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.

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

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

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

Motif Exhibit 1002, Page 611 of 1290

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. 9,943,096

Motif Exhibit 1002, Page 612 of 1290

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	

Motif Exhibit 1002, Page 679 of 1290

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

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

Motif Exhibit 1002, Page 680 of 1290

		$CoCl_2$+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

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

Motif Exhibit 1002, Page 681 of 1290

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

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

Motif Exhibit 1002, Page 682 of 1290

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*, *Rhodosporidium 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.

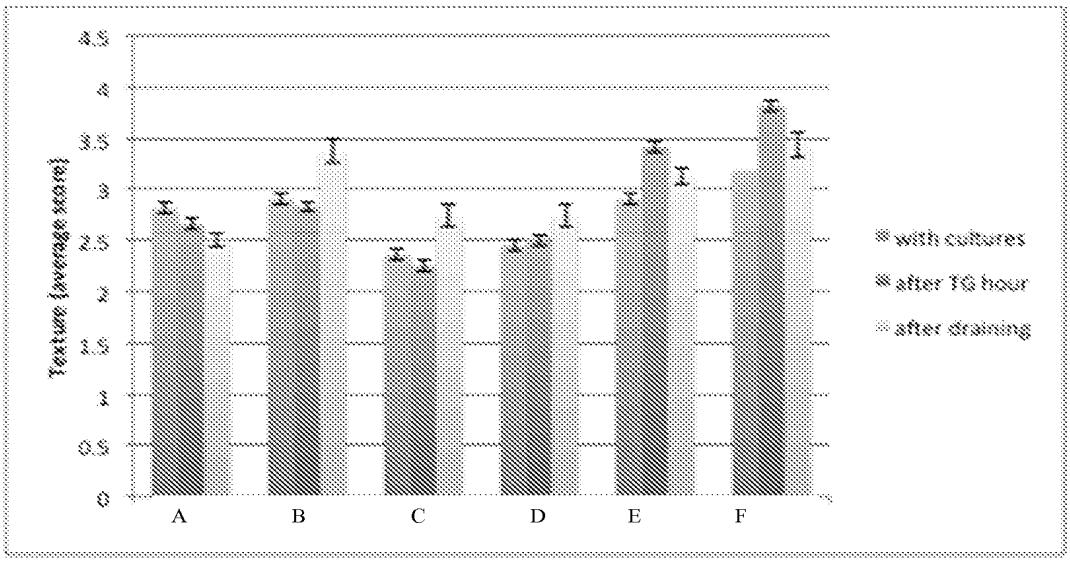

Texture (average score)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
A
B
C
D
E
F
with cultures
after 16 hour
after draining

FIG. 1

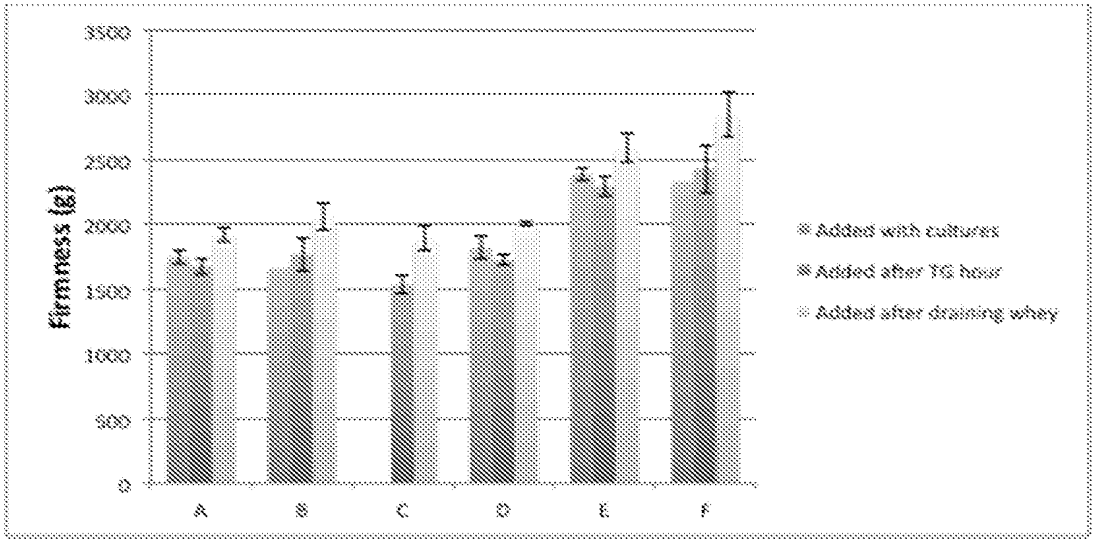

Firmness (g)
3500
3000
2500
2000
1500
1000
500
0
A
B
C
D
E
F
Added with cultures
Added after TG hour
Added after draining whey

