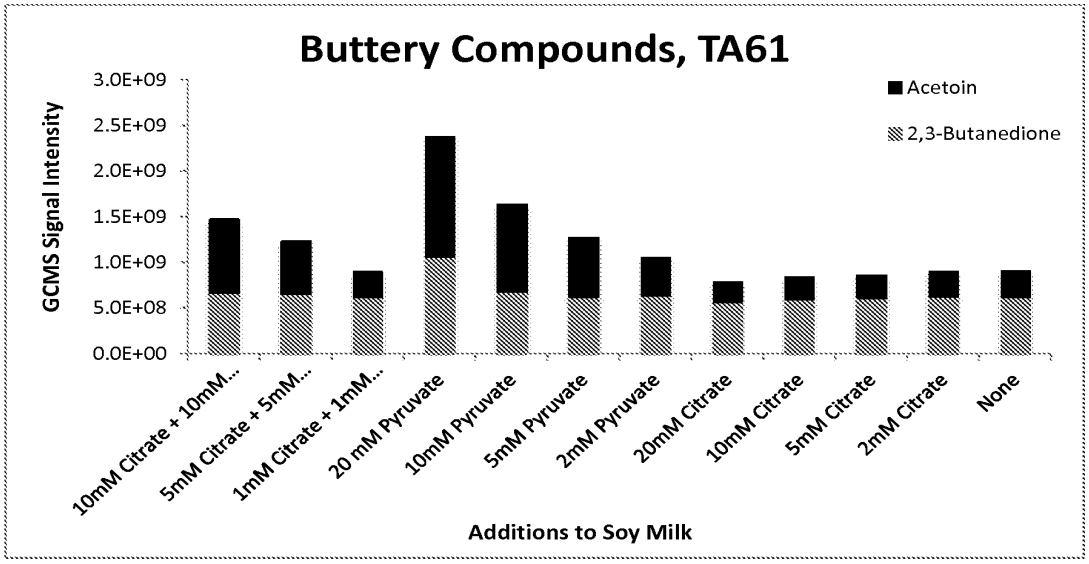


FIGURE 20

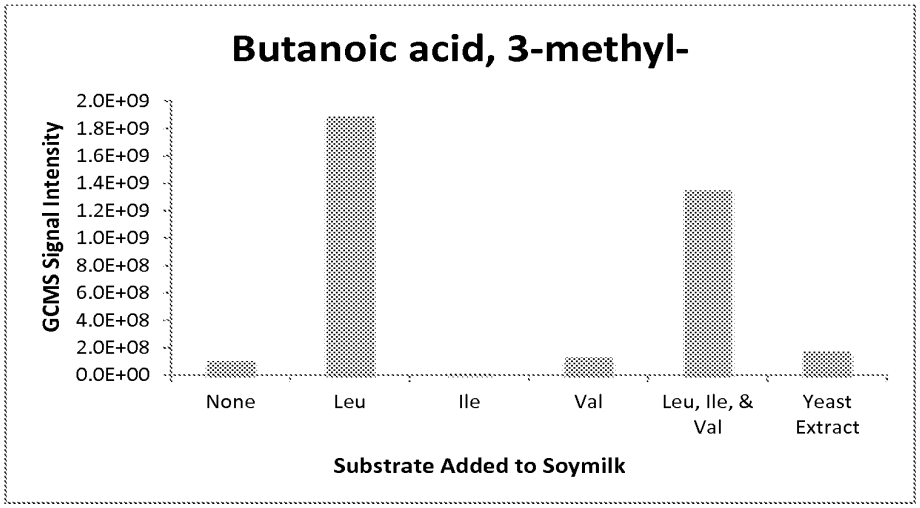


FIGURE 21

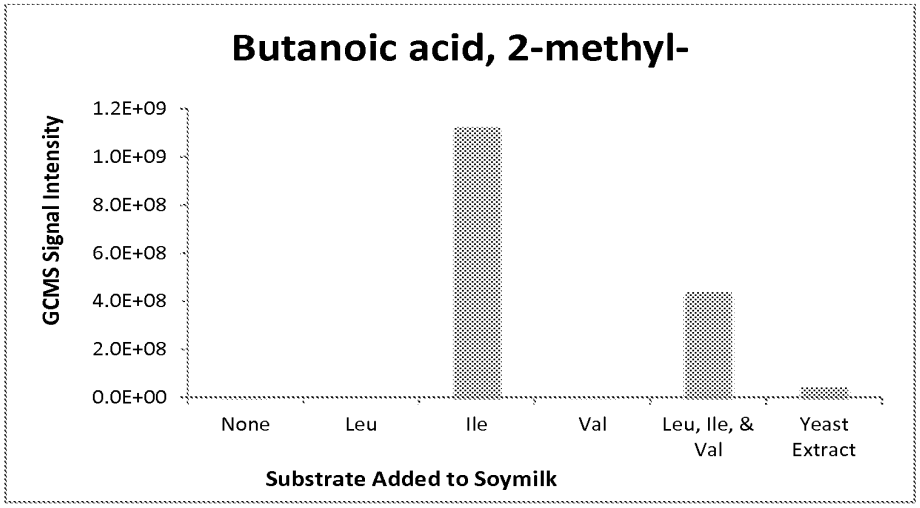


FIGURE 22

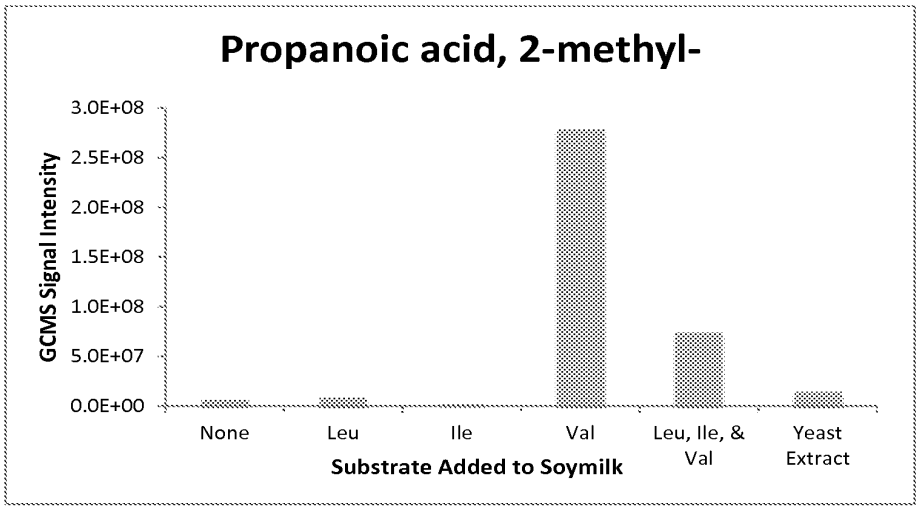


FIGURE 23

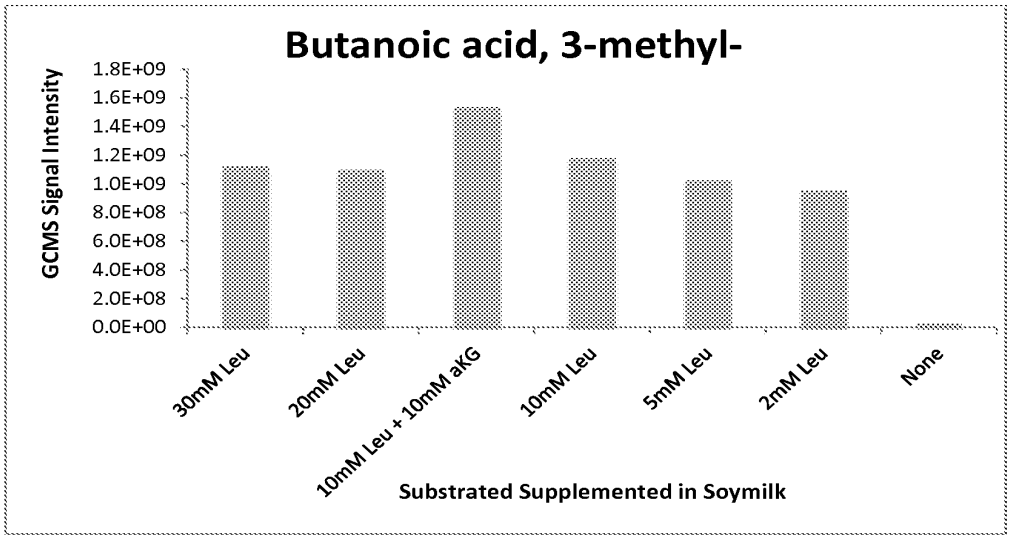


FIGURE 24

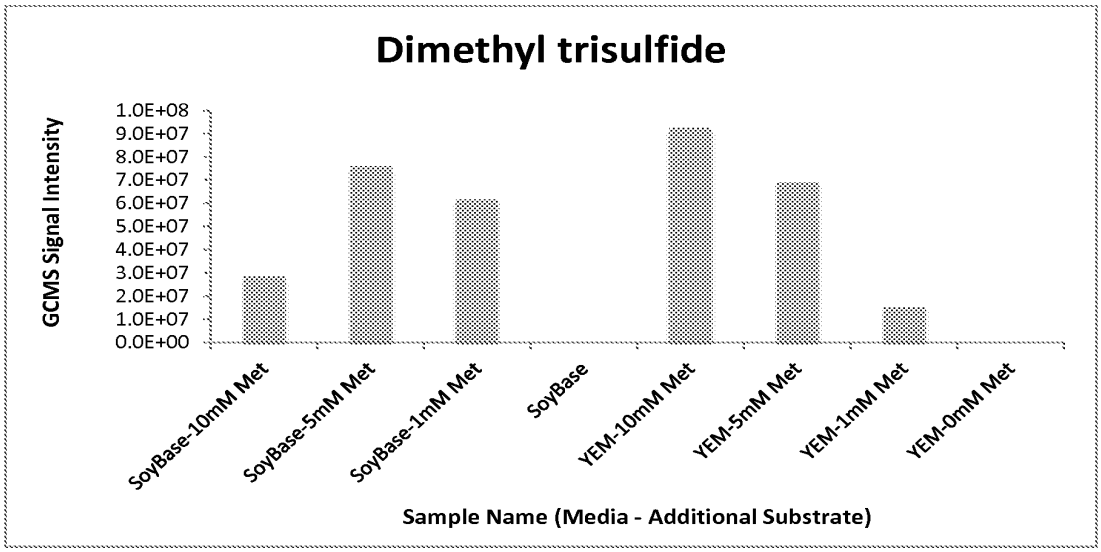


FIGURE 25

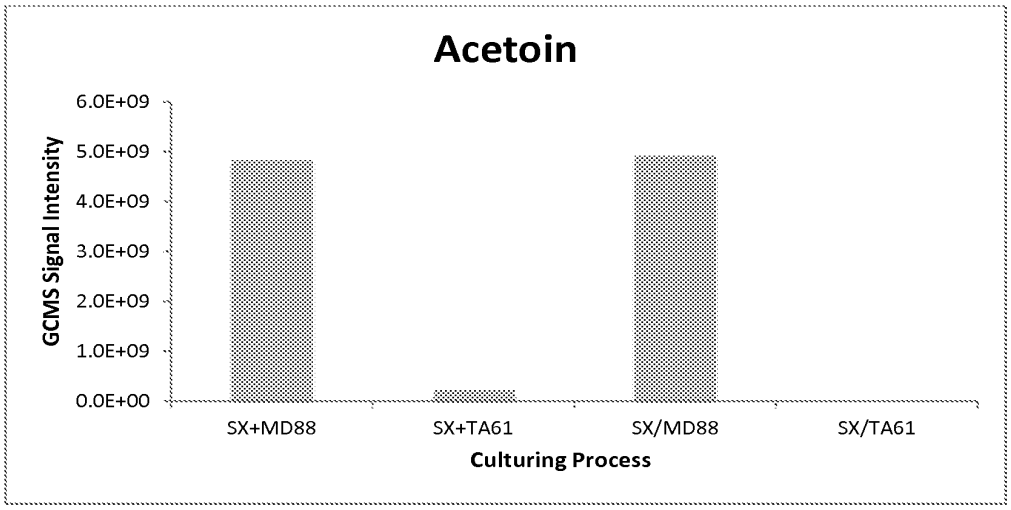


FIGURE 26

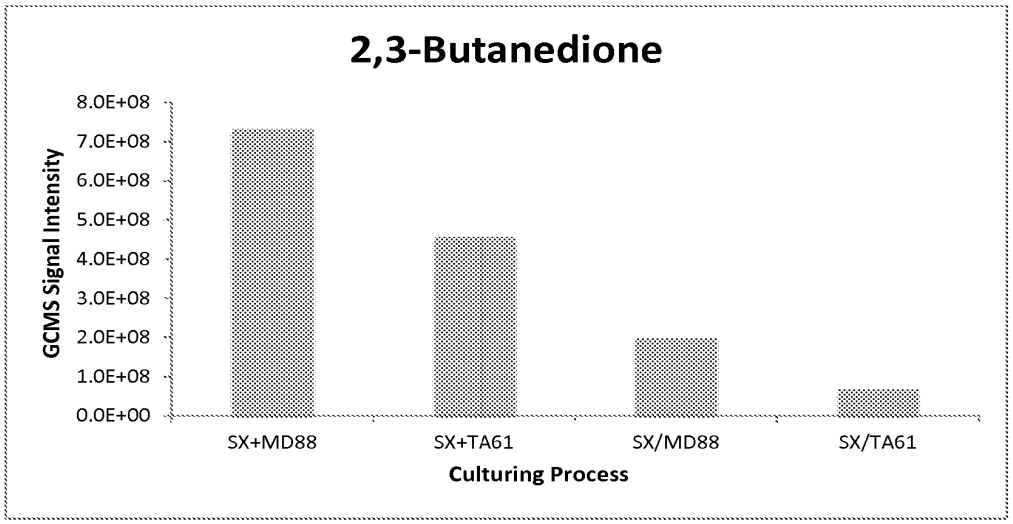
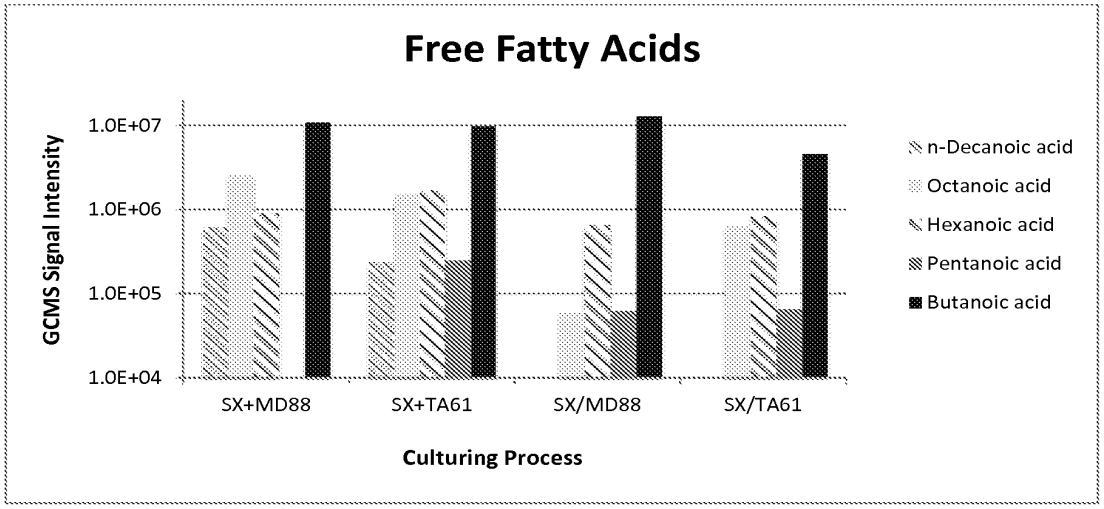


FIGURE 27



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378
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)
IPC(8): A23L 1/052, 1/36; A23C 19/055, 20/02; A23J 3/14 (2014.01)
USPC: 426/615, 634, 656; 530/378

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; non-dairy, non-animal, coacervation, plant protein, chickpea protein, lentil protein, pea protein, lupine protein, pea vicilin, pea legumin, imitation cheese

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2004/0151778 A1 (RICHARD, J et al.) August 5, 2004; paragraphs [0011], [0019], [0022], [0025], [0028], [0039], [0040], [0048]	1-4, 91-93 --- 8/1-4, 73-77, 78/73-77
Y	WO 2008/030089 A1 (BRANDSMA, JB et al.) March 13, 2008; page 5, lines 26-32; page 6, lines 1-3	8/1-4
Y	US 4435438 A (LEHNHARDT, WF et al.) March 6, 1984; column 4, lines 1-5; column 16, lines 20-30	73-77, 78/73-77
Y	US 2008/0226810 A1 (PASSE, D et al.) September 18, 2008; paragraphs [0005], [0007], [0014], [0015]	77, 78/77
A	US 5753295 A (GOLDMAN, MS) May 19, 1998; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93
A	WO 2012/116703 A1 (ANDERSEN, KE et al.) September 7, 2012; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2014 (14.05.2014)	13 JUN 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11362

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.: 9-10, 15-48, 56-67, 72, 79, 89-90
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 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.
Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.
Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.
Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.
Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

-***-Continued Within the Next Supplemental Box-***-

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.:
1-4, 8/1-4, 73-77, 78/73-77, and 91-93

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/US14/11362

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The inventions listed as Groups I-V 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 Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus, and Brevi bacterium linens; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non- animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 August 2015 (27.08.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/127388 A1

(51) International Patent Classification:
A23C 19/00 (2006.01)

(21) International Application Number:
PCT/US2015/017147

(22) International Filing Date:
23 February 2015 (23.02.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/943,172 21 February 2014 (21.02.2014) US
62/058,193 1 October 2014 (01.10.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2015/127388 A1

(54) Title: SOY-BASED CHEESE

(57) Abstract: A soy-based cheese is described that includes microbial cultures or acid. The soy-based cheese is meltable and can be sliced, grated or cubed and used in place of American process cheese. The soy-based cheese can be plant-based.

Soy-based Cheese

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 61/943,172, filed February 21, 2014, and U.S. Provisional Application Serial No. 62/058,193 filed October 1, 2014, which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

This invention relates to soy-based cheese, and more particularly to a soy-based cheese containing microbial cultures or acid.

BACKGROUND

Cheese making has relied on dairy milks as the major ingredient for more than 4000 years. Dairy cheese is usually 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, 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. Commodity cheeses or "processed cheeses" such as bulk cheddar, food-service mozzarella pizza and "cheese products," or "cheese foods" such as American cheese, American singles, Velveeta, and Cheese Whiz are typically produced from dairy-derived ingredients and other additives using industrial processes that little resemble traditional cheese making. A variety of non-dairy cheese analogs are available that contain soy products (e.g., soy milk powder, soy protein, or soy flour), oils, and various thickeners. However, the taste and texture of such products does not match that of dairy-based cheeses, and the amount of protein per

serving is typically a third of that found in their dairy counterparts. Thus, there is a need in the art for an improved method and system for producing non-dairy cheeses.

SUMMARY

In one aspect, this document features a soy-based cheese that includes soy milk; a microbial strain (e.g., a bacterial strain, a yeast strain, or a mold strain) or an acid (e.g., citric acid, lemon juice, HCl, pyruvic acid); a low methyl ester, amidated pectin; an oil; and optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, coconut milk, coconut cream, yeast extract, a dried yeast, one or more proteins, lecithin (e.g., sunflower or soy lecithin), a flour, a fiber, or cellulose. The bacterial strain can be selected from the group consisting of *Lactococcus lactis lactis* (LLL), *Lactococcus lactis cremoris* (LLC), *Lactococcus lactis biovar diacetylactis* (LLBD), *Staphylococcus xylosus* (SX), and *Streptococcus thermophilus* (ST). The soy-based cheese can include two bacterial strains (e.g., ST and SX). The choice of microbial strain(s), and the specific culturing conditions (temperature, aeration, nutritional additives) impacts the flavor profile of the soy cheese. The oil can be canola oil, palm fruit oil, palm oil, palm stearin oil, olive oil, flaxseed oil, sunflower oil, coconut oil, soybean oil, cocoa butter, or a margarine containing a mixture of two or more oils. The soy-based cheeses described herein are meltable. In some embodiments, the soy-based cheese does not include agar-agar. In some embodiments, the soy-based cheese does not include cyanobacteria or *Lactobacillus acidophilus*. In some embodiments, the soy-based cheese does not include agar-agar, cyanobacteria, or *Lactobacillus acidophilus*. In some embodiments, the soy-based cheese can include an animal product, e.g., casein or other non-plant based material. In some embodiments, the soy-based cheese is a plant-based soy-based cheese, i.e., a soy-based cheese containing no animal products. Soy-based cheeses containing no animal products also can be referred to as vegan soy-based cheeses.

Any of the soy-based cheeses described herein can include a salt (e.g., sodium chloride).

Any of the soy-based cheeses described herein can include a starch. The starch can be potato starch, corn starch, tapioca, rice starch, pea starch, arrowroot starch, or a modified food starch. The potato starch can be an amylopectin potato starch.

Any of the soy-based cheeses described herein can include sugar (e.g., one or more of glucose, fructose, sucrose, maltose, ribose, xylose, and mannose). For example, the soy-based cheese can include glucose, fructose, and/or sucrose. In some embodiments, the sugar is cane sugar, cane juice, corn syrup, brown rice syrup, agave syrup, maple syrup, glycerin, inulin, or maltodextrin.

Any of the soy-based cheeses described herein can include a flavoring, either natural or artificial, to provide additional cheesy, buttery, and/or milky/dairy flavor notes, or other desired flavor to the cheese. The flavoring can be a flavoring compound that includes one or more of acetoin, 5,6-decenoic acid, butanoic acid, gamma-undecalactone, 2,3 butanedione, butyl butyryllactate, delta-decacalactone, delta-tetradecalactone, gamma-tetradecalactone, delta-hexadecalatone, gamma-decalactone, gamma-dodecalactone, delta-dodecalactone, delta-tridecalactone, gamma-hexalactone, hexanoic acid, decanoic acid, octanoic acid, 2-tridecanone, caprylic acid, cis-3-hexanol, dimethyl sulfide, furaneol, indole, methional, methyl amyl ketone, thiophenol, trans-2- hexanal, propionic acid, ethyl butyrate, ethyl caproate, ethyl caprylate, ethyl decanoate, ethyl laurate, ethyl myristate, ethyl oleate, ethyl heptanoate, stearic acid, ethyl palmitate, 2-butanol, 2-methyl-butanal, 3-methyl-butanal, isovaleric acid, guaiacol, pyrizines, butyl alcohol, phenylacetic acid, methyl mercaptan, an amino acid, coconut milk or coconut cream. In some embodiments, an amino acid is used, for example, to provide sweetness. In some embodiments, a combination of butanoic acid, 5,6 decenoic acid, and acetoin can be used. In some embodiments, a combination of coconut cream, butanoic acid, 2,3 butanedione, gamma-undecalactone, and acetoin can be used. In some embodiments, the flavoring is an herb, a spice, or a vegetable.

Any of the soy-based cheeses described herein can include an antioxidant (e.g., vitamin E or mixed tocopherols).

Any of the soy-based cheeses described herein can include a coloring agent. For example, the coloring agent can be annatto, apocarotenal, beta-carotene, paprika, vegetable juice, or turmeric.

Any of the soy-based cheeses described herein can include an amino acid (e.g., leucine, alanine, valine, tyrosine, phenylalanine, lysine, isoleucine, glutamic acid and/or glycine). In some embodiments, an amino acid is used, for example, to provide sweetness.

Any of the soy-based cheeses described herein can include a gum. For example, the gum can be carrageenan, xanthan, konjac, guar, gellan, locust bean, or gum arabic. For example, the gum can be guar gum.

Any of the soy-based cheeses described herein can include one or more proteins (e.g., a protein isolate such as soy protein isolate or the soluble portion of soy flour or soy protein isolate, an isolated protein, or a combination of proteins). The protein can be an isolated protein such as a lentil protein, a soy protein, or a pea protein. The isolated protein can be pea vicilin, pea legumin, soy β-conglycinin, soy glycinin, or a dehydrin protein. The term "isolated" indicates that the preparation of the protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The protein content of a soy-based cheese can be up to 10% protein, up to 15% protein, or up to 19% protein. For example, the soy-based cheese can have 10% to 15% protein or 10% to 19% protein. In some embodiments, the soy-based cheese has a protein content of at least 10%, at least 15%, or at least 19%.

The protein content of a soy-based cheese can be similar to that of a dairy based American cheese. For example, the protein content of the soy-based cheese can be 15% to 20% (e.g., 16% to 19%).

The carboyhydrate content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the carbohydrate content of the soy-based cheese can be 4% to 7%.

The fat content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the fat content of the soy-based cheese can be from 16% to 22%.

Any of the soy-based cheeses described herein can include a preservative. For example the preservative can be sorbic acid, natamycin, "cultured dextrose," or olive leaf extract.

In another aspect, this document features a method of making a soy-based cheese. The method includes incubating a mixture of (i) soy milk, (ii) an optional sugar and optional one or more proteins, and (iii) a microbial strain (e.g., a bacterial strain) or an acid for a period of time sufficient to reach a pH of 5.4 to 6.4 (e.g., 5.4 to 6.2, 5.4 to 5.7, 5.8 to 6.2, or 5.4 to 5.6); blending the mixture; adding to the blended mixture, a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending; heating the mixture to a temperature from about 60°C to about 100°C (e.g., at least 65°C, at least 70°C, at least 80°C, at least 85°C, at least 90°C, 60°C to 80°C, 65°C to 85°C, 70°C to 90°C, 75°C to 99°C, 80°C to 100°C, or 85°C to 100°C); and adding to the heated mixture, one or more optional sugars and one or more flavorings to produce the soy-based cheese. The coloring agent also can be added after the heating step, and the sugar can be optionally added before the heating step. The method further can include forming the soy-based cheese in a mold.

In yet another aspect, this document features a method of making a soy-based cheese. The method includes incubating a mixture of (i) soy milk, (ii) an optional sugar and optional one or more proteins, and (iii) a microbial strain (e.g., a bacterial strain) or an acid for a period of time sufficient to reach a pH of 5.4 to 5.7; heating the mixture to a temperature between 70°C and 90°C; separating the curd and whey in the heated mixture and optionally combining the curd:whey to obtain a ratio of 35:65 to 100:0 curd:whey (e.g., 60:40, 65:35, 70:30, or 75:25); adding to the curd:whey combination, a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative such as sorbic acid; cooking the mixture to a temperature between 80°C and 95°C with blending; adding one or more flavorings to the cooked mixture; and blending the cooked mixture to obtain the soy-based cheese. The method further can include forming the soy-based cheese in a mold.

The document also features a soy-based cheese that includes soy milk; an acid (e.g., citric acid, lemon juice, HCl, and pyruvic acid); a low methyl ester, amidated pectin; an oil; and optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose.

In another aspect, this document features a soy-based cheese that includes soy milk; a microbial strain; a low methyl ester, amidated pectin; one or more proteins; an oil; a sugar (glucose, fructose, and/or sucrose); a gum (e.g., carrageenan, xanthan, konjac, guar gum, gellan, locust bean, or gum Arabic); a coloring agent (e.g., annatto, apocarotenal, beta-carotene, paprika, vegetable juice, or turmeric); and a flavoring. The soy-based cheese further can include a salt such as sodium chloride or a preservative such as sorbic acid, natamycin, cultured dextrose, olive leaf extract or a mixture thereof. The one or more proteins can include soy protein isolate or the soluble proteins from soy flour or soy protein isolate, or an isolated protein (e.g., a lentil protein, a soy protein, or a pea protein). For example, the protein can be pea vicilin, pea legumin, soy β-conglycinin, soy glycinin, or a dehydrin protein. The protein content of a soy-based cheese can be up to 10% protein, up to 15% protein, or up to 19% protein. For example, the soy-based cheese can have 10% to 15% protein or 10% to 19% protein. In some embodiments, the soy-based cheese has a protein content of at least 10%, at least 15%, or at least 19%. The protein content of a soy-based cheese can be similar to that of a dairy based American cheese. For example, the protein content of the soy-based cheese can be 15% to 20% (e.g., 16% to 19%). The carboyhydrate content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the carbohydrate content of the soy-based cheese can be 4% to 7%. The fat content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the fat content of the soy-based cheese can be from 16% to 22%. The soy-based cheese can be meltable. The soy-based cheese can be plant-based.

In another aspect, this document features a soy-based cheese that includes soy milk; a low methyl ester, amidated pectin; one or more proteins; an oil, and optionally

one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose. The one or more proteins can include soy protein isolate or the soluble proteins from soy flour or soy protein isolate, or an isolated protein (e.g., a lentil protein, a soy protein, or a pea protein). For example, the protein can be pea vicilin, pea legumin, soy β-conglycinin, soy glycinin, or a dehydrin protein. The protein content of a soy-based cheese can be up to 10% protein, up to 15% protein, or up to 19% protein. For example, the soy-based cheese can have 10% to 15% protein or 10% to 19% protein. In some embodiments, the soy-based cheese has a protein content of at least 10%, at least 15%, or at least 19%. The protein content of a soy-based cheese can be similar to that of a dairy based American cheese. For example, the protein content of the soy-based cheese can be 15% to 20% (e.g., 16% to 19%). The carboyhydrate content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the carbohydrate content of the soy-based cheese can be 4% to 7%. The fat content of a soy-based cheese can be less than that of a dairy based American cheese. For example, the fat content of the soy-based cheese can be from 16% to 22%. The soy-based cheese can be meltable. The soy-based cheese can be plant-based.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from

the claims. The word "comprising" in the claims may be replaced by "consisting essentially of" or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF DRAWINGS

FIG. 1 is a representative photograph of soy-based cheese after melting at 325°F for five minutes.

FIG. 2 is a representative photograph of soy-based cheese produced using the method of Example 2.

FIG. 3 is a representative photograph of the soy-based cheese produced in Example 3.

FIG. 4 is a representative photograph of a sliced block of soy-based cheese produced in Example 3.

FIG. 5 is a representative photograph of the cheese after grating in a Cuisinart food processor a few minutes after removal from refrigerator.

FIG. 6 is a representative photograph of a grilled cheese sandwich made with the soy-based cheese of Example 4.

FIG. 7 is a representative photograph showing the meltability of the soy-based cheese prepared in Example 7 with the soluble protein pellet (SPP) from soy flour. The left panel shows a 2 g sample of cheese prior to heating. The right panel shows that the cheese melted at 70°C.

DETAILED DESCRIPTION

In general, this document provides methods and materials for producing meltable soy-based cheeses, i.e., the soy-based cheeses are able to become liquefied with heating. Broadly, the document provides methods for making soy based cheeses by incubating soy milk with one or more microbial strains (e.g., bacterial, yeast or mold strains) or acids, and optionally, a sugar (e.g., glucose or other sugar), one or more proteins (e.g., one or more isolated proteins, a protein isolate such as soy protein isolate, or the soluble portion of soy flour or soy protein isolate (referred to as the soluble protein pellet)) and/or flavor enhancers such as yeast extract or calcium pyruvate, for a sufficient period of time to

obtain the desired pH, which can range from about 4.5 to 6.4 (e.g., pH 5.4 to 5.7, 5.5 to 5.6, or 5.8 to 6.2). The length of the incubation period may vary depending on the temperature in which the soy milk and cultures are maintained. For example, the soy milk, microbial cultures, and optional ingredients can be incubated at temperatures ranging from room temperature (e.g., approximately 25°C) up to about 40°C (e.g., 25°C-37°C or 25°C-40°C). It will be appreciated that the incubation time may vary depending on the size of the batch, amount of inoculum, and culture temperature.

Once the desired pH is reached, the resulting mixture of curds and whey (i.e., the liquid remaining after curdling) can be processed (e.g., by high speed shearing or blending to integrate the flavors, resulting in a soy based cheese with a mellower flavor) and one or more additional ingredients (e.g., oil, low methylester, amidated pectin such as pectin NH, and optional salts, starches, gums, coloring agents, sugars, flavorings, antioxidants, amino acids, quinoa, pyruvic acid or a salt form thereof, preservative, coconut milk, coconut cream, yeast extract, a dried yeast, protein, lecithin, a flour, a fiber, or cellulose) can be added. For example, the oil, salt, pectin, and coloring agent can be added to the blended mixture and the mixture heated to a temperature of about 60°C to about 100°C (e.g., at least 65°C, at least 70°C, at least 80°C, at least 85°C, at least 90°C, 60°C to 80°C, 65°C to 85°C, 70°C to 90°C, 75°C to 99°C, 80°C to 100°C, or 85°C to 100°C). One or more sugars and one or more flavorings then can be added to produce the soy-based cheese. Cheese molds can be used to form the soy-based cheese to the desired shape.

In some embodiments, the curds and whey can be separated and the individual components processed. For example, the curds and whey can be separated by draining the whey from the curd. For example, the mixture of curds and whey can be heated (e.g., 70°C to 90°C, 75°C, 80°C, or 85°C) to aid the separation of the curd from the whey and to reduce the number of viable microorganisms. The curds and whey can be combined in various ratios ranging from 50:50 curd:whey to 80:20 curd:whey (e.g., 60% curd : 40% whey; 65% curd : 35% whey; 70% curd: 30% whey, or 75% curd : 25% whey). In some embodiments, the curd may be combined with water instead of whey.

A low methylester, amidated (LMA) pectin, an oil, and one or more optional ingredients (e.g., a salt, a starch, sugar, coloring agent, and/or a preservative such as sorbic acid) can be added to the curd:whey combination and the resulting mixture cooked to a temperature between 60°C and 100°C (e.g., at least 65°C, at least 70°C, at least 80°C, at least 85°C, at least 90°C, 60°C to 80°C, 65°C to 85°C, 70°C to 90°C, 75°C to 99°C, 80°C to 100°C, or 85°C to 100°C) with blending. One or more flavorings can be added to the cooked mixture then blended to obtain the soy-based cheese. Cheese molds can be used to form the soy-based cheese to the desired shape.

As described herein, the color, texture, and/or flavor of the soy-based cheese is similar to American process cheese and has similar melting characteristics and gooeyness, as well as a similar dairy character (e.g., buttery, cheesy, and milky). The soy-based cheese can be sliced, grated or cubed, and used, for example, to create grilled soy-cheese sandwiches, soy-cheese sauce (e.g., for macaroni and cheese), nachos, and/or soy-cheese burgers.

In some embodiments, soy protein isolate is used as the optional protein source when making the soy-based cheese. Soy protein isolate refers to the proteins extracted from defatted soy flour and is commercially available from many sources. In some embodiments, insoluble material (e.g., insoluble proteins or other insoluble material) is removed from soy flour or soy protein isolate, and the remaining soluble portion of the soy flour or soy protein isolate is used to prepare the soy-based cheese replica. Removing the insoluble material before making the soy-based cheese replica improves the appearance (including both color and texture) as well as improves the mouth-feel of the soy-based cheese.

In embodiments in which the optional proteins are used in making the soy-based cheese, the protein content can be similar or more than that of a dairy based American cheese single. In some embodiments, the soy-based cheese can have a protein content that is similar or more than that of a dairy based American cheese (e.g., a block of dairy based American cheese or a dairy based American cheese single), and can have a carbohydrate content and/or a fat content that is similar or less than that of a dairy based American cheese (e.g., a block of dairy based American cheese or a dairy based

American cheese single). For example, a soy-based cheese can have a protein content that is similar or higher than that of a dairy based American cheese, with a fat content that is less than that of a dairy based American cheese and a carbohydrate content that is similar to that of a dairy based American cheese. For example, a soy-based cheese can have a protein content that is similar or higher than that of a dairy based American cheese, with a carbohydrate content that is less than that of a dairy based American cheese and a fat content that is similar to that of a dairy based American cheese. For example, a soy-based cheese can have a protein content that is similar or higher than that of a dairy based American cheese, with a carbohydrate content and a fat content that is less than that of a dairy based American cheese. Typically, a dairy based cheese has a protein content of 18%, a carbohydrate content of 8%, and a fat content of 23%. For example, a soy-based cheese can have a protein content between 10% and 20% such as 10% and 19%, 10% to 15%, 15% to 20%, 15% to 19%, 15%, 16%, 17%, 18%, 19%, or 20%. For example, the soy-based cheese can have a carbohydrate content between 4% and 8% (e.g., 4% to 7%, 5% to 6%, 4%, 5%, 6%, or 7%). For example, the soy-based cheese can have a fat content between 16% and 23% (e.g., 16% to 18%, 19% to 22%, or 20% to 22%).

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

EXAMPLE 1

A meltable soy-based cheese was prepared using the following ingredients:

a) soy milk composed of soybeans and water (no other additives)

b) glucose

c) bacterial cultures: strains *Streptococcus thermophilus* (ST) (as TA61, a commercial mix) and *Staphlococcus xylosus* (SX)

d) LMA pectin (pectin NH)

e) potato starch

f) salt

g) canola oil

h) fructose

i) annatto

j) natural flavor

One liter of soy milk (SunOpta organic soy base) was heated to 30°C in a clean sanitized container, and glucose was added to 50 mM (0.9%) along with cultures of SX and ST (each at a starting cell density of 1.25 x 10^7 cfu/ml). The mixture was incubated until a pH of 5.5 +/- 0.1 was reached. The incubation typically takes 3-6 hours to reach the target pH. (At this point, the coagulated soy milk can be placed on ice and refrigerated for up to 24 hours, before proceeding. pH may drop a few tenths of a percent during this time.) The mixture was transferred to a food processor and sheared/blended for 3 minutes, then pectin NH at 3.2%, potato starch (Eliane 100) at 0.4%, salt at 1.4%, canola oil at 14%, and annatto at 0.08% (or as needed to achieve desired orange color) was added and the mixture blended again in the food processor for an additional 2 minutes. After blending, the mixture was transferred to a pot and boiled for about 2 minutes per each 200 ml volume. This step allows the pectin NH to dissolve and also acts as a "kill step" to reduce the inoculated bacterial count. The pot was removed from the heat, and 0.09% fructose and 0.18% glucose (for added sweetness) was whisked into the mixture. At this point, due to evaporation during boiling, the volume of the mixture is about 70% of the starting volume. The mixture was transferred to a food processor and cooled to about 70°C, and then coconut cream at 10%, butanoic acid in oil at 0.01%, 2,3 butanedione in oil at 0.0003%, gamma-undecalactone in oil at 0.00003%, and acetoin in water at 0.015% was added and the mixture blended for an additional minute to incorporate flavors into the mixture. Other combinations of natural flavors and/or artificial also can be used to flavor the cheese. The blended mixture was poured into cheese molds, and immediately covered with a plastic lid or wrap. The molds were refrigerated overnight to several days then removed. Once removed from the molds, the soy-based cheese can be sliced, grated or cubed, and used, for example, to create grilled soy-cheese sandwiches, soy-cheese sauce, and/or soy-cheese burgers. As shown in FIG. 1, the soy-based cheese is meltable (325°F x 5 minutes).

EXAMPLE 2

A soy-based cheese was prepared using the following ingredients:

a) soy milk, composed of soybeans and water (no other additives)

b) glucose

c) bacterial cultures: strains ST (as TA61, a commercial mix) and SX

d) LMA pectin (pectin NH)

e) potato starch

f) salt

g) canola oil

h) fructose

i) annatto

j) natural and artificial flavors

k) sorbic acid (or other preservative):

Fifteen (15) liters (L) of soy milk (SunOpta organic soy base) was heated to 30°C in two clean sanitized pots of 7.5 L each, in a heated water bath, and glucose was added to 50 mM (0.9%) along with cultures of SX and ST (each at a starting cell density of 1.25 x 10^7 cfu/ml). The mixture was incubated until a pH of 5.5 +/- 0.1 was reached. The incubation typically takes 3-6 hours to reach the target pH. Once the desired pH was reached, the pots were transferred to a double boiler set-up, and heated until the soy-milk mixture reached 75°C. After holding at 75° C for one minute, the pots were removed from the heat source. The heating serves as a "kill step" (allowing us to maintain the mixture in the refrigerator for at least one week) and aids in the separation of curd from whey. The mixture was transferred to cheese-draining bags, and the curd was allowed to separate from the whey until a 70:30 curd:whey ratio was achieved. The 70:30 curd/whey mixture can be stored covered in the refrigerator for up to one week.

A 10-lb (4.5-kg) cooker run was performed by blending the curd/whey mixture at ~75%, pectin NH at 3.2%, potato starch (Eliane 100) at 0.4%, salt at 1.4%, canola oil at 14%, annatto at 0.08% or as needed to achieve desired orange color, glucose at 0.18%, fructose at 0.09%, and sorbic acid at 0.2% (optional, as a preservative) in a mixer

(alternatively a food processor) until well-blended and smooth. The blending step can be up to 30 minutes in length. The entire mixture was transferred to a pre-heated steam-cleaned chamber of a 10-pound cheese cooker and the temperature brought up to 180°C by steam injection, with continuous mixing. The mixture was cooked for 4 minutes. The steam was turned off and flavors were added as follows (at the final concentrations shown): butanoic acid in oil at 0.01%, 5,6 decenoic acid in oil at 0.005%, and acetoin in water at 0.015%. The butanoic acid and 5,6 decenoic acid were added together in a 5-ml volume of canola oil, and the acetoin is in 11.2 ml of water. The cooker was run for an additional minute without steam injection. Note that the steam injection contributes an additional ~5% water (or about 225 ml per 4.5-kg batch) to the mixture.

The cooked soy-cheese mixture was decanted and transferred to a mixer (e.g., KitchenAid), and sheared/blended at high speed for 5 minutes. This post-cooking blending step helps prevent syneresis of oil. The blended mixture was poured into cheese molds, and immediately covered with a plastic lid or wrap. The molds were refrigerated overnight to several days, then removed. FIG. 2 contains a photograph of the soy-based cheese. Once removed from the molds, the soy-based cheese can be sliced, grated or cubed, and used, for example, to create grilled soy-cheese sandwiches, soy-cheese sauce, and/or soy-cheese burgers.

EXAMPLE 3

A soy-based cheese was prepared using the ingredients in Example 1, with the following additions: calcium pyruvate, yeast extract, Earth Balance buttery spread, glycine, and mixed tocopherols. The presence of calcium pyruvate contributes to a milder, less sour cheese replica. Yeast extract promotes bacterial strain growth and contributes some savory flavor to the cheese replica. Glycine may reduce sourness in the soy cheese.

The soy cheese was prepared as described in Example 1, with the calcium pyruvate (~pH 2) added to 10 mM final concentration and yeast extract added at 0.5%, at the same time the cultures were added. After the mixture reached the desired pH, the pectin NH at 3.2%, potato starch (Eliane 100) at 0.4%, salt at 1.3%, canola oil at 7%, and

annatto at 0.2% (or as needed to achieve desired orange color), glucose at 10 mM, and glycine at 5 mM was added. After pulsing 10 times to mix in a mini-chopper, the mixture was blended for 1 minute, then transferred to a pot. The mixture was boiled for 2 minutes, and then the following ingredients were added: Earth Balance buttery spread at 7% and mixed tocopherols at 0.5% (0.75 mg in canola oil per 150 ml mixture). The mixture was whisked until blended and smooth, and then immediately poured into cheese molds, and covered in plastic wrap and refrigerated overnight. The soy-based cheeses were un-molded and transferred to plastic containers and wrapped in plastic wrap (FIG. 3) and returned to the refrigerator until used. The cheeses were smooth and shiny when unwrapped, and had a dairy cheesy aroma. The cheese replicas were sliced (FIG. 4), and shredded in a Cuisinart (FIG. 5).

EXAMPLE 4

A meltable plant-based soy-based cheese was prepared using the following ingredients:

a) soy milk, composed of soybeans and water (no other additives)

b) soy protein isolate (Supro XT 219D, Solae)

c) glucose

d) bacterial cultures: strains *Streptococcus thermophilus* (ST) (as TA61, a commercial mix) and *Staphlococcus xylosus* (SX)

e) LMA pectin (pectin NH)

f) potato starch

g) salt

h) coconut oil

i) coconut cream

j) guar gum

k) fructose

l) annatto

m) natural flavors

Soy milk (SunOpta organic soy base) was heated to 30°C in a clean sanitized container. Soy protein isolate (Supro XT 219D) was added at 15%, glucose was added to 50 mM (0.9%), and cultures of SX and ST were each added at a starting cell density of approximately 1.25 x 10^7 cfu/ml. The mixture was incubated until a pH of approximately 5.6 was reached. The incubation typically takes 3-6 hours to reach the target pH. (At this point, the coagulated soy milk can be placed on ice and refrigerated for up to 24 hours, before proceeding. pH may drop a few tenths of a percent during this time.) The mixture was transferred to a food processor and sheared/blended for 1 minute, then coconut cream (Kara, 5%), coconut oil (Shay and Co., 15%) and annatto (0.1 – 0.2%) were added, and the mixture was pulsed to thoroughly blend. Next, Pectin NH at 3.2%, potato starch (Eliane 100) at 0.4%, salt at 1.4%, guar gum at 0.3%, glucose at 0.2% and fructose at 0.1% were added and the mixture blended again in the food processor for an additional 2 minutes. (Note: guar gum also may be pre-hydrated in water prior to blending.) After blending, the mixture was transferred to a pot and boiled for about 2 minutes per each 200 ml volume, to achieve an approximate weight loss of 15% due to water evaporation. This step allows the pectin to dissolve and also acts as a "kill step" to reduce the inoculated bacterial count. The pot was removed from the heat, and its contents were transferred to a mixer. Flavors were added: butanoic acid in oil at 0.01%, 2,3 butanedione in oil at 0.0003%, gamma-undecalactone in oil at 0.00003%, and acetoin in water at 0.015%, and the mixture blended for 30 seconds to incorporate these flavors into the mixture. The blended mixture was poured into cheese molds, and immediately covered with plastic wrap. The molds were refrigerated overnight to several days. Once removed from the molds, the soy-based cheese can be sliced, grated or cubed, and used, for example, to create grilled soy-cheese sandwiches, soy-cheese sauce, and/or soy-cheese burgers. The protein content of the soy-based cheese (analysis by Silliker) was about 15 – 16%. Soy-based cheese prepared with added soy-protein isolate and guar gum has a more cohesive and gooey melt than otherwise comparable soy-based cheese prepared without added soy protein isolate or guar gum. FIG. 6 provides a representative photograph of a grilled cheese prepared with the soy based cheese of this example.

EXAMPLE 5

A meltable plant-based soy-based cheese with a protein content that matches that of dairy American cheese was prepared using the following ingredients:

a) soy milk, composed of soybeans and water (no other additives)

b) soy protein isolate (Supro XT 219D, Solae)

c) glucose

d) bacterial cultures: strains *Streptococcus thermophilus* (ST) (as TA61, a commercial mix) and *Staphlococcus xylosus* (SX)

e) LMA pectin (pectin NH)

f) salt

g) coconut oil

h) canola oil

i) coconut milk

j) guar gum

k) sucrose

l) annatto

m) natural and/ or artificial flavors

Soy milk (SunOpta organic soy base) was heated to 30°C in a clean sanitized container. Soy protein isolate (Supro XT 219D) was added at 23%, glucose was added to 50 mM (0.9%), and cultures of SX and ST were each added at a starting cell density of approximately 1.25×10^7 cfu/ml. The mixture was incubated until a pH of approximately 6.2 was reached. The incubation typically takes 3-4 hours to reach the target pH. The mixture was transferred to a food processor. The following ingredients were added, and the mixture was sheared/blended for 3 minutes: coconut milk (5%), coconut oil (14%), canola oil (4%), pectin NH (3.2%), salt (1.4%), guar gum (0.01%), and sucrose (0.9%). After blending, the mixture was transferred to a pan set atop a pot of boiling water and heated until the mixture reached 80°C, and achieved an approximate weight loss of 4% ± 1% due to water evaporation. This step allows the pectin to dissolve and also acts as a "kill step" to reduce the inoculated bacterial count. (If moisture loss exceeds 5%, water may be added back to the mixture to achieve a final moisture loss of about 4%.) The pot

was removed from the heat, and its contents were transferred to a mixer. Flavors were added, followed by annatto (67% solution at 0.1%) and the mixture was blended for 3 minutes. The blended mixture was poured into cheese molds, and immediately covered with plastic wrap. The molds were refrigerated overnight to several days. Once removed from the molds, the soy-based cheese can be sliced, grated or cubed, and used, for example, to create grilled soy-cheese sandwiches, soy-cheese sauce, and/or soy-cheese burgers. The protein content of the soy-based cheese is comparable to that of a dairy-based American cheese, Kraft American singles, but with slightly lower fat (and no cholesterol) and carbohydrates (see Table 1, nutritional analysis by Silliker Inc. for the dairy based cheese).