FIG. 2

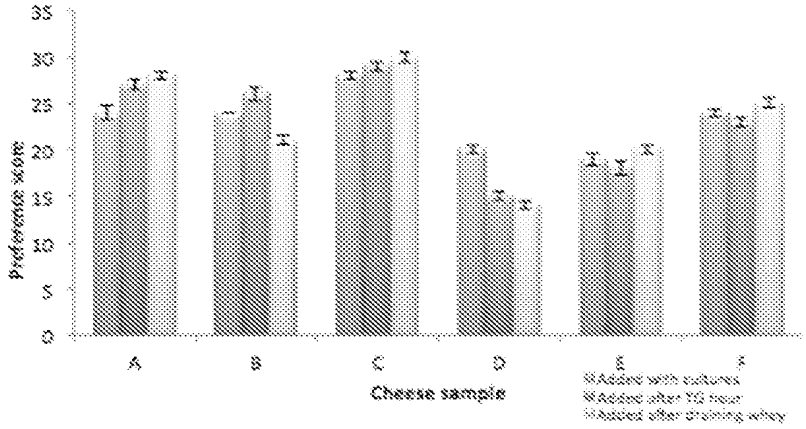

Preference score
Cheese sample
A
B
C
D
E
F

FIG. 3A

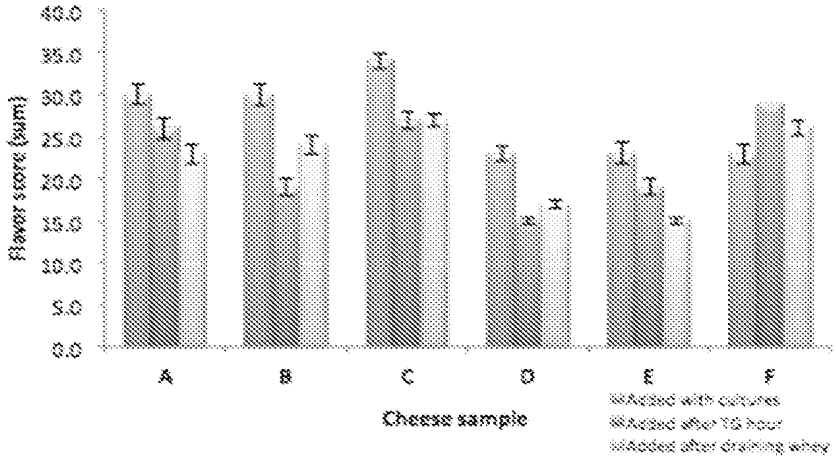

Flavor score (sum)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
A
B
C
D
E
F
Cheese sample

FIG 3B.

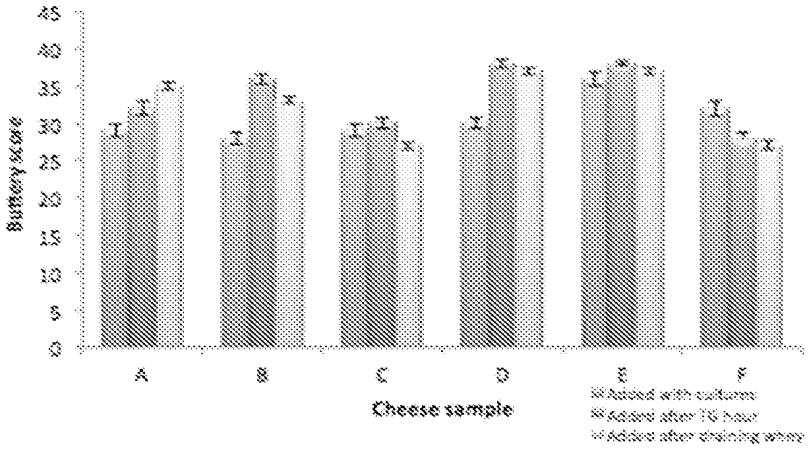


FIG. 4.

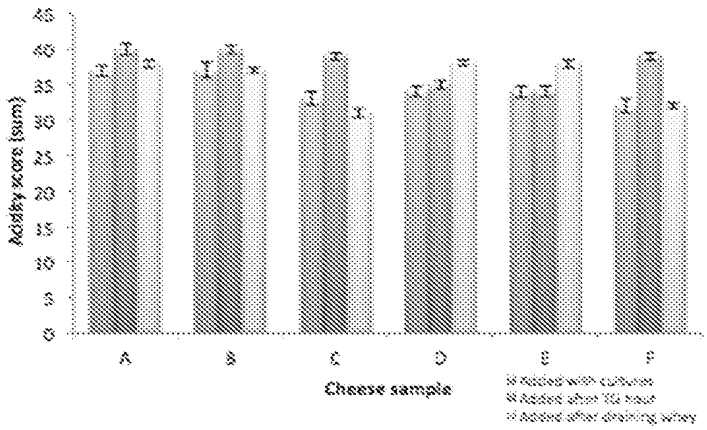


FIG. 5

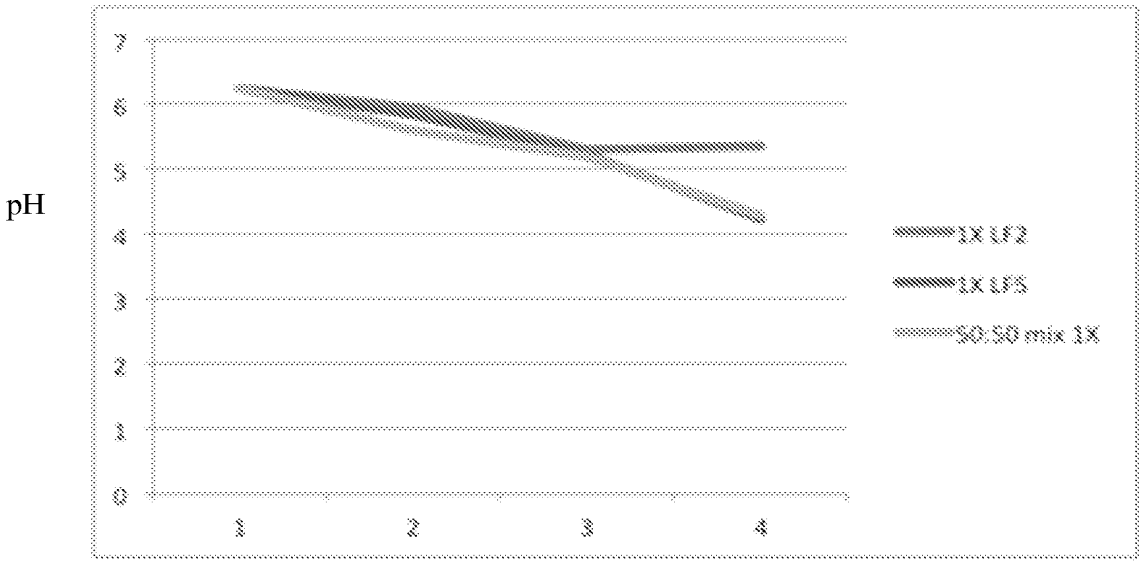
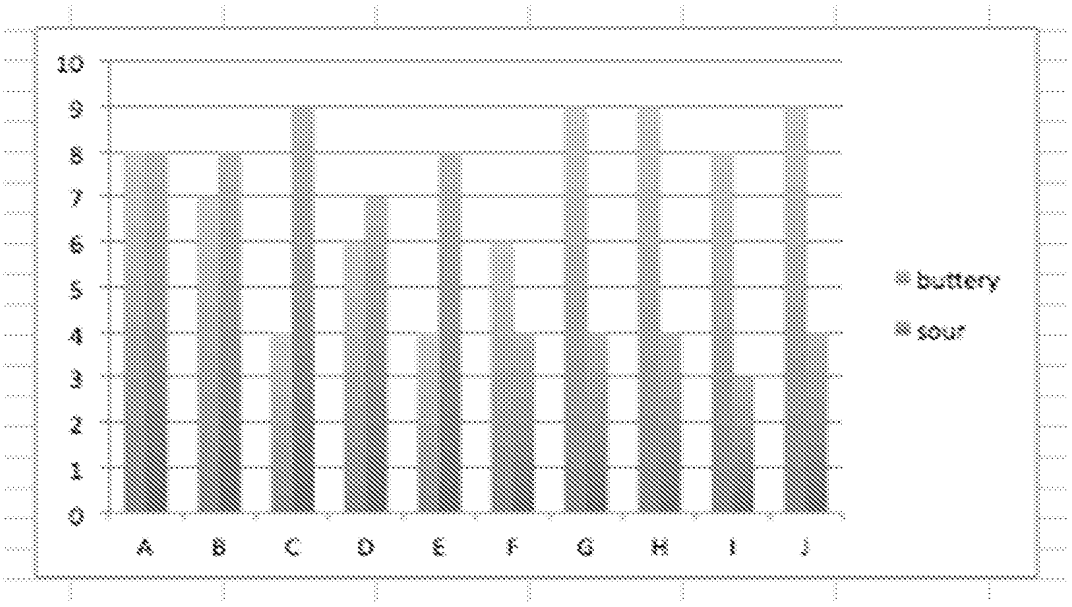