Table 1

Sample	% protein	% fat	% carbohydrates
Kraft American singles cheese	18	23	8
soy-based cheese from Example 5	18	22	6

EXAMPLE 6

A soy-based cheese replica was prepared with the following ingredients:

a) soy milk composed of soybeans and water (no other additives)

b) glucose

c) bacterial cultures: strains *Streptococcus thermophilus* (ST) (as TA61, a commercial mix) and *Staphlococcus xylosus* (SX)

d) LMA pectin (pectin NH)

e) potato starch

f) salt

g) coconut oil

h) coconut cream

i) guar gum

j) fructose

k) annatto

l) natural flavors

Soy milk (SunOpta organic soy base) was heated to 30°C in a clean sanitized container. Glucose was added to 50 mM (0.9%), and cultures of ST and SX were each added at a starting cell density of approximately 1.25 x 10^7 cfu/ml. The mixture was incubated until a pH of approximately 5.5 was reached. The mixture was transferred to a food processor. The following ingredients were added, and the mixture was sheared/blended for 2 minutes: coconut cream (10%), coconut oil (16.4%), Pectin NH (3.2%), potato starch (Eliane 100) (0.4%), salt (1.4%), guar gum (0.1%), and annatto (0.2% of a 67% solution in water). After blending, the mixture was transferred to a pot and boiled for about 2 minutes per each 200 ml volume, with continuous whisking. This step allows the LMA pectin to dissolve, reduces the moisture content, and also acts as a "kill step" to reduce the inoculated bacterial count. The pot was removed from the heat, and its contents transferred to a blender. 0.09% fructose, 0.18% glucose and 0.33% natural flavors were added, and the mixture was sheared/blended for 1 minute. The blended mixture was poured into cheese molds, and immediately covered with plastic wrap. The molds were refrigerated overnight to several days.

Once removed from the molds, the soy-based cheese replica was sliced, packaged in plastic containers and shipped on ice, along with packages of Tofutti American soy cheese slices (ingredients: water, may contain one or more of the following oils: soy, corn or palm, non-GMO [tofu, soy protein), carrageenan, maltodextrin, vinegar, corn starch, calcium phosphate, potato flakes, salt, potassium phosphate, non-dairy lactic acid, adipic acid, soy, natural colors and potassium sorbate (added as a preservative)] to the sensory test site. 98 subjects were served the two sliced samples in random order, and then the two samples in the form of grilled cheese sandwiches (GC) in random order. A 9-point degree of liking (DOL) scale was used to rate the samples for overall liking, appearance, flavor and texture. A 5-point DOL scale was used to rate purchase intent.

As shown in Tables 2A and 2B, the soy-based American cheese replica (Test sample) was preferred to Tofutti when the two samples were compared as slices or as GC sandwiches. There was a significant difference ($p<0.05$) between the samples in DOL on

overall liking (Test GC was rated higher than Tofutti GC and Test Single was rated higher than Tofutti Single), appearance (Test GC was rated higher Tofutti GC), flavor (Test GC was rated higher than Tofutti GC and Test Single was rated higher than Tofutti Single), texture (Test GC was rated higher than Tofutti GC and Test Single was rated higher than Tofutti Single) and purchase intent (Test GC was rated higher than Tofutti GC and Test Single was rated higher than Tofutti Single). The only non-significant difference between slices was for appearance.

Table 2a: Mean scores for comparison of single slices

Attribute	Test mean: slice	Tofutti mean: slice
Overall liking	4.92	3.63
Appearance	5.72	5.88
Flavor	5.09	3.20
Texture	4.74	3.98
Purchase Intent	2.13	1.49

Table 2b: Mean scores for comparison of grilled cheese sandwiches

Attribute	**Test mean: GC**	**Tofutti mean: GC**
Overall liking	6.21	4.73
Appearance	6.53	5.50
Flavor	6.00	4.52
Texture	6.28	4.87
Purchase Intent	2.95	2.00

EXAMPLE 7

A meltable soy-based cheese was prepared using the following ingredients:

a) soy milk composed of soybeans and water (no other additives)

b) soluble soy protein, isolated from CHS HoneySoy flour
c) glucose
d) bacterial cultures: strains *Streptococcus thermophilus* (ST) (as TA61, a commercial mix) and *Staphlococcus xylosus* (SX)
e) LMA pectin
f) salt
g) sucrose
h) coconut oil
i) coconut cream
j) guar gum
k) annatto
l) natural & artificial flavors

Soluble soy protein was isolated from CHS Honeysoy flour using acid precipitation. Soy flour was suspended in water at a 1:5 ratio. The pH of the mixture was adjusted to pH 8.0 using 2 N NaOH. The protein slurry was stirred for 1 hour at 4 °C. The insoluble starch and protein were removed using centrifugation at 15,000xg at 19 °C for 15 minutes. The insolubles (pellet) were discarded and the pH of the supernatant was adjusted to pH 4.5 using 2 M H_2SO_4 to precipitate out the soluble protein. The supernatant was stirred for 1 hour at 4 °C. A second centrifugation (15,000 xg, 19°C, 15 minutes) separated the soluble protein (pellet) from solution. The pellet was stored at 4°C until further use.

To prepare the soy-based cheese using the soluble protein pellet (SPP), the wet SPP (33.50%) recovered from the protein extraction was slowly added to soymilk (64.60%). Due to the large pH differences between the protein pellet (pH 4.5) and soymilk (pH 6.76) the pH was maintained between 6.0-6.8 using 2 M NaOH. An immersion blender was used to ensure a homogeneous mixture. The final pH of the mixture was 6.85. The mixture was stored overnight. The following day the pH was re-adjusted to pH 6.85 using 2N NaOH. The mixture was heated to 30°C in a water bath. Glucose (0.90 %) was added, followed by the bacterial cultures (~ 1.0 %, starting cell

density of 1.25 x 10^7 cfu/ml each for SX and ST). The mixture was incubated at 30-35°C until the pH reached 5.95, approximately 4 hours. The mixture was immediately put on ice to slow the microbial growth and maintain pH, and stored overnight at 4 °C.

The mixture then was added to a food processor and sheared for 1 minute. The dry ingredients (LMA pectin (3.20%), guar gum (0.10%), salt (1.40%) and sucrose (0.9 %)) were added and the mixture was sheared for 1 minute. The wet ingredients, coconut oil (13.0%) and coconut cream (5.00%), were added and the mixture was sheared for an additional minute or until all ingredients were well incorporated. The mixture was heated over a double broiler until the temperature of the mixture reached 80°C. The hot mixture was then returned to a clean food processor and sheared for 30 seconds. The natural and artificial flavors (2.63 μL/g of cheese) were added and the mixture was sheared for 1 minute. Finally the annatto (0.10 %) was added and the mixture was sheared until the color was uniform, approximately 1 minute. The mixture then was poured into molds, covered and stored at 4°C. This soy-based cheese was meltable after heating to 70°C as demonstrated in FIG. 7.

The color of the soy-based cheese prepared with SPP was brighter, shinier, and closer in color to dairy American cheese (e.g., Kraft singles) than soy-based cheese prepared with soy protein isolate (SPI). Colorimetry confirmed that the sample made with SPP was brighter (L* = 71.96) and closer to Kraft (L* = 74.83) than the control (L* = 67.73), where L* of 0 = darkest black and L* of 100 = brightest white. A tasting of the two soy-based cheeses in the form of grilled cheese sandwiches demonstrated that using SPP instead of SPI yields a smoother, creamier mouthfeel.

EXAMPLE 8

Soy milk (SunOpta organic soy base) was heated to 30ºC in a clean sanitized container. Soy protein isolate (Supro XT 219D) was added at 18% and glucose was added at 0.9%. A sample was removed for gas chromatography mass spectrometry (GCMS) analysis of the volatile flavor compounds present in the starting material, which was ~pH 7.0. Cultures of SX and ST were each added at a starting cell density of approximately 1.25 x 10^7 cfu/ml. The mixture was incubated at 30ºC and samples were

removed at various points for GCMS analysis including at pH 6.5, 6.2, 6.0, and 5.8, and after overnight incubation.

The GCMS data indicated that several dairy aroma compounds were created during the soy mixture fermentation, including acetoin, propanoic acid, 3-methyl-butanal, acetic acid, butanoic acid, and hexanoic acid. The signal intensity of each of acetoin, propanoic acid, 3-methyl-butanal, acetic acid, and hexanoic acid was similar to that found in a dairy American cheese (Kraft American singles). See Table 3.

Table 3: GCMS signal intensity of volatile flavor compounds

Compound	Kraft American singles	Fermented soy mixture, pH 5.8
Acetoin (sweet, buttery, creamy, dairy, milky, fatty)	2.36E+08	4.29E+08
Propanoic acid (pungent, acidic, cheesy, vinegar)	3.48E+04	9.00E+05
3-methyl-butanal (ethereal, aldehydic, chocolate, peachy, fatty)	8.99E+06	2.28E+07
Acetic acid (sharp, pungent, sour, vinegar)	1.22E+08	8.98E+07
Hexanoic acid (sour, fatty, sweat, cheese)	2.44E+07	1.68E+07

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A soy-based cheese comprising:

a) soy milk;

b) a microbial strain;

c) a low methyl ester, amidated pectin;

d) an oil; and

e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, coconut milk, coconut cream, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose.

2. The soy-based cheese of claim 1, wherein said microbial strain is a bacterial strain, a yeast strain, or a mold strain.

3. The soy-based cheese of claim 2, wherein said bacterial strain is selected from the group consisting of *Lactococcus lactis lactis* (LLL), *Lactococcus lactis cremoris* (LLC), *Lactococcus lactis biovar diacetylactis* (LLBD), *Staphylococcus xylosus* (SX), and *Streptococcus thermophilus* (ST).

4. The soy-based cheese of claim 3, said soy-based cheese comprising two bacterial strains.

5. The soy-based cheese of claim 4, wherein said bacterial strains are ST and SX.

6. The soy-based cheese of any one of claims 1-5, said soy-based cheese comprising said starch.

7. The soy-based cheese of claim 6, wherein said starch is potato starch, corn starch, tapioca, rice starch, pea starch, arrowroot starch, or a modified food starch.

8. The soy-based cheese of any one of claims 1-7, wherein said oil is canola oil, palm fruit oil, palm oil, palm stearin oil, olive oil, flaxseed oil, sunflower oil, coconut oil, soybean oil, cocoa butter, or a margarine containing a mixture of two or more oils.

9. The soy-based cheese of any one of claims 1-8, wherein said soy-based cheese does not include agar-agar.

10. The soy-based cheese of any one of claims 1-9, wherein said soy-based cheese does not include cyanobacteria or *Lactobacillus acidophilus*.

11. The soy-based cheese of any one of claims 1-10, said soy-based cheese comprising said sugar.

12. The soy-based cheese of claim 11, wherein said sugar is glucose, fructose, and/or sucrose.

13. The soy-based cheese of claim 11, wherein said sugar is cane sugar, cane juice, corn syrup, brown rice syrup, agave syrup, maple syrup, glycerin, inulin, or maltodextrin.

14. The soy-based cheese of any one of claims 1-13, said soy-based cheese comprising said flavoring.

15. The soy-based cheese of claim 14, wherein said flavoring is a flavoring compound.

16. The soy-based cheese of claim 15, wherein said flavoring compound comprises one or more of acetoin, 5,6-decenoic acid (milk lactone), butanoic acid (butyric acid), gamma-undecalactone, 2,3 butanedione, butyl butyryllactate, delta-decacalactone, delta-tetradecalactone, gamma-tetradecalactone, delta-hexadecalatone, gamma-decalactone, gamma-dodecalactone, delta-dodecalactone, delta-tridecalactone, gamma-hexalactone, hexanoic acid, decanoic acid, octanoic acid, 2-tridecanone, caprylic acid, cis-3-hexanol, dimethyl sulfide, furaneol, indole, methional, methyl amyl ketone, thiophenol, trans-2- hexanal, propionic acid, ethyl butyrate, ethyl caproate, ethyl caprylate, ethyl decanoate, ethyl laurate, ethyl myristate, ethyl oleate, ethyl heptanoate, stearic acid, ethyl palmitate, 2-butanol, 2-methyl-butanal, 3-methyl-butanal, isovaleric acid, guaiacol, pyrizines, butyl alcohol, phenylacetic acid, methyl mercaptan, coconut milk, and coconut cream.

17. The soy-based cheese of claim 14, wherein said flavoring is an herb, a spice, or a vegetable.

18. The soy-based cheese of any one of claims 1-17, said soy-based cheese comprising said antioxidant.

19. The soy-based cheese of claim 18, wherein said antioxidant is vitamin E.

20. The soy-based cheese of any one of claims 1-19, said soy-based cheese comprising said coloring agent.

21. The soy-based cheese of claim 20, wherein said coloring agent is annatto, apocarotenal, beta-carotene, paprika, vegetable juice, or turmeric.

22. The soy-based cheese of any one of claims 1-21, said soy-based cheese comprising said amino acid.

23. The soy-based cheese of claim 22, wherein said amino acid is leucine, alanine, valine, tyrosine, phenylalanine, lysine, isoleucine, glutamic acid, and/or glycine.

24. The soy-based cheese of any one of claims 1-23, said soy-based cheese comprising said gum.

25. The soy-based cheese of claim 24, wherein said gum is carrageenan, xanthan, konjac, guar, gellan, locust bean, or gum arabic.

26. The soy-based cheese of claim 24, wherein said gum is guar gum.

27. The soy-based cheese of any one of claims 1-26, said soy-based cheese comprising said one or more proteins.

28. The soy-based cheese of claim 27, wherein said one or more proteins comprise soy protein isolate or the soluble proteins from soy flour or soy protein isolate.

29. The soy-based cheese of claim 27, wherein said one or more proteins is an isolated protein.

30. The soy-based cheese of claim 29, wherein said isolated protein is selected from the group consisting of a lentil protein, a soy protein, or a pea protein.

31. The soy-based cheese of claim 30, wherein said protein is pea vicilin, pea legumin, soy β-conglycinin, soy glycinin, or a dehydrin protein.

32. The soy-based cheese of any one of claims 27-31, wherein the protein content of said soy-based cheese is similar to that of a dairy based American cheese.

33. The soy-based cheese of claim 32, wherein the protein content of said soy-based cheese is 15% to 20%.

34. The soy-based cheese of claim 33, wherein the protein content of said soy-based cheese is 16% to 19%.

35. The soy-based cheese of any one of claims 1-34, wherein the carboyhydrate content of said soy-based cheese is less than that of a dairy based American cheese.

36. The soy-based cheese of claim 35, wherein the carbohydrate content of said soy-based cheese is 4% to 7%.

37. The soy-based cheese of any one of claims 1-36, wherein the fat content of said soy-based cheese is less than that of a dairy based American cheese.

38. The soy-based cheese of claim 37, wherein the fat content of said soy-based cheese is from 16% to 22%.

39. The soy-based cheese of any one of claims 1-38, wherein said soy-based cheese is meltable.

40. The soy-based cheese of any one of claim 1-39, wherein said soy-based cheese is plant-based.

41. A method of making a soy-based cheese, said method comprising:

a) incubating a mixture of (i) soy milk, (ii) an optional sugar and/or optional one or more proteins, and (iii) a microbial strain or an acid for a period of time sufficient to reach a pH of 5.4 to 6.4;

b) blending said mixture;

c) adding to said blended mixture of b), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending;

d) heating said mixture of c) to a temperature of 60°C to 100°C; and

e) adding to the heated mixture of d), one or more optional sugars and one or more flavorings to produce said soy-based cheese.

42. The method of claim 41, further comprising forming said soy-based cheese in a mold.

43. The method of claim 41 or claim 42, wherein said microbial strain is a bacterial strain.

44. A method of making a soy-based cheese, said method comprising:

a) incubating a mixture of (i) soy milk, (ii) an optional sugar and/or optional one or more proteins, and (iii) a microbial strain or an acid for a period of time sufficient to reach a pH of 5.4 to 5.6;

b) heating said mixture to a temperature between 70°C and 90°C;

c) separating the curd and whey in said heated mixture of b) and optionally combining the curd:whey to obtain a ratio of 35:65 to 100:0 curd:whey;

d) adding to said curd:whey of c), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative;

e) cooking said mixture of d) to a temperature between 80°C and 100°C with blending;

f) adding to the cooked mixture of d), one or more flavorings; and

g) blending said cooked mixture of f) to obtain said soy-based cheese.

45. The method of claim 44, further comprising forming said soy-based cheese in a mold.

46. The method of claim 44 or claim 45, wherein said microbial strain is a bacterial strain.

47. A soy-based cheese comprising:

a) soy milk;

b) an acid;

c) a low methyl ester, amidated pectin;

d) an oil; and

e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose.

48. The soy-based cheese of claim 47, wherein said acid is selected from the group consisting of citric acid, lemon juice, HCl, and pyruvic acid.

49. A soy-based cheese comprising:

a) soy milk;

b) a microbial strain;

c) a low methyl ester, amidated pectin;

d) one or more proteins;

e) an oil;

f) a salt;

g) a sugar;

h) a gum;

i) a coloring agent; and

j) a flavoring.

50. A soy-based cheese comprising:

a) soy milk;

b) one or more proteins;

c) a low methyl ester, amidated pectin;

d) an oil; and

e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, lecithin, a flour, a fiber, or cellulose.

51. The soy-based cheese of claim 49 or claim 50, wherein said one or more proteins comprise soy protein isolate or the soluble proteins from soy flour or soy protein isolate.

52. The soy-based cheese of claim 49 or claim 50, wherein said one or more proteins is an isolated protein.

53. The soy-based cheese of claim 52, wherein said isolated protein is selected from the group consisting of a lentil protein, a soy protein, or a pea protein.

54. The soy-based cheese of claim 53, wherein said protein is pea vicilin, pea legumin, soy β-conglycinin, soy glycinin, or a dehydrin protein.

55. The soy-based cheese of any one of claims 49-54, wherein the protein content of said soy-based cheese is at least 15%.

56. The soy-based cheese of any one of claims 49-54, wherein the protein content of said soy-based cheese is similar to a dairy based American cheese.

57. The soy-based cheese of claim 56, wherein the protein content of said soy-based cheese is 15% to 20%.

58. The soy-based cheese of claim 56, wherein the protein content of said soy-based cheese is 16% to 19%.

59. The soy-based cheese of any one of claims 49-58, wherein the carboyhydrate content of said soy-based cheese is less than that of a dairy based American cheese.

60. The soy-based cheese of claim 59, wherein the carbohydrate content of said soy-based cheese is 4% to 7%.

61. The soy-based cheese of any one of claims 49-60, wherein the fat content of said soy-based cheese is less than that of a dairy based American cheese.

62. The soy-based cheese of claim 61, wherein the fat content of said soy-based cheese is from 16% to 22%.

63. The soy-based cheese of any one of claims 49-62, wherein said sugar comprises glucose, fructose, and/or sucrose.

64. The soy-based cheese of any one of claims 49-63, wherein said coloring agent is annatto, apocarotenal, beta-carotene, paprika, vegetable juice, or turmeric.

65. The soy-based cheese of any one of claims 49-64, said soy-based cheese further comprising a preservative.

66. The soy-based cheese of claim 65, wherein said preservative is selected from the group consisting of sorbic acid, natamycin, cultured dextrose, and olive leaf extract.

67. The soy-based cheese of any one of claims 49-66, wherein said soy-based cheese is meltable.

68. The soy-based cheese of any one of claim 49-67, wherein said soy-based cheese is plant-based.

FIGURE 1



FIGURE 2

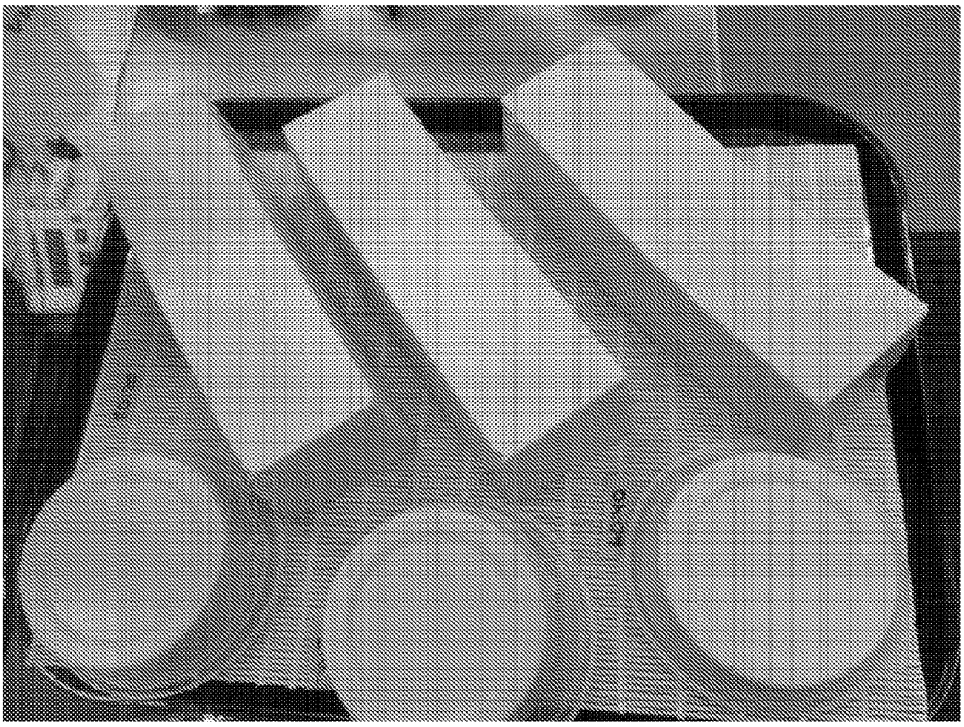


FIGURE 3

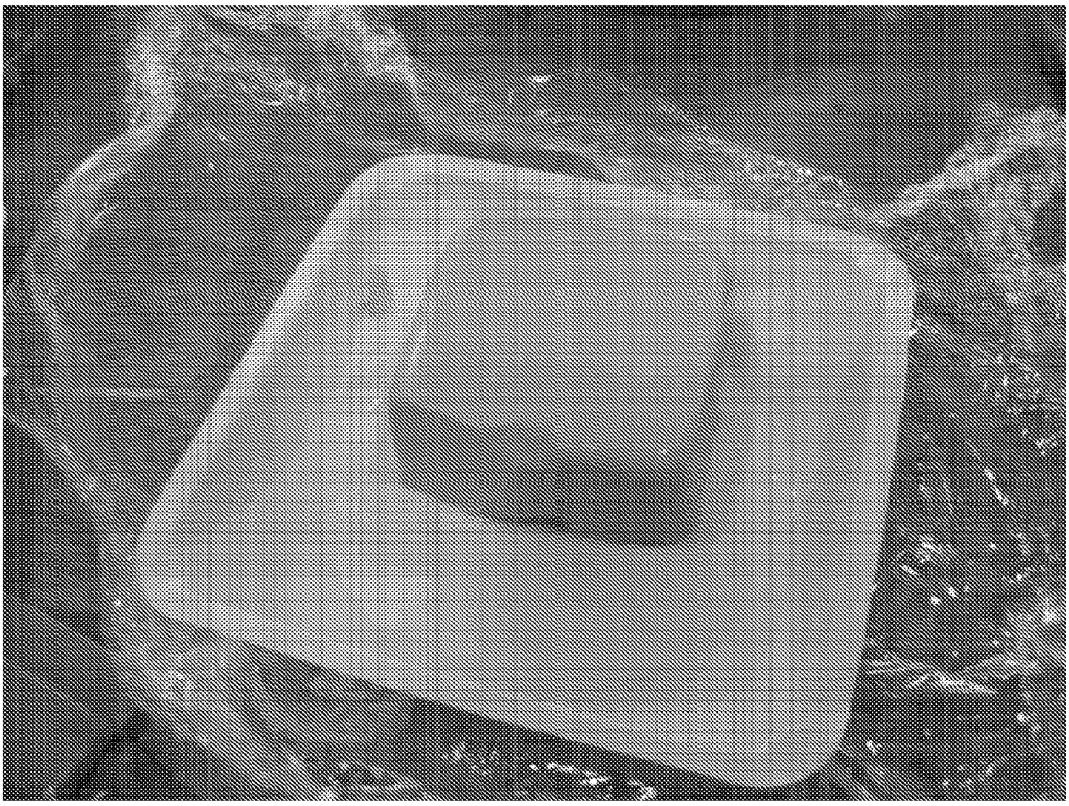


FIGURE 4

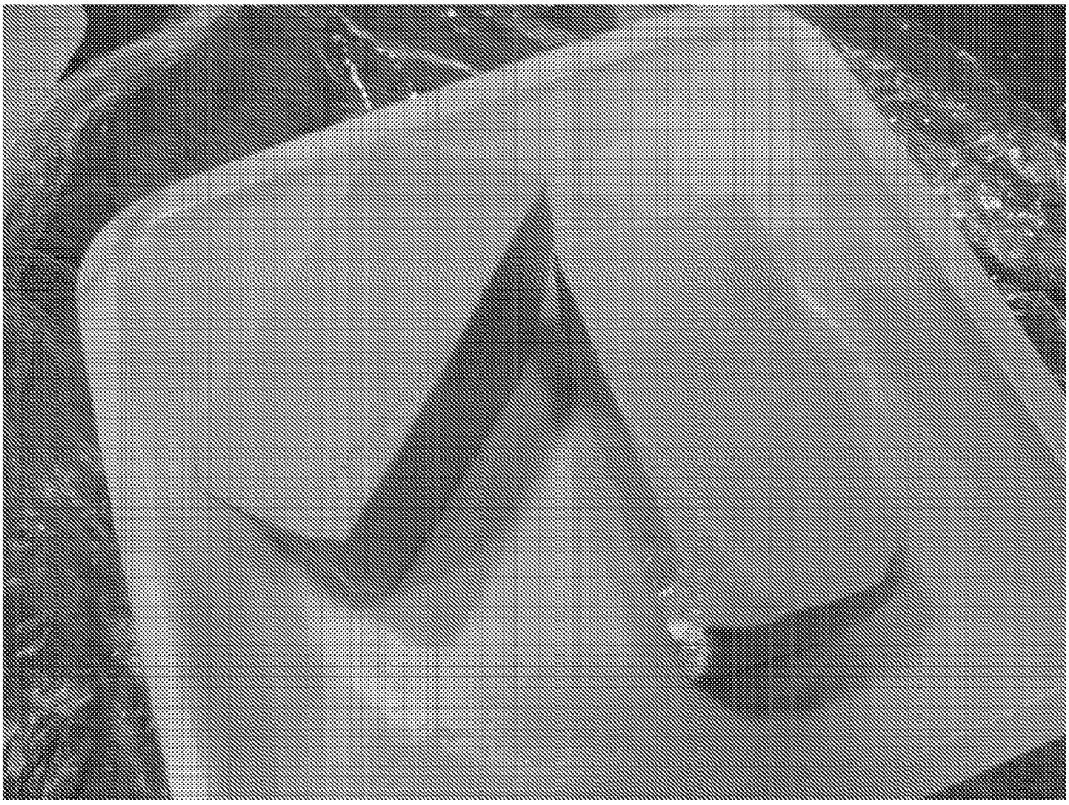


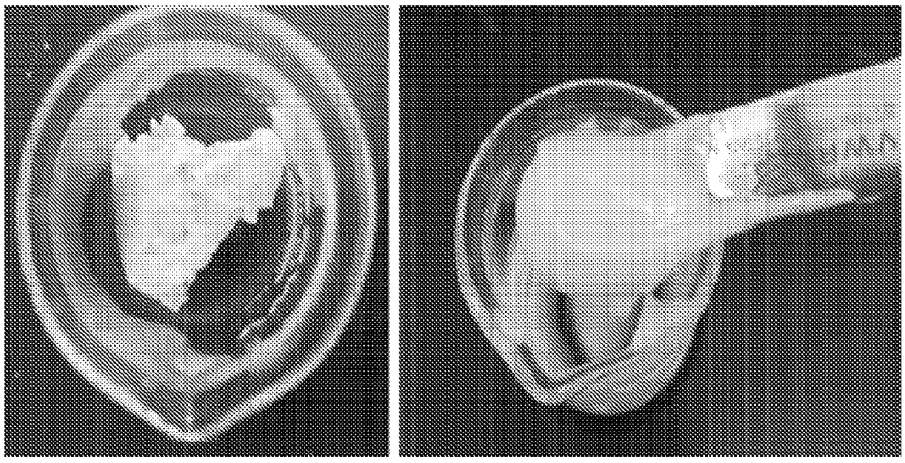
FIGURE 5





FIGURE 6

FIGURE 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/17147

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23C 19/00 (2015.01)
CPC - A23C 19/082; A23C 19/093; A23C20/00
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)
cpc-A23C19/082; A23C19/093; A23C20/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-426/582 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWest, ProQuest Dialog, Google Scholar, Google
Search Terms: Soy, bean, milk, cheese, microbial, yeast, mold, strain, bacterial, micro, acid, amidated pectin, oil, sugar, starch, Lactococcus lactis lac tis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylos

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/013292 A1 (Lin et al.) 31 January 2013 (31.01.2013) pg2, ln7-8; pg4, ln1-2, 15-16; pg8, ln12, 17; pg11, ln26-29; pg12, ln12, 25, 32; pg14, ln22;	1-7, 47-54
Y	Herbstreith&Fox. Low Methylester, Amidated Pectins. Technical Application Information. 18 January 2007; pg1, col1, ln1-5	1-7, 41-54
Y	US 5,597,594 A (Matsuura et al.) 28 January 1997 (28.01.1997) abstract, col3, ln29, 35-40, 46-48; col4, ln3; col5, ln53-54	3-7, 41-46
Y	US 2010/0196575 A1 (Sanchez et al.) 5 August 2010 (05.08.2010) para [0065], [0098]	41-46
Y,E	WO 2014/110540 A1 (Holz-Schietinget et al.) 17 July 2014 (17.07.2014) abstract, pg4, ln1-30	1-7

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2015 (17.04.2015)	01 MAY 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/17147

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.: 8-40, 55-68
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:

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.:

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.

PATENT COOPERATION TREATY

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
PRELIMINARY REPORT ON PATENTABILITY
(CHAPTER I OF THE PATENT COOPERATION
TREATY)
(PCT Rule 44bis.1(c))

To:

McCORMICK GRAHAM, Monica
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440-1022
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 23 January 2014 (23.01.2014)	
Applicant's or agent's file reference 38767-0012WO1	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/046560	12 July 2012 (12.07.2012)	12 July 2011 (12.07.2011)

Applicant
MARAXI, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Mineko Mohri

e-mail: pt08.pct@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 38767-0012WO1	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/046560	International filing date *(day/month/year)* 12 July 2012 (12.07.2012)	Priority date *(day/month/year)* 12 July 2011 (12.07.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant MARAXI, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 14 January 2014 (14.01.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL A. WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	14 DEC 2012
Applicant's or agent's file reference 41751-701601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/46560	12 July 2012 (12.07.2012)	12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23J 1/00 (2012.01)
USPC - 426/656

Applicant SAND HILL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	05 December 2012 (05.12.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 21-26, 47-63, 70-71, and 88-104

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 21-26, 47-63, 70-71, 88-104 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 21-26, 47-63, 70-71, and 88-104

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked 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 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

----------(Continued in Supplemental Box)----------

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-20

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	5-6, 11, 16-17, 19, 20	YES
	Claims	1-4, 7-10, 12-15, 18	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 7-10, 12-15, and 18 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0035003 A1 to McMindes et al. (hereinafter "McMindes").

As to claims 1, 3-4 and 12, McMindes teaches a meat substitute composition comprising a protein content (para [0033]), wherein one or more isolated and purified proteins accounts for 50% or more of said protein content by weight (para [0054] - 10-50% gluten), wherein said meat substitute accurately mimics the taste, texture, or color of a meat product derived from animal sources (para [0004] - "a need exists for structured meat products which closely simulate natural steaks and roasts in appearance, taste, texture and nutritional value, but which cost less. One object of the present invention is to provide such structured meat products."; para [0033]) wherein said one or more isolated and purified proteins is gluten (para [0054] - 10-50% gluten).

As to claim 2, McMindes teaches that said composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking (para [0033]).

As to claim 7, McMindes teaches that gluten does not account for 10% or more of said protein content by weight (para [0052]).

As to claim 8, McMindes teaches that each of said isolated, purified proteins is separately isolated and purified (para [0039], [0047]).

As to claim 9, McMindes teaches that said composition comprises 1-7 isolated and purified proteins (para [0039], [0047], [0054]).

As to claim 10, McMindes teaches that said 1-7 isolated and purified proteins are each isolated from different plant species (para [0039], [0047], [0054] - soy, wheat).

As to claim 13, McMindes teaches that said one or more isolated and purified proteins are not isolated from an animal (para [0039], [0047], [0054]).

As to claim 14, McMindes teaches that said one or more isolated and purified proteins are isolated from a single plant source (para [0053]).

As to claim 15, McMindes teaches that said one or more isolated and purified proteins are isolated from multiple plant sources (para [0039], [0047], [0054]).

As to claim 18, McMindes teaches that the isolated, purified protein has been formed into fibers (para [0050], [0058], [0065] - formation of protein fibers)

Claims 5-6, 11, 16-17, 19, and 20 lack an inventive step under PCT Article 33(3) as being obvious over McMindes.

As to claims 5-6, McMindes teaches a meat substitute composition, wherein said one or more isolated and purified proteins accounts for 50% of said protein content by weight (see explanation above for claim 4). McMindes does not teach that said one or more isolated and purified proteins accounts for 75% or 90% or more of said protein content by weight. However, methods for further optimizing the protein content of the disclosed composition were routine in the art, and one of ordinary skill in the art would have arrived at the claimed percentages via routine experimentation.

As to claim 11, McMindes teaches a meat substitute composition (see explanation above for claim 10). McMindes does not expressly teach that said protein content comprises no more than trace amounts of any other proteins derived from different plant species. However, it would have been obvious to one of ordinary skill in the art that, absent any express teachings of other added proteins, it could be assumed that said protein content comprises no more than trace amounts of any other proteins derived from different plant species.

----------(Continued in Second Supplemental Box)----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box IV.3. Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single inventive concept under 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 Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat (" In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

++++++++++++++++

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box V.2. Citations and Explanations:

As to claim 16, McMindes teaches a meat substitute composition (see explanation above for claim 13). McMindes further teaches that a component of the composition may be isolated from a genetically modified organism (para [0110], [0118]). Although McMindes does not specifically teach that the one or more isolated, purified proteins are isolated from a genetically modified organism, McMindes does teach that said proteins may be soy or wheat (para [0039], [0047], [0054]), and it would have been obvious to one of ordinary skill in the art that genetically modified soy and wheat plants were well-known in the art and routinely used to provide the materials (e.g., protein) for food production. Accordingly, it would have been obvious to isolate and purify the disclosed proteins from a genetically modified plant.

As to claim 17, McMindes does not teach that said genetically modified organism is a genetically modified bacteria or yeast organism. However, genetically modified bacteria and yeast were well-known and routinely used to inexpensively produce proteins in large volumes for a wide variety of uses, and it thus would have been obvious to one of ordinary skill in the art that the isolated and purified proteins taught by McMindes could be substituted by or supplemented with isolated, purified proteins isolated from a genetically modified bacteria or yeast organism.

As to claims 19-20, McMindes teaches a meat substitute composition, wherein said isolated, purified protein has been formed into fibers (see explanation above for claim 18). McMindes does not teach that said fibers resemble skeletal muscle fibers, or that said fibers are asymmetric fibers. However, McMindes does teach that the disclosed composition simulates the texture of natural meat products comprising skeletal muscle (para [0004]). In order to achieve such simulation of natural meat products, it would have been obvious to one of ordinary skill in the art that the fibers comprising the isolated, purified protein would need to resemble natural skeletal muscle fibers, including asymmetric fibers.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 41778-701601	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/046552	International filing date *(day/month/year)* 12 July 2012 (12.07.2012)	Priority date *(day/month/year)* 12 July 2011 (12.07.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant LYRICAL FOODS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 14 January 2014 (14.01.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Yukari Nakamura e-mail: pt07.pct@wipo.int

Form PCT/IB/373 (January 2004)

PCT/US2012/046552 19.11.2012

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	19 NOV 2012
Applicant's or agent's file reference 41778-701601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/46552	12 July 2012 (12.07.2012)	12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)
USPC - 426/573, 426/602, 426/632

Applicant LYRICAL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	31 October 2012 (31.10.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

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.

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.

- Please see first supplemental box for continuation -

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-33 and 89

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-33 and 89	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-33 and 89	NO
Industrial applicability (IA)	Claims	1-33 and 89	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-7, 17-19, 22-24, and 28-32 lack an inventive step under PCT Article 33(3) as being obvious over US 5,055,310 A to Nonaka et al. (hereinafter 'Nonaka') in view of US 4,678,676 A to Ishizuka et al. (hereinafter 'Ishizuka').

Regarding claim 1, Nonaka teaches a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources (col 2, ln 9-43: 'soy milk'); and (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins (col 2, ln 44-59). Nonaka fails to expressly teach wherein the method further comprises: (c) producing a cheese-replica from the gel. However, in a related disclosure, Ishizuka teaches a method of forming a similar soy-milk-based gel by coagulating soybean solids (col 1, ln 60 to col 2, ln 14) and using a gelling agent to form moldable soybean-milk-based gels (col 2, ln 50 to col 3, ln 2); and the specific formation of 'cheese-like emulsified foods' from the soybean-milk-based gels (col 3, ln 45-56). In light of the soybean-milk-based cheese-like emulsified foods taught in Ishizuka, one with ordinary skill in the art could confirm without undue experimentation the effectiveness of forming the soybean-milk-based gel taught in Nonaka into a cheese-replica product, as taught in Ishizuka.

Regarding claims 2 and 3, claim 1 is obvious as above. Nonaka further teaches wherein the emulsion contains no animal products (col 1, ln 35: soy-milk was the source of proteins and fats in the product).

Regarding claims 4, 5 and 6, claim 1 is obvious as above. Nonaka also teaches wherein the inducing step comprises adding an enzyme (col 1, ln 36); more specifically a transglutaminase from Streptoverticillium mobaraense (col 4, ln 15).

Regarding claim 7, claim 1 is obvious as above. Nonaka additionally teaches wherein the emulsion is a non-dairy milk (col 1, ln 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claims 17, 18 and 19, claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a sugar to the emulsion; more specifically a monosaccharide or a disaccharide. However, Nonaka does teach the use of various monosaccharides and disaccharides as carbon sources for the incubation of the transglutaminase-forming microorganisms (col 4, ln 19-46). Ishizuka also teaches the inclusion of a 'sweetener' component within the cheese-like composition (col 3, ln 60), wherein the use of monosaccharides and disaccharides as sweetener components in a food product is known and routine to those with ordinary skill in the art. A person of ordinary skill in the art would have desired to produce a flavor similar to dairy-based cheese, and would have known to use sweeteners, particularly sugars, based on the combined teachings of Nonaka and Ishizuka.

Regarding claim 22, claim 1 is obvious as above. Nonaka also discloses wherein the method further comprises cutting the gel (col 8, ln 48 -49).

Regarding claim 23, claim 22 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises draining and shaping the cut gel. However, the processing of draining and shaping a food product, especially gel-based food products, was known and routine to those with ordinary skill in the art as an effective way of optimizing the texture, palatibility, and marketability of a gel-based food product, such as a cheese-like product; such that one with ordinary skill could have devised, without undue experimentation, amethod of producing a gel-based food product comprising a step of draining and shaping the cut gel product, such as a product taught in Nonaka, such as to achieve a desired likeness to a cheese product.

Regarding claim 24, Ishizuka teaches the method further comprising adding a flavoring component (claim 6).

Regarding claim 28, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein at least one of the plant sources is a legume (col 1, ln 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claim 29, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein the proteins are concentrated prior to preparing the emulsion (col 2, ln 61-62).

---------- continued in Second Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box IV: Lack of Unit of Invention

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.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2. Citations and explanations:

Regarding claim 30, claim 29 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are each essentially homogeneous proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art could have produced without undue experimentation a food product using a mixture as taught by Nonaka using homogeneous proteins.

Regarding claim 31, claim 1 is obvious as above. Nonaka further discloses wherein the method further comprises concentrating, isolating, or purifying the proteins and fats from one or more plant sources (col 2, ln 9-43).

Regarding claim 32, claim 30 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are all the same essentially homogeneous plant-derived proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art would have been able, without undue experimentation, to produce the food product mixture taught by Nonaka using a homogeneous mixture of proteins.

Claims 20-21 and 25-26 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0299254 A1 to Kim et al. (hereinafter 'Kim').

Regarding claim 20, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises inoculating with lactic acid bacteria. However, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Regarding claim 21, claim 20 is obvious as above. Kim teaches wherein the method further comprises incubating to allow growth of the bacterial cultures (para [0013]: 'aging the resulting composition for a period sufficient for fermentation by the microorganism').

Regarding claims 25 and 26, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a lactic acid bacteria such as Lactobacillus species, however, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0292749 A1 to Goodwins et al. (hereinafter "Goodwins").

Regarding claim 27; claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a halophile bacterium or achaea. However, in a related disclosure, Goodwins teaches the production of cheeses (para [0034]) using at least one bacterium producing bateriocin (para [0029]), wherein the bacterium may be a halophilic bacterium (pediococcus halophiles; para [0021]). It would have been obvious to a person of ordinary skill in the art to apply a method of enhancing cheese production, as taught by Goodwin, to the cheese substitute taught by the combination of Nonaka and ishizuka without undue experimentation, in order to improve the cheese substitute product and make it more cheese-like to a consumer.

Claim 89 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2003/0224476 A1 (Chou).

Regarding claim 89; claim 4 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the enzyme is lysyl oxidase, in a related disclosure, Chou teaches transglutaminase-reactive compounds (abstract), including the use of cheesegelling of proteins (para [0003]); wherein the transglutaminase-reactive compund is produced by denaturing a compound and refolding the compound (para [0008]). The compound may also be cross-linked with a biological agent (para [0042]), wherein the biological agent may be a lysyl oxidase (para [0050]), and may be used in the gelling of cheese (para [0176]). It would have been obvious to a person of ordinary skill in the art to substitute lysyl oxidsae for transglutaminase to gel a proteinaceous product, as taught by Chou, wherein the product was a substitute cheese product, as taught by the combination of Nonaka and Ishizuka, in order to provide a cheese substitute with a flavor that was not affected by the presence of the transglutaminase enzyme.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box

Claims 8-16 and 33 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2011/0064847 A1 to McCready et al. (hereinafter 'McCready'), and further in view of US 5,807,601 A to Carpenter et al. (hereinafter "Carpenter").

Regarding claim 8; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the ultility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies.

Regarding claim 9; claim 8 is obvious as above. Further, McCready teaches wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]).

Regarding claim 10; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the ultility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies. Niether Nonaka, Ishizuka, nor Carpenter teaches wherein the method further comprises 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. However, McCready further teaches wherein preparation further includes the steps of: (a) obtaining nuts (para [0016]), and (b) surface sterilizing the nuts (para [0016]: 'blanching'). It would have been obvious to one with ordinary skill in the art to obtain and prepare the nuts for a nut milk, as taught by McCready, in order to minimize contamination of the nuts with known pathogens, such as salmonella, or fungi that produced known carcinogens, such as aflatoxin. A person of skill in the art would have desired to appropriately sterilize the raw materials used in the process as a first step.

Regarding claim 11; claim 10 is obvious as above. Furthermore, McCready teaches wherein the surface sterilization is blanching (para [0016]).

Regarding claim 12; claim 8 is obvious as above. Furthermore, McCready teaches wherein the method further comprises washing the nuts (para [0016]: 'blanching').

Regarding claims 13 and 14; claim 10 is obvious as above. Furthermore, McCready teaches wherein the preparation further comprising decompounding the nuts; more specifically wherein the decompounding is blending (para [0017]).

Regarding claim 15 and 16; claim 10 is obvious as above. Ishizuka further teaches centrifuging the soy materials (col 4, ln 38-44). Although Ishizuka does not explicitly recite wherein the centrifuging removes at least 85% of the suspended solids, it would have been obvious to a person of ordinary skill in the art to determine an appropriate centrifugation speed and duration to separate or remove a desired amount of solids that varied in density from the solvent from any solution without undue experimentation.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box

Regarding claim 33; claim 30 is obvious as above, Neither Nonaka nor Ishizuka explicitly teaches wherein each concentrated protein is from a separate plant species. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use concentrated proteins from a variety of separate plant species, particularly different nut species in combination with soy, based on combining the disclosure of Carpenter regarding using a nut product to produce a cheese substitute, wherein the nut product could have been any of a number of nut milks, based on the disclosure of McCready, processed according to the method disclosed by the combination of Nonaka and Ishizuka. A person of skill in the art would have recognized the potential of the different nut proteins to alter and contribute to the flavor and texture of the product, and would have desired to produce different combinations thereof, either to identify a composition most resembling a known cheese product, or to create a series of imitation cheese products with varying flavors.

Claims 1-33 and 89 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 387670015WO1	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2014/011347	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)	Priority date *(day/month/year)* 11 January 2013 (11.01.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant IMPOSSIBLE FOODS INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 14 July 2015 (14.07.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Teresa Lavoie
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	03 JUL 2014
Applicant's or agent's file reference 387670015WO1	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/11347	13 January 2014 (13.01.2014)	11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23D 7/005, 9/013; A23L 1/30 (2014.01)
USPC - 426/92, 641, 647

Applicant Maraxi, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	09 June 2014 (09.06.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

a. (means)

☐ on paper

☒ in electronic form

b. (time)

☐ in the international application as filed

☐ together with the international application in electronic form

☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

-***-Please See Supplemental Page-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts
   - ☒ the parts relating to claims Nos. 1-10, 28-37, 39, phenylacetaldehyde (flavor compound, not the precursor molecule)

Form PCT/ISA/237 (Box No. IV) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	28-34	YES
	Claims	1-10, 35-37, 39	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-10, 28-37, 39	NO
Industrial applicability (IA)	Claims	1-10, 28-37, 39	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-10, 35-37 and 39 lack novelty under PCT Article 33(2) as being anticipated by US 2009/0274817 A1 to Yamaguchi, et al. (hereinafter 'Yamaguchi').

As per Claim 1, Yamaguchi discloses a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]) comprising: a) a highly conjugated heterocyclic ring complexed to iron ion (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferrous sulfate, ferrous citrate, ferric citrate, ferrous gluconate, ferric chloride, and ferrous lactate; and heme iron; plasma powder; and blood cell powder; paragraph [0029]); and b) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

As per Claim 2, Yamaguchi discloses the food product of claim 1, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]), and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, and an isobacteriochlorin moiety complexed to iron ion.

As per Claim 3, Yamaguchi discloses the food product of claim 2, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]).

As per Claim 4, Yamaguchi discloses the food product of claim 2, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts such as ferric chloride (ferrous chlorin); paragraph [0029]).

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

As per Claim 5, Yamaguchi discloses a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]) comprising: a) a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]); and b) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

As per Claims 6/1-6/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to fifty flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to fifty flavor precursors); paragraph [0035]).

As per Claims 7/1-7/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to forty flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to fifty flavor precursors; paragraph [0035]).

As per Claims 8/1-8/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to thirty-five flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to thirty-five flavor precurors; paragraph [0035]).

As per Claims 9/1-9/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to ten flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind; (two to ten flavor precurors; paragraph [0035]).

As per Claims 10/1-10/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to six flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to six flavor precursors; paragraph [0035]).

As per Claim 35, Yamaguchi discloses a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition (flavor of Pork Cutlet that was prepared by seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper, coating it with wheat flour, 20% egg solution and bread crumb, followed by deep-frying at 180 degrees C, for 30 min. in the above fat and oil preparations; paragraph [0078]; Table 9), said flavoring composition comprising i) a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]); and ii) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product (flavor of Pork Cutlet that was prepared by seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper, coating it with wheat flour, 20% egg solution and bread crumb, followed by deep-frying at 180 degrees C, for 30 min. in the above fat and oil preparations; paragraph [0078]; Table 9).

As per Claim 36, Yamaguchi discloses the method of claim 35, and Yamaguchi further discloses wherein said meat-like flavor is a beef-like, chicken-like, pork-like (there is no limitation on the kind of subject foods, which include foods cooked with oil, such as various fried foods and stir-fry; and fat-containing foods, such as Chinese-style pork dumplings and steamed Chinese-style pork dumplings; paragraphs [0053], [0078]), lamb-like flavor, or turkey-like.

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
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As per Claims 37/35 and 37/36, Yamaguchi discloses the method of claims 35 and 36, and Yamaguchi discloses wherein said flavoring composition further comprises a seasoning agent (seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper; paragraph [0078]), a flavoring agent, a protein, a protein concentrate, or an emulsifier (the body taste improver of the present invention may additionally contains other optional components known to those skilled in the art, such as an emulsifier; paragraph [0040]).