FIG. 6



LM LLBD

FIG 7

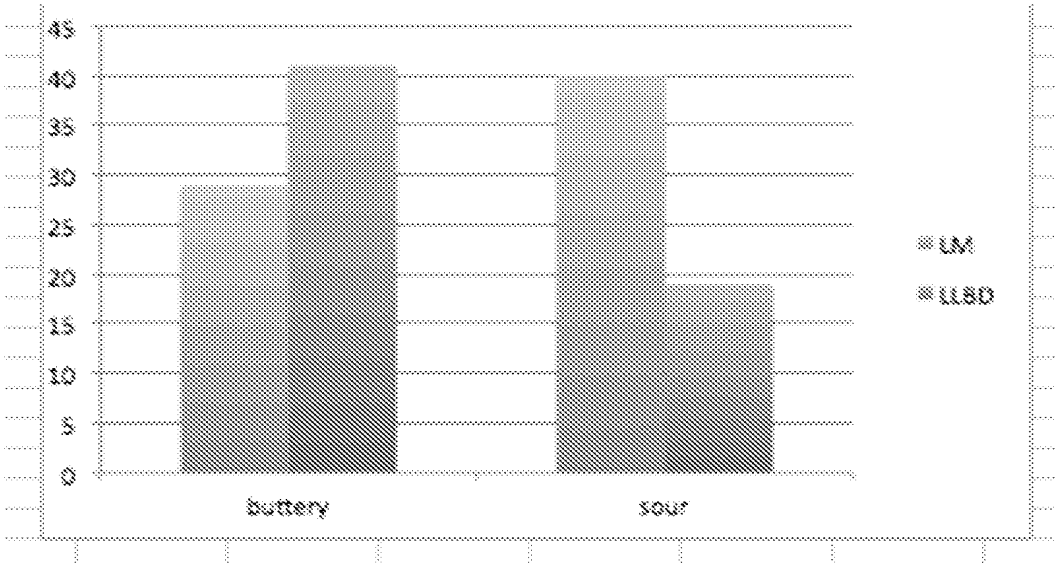


FIG. 8

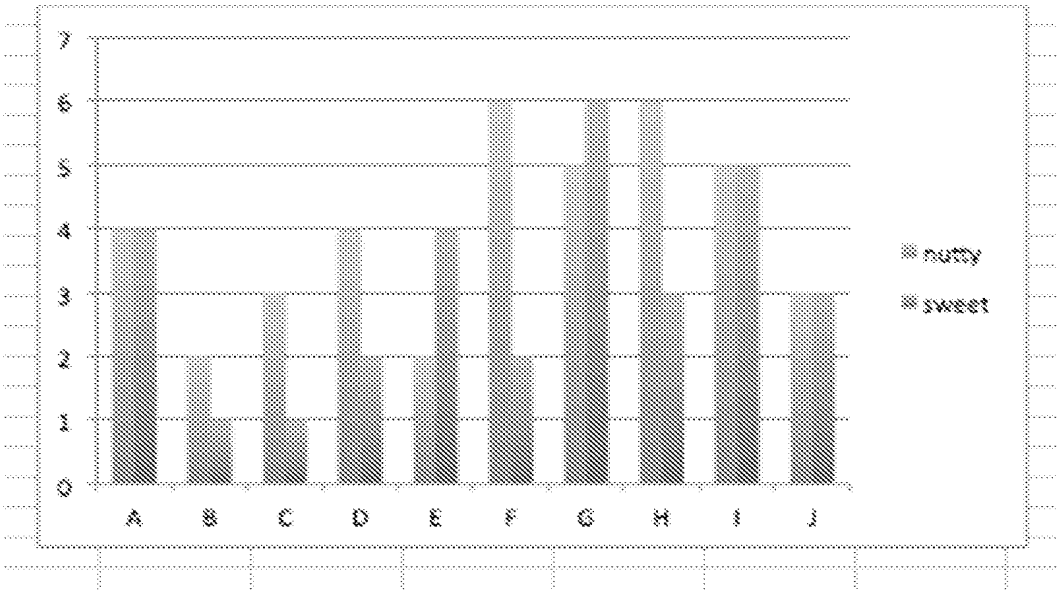


FIG. 9



FIG. 10

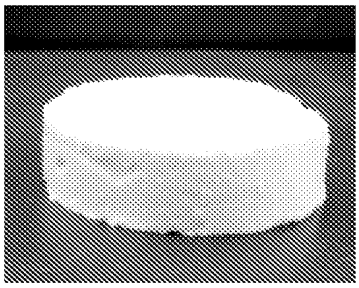


FIG. 11

FIGURE 12

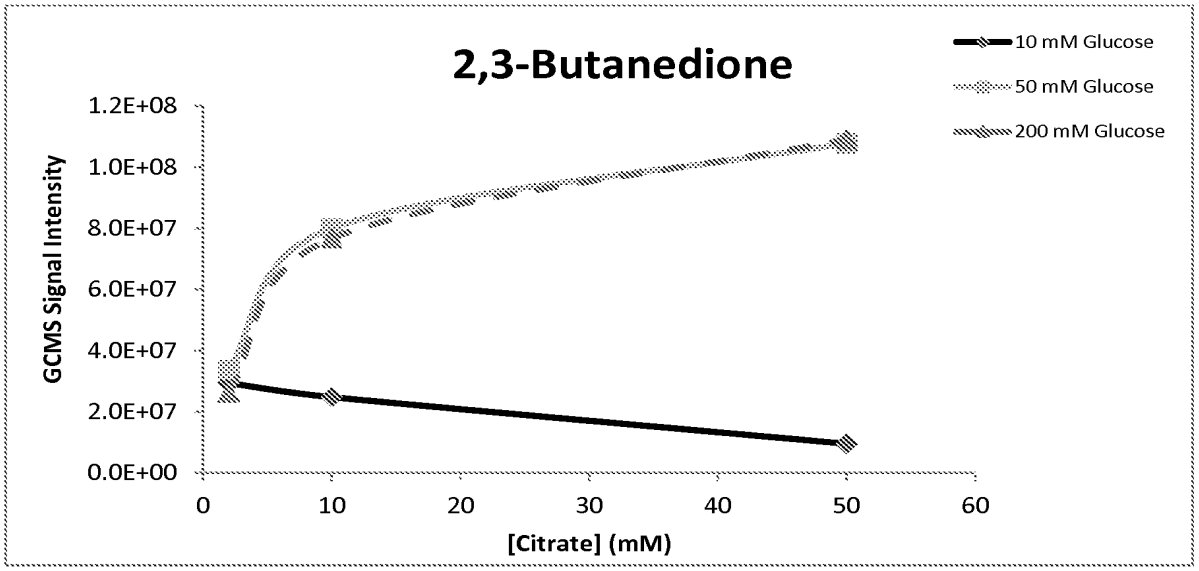

2,3-Butanedione
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 13

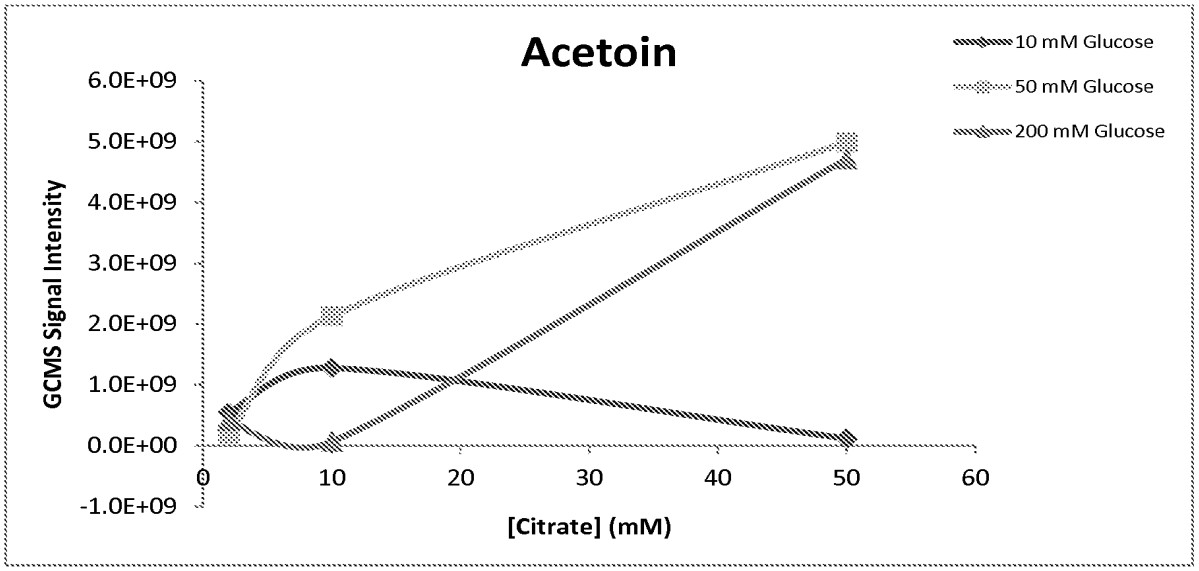

Acetoin