As per Claim 39, Yamaguchi discloses a method of making a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]), said method comprising combining an isolated heme-containing protein and one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture (there is no limitation on a method for the oxidization treatment, which, for example, includes heating treatment (heating the mixture); paragraph [0041]).

Claims 28 and 34 lack an inventive step under PCT Article 33(3) as being obvious over Yamaguchi in view of US 3,829,582 A to Guadagni, et al. (hereinafter 'Guadagni').

As per Claim 28, Yamaguchi discloses a method for producing a flavor compound (method for producing a body taste improver (flavor compound); paragraphs [0024], [0040], [0045]), said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferrous sulfate, ferrous citrate, ferric citrate, ferrous gluconate, ferric chloride, and ferrous lactate; and heme iron; plasma powder; and blood cell powder; paragraph [0029]) with one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. Yamaguchi does not disclose b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde. Guadagni discloses b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, for imparting a fatty-fried flavor to potato and other food products (a mixture containing methional, 2-acetylthiazoline, deca-2,4-dienal, 2-octenal, 2-acetyl-l ,4,5,6-tetrahydropyridine, 2-ethyl-3,6-dimethylpyrazine, 2,6-diethylpyrazine, and 2-phenylacetaldehyde is used to impart a fatty-fried flavor to potato and other food products; abstract; Claims 1-3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with a mixture containing 2-phenylacetaldehyde used to impart a fatty-fried flavor to potato and other food products (Guadagni; abstract; Claims 1-3), as previously disclosed by Guadagni, as the previous disclosure by Yamaguchi includes a method of deep-frying pork loin in vegetable oil for improving taste, Guadagni discloses adding a fatty-fried flavor to food products, and a combination of these references would have resulted in an improved fatty-fried taste for pork.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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As per Claim 34, Yamaguchi discloses a method for producing a flavor compound (body taste improvers (flavor compounds); paragraphs [0024], [0040], [0045]), said method comprising: a) combining a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), and one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. Yamaguchi does not disclose cysteine, and a sugar; and b) heating the mixture to form one or more flavor compounds set forth in Table 3 (phenylacetaldehyde). Guadagni discloses cysteine, and a sugar (to obtain a given quantity of protein (cysteine), starch, and sugar, or other non-fat nutrient in addition to a pleasing flavor, the consumer necessarily receives a high proportion of fat; column 1, lines 30-35); and b) heating the mixture to form one or more flavor compounds set forth in Table 3 (phenylacetaldehyde) (a mixture containing methional, 2-acetylthiazoline, deca-2,4-dienal, 2-octenal, 2-acetyl-l ,4,5,6-tetrahydropyridine, 2-ethyl-3 ,6-dimethylpyrazine, 2,6-diethylpyrazine, and 2-phenylacetaldehyde is used to impart a fatty-fried flavor to potato and other food products; abstract; Claims 1-3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with a mixture containing 2-phenylacetaldehyde, protein, and sugar used to impart a fatty-fried flavor to potato and other food products (Guadagni; abstract; column 1, lines 30-35; Claims 1-3), as previously disclosed by Guadagni, as the previous disclosure by Yamaguchi includes the method of deep-frying pork loin in vegetable oil for improving the taste, Guadagni discloses the adding a fatty-fried flavor to food products, and a combination of these references would have resulted in an improved fatty-fried taste for pork.

Claims 29-33 lack an inventive step under PCT Article 33(3) as being obvious over Yamaguchi and Guadagni and further in view of US 5,443,852 A to Shahidi, et al. (hereinafter 'Shahidi').

As per Claim 29, Yamaguchi and Guadagni, in combination, discloses the method of claim 28, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]). Yamaguchi does not disclose a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a achlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to iron ion. Shahidi discloses an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, for effectively preserving cured meats in the absence of nitrites and nitrates (Shahidi; column 8, lines 10-25; column 8, lines 50-60), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes the method of preparing pork in vegetable oil to improve the taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and a combination of these references would have resulted in an improved fatty-fried taste for pork that lacks nitrites and nitrates.

As per Claim 30, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 29, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]).

As per Claim 31, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 30, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]).

As per Claim 32, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 29, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is ferrous chlorin (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferric chloride (ferrous chlorin); paragraph [0029]).

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

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In case **the space in any of the preceding boxes is not sufficient.**
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As per Claim 33/28, Yamaguchi and Guadagni, in combination, discloses the method of claim 28, but Yamaguchi does not disclose said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron. Shahidi discloses said method comprising combining lactic acid (lactic acid, its sodium or potassium salts, or lactic acid-producing microorganisms may also be used in providing microbial stability to muscle food; column 42, lines 5-15) with said highly conjugated heterocyclic ring complexed to iron (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells and lactic acid, for preserving cured meats in the absence of nitrites and nitrates, and for providing microbial stability (Shahidi; column 8, lines 10-25; column 8, lines 50-60, column 42, lines 5-15), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes a method of preparing pork in vegetable oil for preserving taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and providing microbial stability, and a combination of these references would have resulted in an improved fatty-fried taste for pork which lacks nitrites and nitrates, while having enhanced microbial stability.

As per Claims 33/29-33/32, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claims 29-32, but Yamaguchi does not disclose said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron. Shahidi discloses said method comprising combining lactic acid (lactic acid, its sodium or potassium salts, or lactic acid-producing microorganisms may also be used in providing microbial stability to muscle food; column 42, lines 5-15) with said highly conjugated heterocyclic ring complexed to iron (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells and lactic acid, for preserving cured meats in the absence of nitrites and nitrates, and providing enhanced microbial stability (Shahidi; column 8, lines 10-25; column 8, lines50-60, column 42, lines 5-15), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes the method of preparing pork in vegetable oil to improve the taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and providing enhanced microbial stability, and a combination of these references would have resulted in an improved fatty-fried taste for pork that lacks nitrites and nitrates, while having enhanced microbial stability.

Claims 1-10, 28-37 and 39 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 387670016WO1	**FOR FURTHER ACTION** See item 4 below	
International application No. PCT/US2014/011362	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)	Priority date *(day/month/year)* 11 January 2013 (11.01.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant IMPOSSIBLE FOODS INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 14 July 2015 (14.07.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

PCT/US2014/011362 13.06.2014

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 JUN 2014
Applicant's or agent's file reference 387670016WO1	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/11362	13 January 2014 (13.01.2014)	11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378

Applicant MARAXI, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 May 2014 (14.05.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9-10, 15-48, 56-67, 72,79,89-99 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

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 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.

Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.

Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.

Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.

Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

-***-Continued Within Supplemental Box-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-4, 8/1-4, 73-77, 78/73-77, and 91-93

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	8/1-4, 73-77, 78/73-77	YES
	Claims	1-4, 91-93	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	NO
Industrial applicability (IA)	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-4 and 91-93 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0151778 A1 to Richard, et al. (hereafter 'Richard').

As per claim 1, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated and purified proteins from a non-animal source (solubilization of at least one plant protein in an aqueous medium; the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used, are extracted from plants (extraction comprise process for isolation of pure proteins from the mixture); paragraphs [0022], [0025], [0028]; [0039], [0040]).

As per claim 2, Richard discloses the non-dairy cheese replica of claim 1, and Richard further discloses wherein said one or more isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 3, Richard discloses the non-dairy cheese replica of claim 2, and Richard further discloses wherein said one or more isolated and purified plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus); paragraph [0040]).

As per claim 4, Richard discloses the non-dairy cheese replica of claim 3, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claim 91, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated proteins from a non-animal source (the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used in the context of the invention are extracted from plants; paragraphs [0022], [0025], [0039], [0040]).

As per claim 92, Richard discloses the non-dairy cheese replica of claim 91, and Richard further discloses wherein said one or more isolated proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 93, Richard discloses the non-dairy cheese replica of claim 92, and Richard further discloses wherein said one or more isolated plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus); paragraph [0040]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

Claims 8/1-4 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of WO 2008/030089 A1 to Brandsma, et al. (hereafter 'Brandsma').

As per claims 8/1-4, Richard discloses the non-dairy cheese replica of any one of claims 1-4, but Richard does not disclose further comprising one or more microbes selected from bacteria, molds, and yeast. However, Brandsma discloses comprising one or more microbes selected from bacteria and yeast (adding at least two live food grade micro-organisms, said two micro-organisms are at least one a yeast strain selected from the genus Kluyveromyces and at least one bacterial strain selected from the genus Propionibacterium; to form a cheese; page 5, lines 26-32 to page 6, lines 1-3; claim 1). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide microbes such as bacteria or yeast, as disclosed by Brandsma, in order to have flavor formation in manufacture of fermented products, in order to provide non-dairy consumables.

Claims 73-76 and 78/73-76 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of US 4,435,438 A to Lehnhardt, et al. (hereafter 'Lehnhardt').

As per claim 73, Richard discloses a method of making a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; paragraph [0025]), said method comprising acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5 (method comprises, solubilization of at least one plant protein in an aqueous medium at a pH of between 2 and 7, and below the isoelectric pH of the protein; the pH of the medium is decreased, by adding IN hydrochloric acid; plant proteins used in the context of the invention are extracted from plants; paragraphs [0028],[0040], [0048]) and isolating said coacervate from said solution (encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; the wall formed is hardened by cooling the medium and crosslinked by the action of a crosslinking agent; paragraph [0011]). Richard does not disclose wherein said solution comprises 100 mM or less of a salt. However, Lehnhardt discloses wherein said solution comprises 100 mM or less of a salt (salt (sodium chloride); 2 parts by weight (2 pbw corresponds to 0.66 mM of sodium chloride); column 4, lines 1-5; column 16, lines 20-30). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein said solution comprises 100 mM or less of a salt, as disclosed by Lehnhardt, in order to provide plant protein coacervates having low salt content, in order to provide non-dairy protein consumables.

As per claim 74, Richard and Lehnhardt, in combination, disclose the method of claim 73, and Richard further discloses wherein said isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus) and cereals such as wheat, maize, barley, malt or oats; paragraph [0040]).

As per claim 75, Richard and Lehnhardt, in combination, disclose the method of claim 74, and Richard further discloses wherein said plant proteins are seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus) and cereals such as wheat, maize, barley, malt or oats; plant proteins SWP100 and SWP50 and those marketed under the name Supro 670 and PISANE; paragraph [0040]).

As per claim 76, Richard and Lehnhardt, in combination, disclose the method of claim 75, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claims 78/73-76, Richard and Lehnhardt, in combination, disclose the method of claims 73-76, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the pH of the medium is decreased, by adding in hydrochloric acid; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
-***-Continued from Previous Supplemental Box-***-

Claims 77 and 78/77 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of Lehnhardt further in view of US 2008/0226810 A1 to Passe, et al (hereafter 'Passe').

As per claim 77, Richard and Lehnhardt, in combination, disclose the method of claim 76, but Richard does not disclose wherein said pea vicilins comprises convicilins. However, Passe discloses wherein said pea proteins comprise pea vicilins and/or pea legumins (pea proteins, like all proteins of leguminous plants, are constituted of three classes of proteins: globulins, albumins and insoluble proteins, the globulins (legumins, vicilins and convicilins) are the main storage proteins of the plant; legumins are hexameric proteins of 360 to 410 kDa composed of 6 double-stranded subunits of 60 kDa; vicilins are trimeric proteins whose mass ranges from 150 to 200 kDa; paragraphs [0005], [0007], [0014], [0015]). It would be obvious to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein pea proteins comprise pea vicilins and/or pea legumins, as disclosed by Passe, in order to provide other legumes, in order provide other suitable plant based proteins, in order to manufacture non-animal based imitation cheese.

As per claim 78/77, Richard, Lehnhardt, and Passe, in combination, disclose the method of claim 77, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
-***-Continued from Box No. IV: Lack of unity of invention-***-

The inventions listed as Groups I-V 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 Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus, and Brevi bacterium linens; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non- animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

PCT/US2012/046560 14.12.2012

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 41751-701601	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/46560	International filing date *(day/month/year)* 12 July 2012 (12.07.2012)	(Earliest) Priority Date *(day/month/year)* 12 July 2011 (12.07.2011)
Applicant SAND HILL FOODS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46560

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.: 21-26, 47-63, 70-71, and 88-104
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 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 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

----------(Continued on extra sheet)----------

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.:
1-20

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 12/46560

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23J 1/00 (2012.01)
USPC - 426/656
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: 426/656

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 426/656, 574, 573, 531, 615
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase; Google; PubMed
Search terms: meat, substitute, isolated, purified, protein, gluten, raw, cook, recombinant, yeast

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0035003 A1 (MCMINDES et al.) 16 February 2006 (16.02.2006) para [0004], [0033], [0039], [0047], [0050], [0052]-[0054], [0058], [0065], [0110], [0118]	1-20
Y	US 2007/0269571 A1 (AKITA et al.) 22 November 2007 (22.11.2007) entire document.	1-20
Y	US 2010/0074998 A1 (ESPELETA VEGA et al.) 25 March 2010 (25.03.2010) entire document.	1-20
Y	US 2004/0161513 A1 (AKASHE et al.) 19 August 2004 (19.08.2004) entire document.	1-20
Y	US 2011/0008502 A1 (HOSOMI et al.) 13 January 2011 (13.01.2011) entire document.	1-20

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 December 2012 (05.12.2012)	14 DEC 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46560

Continuation of Box III - observations where unity of invention is lacking:

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

The inventions listed as Groups I-XI do not relate to a single inventive concept under 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 Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat (" In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

+++++++

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL A. WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	14 DEC 2012
Applicant's or agent's file reference 41751-701601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/46560	12 July 2012 (12.07.2012)	12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23J 1/00 (2012.01)
USPC - 426/656

Applicant SAND HILL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	05 December 2012 (05.12.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 21-26, 47-63, 70-71, and 88-104

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 21-26, 47-63, 70-71, 88-104 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 21-26, 47-63, 70-71, and 88-104

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

Form PCT/ISA/237 (Box No. III) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked 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 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

----------(Continued in Supplemental Box)----------

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-20

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	5-6, 11, 16-17, 19, 20	YES
	Claims	1-4, 7-10, 12-15, 18	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 7-10, 12-15, and 18 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0035003 A1 to McMindes et al. (hereinafter "McMindes").

As to claims 1, 3-4 and 12, McMindes teaches a meat substitute composition comprising a protein content (para [0033]), wherein one or more isolated and purified proteins accounts for 50% or more of said protein content by weight (para [0054] - 10-50% gluten), wherein said meat substitute accurately mimics the taste, texture, or color of a meat product derived from animal sources (para [0004] - "a need exists for structured meat products which closely simulate natural steaks and roasts in appearance, taste, texture and nutritional value, but which cost less. One object of the present invention is to provide such structured meat products."; para [0033]) wherein said one or more isolated and purified proteins is gluten (para [0054] - 10-50% gluten).

As to claim 2, McMindes teaches that said composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking (para [0033]).

As to claim 7, McMindes teaches that gluten does not account for 10% or more of said protein content by weight (para [0052]).

As to claim 8, McMindes teaches that each of said isolated, purified proteins is separately isolated and purified (para [0039], [0047]).

As to claim 9, McMindes teaches that said composition comprises 1-7 isolated and purified proteins (para [0039], [0047], [0054]).

As to claim 10, McMindes teaches that said 1-7 isolated and purified proteins are each isolated from different plant species (para [0039], [0047], [0054] - soy, wheat).

As to claim 13, McMindes teaches that said one or more isolated and purified proteins are not isolated from an animal (para [0039], [0047], [0054]).

As to claim 14, McMindes teaches that said one or more isolated and purified proteins are isolated from a single plant source (para [0053]).

As to claim 15, McMindes teaches that said one or more isolated and purified proteins are isolated from multiple plant sources (para [0039], [0047], [0054]).

As to claim 18, McMindes teaches that the isolated, purified protein has been formed into fibers (para [0050], [0058], [0065] - formation of protein fibers)

Claims 5-6, 11, 16-17, 19, and 20 lack an inventive step under PCT Article 33(3) as being obvious over McMindes.

As to claims 5-6, McMindes teaches a meat substitute composition, wherein said one or more isolated and purified proteins accounts for 50% of said protein content by weight (see explanation above for claim 4). McMindes does not teach that said one or more isolated and purified proteins accounts for 75% or 90% or more of said protein content by weight. However, methods for further optimizing the protein content of the disclosed composition were routine in the art, and one of ordinary skill in the art would have arrived at the claimed percentages via routine experimentation.

As to claim 11, McMindes teaches a meat substitute composition (see explanation above for claim 10). McMindes does not expressly teach that said protein content comprises no more than trace amounts of any other proteins derived from different plant species. However, it would have been obvious to one of ordinary skill in the art that, absent any express teachings of other added proteins, it could be assumed that said protein content comprises no more than trace amounts of any other proteins derived from different plant species.

----------(Continued in Second Supplemental Box)----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box IV.3. Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single inventive concept under 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 Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat (" In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box V.2. Citations and Explanations:

As to claim 16, McMindes teaches a meat substitute composition (see explanation above for claim 13). McMindes further teaches that a component of the composition may be isolated from a genetically modified organism (para [0110], [0118]). Although McMindes does not specifically teach that the one or more isolated, purified proteins are isolated from a genetically modified organism, McMindes does teach that said proteins may be soy or wheat (para [0039], [0047], [0054]), and it would have been obvious to one of ordinary skill in the art that genetically modified soy and wheat plants were well-known in the art and routinely used to provide the materials (e.g., protein) for food production. Accordingly, it would have been obvious to isolate and purify the disclosed proteins from a genetically modified plant.

As to claim 17, McMindes does not teach that said genetically modified organism is a genetically modified bacteria or yeast organism. However, genetically modified bacteria and yeast were well-known and routinely used to inexpensively produce proteins in large volumes for a wide variety of uses, and it thus would have been obvious to one of ordinary skill in the art that the isolated and purified proteins taught by McMindes could be substituted by or supplemented with isolated, purified proteins isolated from a genetically modified bacteria or yeast organism.

As to claims 19-20, McMindes teaches a meat substitute composition, wherein said isolated, purified protein has been formed into fibers (see explanation above for claim 18). McMindes does not teach that said fibers resemble skeletal muscle fibers, or that said fibers are asymmetric fibers. However, McMindes does teach that the disclosed composition simulates the texture of natural meat products comprising skeletal muscle (para [0004]). In order to achieve such simulation of natural meat products, it would have been obvious to one of ordinary skill in the art that the fibers comprising the isolated, purified protein would need to resemble natural skeletal muscle fibers, including asymmetric fibers.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PCT/US2012/046552 19.11.2012

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 41778-701601	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/46552	International filing date *(day/month/year)* 12 July 2012 (12.07.2012)	(Earliest) Priority Date *(day/month/year)* 12 July 2011 (12.07.2011)
Applicant LYRICAL FOODS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

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

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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2012 (31.10.2012)	19 NOV 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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.

PCT/US2012/046552 19.11.2012

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	19 NOV 2012
Applicant's or agent's file reference 41778-701601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/46552	12 July 2012 (12.07.2012)	12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)
USPC - 426/573, 426/602, 426/632

Applicant LYRICAL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	31 October 2012 (31.10.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

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.

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.

- Please see first supplemental box for continuation -

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-33 and 89

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-33 and 89	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-33 and 89	NO
Industrial applicability (IA)	Claims	1-33 and 89	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-7, 17-19, 22-24, and 28-32 lack an inventive step under PCT Article 33(3) as being obvious over US 5,055,310 A to Nonaka et al. (hereinafter 'Nonaka') in view of US 4,678,676 A to Ishizuka et al. (hereinafter 'Ishizuka').

Regarding claim 1, Nonaka teaches a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources (col 2, ln 9-43: 'soy milk'); and (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins (col 2, ln 44-59). Nonaka fails to expressly teach wherein the method further comprises: (c) producing a cheese-replica from the gel. However, in a related disclosure, Ishizuka teaches a method of forming a similar soy-milk-based gel by coagulating soybean solids (col 1, ln 60 to col 2, ln 14) and using a gelling agent to form moldable soybean-milk-based gels (col 2, ln 50 to col 3, ln 2); and the specific formation of 'cheese-like emulsified foods' from the soybean-milk-based gels (col 3, ln 45-56). In light of the soybean-milk-based cheese-like emulsified foods taught in Ishizuka, one with ordinary skill in the art could confirm without undue experimentation the effectiveness of forming the soybean-milk-based gel taught in Nonaka into a cheese-replica product, as taught in Ishizuka.

Regarding claims 2 and 3, claim 1 is obvious as above. Nonaka further teaches wherein the emulsion contains no animal products (col 1, ln 35: soy-milk was the source of proteins and fats in the product).

Regarding claims 4, 5 and 6, claim 1 is obvious as above. Nonaka also teaches wherein the inducing step comprises adding an enzyme (col 1, ln 36); more specifically a transglutaminase from Streptoverticillium mobaraense (col 4, ln 15).

Regarding claim 7, claim 1 is obvious as above. Nonaka additionally teaches wherein the emulsion is a non-dairy milk (col 1, ln 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claims 17, 18 and 19, claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a sugar to the emulsion; more specifically a monosaccharide or a disaccharide. However, Nonaka does teach the use of various monosaccharides and disaccharides as carbon sources for the incubation of the transglutaminase-forming microorganisms (col 4, ln 19-46). Ishizuka also teaches the inclusion of a 'sweetener' component within the cheese-like composition (col 3, ln 60), wherein the use of monosaccharides and disaccharides as sweetener components in a food product is known and routine to those with ordinary skill in the art. A person of ordinary skill in the art would have desired to produce a flavor similar to dairy-based cheese, and would have known to use sweeteners, particularly sugars, based on the combined teachings of Nonaka and Ishizuka.

Regarding claim 22, claim 1 is obvious as above. Nonaka also discloses wherein the method further comprises cutting the gel (col 8, ln 48 -49).

Regarding claim 23, claim 22 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises draining and shaping the cut gel. However, the processing of draining and shaping a food product, especially gel-based food products, was known and routine to those with ordinary skill in the art as an effective way of optimizing the texture, palatibility, and marketability of a gel-based food product, such as a cheese-like product; such that one with ordinary skill could have devised, without undue experimentation, amethod of producing a gel-based food product comprising a step of draining and shaping the cut gel product, such as a product taught in Nonaka, such as to achieve a desired likeness to a cheese product.

Regarding claim 24, Ishizuka teaches the method further comprising adding a flavoring component (claim 6).

Regarding claim 28, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein at least one of the plant sources is a legume (col 1, ln 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claim 29, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein the proteins are concentrated prior to preparing the emulsion (col 2, ln 61-62).

---------- continued in Second Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box IV: Lack of Unit of Invention

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.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2. Citations and explanations:

Regarding claim 30, claim 29 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are each essentially homogeneous proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art could have produced without undue experimentation a food product using a mixture as taught by Nonaka using homogeneous proteins.

Regarding claim 31, claim 1 is obvious as above. Nonaka further discloses wherein the method further comprises concentrating, isolating, or purifying the proteins and fats from one or more plant sources (col 2, ln 9-43).

Regarding claim 32, claim 30 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are all the same essentially homogeneous plant-derived proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art would have been able, without undue experimentation, to produce the food product mixture taught by Nonaka using a homogeneous mixture of proteins.

Claims 20-21 and 25-26 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0299254 A1 to Kim et al. (hereinafter 'Kim').

Regarding claim 20, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises inoculating with lactic acid bacteria. However, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Regarding claim 21, claim 20 is obvious as above. Kim teaches wherein the method further comprises incubating to allow growth of the bacterial cultures (para [0013]: 'aging the resulting composition for a period sufficient for fermentation by the microorganism').

Regarding claims 25 and 26, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a lactic acid bacteria such as Lactobacillus species, however, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0292749 A1 to Goodwins et al. (hereinafter "Goodwins").

Regarding claim 27; claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a halophile bacterium or achaea. However, in a related disclosure, Goodwins teaches the production of cheeses (para [0034]) using at least one bacterium producing bateriocin (para [0029]), wherein the bacterium may be a halophilic bacterium (pediococcus halophiles; para [0021]). It would have been obvious to a person of ordinary skill in the art to apply a method of enhancing cheese production, as taught by Goodwin, to the cheese substitute taught by the combination of Nonaka and ishizuka without undue experimentation, in order to improve the cheese substitute product and make it more cheese-like to a consumer.

Claim 89 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2003/0224476 A1 (Chou).

Regarding claim 89; claim 4 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the enzyme is lysyl oxidase, in a related disclosure, Chou teaches transglutaminase-reactive compounds (abstract), including the use of cheesegelling of proteins (para [0003]); wherein the transglutaminase-reactive compund is produced by denaturing a compound and refolding the compound (para [0008]). The compound may also be cross-linked with a biological agent (para [0042]), wherein the biological agent may be a lysyl oxidase (para [0050]), and may be used in the gelling of cheese (para [0176]). It would have been obvious to a person of ordinary skill in the art to substitute lysyl oxidsae for transglutaminase to gel a proteinaceous product, as taught by Chou, wherein the product was a substitute cheese product, as taught by the combination of Nonaka and Ishizuka, in order to provide a cheese substitute with a flavor that was not affected by the presence of the transglutaminase enzyme.

---------- continued in next Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box

Claims 8-16 and 33 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2011/0064847 A1 to McCready et al. (hereinafter 'McCready'), and further in view of US 5,807,601 A to Carpenter et al. (hereinafter "Carpenter").

Regarding claim 8; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the ultility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies.

Regarding claim 9; claim 8 is obvious as above. Further, McCready teaches wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]).

Regarding claim 10; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the ultility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies. Niether Nonaka, Ishizuka, nor Carpenter teaches wherein the method further comprises 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. However, McCready further teaches wherein preparation further includes the steps of: (a) obtaining nuts (para [0016]), and (b) surface sterilizing the nuts (para [0016]: 'blanching'). It would have been obvious to one with ordinary skill in the art to obtain and prepare the nuts for a nut milk, as taught by McCready, in order to minimize contamination of the nuts with known pathogens, such as salmonella, or fungi that produced known carcinogens, such as aflatoxin. A person of skill in the art would have desired to appropriately sterilize the raw materials used in the process as a first step.

Regarding claim 11; claim 10 is obvious as above. Furthermore, McCready teaches wherein the surface sterilization is blanching (para [0016]).

Regarding claim 12; claim 8 is obvious as above. Furthermore, McCready teaches wherein the method further comprises washing the nuts (para [0016]: 'blanching').

Regarding claims 13 and 14; claim 10 is obvious as above. Furthermore, McCready teaches wherein the preparation further comprising decompounding the nuts; more specifically wherein the decompounding is blending (para [0017]).

Regarding claim 15 and 16; claim 10 is obvious as above. Ishizuka further teaches centrifuging the soy materials (col 4, ln 38-44). Although Ishizuka does not explicitly recite wherein the centrifuging removes at least 85% of the suspended solids, it would have been obvious to a person of ordinary skill in the art to determine an appropriate centrifugation speed and duration to separate or remove a desired amount of solids that varied in density from the solvent from any solution without undue experimentation.

---------- continued in next Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box

Regarding claim 33; claim 30 is obvious as above, Neither Nonaka nor Ishizuka explicitly teaches wherein each concentrated protein is from a separate plant species. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use concentrated proteins from a variety of separate plant species, particularly different nut species in combination with soy, based on combining the disclosure of Carpenter regarding using a nut product to produce a cheese substitute, wherein the nut product could have been any of a number of nut milks, based on the disclosure of McCready, processed according to the method disclosed by the combination of Nonaka and Ishizuka. A person of skill in the art would have recognized the potential of the different nut proteins to alter and contribute to the flavor and texture of the product, and would have desired to produce different combinations thereof, either to identify a composition most resembling a known cheese product, or to create a series of imitation cheese products with varying flavors.

Claims 1-33 and 89 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670015WO1	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/11347	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)	(Earliest) Priority Date *(day/month/year)* 11 January 2013 (11.01.2013)
Applicant Maraxi, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 9 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

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:

-***-Please See Supplemental Page-***-

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.:

Groups I+: Claims 1-10, 28-37, 39, phenylacetaldehyde (flavor compound, not the precursor molecule)

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.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23D 7/005, 9/013; A23L 1/30 (2014.01)
USPC - 426/92, 641, 647
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)
IPC(8): A23D 7/00, 59/013, 9/00, 9/06, 7/02; A23L 1/30, 1/226, 1/226, 1/217, 1/235 (2014.01)
USPC: 426/613, 506, 535, 808, 537, 92, 540, 96, 644, 643, 641, 647, 250, 646

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; PubMed; ProQuest; flavor, taste, precursor, iron, 'heterocycline ring,' heme, phenylacetaldehyde, food, consumable, beef, chicken, pork, lamb, turkey

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0274817 A1 (YAMAGUCHI, S et al.); November 05, 2009: paragraphs [0024], [0029], [0035], [0040], [0045], [0053], [0078]; Table 9	1-10, 35-37, 39 ----- 28-34
Y	US 3829582 A (GUADAGNI, DG et al.); August 13, 1974; abstract; column 1, lines 30-35; Claims 1-3	28-34
Y	US 5443852 A (SHAHIDI, F et al.); August 22, 1995; column 8, lines 10-25; column 8, lines 50-60; column 42, lines 5-15	29-33

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 June 2014 (09.06.2014)	03 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

-***-Continued from Box III -***-

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 1-10, 28-37, 39, and Tables 3 , 8, 9, 12, 14, 16, and 17 (flavor compounds) are directed toward a food product comprising: a) a highly conjugated heterocyclic ring complexed to iron ion; and b) one or more flavor precursor molecules; and a method for producing a flavor compound, said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules; a method for producing a flavor compound, said method comprising: a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; and a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

Group II: Claim 40 is directed toward a method for producing a flavor compound, said method comprising: a) incubating a heme-moiety bound to a solid support with one or more flavor precursor molecules and b) heating the heme-moiety bound to said solid support and one or more flavor precursors to form one or more flavor compounds.

The food product, the methods for producing a flavor compound, and the method for imparting a meat-like flavor to a food product will be searched to the extent that they encompass the first item in Table 3, phenylacetaldehyde (flavor compound). It is believed that Claims 1-10, 28-37 and 39 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass the first item in Table 3, phenylacetaldehyde (flavor compound). Applicants must indicate, if applicable, the claims which encompass this first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" groups will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: the second item in Table 3, 1-octen-3-one (flavor compound).

The inventions listed as Groups I+-II 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 Group I+ include a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product, which are not present in Group II, the special technical features of Group II including a heme-moiety bound to a solid support.

Groups I+-II share the technical features including a method for producing a flavor compound, said method comprising: a) combining a heme-moiety with one or more flavor precursor molecules selected from a group including a sugar and cysteine; and b) heating the heme-moiety and one or more flavor precursors to form one or more flavor compounds selected from a group including phenylacetaldehyde. Groups I+ share the technical features including a food product comprising: a) a highly conjugated heterocyclic ring complexed to iron ion; and b) one or more flavor precursor molecules; and a method for producing a flavor compound, said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules; a method for producing a flavor compound, said method comprising: a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; and a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

-***-Continued Within the Next Supplemental Box-***-

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

-***-Continued from Previous Supplemental Box-***-

However, these shared technical features are previously disclosed by US 2009/0274817 A1 to Yamaguchi, et al. (hereinafter 'Yamaguchi') in view of the publication entitled 'The Chemistry Of Beef Flavor: Executive Summary' by Brewer, and the publication entitled 'Prediction Of Beef Flavor By Precursor And Volatile Compounds' by Brooks, et al. (hereinafter 'Brooks'). Yamaguchi discloses a method for producing a flavor compound (creating a body taste-improver; paragraphs [0023]-[0027]), said method comprising: a) combining a heme-moiety with one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029]); and b) heating the heme-moiety and one or more flavor precursors (heating the body taste improver, comprising heme iron with another flavor component, enhances the taste enhancing effect; paragraphs [0041], [0043], [0053]); a food product (a food with an improved body taste; paragraph [0053]) comprising: a) a highly conjugated heterocyclic ring complexed to iron ion (a body taste-increasing component consists of heme iron; paragraphs [0027], [0029]); and b) one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029])]; and a method for producing a flavor compound (a method of creating a body taste-improver; paragraphs [0023]-[0027]), said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029]) to form a mixture (a composition comprising various body taste improving components (a mixture); paragraph [0024]), and b) heating the mixture (heating the body taste improver, comprising heme iron with another flavor component, enhances the taste enhancing effect; paragraphs [0041], [0043], [0053]). Yamaguchi does not disclose flavor precursor molecules selected from a group including a sugar and cysteine; forming one or more flavor compounds selected from the group including phenylacetaldehyde; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules selected from a group including a sugar and cysteine; a method comprising: a) combining a heme-containing protein, cysteine, and a sugar; or a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from a group including a sugar, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product. Brewer discloses flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1); a food product (beef (food product); page 2, left column, first paragraph to right column, first paragraph) comprising: a) a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph); and b) one or more flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars, such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1); a method comprising: a) combining a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph), cysteine (precursor compounds include amino acids, such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and a sugar (precursor compounds include sugars, such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and one or more optional flavor precursor molecules to form a mixture (optional language not analyzed); and a method for imparting a meat-like flavor to a food product (developing a beef flavor in beef (food product); page 2, left column, first paragraph to right column, first paragraph) comprising contacting said food product with a flavoring composition (precursor compounds including flavor-active volatiles embedded in a matrix (composition); page 2, left column, first paragraph), said flavoring composition comprising i) a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph); and ii) one or more flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product (beef flavor is developed by heating the beef; page 2, left column, first paragraph to right column, first paragraph). Brooks discloses a flavor compound selected from the group including phenylacetaldehyde (phenylacetaldehyde is a volatile flavor compound present in cooked steak; page 4, Table 8). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods for producing a flavor compound and the food product, as previously disclosed by Yamaguchi, for including the use of the flavor precursor molecules and the method for imparting a meat-like flavor to a food product, as previously disclosed by Brewer, as the previous disclosure by Yamaguchi includes the use of compounds which when heated oxidize and enhance the flavor of foods, including meats (Yamaguchi; paragraphs [0022], [0041], [0043], [0053]), Brewer discloses flavor precursor molecules which, when heated, impart various flavors to beef (Brewer; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and a combination of these references would have resulted in an improved flavor enhancer for imparting meat flavors to foods when cooked. Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods for producing a flavor compound and the food product, as previously disclosed by Yamaguchi, for including forming flavor compounds, such as phenylacetaldehyde, as previously disclosed by Brooks, as the previous disclosure by Yamaguchi includes the use of volatile compounds which, when heated, oxidize and enhance the flavor of foods, including meats (Yamaguchi; paragraphs [0022], [0041], [0043], [0044], [0053]), Brooks discloses volatile flavor compounds present in cooked steaks, including phenylacetaldehyde, which is present in higher concentrations in Prime quality grade steaks (Brooks; page 4, Table 8), and a combination of these references would have resulted in an improved method for imparting meat flavors to food, based on the formation of desirable volatile compounds, when the food is cooked.

Since none of the special technical features of the Groups I-II+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by Yamaguchi, Brewer and Brooks references, unity of invention is lacking.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Teresa Lavoie
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	03 JUL 2014
Applicant's or agent's file reference 387670015WO1	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/11347	13 January 2014 (13.01.2014)	11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23D 7/005, 9/013; A23L 1/30 (2014.01)
USPC - 426/92, 641, 647

Applicant Maraxi, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	09 June 2014 (09.06.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

a. (means)

☐ on paper

☒ in electronic form

b. (time)

☐ in the international application as filed

☐ together with the international application in electronic form

☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

-***-Please See Supplemental Page-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts
   - ☒ the parts relating to claims Nos. 1-10, 28-37, 39, phenylacetaldehyde (flavor compound, not the precursor molecule)

Form PCT/ISA/237 (Box No. IV) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	28-34	YES
	Claims	1-10, 35-37, 39	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-10, 28-37, 39	NO
Industrial applicability (IA)	Claims	1-10, 28-37, 39	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-10, 35-37 and 39 lack novelty under PCT Article 33(2) as being anticipated by US 2009/0274817 A1 to Yamaguchi, et al. (hereinafter 'Yamaguchi').

As per Claim 1, Yamaguchi discloses a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]) comprising: a) a highly conjugated heterocyclic ring complexed to iron ion (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferrous sulfate, ferrous citrate, ferric citrate, ferrous gluconate, ferric chloride, and ferrous lactate; and heme iron; plasma powder; and blood cell powder; paragraph [0029]); and b) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

As per Claim 2, Yamaguchi discloses the food product of claim 1, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]), and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, and an isobacteriochlorin moiety complexed to iron ion.

As per Claim 3, Yamaguchi discloses the food product of claim 2, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]).

As per Claim 4, Yamaguchi discloses the food product of claim 2, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts such as ferric chloride (ferrous chlorin); paragraph [0029]).

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11347

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

As per Claim 5, Yamaguchi discloses a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]) comprising: a) a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]); and b) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

As per Claims 6/1-6/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to fifty flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to fifty flavor precursors); paragraph [0035]).

As per Claims 7/1-7/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to forty flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to fifty flavor precursors; paragraph [0035]).

As per Claims 8/1-8/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to thirty-five flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to thirty-five flavor precurors; paragraph [0035]).

As per Claims 9/1-9/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to ten flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind; (two to ten flavor precurors; paragraph [0035]).

As per Claims 10/1-10/5, Yamaguchi discloses the food product of claims 1-5, and Yamaguchi further discloses wherein said food product comprises two to six flavor precursors (it is possible to mix and use together two or more kinds of the long-chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins, but belong to the same kind (two to six flavor precursors; paragraph [0035]).

As per Claim 35, Yamaguchi discloses a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition (flavor of Pork Cutlet that was prepared by seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper, coating it with wheat flour, 20% egg solution and bread crumb, followed by deep-frying at 180 degrees C, for 30 min. in the above fat and oil preparations; paragraph [0078]; Table 9), said flavoring composition comprising i) a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]); and ii) one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product (flavor of Pork Cutlet that was prepared by seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper, coating it with wheat flour, 20% egg solution and bread crumb, followed by deep-frying at 180 degrees C, for 30 min. in the above fat and oil preparations; paragraph [0078]; Table 9).

As per Claim 36, Yamaguchi discloses the method of claim 35, and Yamaguchi further discloses wherein said meat-like flavor is a beef-like, chicken-like, pork-like (there is no limitation on the kind of subject foods, which include foods cooked with oil, such as various fried foods and stir-fry; and fat-containing foods, such as Chinese-style pork dumplings and steamed Chinese-style pork dumplings; paragraphs [0053], [0078]), lamb-like flavor, or turkey-like.

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

As per Claims 37/35 and 37/36, Yamaguchi discloses the method of claims 35 and 36, and Yamaguchi discloses wherein said flavoring composition further comprises a seasoning agent (seasoning pork loin (75 g) with salt (0.6 g) and a small amount of pepper; paragraph [0078]), a flavoring agent, a protein, a protein concentrate, or an emulsifier (the body taste improver of the present invention may additionally contains other optional components known to those skilled in the art, such as an emulsifier; paragraph [0040]).

As per Claim 39, Yamaguchi discloses a method of making a food product (a food (food product) having an improved body taste; paragraphs [0024], [0029], [0040], [0045]), said method comprising combining an isolated heme-containing protein and one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture (there is no limitation on a method for the oxidization treatment, which, for example, includes heating treatment (heating the mixture); paragraph [0041]).

Claims 28 and 34 lack an inventive step under PCT Article 33(3) as being obvious over Yamaguchi in view of US 3,829,582 A to Guadagni, et al. (hereinafter 'Guadagni').

As per Claim 28, Yamaguchi discloses a method for producing a flavor compound (method for producing a body taste improver (flavor compound); paragraphs [0024], [0040], [0045]), said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferrous sulfate, ferrous citrate, ferric citrate, ferrous gluconate, ferric chloride, and ferrous lactate; and heme iron; plasma powder; and blood cell powder; paragraph [0029]) with one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. Yamaguchi does not disclose b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde. Guadagni discloses b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, for imparting a fatty-fried flavor to potato and other food products (a mixture containing methional, 2-acetylthiazoline, deca-2,4-dienal, 2-octenal, 2-acetyl-l ,4,5,6-tetrahydropyridine, 2-ethyl-3,6-dimethylpyrazine, 2,6-diethylpyrazine, and 2-phenylacetaldehyde is used to impart a fatty-fried flavor to potato and other food products; abstract; Claims 1-3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with a mixture containing 2-phenylacetaldehyde used to impart a fatty-fried flavor to potato and other food products (Guadagni; abstract; Claims 1-3), as previously disclosed by Guadagni, as the previous disclosure by Yamaguchi includes a method of deep-frying pork loin in vegetable oil for improving taste, Guadagni discloses adding a fatty-fried flavor to food products, and a combination of these references would have resulted in an improved fatty-fried taste for pork.

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box

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As per Claim 34, Yamaguchi discloses a method for producing a flavor compound (body taste improvers (flavor compounds); paragraphs [0024], [0040], [0045]), said method comprising: a) combining a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), and one or more flavor precursor molecules (one or more body taste improvers (flavor precursor molecules); paragraphs [0024], [0040], [0045]) selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid (phospholipids; paragraph [0040]), a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil (vegetable fat and oil composition; paragraphs [0024], [0045]), algal oil, sunflower oil (sunflower oil; paragraph [0045]), corn oil (corn oil; paragraph [0045]), soybean oil (soybean oil; paragraph [0045]), palm fruit oil (palm oil; paragraph [0045]), palm kernel oil (palm oil; paragraph [0045]), safflower oil, flaxseed oil, rice bran oil (rice oil; paragraph [0045]), cottonseed oil, olive oil, sunflower oil (sunflower oil; paragraph [0045]), canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid (fatty acids; paragraph [0040]), cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. Yamaguchi does not disclose cysteine, and a sugar; and b) heating the mixture to form one or more flavor compounds set forth in Table 3 (phenylacetaldehyde). Guadagni discloses cysteine, and a sugar (to obtain a given quantity of protein (cysteine), starch, and sugar, or other non-fat nutrient in addition to a pleasing flavor, the consumer necessarily receives a high proportion of fat; column 1, lines 30-35); and b) heating the mixture to form one or more flavor compounds set forth in Table 3 (phenylacetaldehyde) (a mixture containing methional, 2-acetylthiazoline, deca-2,4-dienal, 2-octenal, 2-acetyl-l ,4,5,6-tetrahydropyridine, 2-ethyl-3 ,6-dimethylpyrazine, 2,6-diethylpyrazine, and 2-phenylacetaldehyde is used to impart a fatty-fried flavor to potato and other food products; abstract; Claims 1-3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with a mixture containing 2-phenylacetaldehyde, protein, and sugar used to impart a fatty-fried flavor to potato and other food products (Guadagni; abstract; column 1, lines 30-35; Claims 1-3), as previously disclosed by Guadagni, as the previous disclosure by Yamaguchi includes the method of deep-frying pork loin in vegetable oil for improving the taste, Guadagni discloses the adding a fatty-fried flavor to food products, and a combination of these references would have resulted in an improved fatty-fried taste for pork.

Claims 29-33 lack an inventive step under PCT Article 33(3) as being obvious over Yamaguchi and Guadagni and further in view of US 5,443,852 A to Shahidi, et al. (hereinafter 'Shahidi').

As per Claim 29, Yamaguchi and Guadagni, in combination, discloses the method of claim 28, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]). Yamaguchi does not disclose a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a achlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to iron ion. Shahidi discloses an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, for effectively preserving cured meats in the absence of nitrites and nitrates (Shahidi; column 8, lines 10-25; column 8, lines 50-60), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes the method of preparing pork in vegetable oil to improve the taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and a combination of these references would have resulted in an improved fatty-fried taste for pork that lacks nitrites and nitrates.

As per Claim 30, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 29, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]), a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art, including ion salts, such as heme iron (heme moiety); plasma powder; and blood cell powder; paragraph [0029]).

As per Claim 31, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 30, and Yamaguchi further discloses wherein said heme moiety is a heme-containing protein (pork (heme-containing protein); paragraphs [0053], [0078]).

As per Claim 32, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claim 29, and Yamaguchi further discloses wherein said highly conjugated heterocyclic ring complexed to an iron ion is ferrous chlorin (the iron component may be provided from any commercially available iron-containing materials know to those skilled in the art (highly-conjugated heterocyclic ring complexed to iron ion), including ion salts, such as ferric chloride (ferrous chlorin); paragraph [0029]).

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
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Box No. V:
-Continued from Citations and Explanations:

As per Claim 33/28, Yamaguchi and Guadagni, in combination, discloses the method of claim 28, but Yamaguchi does not disclose said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron. Shahidi discloses said method comprising combining lactic acid (lactic acid, its sodium or potassium salts, or lactic acid-producing microorganisms may also be used in providing microbial stability to muscle food; column 42, lines 5-15) with said highly conjugated heterocyclic ring complexed to iron (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells and lactic acid, for preserving cured meats in the absence of nitrites and nitrates, and for providing microbial stability (Shahidi; column 8, lines 10-25; column 8, lines 50-60, column 42, lines 5-15), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes a method of preparing pork in vegetable oil for preserving taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and providing microbial stability, and a combination of these references would have resulted in an improved fatty-fried taste for pork which lacks nitrites and nitrates, while having enhanced microbial stability.

As per Claims 33/29-33/32, Yamaguchi, Guadagni and Shahidi, in combination, discloses the method of claims 29-32, but Yamaguchi does not disclose said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron. Shahidi discloses said method comprising combining lactic acid (lactic acid, its sodium or potassium salts, or lactic acid-producing microorganisms may also be used in providing microbial stability to muscle food; column 42, lines 5-15) with said highly conjugated heterocyclic ring complexed to iron (an edible muscle food product comprising: (A) an encapsulated powdered cooked cured-meat pigment (PCCMP) comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells, from a hemin intermediate; column 8, lines 50-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for producing a body taste improver (flavor compound) comprising a heme iron or pork loin prepared in a vegetable fat and oil composition (Yamaguchi; paragraphs [0024], [0029], [0040], [0045], [0053], [0078]), as previously disclosed by Yamaguchi, with an edible cured meat product comprising a mononitric oxide complex of protoporphrin (IX) Fe (II) compound derived from red blood cells and lactic acid, for preserving cured meats in the absence of nitrites and nitrates, and providing enhanced microbial stability (Shahidi; column 8, lines 10-25; column 8, lines50-60, column 42, lines 5-15), as previously disclosed by Shahidi, as the previous disclosure by Yamaguchi includes the method of preparing pork in vegetable oil to improve the taste, Shahidi discloses a method for preserving meats in the absence of nitrites and nitrates, and providing enhanced microbial stability, and a combination of these references would have resulted in an improved fatty-fried taste for pork that lacks nitrites and nitrates, while having enhanced microbial stability.

Claims 1-10, 28-37 and 39 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY PCT/US2014/011362 13.06.2014

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670016WO1	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/11362	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)	(Earliest) Priority Date *(day/month/year)* 11 January 2013 (11.01.2013)
Applicant MARAXI, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

NON-DAIRY CHEESE REPLICA COMPRISING A COACERVATE

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 11

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT PCT/US2014/011362 13.06.2014

International application No.
PCT/US14/11362

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.: 9-10, 15-48, 56-67, 72, 79, 89-90
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 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.
Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.
Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.
Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.
Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

-***-Continued Within the Next Supplemental Box-***-

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.:
1-4, 8/1-4, 73-77, 78/73-77, and 91-93

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 PCT/US2014/011362 13.06.2014

International application No.
PCT/US14/11362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378
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)
IPC(8): A23L 1/052, 1/36; A23C 19/055, 20/02; A23J 3/14 (2014.01)
USPC: 426/615, 634, 656; 530/378

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; non-dairy, non-animal, coacervation, plant protein, chickpea protein, lentil protein, pea protein, lupine protein, pea vicilin, pea legumin, imitation cheese

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2004/0151778 A1 (RICHARD, J et al.) August 5, 2004; paragraphs [0011], [0019], [0022], [0025], [0028], [0039], [0040], [0048]	1-4, 91-93 --- 8/1-4, 73-77, 78/73-77
Y	WO 2008/030089 A1 (BRANDSMA, JB et al.) March 13, 2008; page 5, lines 26-32; page 6, lines 1-3	8/1-4
Y	US 4435438 A (LEHNHARDT, WF et al.) March 6, 1984; column 4, lines 1-5; column 16, lines 20-30	73-77, 78/73-77
Y	US 2008/0226810 A1 (PASSE, D et al.) September 18, 2008; paragraphs [0005], [0007], [0014], [0015]	77, 78/77
A	US 5753295 A (GOLDMAN, MS) May 19, 1998; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93
A	WO 2012/116703 A1 (ANDERSEN, KE et al.) September 7, 2012; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2014 (14.05.2014)	13 JUN 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT PCT/US2014/011362 13.06.2014

International application No.
PCT/US14/11362

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The inventions listed as Groups I-V 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 Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus, and Brevi bacterium linens; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non- animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

PCT/US2014/011362 13.06.2014

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 JUN 2014
Applicant's or agent's file reference 387670016WO1	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/11362	13 January 2014 (13.01.2014)	11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378

Applicant MARAXI, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 May 2014 (14.05.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9-10, 15-48, 56-67, 72,79,89-99 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

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 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.

Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.

Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.

Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.

Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

-***-Continued Within Supplemental Box-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-4, 8/1-4, 73-77, 78/73-77, and 91-93

PCT/US2014/011362 13.06.2014

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	8/1-4, 73-77, 78/73-77	YES
	Claims	1-4, 91-93	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	NO
Industrial applicability (IA)	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-4 and 91-93 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0151778 A1 to Richard, et al. (hereafter 'Richard').

As per claim 1, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated and purified proteins from a non-animal source (solubilization of at least one plant protein in an aqueous medium; the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used, are extracted from plants (extraction comprise process for isolation of pure proteins from the mixture); paragraphs [0022], [0025], [0028]; [0039], [0040]).

As per claim 2, Richard discloses the non-dairy cheese replica of claim 1, and Richard further discloses wherein said one or more isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 3, Richard discloses the non-dairy cheese replica of claim 2, and Richard further discloses wherein said one or more isolated and purified plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus); paragraph [0040]).

As per claim 4, Richard discloses the non-dairy cheese replica of claim 3, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claim 91, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated proteins from a non-animal source (the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used in the context of the invention are extracted from plants; paragraphs [0022], [0025], [0039], [0040]).

As per claim 92, Richard discloses the non-dairy cheese replica of claim 91, and Richard further discloses wherein said one or more isolated proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 93, Richard discloses the non-dairy cheese replica of claim 92, and Richard further discloses wherein said one or more isolated plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus); paragraph [0040]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

Claims 8/1-4 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of WO 2008/030089 A1 to Brandsma, et al. (hereafter 'Brandsma').

As per claims 8/1-4, Richard discloses the non-dairy cheese replica of any one of claims 1-4, but Richard does not disclose further comprising one or more microbes selected from bacteria, molds, and yeast. However, Brandsma discloses comprising one or more microbes selected from bacteria and yeast (adding at least two live food grade micro-organisms, said two micro-organisms are at least one a yeast strain selected from the genus Kluyveromyces and at least one bacterial strain selected from the genus Propionibacterium; to form a cheese; page 5, lines 26-32 to page 6, lines 1-3; claim 1). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide microbes such as bacteria or yeast, as disclosed by Brandsma, in order to have flavor formation in manufacture of fermented products, in order to provide non-dairy consumables.

Claims 73-76 and 78/73-76 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of US 4,435,438 A to Lehnhardt, et al. (hereafter 'Lehnhardt').

As per claim 73, Richard discloses a method of making a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; paragraph [0025]), said method comprising acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5 (method comprises, solubilization of at least one plant protein in an aqueous medium at a pH of between 2 and 7, and below the isoelectric pH of the protein; the pH of the medium is decreased, by adding IN hydrochloric acid; plant proteins used in the context of the invention are extracted from plants; paragraphs [0028],[0040], [0048]) and isolating said coacervate from said solution (encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; the wall formed is hardened by cooling the medium and crosslinked by the action of a crosslinking agent; paragraph [0011]). Richard does not disclose wherein said solution comprises 100 mM or less of a salt. However, Lehnhardt discloses wherein said solution comprises 100 mM or less of a salt (salt (sodium chloride); 2 parts by weight (2 pbw corresponds to 0.66 mM of sodium chloride); column 4, lines 1-5; column 16, lines 20-30). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein said solution comprises 100 mM or less of a salt, as disclosed by Lehnhardt, in order to provide plant protein coacervates having low salt content, in order to provide non-dairy protein consumables.

As per claim 74, Richard and Lehnhardt, in combination, disclose the method of claim 73, and Richard further discloses wherein said isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus) and cereals such as wheat, maize, barley, malt or oats; paragraph [0040]).

As per claim 75, Richard and Lehnhardt, in combination, disclose the method of claim 74, and Richard further discloses wherein said plant proteins are seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus Lupinus), soybean (genus Glycine), pea (genus Pisum), chickpea (Cicer), alfalfa (Medicago), broad bean (Vicia), lentil (Lens), bean (Phaseolus), rapeseed (Brassica), sunflower (Helianthus) and cereals such as wheat, maize, barley, malt or oats; plant proteins SWP100 and SWP50 and those marketed under the name Supro 670 and PISANE; paragraph [0040]).

As per claim 76, Richard and Lehnhardt, in combination, disclose the method of claim 75, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claims 78/73-76, Richard and Lehnhardt, in combination, disclose the method of claims 73-76, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the pH of the medium is decreased, by adding in hydrochloric acid; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
-***-Continued from Previous Supplemental Box-***-

Claims 77 and 78/77 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of Lehnhardt further in view of US 2008/0226810 A1 to Passe, et al (hereafter 'Passe').

As per claim 77, Richard and Lehnhardt, in combination, disclose the method of claim 76, but Richard does not disclose wherein said pea vicilins comprises convicilins. However, Passe discloses wherein said pea proteins comprise pea vicilins and/or pea legumins (pea proteins, like all proteins of leguminous plants, are constituted of three classes of proteins: globulins, albumins and insoluble proteins, the globulins (legumins, vicilins and convicilins) are the main storage proteins of the plant; legumins are hexameric proteins of 360 to 410 kDa composed of 6 double-stranded subunits of 60 kDa; vicilins are trimeric proteins whose mass ranges from 150 to 200 kDa; paragraphs [0005], [0007], [0014], [0015]). It would be obvious to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein pea proteins comprise pea vicilins and/or pea legumins, as disclosed by Passe, in order to provide other legumes, in order provide other suitable plant based proteins, in order to manufacture non-animal based imitation cheese.

As per claim 78/77, Richard, Lehnhardt, and Passe, in combination, disclose the method of claim 77, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
-***-Continued from Box No. IV: Lack of unity of invention-***-

The inventions listed as Groups I-V 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 Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of Pediococcus pentosaceus, Clostridium butyricum, Lactobacillus delbrueckii lactis, Lactobacillus delbrueckii bulgaricus, Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus, Staphylococcus xylosus, and Brevi bacterium linens; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non- animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: MONICA MCCORMICK GRAHAM
FISH & RICHARDSON P.C.
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 01 MAY 2015
Applicant's or agent's file reference 387670011WO1	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 15/17147	International filing date *(day/month/year)* 23 February 2015 (23.02.2015)
Applicant IMPOSSIBLE FOODS INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide,* International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 *months from the priority date, the international application will be published*** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670011WO1	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 15/17147	International filing date *(day/month/year)* 23 February 2015 (23.02.2015)	(Earliest) Priority Date *(day/month/year)* 21 February 2014 (21.02.2014)
Applicant IMPOSSIBLE FOODS INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/17147

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.: 8-40, 55-68
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:

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.:

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 15/17147

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23C 19/00 (2015.01)
CPC - A23C 19/082; A23C 19/093; A23C20/00
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)
cpc-A23C19/082; A23C19/093; A23C20/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-426/582 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWest, ProQuest Dialog, Google Scholar, Google
Search Terms: Soy, bean, milk, cheese, microbial, yeast, mold, strain, bacterial, micro, acid, amidated pectin, oil, sugar, starch, Lactococcus lactis lac tis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylos

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/013292 A1 (Lin et al.) 31 January 2013 (31.01.2013) pg2, ln7-8; pg4, ln1-2, 15-16; pg8, ln12, 17; pg11, ln26-29; pg12, ln12, 25, 32; pg14, ln22;	1-7, 47-54
Y	Herbstreith&Fox. Low Methylester, Amidated Pectins. Technical Application Information. 18 January 2007; pg1, col1, ln1-5	1-7, 41-54
Y	US 5,597,594 A (Matsuura et al.) 28 January 1997 (28.01.1997) abstract, col3, ln29, 35-40, 46-48; col4, ln3; col5, ln53-54	3-7, 41-46
Y	US 2010/0196575 A1 (Sanchez et al.) 5 August 2010 (05.08.2010) para [0065], [0098]	41-46
Y,E	WO 2014/110540 A1 (Holz-Schietinget et al.) 17 July 2014 (17.07.2014) abstract, pg4, ln1-30	1-7

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2015 (17.04.2015)	01 MAY 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MONICA MCCORMICK GRAHAM
FISH & RICHARDSON P.C.
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	01 MAY 2015

Applicant's or agent's file reference	FOR FURTHER ACTION
387670011WO1	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 15/17147	23 February 2015 (23.02.2015)	21 February 2014 (21.02.2014)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23C19/00 (2015.01)
CPC - A23C19/082; A23C19/093; A23C20/00

Applicant IMPOSSIBLE FOODS INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	16 April 2015 (16.04.2015)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/17147

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/17147

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 8-40, 55-68

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 8-40, 55-68 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 8-40, 55-68

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/17147

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-7, 41-54	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-7, 41-54	NO
Industrial applicability (IA)	Claims	1-7, 41-54	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-2, 47-54 lack inventive step under PCT Article 33(3) as being obvious over WO 2013/013292 A1 to Lin et al. (hereinafter 'Lin') in view of the article entitled "Low Methylester, Amidated Pectins" to Herbstreith&Fox (hereinafter 'Herbstreith').

As per claim 1, Ln teaches a soy-based cheese (Lin-pg2, ln7-8) comprising: a) soy milk (Lin-pg2, ln7-8) b) a microbial strain (Lin-pg4, ln15 -16) d) an oil (Lin-pg11, ln28) e) optionally one or more of a salt, a starch, a sugar, a flavoring (Lin-pg11, ln27), an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, coconut milk, coconut cream, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose. Althougt Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach a soy-based cheese comprising a pectin as a stablizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese-making because it offers a very high product safety

As per claim 2, Lin teaches the soy-based cheese of claim 1, wherein said microbial strain is a bacterial strain (Lin-pg4, ln15-16) a yeast strain, or a mold strain.

As per claim 47, Lin teaches a soy-based cheese comprising: a) soy milk (Lin pg2, ln7-8); b) an acid (Lin-pg4, ln1-2); d) an oil (Lin-pg14, ln22); and e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose. Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach a cheese comprising a pectin as a stablizer (Lin-pg12, ln32) and Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to incorporate a low methyl ester, amidated pectin as taught by Herbstreith into the cheese as taught by Lin because it offers a very high product safety.

As per claim 48, Lin teaches the soy-based cheese of claim 47, wherein said acid is selected from the group consisting of citric acid (Lin-pg4, ln2), lemon juice, HCl, and pyruvic acid.

As per claim 49, Lin teaches a soy-based cheese comprising: a) soy milk (Lin-pg2, ln7-8); b) a microbial strain (Lin-pg4, ln15-16); d) one or more proteins (Lin-pg8, ln12-two proteins); e) an oil (Lin-pg11, ln28; f) a salt (Lin-pg11, ln28); g) a sugar (Lin-pg11, ln29); h) a gum (Lin-pg12, ln25); i) a coloring agent (Lin-pg11, ln26); and j) a flavoring (Lin-pg11, ln27). Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach the cheese comprising a pectin as a stablizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese-making because it offers a very high product safety.

As per claim 50, Lin teaches a soy-based cheese comprising: a) soy milk (Lin pg2, ln7-8); b) one or more proteins (Lin-pg8, ln12, two proteins) d) an oil (Lin-pg11, ln28); and e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, lecithin, a flour, a fiber, or cellulose. Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach the cheese comprising a pectin as a stablizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese making because of it offers a very high product safety.

----------*continued in Supplemental Box*----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/17147

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box No. V 2. Citations and explanations:

As per claim 51, Lin teaches the soy-based cheese of claim 49 or claim 50, wherein said one or more proteins comprise soy protein isolate or the soluble proteins from soy flour or soy protein (Lin-pg8, ln29) isolate.

As per claim 52, Lin teaches the soy-based cheese of claim 49 or claim 50, wherein said one or more proteins is an isolated protein (Lin-pg8, ln22).

As per claim 53, Lin teaches the soy-based cheese of claim 52, wherein said isolated protein is selected from the group consisting of a lentil protein, a soy protein (Lin-pg8, ln29), or a pea protein.

As per claim 54, Lin teaches the soy-based cheese of claim 53, wherein said protein is pea vicilin, pea legumin, soy B-conglycinin, soy glycinin (Lin-pg8, ln22), or a dehydrin protein.

Claims 3-7 lack inventive step under PCT Article 33(3) as being obvious over Lin in view of Herbstreith in furthe view of US 5,597,594 A to Matsuura et al. (hereinafter 'Matsuura').

As per claim 3, although neither Lin nor Herbstreith teaches the soy-based cheese of claim 2, wherein said bacterial strain is selected from the group consisting of Lactococcus lactis lactis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), and Streptococcus thermophilus (ST). Matsuura teaches the soy-based cheese of claim 2, wherein said bacterial strain is selected from the group consisting of Lactococcus lactis lactis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), and Streptococcus thermophilus (ST) (Matsuura-col3, ln37-38). It would have been obvious to one skilled in thart to incorporate Streptococcus thermophilus (ST) as taught by Matsuura into the cheese as taught by Lin and Herbstreith since Matsuura teaches Streptococcus thermophilus (ST) is good for making cheese.

As per claim 4, Matsuura further teaches the soy-based cheese of claim 3, said soy-based cheese comprising two bacterial strains (Matsuura-col3, ln36, mixed bacteria).

As per claim 5, Matsuura teaches the soy-based cheese of claim 4, wherein said bacterial strains are ST (Matsuura-col3, ln37-38), but no mentioning SX. It would have been obvious to one skilled in the art to used ST and SX togather since both are common strains for fermentation and may achieve synergetic effect when using togather.

As per claim 6, Lin teaches the soy-based cheese of any one of claims 1-5, said soy-based cheese comprising said starch (Lin-pg12, ln12).

As per claim 7, Lin teaches further teaches the soy-based cheese of claim 6, wherein said starch is potato starch (Sanchez-para [0085]), com starch, tapioca, rice starch (Lin-pg12, ln17), pea starch, arrowroot starch, or a modified food starch.

----------continued in Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/17147

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
provious box

Claims 41-46 lack inventive step under PCT Article 33(3) as being obvious over Matsuura in view of US 2010/0196575 A1 to Sanchez et al. (hereinafter 'Sanchez') further in view of Herbstreith

As per claim 41, Matsuura teaches a method of making a soy-based cheese (Matsuura-abstract) teaches, said method comprising: a) incubating (Matsuura-col3, ln46-47) mixture of (i) soy milk (Matsuura-col3, ln26), (ii) an optional sugar (Matsuura-col3, ln29) and/or optional one or more proteins, and (iii) a microbial strain (Matsuura-col3, ln35) or an acid for a period of time sufficient (Matsuura-col3, ln46-48) to reach a pH of 5.4 to 6.4 (Matsuura-col5, ln53); Although Matssuura doesn't explicitly teach b) blending said mixture, c) adding to said blended mixture of b), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending; d) heating said mixture of c) to a temperature of 60C to 100C; and e) adding to the heated mixture of d), one or more optional sugars and one or more flavorings to produce said soy-based cheese, Sanchez teaches b) blending said mixture (Sanchez-para [0098]), c) adding to said blended mixture of b), an oil (Sanchez-para [0098]-coconut oil), an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending (Sanchez-para [0098]-combined); d) heating said mixture of c) to a temperature of 60C to 100C (Sanchez-para [0098]-90C); and e) adding to the heated mixture of d), one or more optional sugars and one or more flavorings to produce said soy-based cheese. Although Sanchez doesn't explicitly teach adding b), a low methylester, amidated pectin, it does teach a pectin can be added as a hydrocolloid (Sanchez-para [0065]). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to add a low methyl ester, amidated pectin to the mixture because it offers a very high product safety.

As per claim 42, Matsuura teaches the method of claim 41, further comprising forming said soy-based cheese in a mold (Matsuura-col4, ln3).

As per claim 43, Matsuura teaches the method of claim 41 or claim 42, wherein said microbial strain is a bacterial strain (Matsuura-col3, ln35-40).

As per claim 44, Matsuura teaches a method of making a soy-based cheese (Matsuura-abstract), said method comprising: a) incubating (Matsuura-col3, ln46-47) a mixture of (i) soy milk (Matsuura-col3, ln26), (ii) an optional sugar (Matsuura-col3, ln29) and/or optional one or more proteins, and (iii) a microbial strain (Matsuura-col3, ln35) or an acid for a period of time sufficient to reach a pH of 5.4 to 5.6 (Matsuura-col3, ln46-48 and col5, ln53-54), c) separating the curd and whey (Matsuura-col3, ln53-67) in said heated mixture of b) and optionally combining the curd:whey to obtain a ratio of 35 :65 to 100:0 curd:whey; Although Matsuura doesn't teach b) heating said mixture to a temperature between 70C and 90C d) adding to said curd:whey of c), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative; e) cooking said mixture of d) to a temperature between 80C and 100C with blending; f) adding to the cooked mixture of d), one or more flavorings; and g) blending said cooked mixture off) to obtain said soy-based cheese, Sanchez teaches b) heating said mixture to a temperature between 70C and 90C (Sanchez-para [0098]-75C) d) adding to said curd:whey of c) a hydrocolloid (Sanchez-para [0065] and [0098]), an oil (Sanchez-para [0098]), an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative; e) cooking said mixture of d) to a temperature between 80C and 100C with blending (Sanchez-para [0098]-90C); f) adding to the cooked mixture of d), one or more flavorings (Sanchez-para [0098]-cheese flavorant); and g) blending said cooked mixture off) to obtain said soy-based cheese (Sanchez-para [0098]). Althought Sanchez doesn't teaches the hydrocolloid is a low methylester, amidated pectin, it doesn't teach a pectin can be used as a hydrocolloid (Sanchez-para [0065]) and Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to add a low methyl ester, amidated pectin to the mixture because it offers a very high product safety.

As per claim 45, Matsuura teaches the method of claim 44, further comprising forming said soy-based cheese in a mold (Matsuura-col4, ln3).

As per claim 46, Matsuura teaches the method of claim 44 or claim 45, wherein said microbial strain is a bacterial strain (Matsuura-col3, ln35-40).

Claims 1-7, and 41-54 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

SEARCH HISTORY

Application Number	PCT/US 15/17147
Search Conducted By	QW
Search Approved By	SV

US/IPC Classifications Searched	IPC (8)- A23C19/00 (2015.01) CPC – A23C19/082; A23C19/093; A23C20/00
Date Conducted	April 14-17, 2015

Documentation Searched	CPC -A23C19/082; A23C19/093; A23C20/00
Search Terms Used	Soy, bean, milk, cheese, microbial, yeast, mold, strain, bacterial, micro, acid, amidated pectin, oil, sugar, starch, Lactococcus lactis lac tis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), Streptococcus thermophilus (ST)
Date Conducted	April 14-17, 2015

Electronic Database Searched	Patbase
Files Searched	Patents: US, WIPO/PCT, EP, JP, CN, IN, KR, GB, FR, DE, CA, BR, IE, HU, GE, FI, IL, HK, MY, PH, AT, BA, BE, BG, CH, CS, CZ, DK, EE, ES, GR, HR, HU, IE, EA, GC, ID, IN, KZ, MN, CR, CU, DO, AR, CL, AU, AP, DZ, EG, KE),
Date Conducted	April 14-17, 2015
Search Logic:	

1) ipc=A23C19/00 (6067)
2) uc=426/582 (1094)
3) cpc=A23C19/082 (511)
4) cpc= A23C19/093 (227)
5) cpc=A23C20/00 (208)
6) 1 or 2 or 3 or 4 or 5 (6668)
7) PA=("IMPOSSIBLE" OR "IMPOSSIBLE_FOODS_INC" OR "IMPOSSIBLE_INC" OR "IMPOSSIBLE_OBJECTS_LLC" OR "IMPOSSIBLE_S_R_O") (16)
8) 6 and (soy or soybean or saya*) (835)
9) 8 and cheese (794)
10) 9 and (bacterial or yeast or mold) (428)

11) 10 and ((Lactococcus lactis lactis) or LLL or (Lactococcus lactis cremoris) or LLC or (Lactococcus lactis biovar diacetylactis) or LLBD or (Staphylococcus xylosus) or SX or (Streptococcus thermophilus) or ST) (153)
12) 11 and (Amidated pectin) (0)
13) 11 and (oil or fat) (144)
14) 13 not 7 (143)
15) 6 and ((soy or soybean or saya*) W10 cheese) (194)
16) 15 and (Amidated pectin) (0)
17) 15 and (microbial or yeast or mold or strain or bacterial or micro*) (146)
18) 17 and (oil or fat) (138)
19) 18 and pectin (24)
20) PN=WO2013/010037 (1)
21) ctf 20 or ctb 20 (10)
22) ((soy or soybean or saya*) W10 cheese) (1584)
23) 22 and (microbial or yeast or mold or strain or bacterial or micro*) (1335)
24) 23 and (Amidated pectin) (2)
25) 22 and acid (1343)
26) 25 and (Amidated pectin) (2)
27) 26 not 24 (0)
28) 23 and pectin (157)
29) 28 not 19 (133)
30) 29 not 11 (132)
31) 25 and pectin (164)
32) 31 not 28 (9)
33) PN=WO13152406 (1)
34) (Staphylococcus xylosus) and (Streptococcus thermophilus) (164)
35) 34 and 22 (2)
36) (soy or soybean or saya*) and 34 (85)
37) 36 and cheese (35)
38) PN=WO14110540 (1)
39) ctf 38 or ctb 38 (6)
40) PN=WO2013/010037 (1)
41) ctf 40 or ctb 40 (10)
42) PN=US5597594 (1)
43) ctf 42 or ctb 42 (14)

Electronic Database Searched	PubWest
Files Searched	(US Pat, PgPub, EPO, JPO: class, keyword)
Date Conducted	April 15, 2015

DATE: Wednesday, April 15, 2015 Purge Queries Printable Copy Create Case

Order By	Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid	Set Name Classification
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD,FPRS; PLUR=YES; OP=OR				
Date	L28	L27 not L14	35	L28	L28	L28
Date	L27	L26 not L17	38	L27	L27	L27

Date	L26	L24 and pectin	38	L26	L26	L26
Date	L25	L24 and (Amidated ADJ1 pectin)	1	L25	L25	L25
Date	L24	L18 and acid	951	L24	L24	L24
Date	L23	L22 not L14	7	L23	L23	L23
Date	L22	L21 not 17	8	L22	L22	L22
Date	L21	L19 and pectin	30	L21	L21	L21
Date	L20	L19 and (Amidated ADJ1 pectin)	1	L20	L20	L20
Date	L19	L18 and (yeast or mold or strain or bacterial or micro$)	880	L19	L19	L19
Date	L18	(soy or soybean or saya$ or bean) near cheese	1212	L18	L18	L18
Date	L17	L16 not L14	28	L17	L17	L17
Date	L16	L15 and pectin	215	L16	L16	L16
Date	L15	L10 and acid	813	L15	L15	L15
Date	L14	L13 not L7	192	L14	L14	L14
Date	L13	L12 and pectin	192	L13	L13	L13
Date	L12	L10 and (yeast or mold or strain or bacterial or micro$)	661	L12	L12	L12
Date	L11	L10 and (Amidated ADJ1 pectin)	0	L11	L11	L11
Date	L10	L9 and cheese	950	L10	L10	L10
Date	L9	L6 and (soy or soybean or saya$ or bean)	1056	L9	L9	L9
Date	L8	L7 and L6	0	L8	L8	L8
Date	L7	IMPOSSIBLE$.as.	14	L7	L7	L7
Date	L6	L5 or L4 or L3 or L2 or L1	7311	L6	L6	L6
Date	L5	A23C20/00.cpc.	1188	L5	L5	L5
Date	L4	A23C19/093.cpc.	1146	L4	L4	L4
Date	L3	A23C19/082.cpc.	2349	L3	L3	L3
Date	L2	426/582.ccls.	2167	L2	L2	L2
Date	L1	A23C19/00.ipc.	1976	L1	L1	L1

END OF SEARCH HISTORY

Electronic Database Searched	ProQuest Dialog
Files Searched	ABI/INFORM® Professional Market Research, ABI/INFORM® Professional Standard, AGRICOLA, AGRIS, Allied & Complementary Medicine™, Analytical Abstracts, British Library Inside Conferences, British Nursing Index, Business & Industry, Business Monitor International, Chemical Business Newsbase, Chemical Engineering & Biotechnology Abstracts, Current Contents® Search, Dialog Global Reporter, Economist Intelligence Unit, Ei EnCompassLIT, Emerging Markets Direct, Energy Science and Technology, ESPICOM Pharmaceutical & Medical Device News, FDAnews, FLUIDEX (Fluid Engineering Abstracts), Gale Group Computer Database™, Gale Group