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
6.0E+09
5.0E+09
4.0E+09
3.0E+09
2.0E+09
1.0E+09
0.0E+00
-1.0E+09
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 14

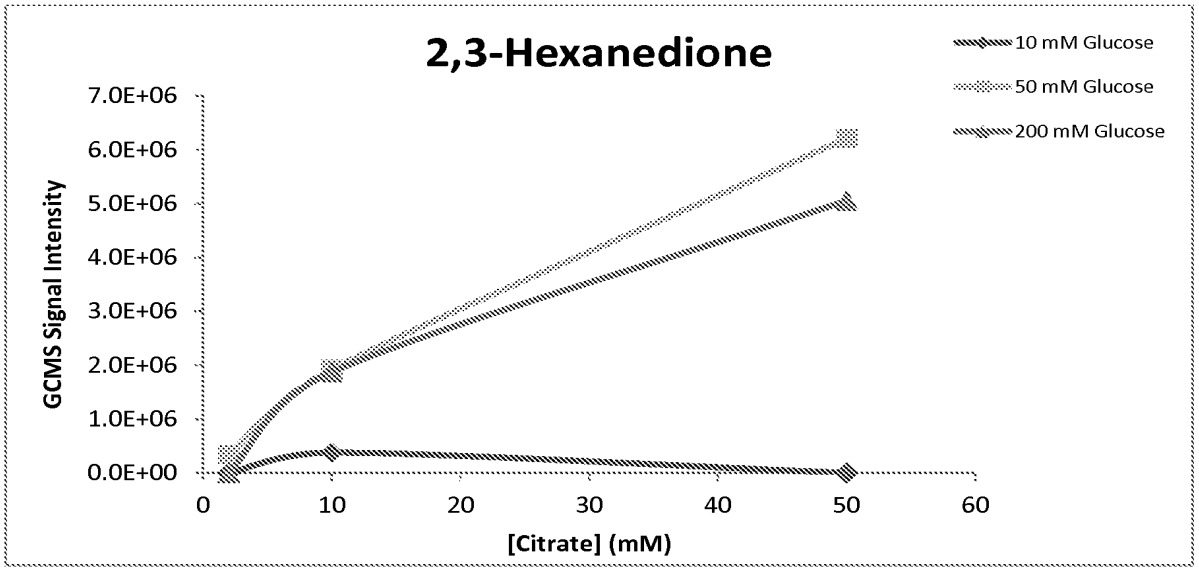

2,3-Hexanedione
10 mM Glucose
50 mM Glucose
200 mM Glucose
GCMS Signal Intensity
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
0
10
20
30
40
50
60
[Citrate] (mM)

FIGURE 15

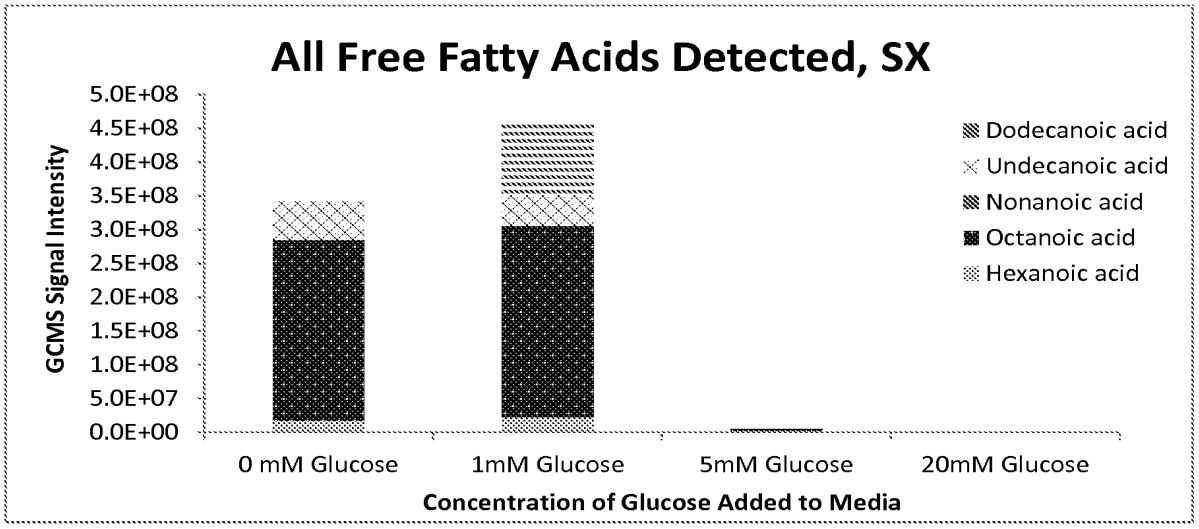

All Free Fatty Acids Detected, SX
GCMS Signal Intensity
5.0E+08
4.5E+08
4.0E+08
3.5E+08
3.0E+08
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
0 mM Glucose
1mM Glucose
5mM Glucose
20mM Glucose
Concentration of Glucose Added to Media
Dodecanoic acid
Undecanoic acid
Nonanoic acid
Octanoic acid
Hexanoic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

FIGURE 16

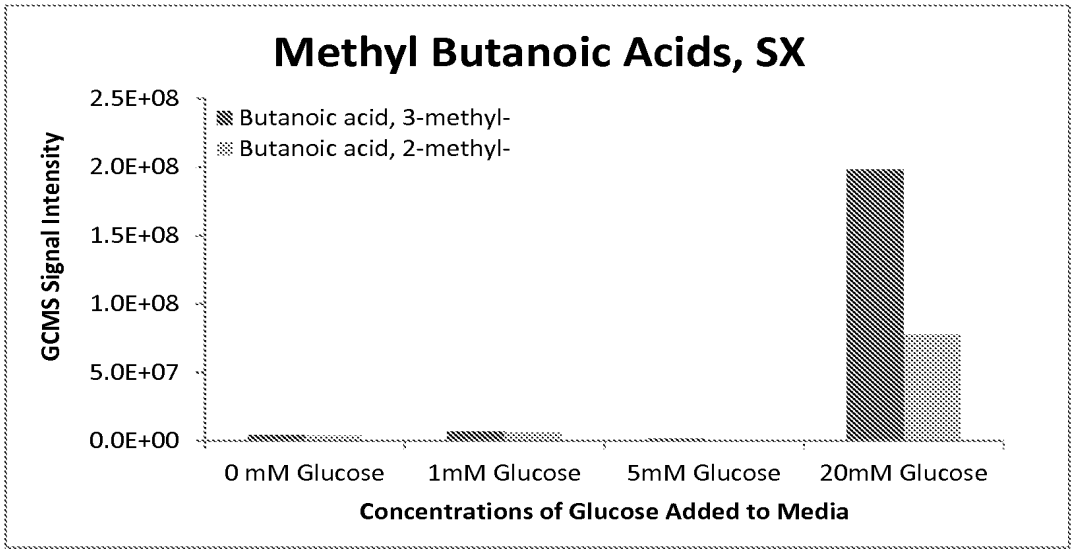

Methyl Butanoic Acids, SX
GCMS Signal Intensity
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
Butanoic acid, 3-methyl-
Butanoic acid, 2-methyl-
0 mM Glucose
1mM Glucose
5mM Glucose
20mM Glucose
Concentrations of Glucose Added to Media