	Health Periodicals Database, Gale Group New Product Announcements / Plus®, Gale Group Newsletter Database™, Gale Group PharmaBiomed Business Journals, Gale Group PROMT®, Gale Group Trade & Industry Database™, GEOBASE, HSELINE: Health and Safety, ICONDA - International Construction Database, Inspec®, Jane's Defense & Aerospace News, Lancet Titles, Material Safety Datasheets -OHS™, MEDLINE®, New England Journal of Medicine, Paperbase, PAPERCHEM, PASCAL, PIRABASE, ProQuest Biological & Health Science Professional, ProQuest Environmental Science Professional, ProQuest Newsstand Professional, ProQuest Research, ProQuest Technology Research Professional, Registry of Toxic Effects of Chemical Substances (RTECS®), Toxfile®, Transport Research International Documentation, TULSA™ (Petroleum Abstracts), UBM Computer Full Text, Weldasearch®, World News
Date Conducted	April 16, 2015
Search Logic:	

Set#: S1
Searched for: (soy or soybean or saya?)
Results: 1589013*
Set#: S2
Searched for: S1 and cheese
Results: 104284*
Set#: S3
Searched for: (yeast or mold or strain or bacterial or micro?)
Results: 12399525*
Set#: S4
Searched for: S2 and S3
Results: 13391*
Set#: S5
Searched for: S4 and pectin
Results: 304°
Set#: S6
Searched for: S5 and oil
Results: 252°
Set#: S7
Searched for: S6 and starch
Results: 160°
Set#: S8
Searched for: S6 and sugar
Results: 202°

Electronic Database Searched	Google web

Files Searched	Google Web
Date Conducted	April 16, 2015
Search Logic:	

1). soy milk cheese bacterial
About 304,000 results
2). soy milk cheese bacterial pectin
About 551,000 results
3). soy milk cheese bacterial low methyl ester, amidated pectin
About 1,160 results
4). soy milk cheese acid oil low methyl ester, amidated pectin
About 1,310 results

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: ELIZABETH N. KAYTOR
FISH & RICHARDSON P.C.
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)*	28 AUG 2015
Applicant's or agent's file reference: 387670026WO1	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US2015/023679	International filing date *(day/month/year)*: 31 March 2015
Applicant: IMPOSSIBLE FOODS INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670026WO1	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2015/023679	International filing date *(day/month/year)* 31 March 2015	(Earliest) Priority Date *(day/month/year)* 31 March 2014
Applicant IMPOSSIBLE FOODS INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/023679

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.: 4-17, 37-74, 84, 95, 96, 105-109
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:

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.:

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.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/023679

Box No. IV ***Text of the abstract (Continuation of item 5 of the first sheet)***

This document relates to ground meat replicas, and more particularly to plant-based products that mimic ground meat, including the fibrousness, heterogeneity in texture, beefy flavor, and red-to-brown color transition during cooking of ground meat. For example, this document provides meat replicas that include proteins that are selected based upon the temperature at which they gel and/or denature to replicate the behavior and qualities of meat during cooking, i.e., the firming, syneresis (water release), chew texture, or mouthfeel.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/023679

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23J 3/22 (2015.01)
CPC - A23J 3/227 (2015.05)
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)
IPC(8) - A23J 3/14, 3/22 (2015.01)
CPC - A23J 3/14, 3/227 (2015.05) (keyword delimited)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 426/573, 574 (keyword delimited)

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

Orbit, Google Patents, Public AppFT and PatFT, Google Scholar
Search terms used: "meat replica" isolated plant protein dough cucumis juice cucumber food flavor meat vegetarian

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2008/0254199 A1 (ORCUTT et al) 16 October 2008 (16.10.2008) entire document	99, 100, 103, 104 --- 28-34, 77-83, 87-94, 101, 102, 113
Y	WO 2013/010042 A1 (SAND HILL FOODS INC) 17 January 2013 (17.01.2013) entire document	1-3, 18-27, 33-36, 85-94, 110-113
Y	US 2006/0204644 A1 (CAVALLINI et al) 14 September 2006 (14.09.2006) entire document	1-3, 18-36, 77-83, 110-113
Y	US 2002/0034570 A1 (KRAMMER et al) 21 March 2002 (21.03.2002) entire document	75, 76, 85, 86, 97, 98
Y	US 2003/0212281 A1 (SINHA et al) 13 November 2003 (13.11.2003) entire document	75, 76, 85, 86, 97, 98
Y	WO 2013/010037 A1 (LYRICAL FOODS INC) 17 January 2013 (17.01.2013) entire document	75, 76, 97, 98
Y	US 2010/0310738 A1 (LUDWIG) 09 December 2010 (09.12.2010) entire document	81, 82, 91, 92, 101, 102

☐ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2015	28 AUG 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: ELIZABETH N. KAYTOR
FISH & RICHARDSON P.C.
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 AUG 2015

Applicant's or agent's file reference	FOR FURTHER ACTION
387670026WO1	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2015/023679	31 March 2015	31 March 2014

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23J 3/22 (2015.01)
CPC - A23J 3/227 (2015.05)

Applicant IMPOSSIBLE FOODS INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	26 May 2015	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2015/023679

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2015/023679

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 4-17, 37-74, 84, 95, 96, 105-109

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. See Below are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 4-17, 37-74, 84, 95, 96, and 105-109 are multiple dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 4-17, 37-74, 84, 95, 96, 105-109

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

Form PCT/ISA/237 (Box No. III) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2015/023679

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-3, 18-36, 75-83, 85-94, 97, 98, 101, 102, 110-113	YES
	Claims	99, 100, 103, 104	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 18-36, 75-83, 85-94, 97-104, 110-113	NO
Industrial applicability (IA)	Claims	1-3, 18-36, 75-83, 85-94, 97-104, 110-113	YES
	Claims	None	NO

2. Citations and explanations:

Claims 99, 100, 103 and 104 lack novelty under PCT Article 33(2) as being anticipated by Orcutt et al.

Regarding claim 99, Orcutt et al. teach a food replica product ("A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], and "methods for preparing acceptable meat-like food products, such as beef, pork, poultry, fish, and shellfish analogs, from a wide variety of plant proteins", Para. [0003]) comprising one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said one or more carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein, trans-beta-apo-8'-carotenal, lycopene, canthaxanthin, and combinations thereof (the composition may comprise "beet juice, beta-carotene (orange), beta-APO 8 carotenal (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]).

Regarding claim 100, Orcutt et al. teach a food replica product comprising a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract at a concentration from 0.0001% to 10% of said food product (the composition may comprise "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]).

Regarding claim 103, Orcutt et al. teach the product of claim 100, wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was cooked or otherwise treated to denature proteins before being added to said food replica product (various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]).

Regarding claim 104, Orcutt et al. teach the product of claim 103, wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product (various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], "The dry pre-mix or the conditioned pre-mix is then fed into an extruder to heat, shear, and ultimately plasticize the mixture.", Para. [0083]).

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 113 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 113 is indefinite for the following reason:

Regarding claim 113, the claim depends from claim 110 which fails to provide antecedent basis for "the denaturation temperature." For the purposes of the Written Opinion purposed, "the denaturation temperature" is best interpreted as "a denaturation temperature," to provide sufficient antecedent basis.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claims 1-3, 18 and 19 lack an inventive step under PCT Article 33(3) as being obvious over Sand Hill Foods Inc [hereinafter Sandhill] in view of Cavallini et al.

Regarding claim 1, Sandhill teaches a meat replica composition ("a plant-based meat replica" composition, Para. [00354]; Claims 163-166) comprising: (a) about 5% to about 88% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat", isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189]; [00244]-[00247];[00332]); (b) about 0% to about 40% by weight of a carbohydrate-based gel ("some or all of the components of the consumable are suspended in a gel ... The gel can be made thick using an agent based on polysaccharides or proteins.", Para. [00332]); (c) about 5% to about 35% by weight of a non-animal fat ("20-80%% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (e) about 0% to about 15% by weight of a binding agent ("crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable", Para. [00331]); and (f) about 0.01% to about 4% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 0.00001% to about 10% by weight of a flavoring agent. However, Cavallini et al. teach (d) about 0.00001% to about 10% by weight of a flavoring agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of flavoring to the meat replica, as taught by Cavallini et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00362]-[00376]).

Regarding claim 2, Sandhill in view of Cavallini et al. teach the composition of claim 1, Sandhill teaches wherein said meat dough comprises a flavoring agent (the meat dough is a combination of "a muscle replica; a fat tissue replica; and a connective tissue replica", Paras. [0006];[00189];[00299];[00246]-[00247];[00332], and the fat replica contains plant oils that "can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking.", Paras. [00313]-[00317]).

Regarding claim 3, Sandhill in view of Cavallini et al. teach the composition of claim 1, Sandhill teaches wherein said non-animal fat comprises a flavoring agent ("The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking.", Para. [00317]).

Regarding claim 18, Sandhill teaches a meat replica composition ("a plant-based meat replica" composition, Para. [00354]; Claims 163-166) comprising: (a) about 5% to about 80% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat", isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189]; [00244]-[00247];[00332]); (b) about 5% to about 35% by weight of a non-animal fat ("20-80%% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (c) about 15% to about 40% by weight of an edible fibrous component (the connective tissue replica dough may be made from fibers that comprise "the one or more isolated, purified protein", Paras. [00299]; [00326]-[00327], "and said connective tissue replica accounts for 1-30% of said product by weight", Para. [00191]); and (g) about 0.1% to about 8% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 0.1% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 0.5% to about 15% by weight of a binding agent. However, Cavallini et al. teach (d) about 0.1% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 0.5% to about 15% by weight of a binding agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of binder, flavor and gel as taught by Cavallini et al. The motivation for doing so would be to mimic the "look, texture, and taste" of meat, (Sandhill, Paras. [00284]-[00285]).

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In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 19, Sandhill in view of Cavallini et al. teach the composition of claim 18, Sandhill teaches comprising: (a) about 20% to about 30% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat" and isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189];[00244]-[00247];[00332]); (b) about 15% to about 25% by weight of a non-animal fat ("20-80%% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (c) about 15% to about 25% by weight of an edible fibrous component (the connective tissue replica dough may be made from fibers that comprise "the one or more isolated, purified protein", Paras. [00299];[00326]-[00327], "and said connective tissue replica accounts for 1-30% of said product by weight", Para. [00191]); and (g) about 2% to about 8% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 7% to about 18% by weight of a carbohydrate-based gel and (f) about 5% to about 15% by weight of a binding agent. However, Cavallini et al. teach (d) about 7% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 5% to about 15% by weight of a binding agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of binder, flavor and gel as taught by Cavallini et al. The motivation for doing so would be to mimic the "look, texture, and taste" of meat, (Sandhill, Paras. [00284]-[00285]).

Claims 20-27, 35, 36 and 110-112 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Sandhill.

Regarding claim 20, Cavallini et al. teach a method of making a ground meat replica (a "ground meat pattie", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) heating a dough to a temperature ranging from 150°F to 250°F, said dough comprising an isolated plant protein (a vegetable dough comprising plant protein, flavorings and plant oil is heated "to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", Paras. [0024]-[0028];[0046]-[0047], where the plant protein is isolated, Para. [0031]), an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat; (b) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (c) combining the dough from step (b) with a carbohydrate-based gel, an optional edible fibrous component, an optional binding agent, and one or more optional flavoring agents to make said ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 21, Cavallini et al. in view of Sandhill teach the method of claim 20, Cavallini et al. teach said method further comprising breaking the dough from step (b) into pieces before combining with said carbohydrate-based gel, said optional edible fibrous component, said optional binding agent, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 22, Cavallini et al. teach a method of flavoring a meat dough ("This invention relates to a "two step" process wherein in the first step a flavored emulsion base and a vegetable base meat analog are prepared. These two components from the first step are combined in the second step with other components to produce a pattie that maintains much of its original weight upon cooking.", Paras. [0022];[0046]-[0047]), said method comprising: (a) combining one or more flavor precursors and an optional non-animal fat ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending *methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high* solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base", Para. [0023]); (b) heating the mixture to form one or more flavor compounds ("and cooking the flavored emulsion base to produce a vegetable base meat analog", Para. [0023]); and (c) making a dough comprising an isolated plant protein, an optional edible fibrous component, and the mixture from step (b) ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the *mixture into patties", Para. [0028], where the vegetable base comprises isolated plant protein, Paras.* [0031];[0046]). *Cavallini et al. do not* explicitly disclose a first highly conjugated heterocyclic ring complexed to an iron ion and/or first iron salt. However, Sandhill teaches a first highly conjugated heterocyclic ring complexed to an iron ion and/or first iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01%nand 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 23, Cavallini et al. in view of Sandhill teach the method of claim 22, Cavallini et al. teach further comprising: (d) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (e) combining the dough of step (d) with a carbohydrate-based gel, an optional binding agent, and one or more optional flavoring agents to make a ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the *vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel,* and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where the vegetable base comprises isolated plant protein, Paras. [0031];[0046], and "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to a second iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of *leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify* Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 24, Cavallini et al. in view of Sandhill teach the method of claim 23, Cavallini et al. teach said method further comprising breaking the dough from step (d) into pieces before combining with said carbohydrate-based gel, said optional binding agent, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 *to 12 mm in diameter to give vegetable base meat analog pieces.* The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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In case the space in any of the preceding boxes is not sufficient.
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Regarding claim 25, Cavallini et al. teach a method of flavoring a meat dough ("This invention relates to a "two step" process wherein in the first step a flavored emulsion base and a vegetable base meat analog are prepared. These two components from the first step are combined in the second step with other components to produce a pattie that maintains much of its original weight upon cooking.", Paras. [0022];[0046]-[0047]), said method comprising: (a) making a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) making a flavored non-animal fat by combining a non-animal fat with one or more flavor precursors and heating the mixture ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil, Paras. [0023]-[0025], and "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]); and (c) combining said dough, after heating, with said flavored non-animal fat ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or a first iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or a first iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 26, Cavallini et al. in view of Sandhill teach the method of claim 25, Cavallini et al. teach further comprising combining the dough of step (c) with a carbohydrate-based gel, an optional binding agent, and one or more optional flavoring agents to make a ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 27, Cavallini et al. in view of Sandhill teach the method of claim 26, Cavallini et al. teach further comprising breaking the dough of step (c) before combining with said carbohydrate-based gel, said optional binding agent, said one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 35, Cavallini et al. in view of Sandhill teach the method of claim 22, Cavallini et al. teach wherein said one or more flavor precursors is a sugar, a sugar alcohol, a sugar acid, a sugar derivative, an oil, a free fatty acid, an amino acid or derivative thereof, a nucleoside, a nucleotide, a vitamin, an acid, a peptide, a phospholipid, a protein hydrolysate, a yeast extract, or a mixture thereof ("The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]).

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Regarding claim 36, Cavallini et al. in view of Sandhill teach the method of claim 35, Cavallini et al. teach wherein said flavor precursor is selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1 ,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, urea, pantothenic acid, ornithine, niacin, glycerol, citrulline, taurine, biotin, borage oil, fungal oil, blackcurrant oil, betaine, beta carotene, B-vitamins, N-Acetyl L-cysteine, iron glutamate and a peptone, or mixtures thereof ("Any vegetable oil such as soybean oil, canola oil, corn oil, or peanut oil is suitable. The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye. The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024], and "a sweetener such as dextrose or sucrose", Para. [0036]).

Regarding claim 110, Cavallini et al. teach a ground meat replica ("a vegetable base meat analog, which may be used in a variety of vegetarian food products", Abstract, such as a "ground meat pattie", Para. [0004]) comprising: (a) a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (c) a carbohydrate-based gel, a binding agent, an optional edible fibrous component, and one or more optional flavoring agents ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025], and "the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 111, Cavallini et al. in view of Sandhill teach the replica of claim 110, Cavallini et al. teach wherein said binding agent is an isolated plant protein (wheat gluten and/or "soy protein concentrate" may be used as a binding agent, Paras. [0023]-[0024];[0040]; [0046]).

Regarding claim 112, Cavallini et al. in view of Sandhill teach the replica of claim 110, Cavallini et al. do not explicitly disclose wherein said isolated plant protein is a RuBisCO, an albumin, a gluten, a conglycinin, or mixtures thereof. However, Sandhill teaches wherein said isolated plant protein is a RuBisCO, an albumin, a gluten, a conglycinin, or mixtures thereof (Sandhill, "one or more isolated and purified proteins", such as "rubisco", "albumins", "gluten", and "conglycinins", Para. [0018]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to use the desired vegetable protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Claims 28-32, 77-80 and 83 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Orcutt et al.

Regarding claim 28, Cavallini et al. teach a method of making a ground meat replica (a "ground meat pattie", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) combining a salt with one or more flavor precursors and an optional non-animal fat; (b) heating the mixture to form one or more flavor compounds ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]); (c) making a dough comprising an isolated plant protein, an optional edible fibrous component, and the mixture from step (b); (d) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (e) combining the dough of step (d) with a carbohydrate-based gel, an optional binding agent, a salt, an optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents to make said ground meat replica ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039], where "the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], and the vegetable base comprises isolated plant protein, Paras. [0031];[0046], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose an iron salt. However, Orcutt et al. teach an iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 29, Cavallini et al. in view of Orcutt et al. teach the method of claim 28, Cavallini et al. teach further comprising breaking the dough from step (d) into pieces before combining with said carbohydrate-based gel, said optional binding agent, said optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al.,"A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 30, Cavallini et al. teach a method of making a ground meat replica (a "ground meat pattie", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) making a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) making a flavored non-animal fat by combining a non-animal fat with a salt and one or more flavor precursors and heating the mixture ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]); (c) combining said dough, after heating, with said flavored non-animal fat ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]); and (d) combining the dough of step (c) with a carbohydrate-based gel, an optional binding agent, an optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents to make said ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose an iron salt. However, Orcutt et al. teach an iron salt (Orcutt et al.,"A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Regarding claim 31, Cavallini et al. in view of Orcutt et al. teach the method of claim 30, Cavallini et al. teach further comprising breaking the dough from step (c) before combining with said carbohydrate-based gel, said optional binding agent, said optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al.,"A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 32, Cavallini et al. in view of Orcutt et al. teach the method of claim 30, Cavallini et al. do not explicitly disclose wherein said iron salt is iron gluconate, iron chloride, iron oxalate, iron nitrate, iron citrate, iron ascorbate, ferrous sulfate, ferric pyrophosphate, or any other aqueous soluble salt. However, Orcutt et al. teach wherein said iron salt is any other aqueous soluble salt *(Orcutt et al., "minerals include, without limitation, ... iron ... Suitable forms of any of the foregoing minerals include soluble mineral salts,* slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non-reactive minerals such as carbonate minerals, reduced minerals, and combinations thereof.", Para. [0064]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 77, Cavallini et al. teach a method of increasing the meat flavor or masking off flavors from plant material in a food product (a "ground meat pattie", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. *[0019]-[0021], where the flavor may be a meat flavor and* "The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]; Claim 7). Caviling et al. do not explicitly disclose said method comprising adding, to said food product, one or more carotenoids at a concentration of between 0. 00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein,trans-beta-apo-8', lycopene, canthaxanthin, and combinations thereof. However, Orcutt et al. teach said method comprising adding, to said food product, one or more carotenoids at a concentration of between 0. 00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, lutein, beta-apo-8', lycopene, canthaxanthin, and combinations thereof (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice, beta-carotene (orange), beta-APO 8 carotenal (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add carotene component, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 78, Cavallini et al. in view of Orcutt et al. teach the method of claim 77, Cavallini et al. teach wherein said food product is a meat replica (a "ground meat pattie", Para. [0004] from "a vegetable base meat analog", Abstract, and "a process for making vegetable burger patties", Paras. [0019]-[0021]).

Regarding claim 79, Cavallini et al. in view of Orcutt et al. teach the method of claim 78, Cavallini et al. teach wherein said meat replica is free of animal products ("a vegetable base meat analog", Abstract, "For making vegetarian burger patties", Para. [0037]).

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Regarding claim 80, Cavallini et al. teach a method of increasing the meat flavor of a meat replica (a "ground meat pattie", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021], where the flavor may be a meat flavor and "The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]; Claim 7). Caviling et al. do not explicitly disclose said method comprising adding, to said meat replica, a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract to said meat replica at a concentration from 0.0001% to 10% of said meat replica. However, Orcutt et al. teach said method comprising adding, to said meat replica, a vegetable juice, a vegetable extract to said meat replica at a concentration from 0.0001% to 10% of said meat replica (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add a vegetable extract or juice component, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 83, Cavallini et al. in view of Orcutt et al. teach the method of claim 80, Cavallini et al. do not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract is cooked or otherwise treated to denature proteins before adding to said meat replica. However, Orcutt et al. teach wherein said vegetable extract is cooked or otherwise treated to denature proteins before adding to said meat replica (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Claims 87-90, 93 and 94 lack an inventive step under PCT Article 33(3) as being obvious over Sandhill in view of Orcutt et al.

Regarding claim 87, Sandhill teaches a food product or food replica product ("a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources", Paras. [0006];[00354]; Claims 163-166) comprising a heme-containing protein ("said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin", Para. [0018]). Sandhill does not explicitly disclose one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein,trans-beta-apo-8', lycopene, canthaxanthin, and combinations thereof. However, Orcutt et al. teach one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, lutein, beta-apo-8', lycopene, canthaxanthin, and combinations thereof (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice, beta-carotene (orange), beta-APO 8 carotenal (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add a carotene component, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 88, Sandhill in view of Orcutt et al. teach the product of claim 87, Sandhill teaches wherein said food replica product is a meat replica (a plant-based meat replica" composition, Para. [00354]; Claims 163-166).

Regarding claim 89, Sandhill in view of Orcutt et al. teach the product of claim 88, Sandhill teaches wherein said meat replica is free of animal products ("the meat substitute compositions contains no animal protein", Para. [00353], and "said meat substitute product contains no animal products", Para. [0065]).

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Regarding claim 90, Sandhill teaches a food product or food replica product comprising (a) a heme-containing protein ("a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources", Paras. [0006];[00354]; Claims 163-166, where "said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin", Para. [0018]). Sandhill does not explicitly disclose (b) a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract at a concentration from 0.0001% to 10% of said food product. However, Orcutt et al. teach (b) a vegetable juice, a vegetable extract at a concentration from 0.0001% to 10% of said food product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add a vegetable extract or juice component, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 93, Sandhill in view of Orcutt et al. teach the product of claim 90, Sandhill does not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was cooked or otherwise treated to denature proteins before being added to said food replica product. However, Orcutt et al. teach wherein said vegetable extract was cooked or otherwise treated to denature proteins before being added to said food replica product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 94, Sandhill in view of Orcutt et al. teach the product of claim 93, Sandhill does not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product. However, Orcutt et al. teach wherein said vegetable extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], "The dry pre-mix or the conditioned pre-mix is then fed into an extruder to heat, shear, and ultimately plasticize the mixture.", Para. [0083]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2015/023679

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claims 101 and 102 lack an inventive step under PCT Article 33(3) as being obvious over Orcutt et al. in view of Ludwig.

Regarding claim 101, Orcutt et al. teach the product of claim 100, Orcutt et al. do not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, a fruit juice, a fruit puree, or a fruit extract is a Cucumis juice, puree, or extract. However, Ludwig teaches wherein said vegetable juice, vegetable puree, vegetable extract is a Cucumis juice, puree, or extract (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Orcutt et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic ground "meat compositions" through use of the optimal plant extracts, (Orcutt et al., Paras. [0028];[0033];[0062]).

Regarding claim 102, Orcutt et al. in view of Ludwig teach the product of claim 101, Orcutt et al. do not explicitly disclose wherein said Cucumis juice, puree, or extract is from a cucumber or a melon. However, Ludwig teaches wherein said Cucumis juice, puree, or extract is from a cucumber (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Orcutt et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic ground "meat compositions" through use of the optimal plant extracts, (Orcutt et al., Paras. [0028];[0033];[0062]).

Claims 33, 34 and 113 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Orcutt et al. and Sandhill.

Regarding claim 33, Cavallini et al. in view of Orcutt et al. teach the method of claim 28, Cavallini et al. teach wherein salt, said one or more flavor precursors, and said non-animal fat before heating said mixture ("blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base; and cooking the flavored emulsion base to produce a vegetable base meat analog", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al.,"A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion. However, Sandhill teaches said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]).