FIGURE 17

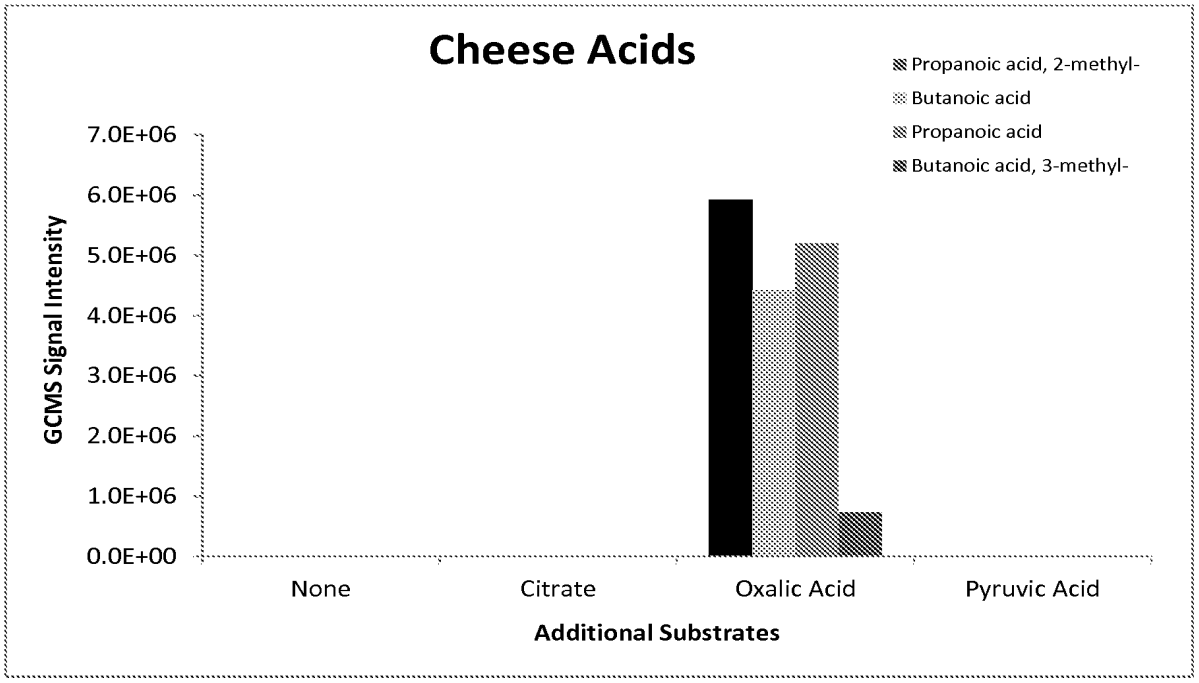

Cheese Acids
Propanoic acid, 2-methyl-
Butanoic acid
Propanoic acid
Butanoic acid, 3-methyl-
GCMS Signal Intensity
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
None
Citrate
Oxalic Acid
Pyruvic Acid
Additional Substrates

FIGURE 18

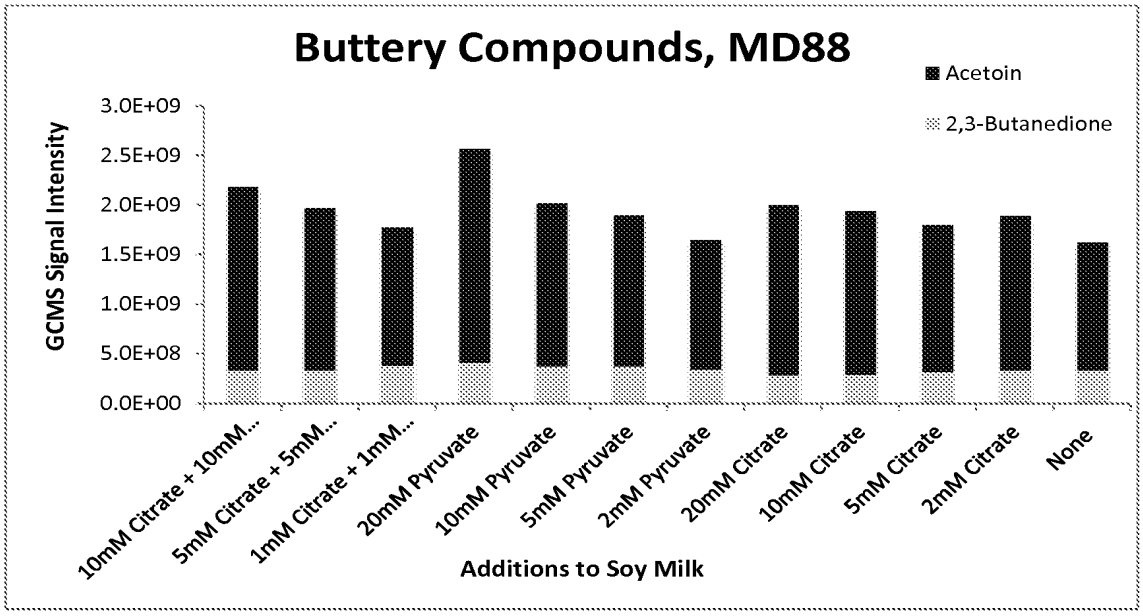


FIGURE 19

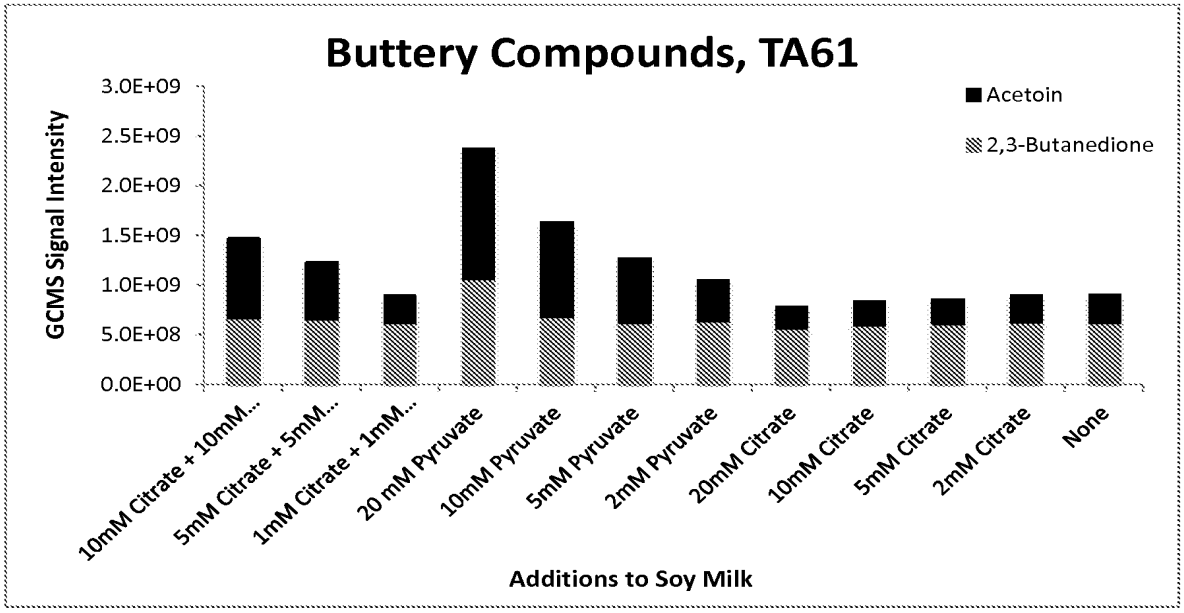


FIGURE 20

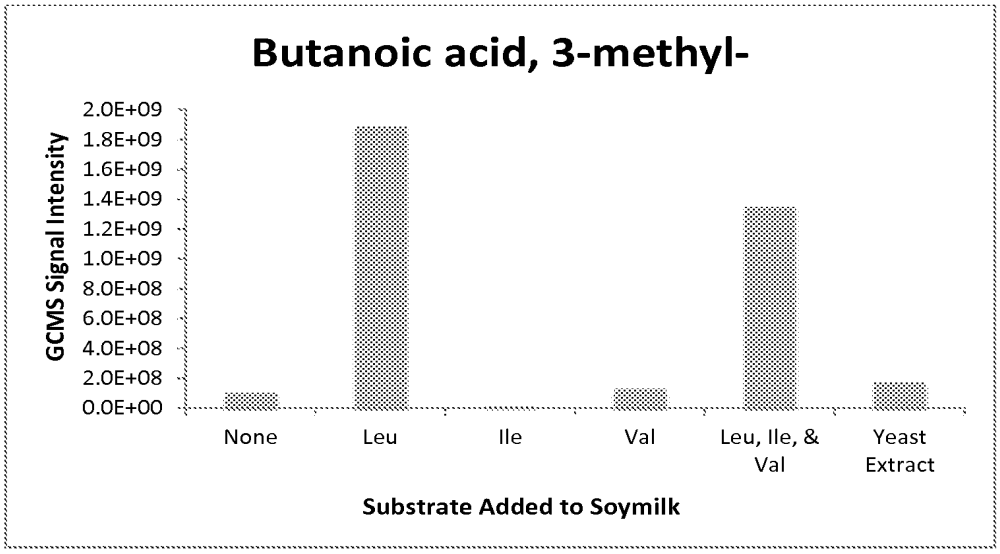


FIGURE 21

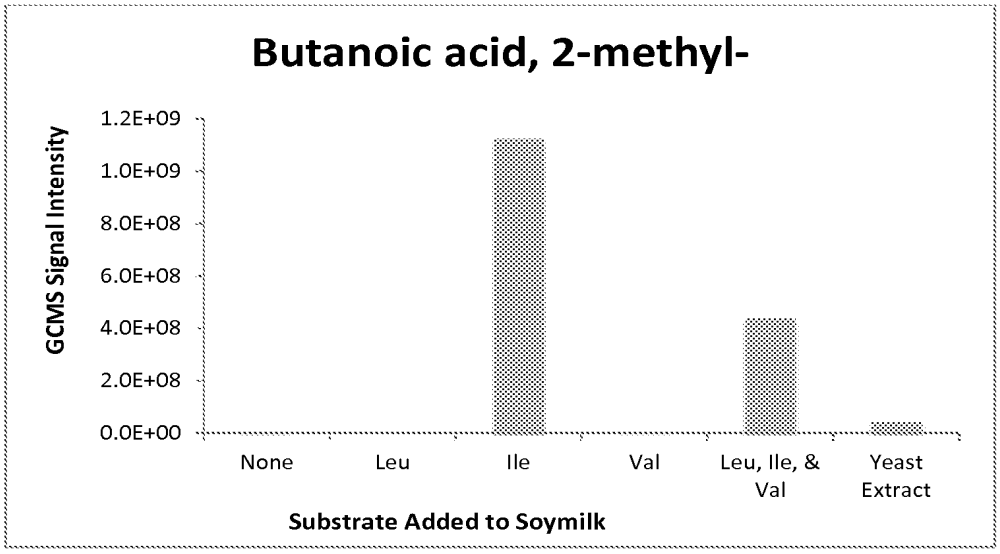


FIGURE 22

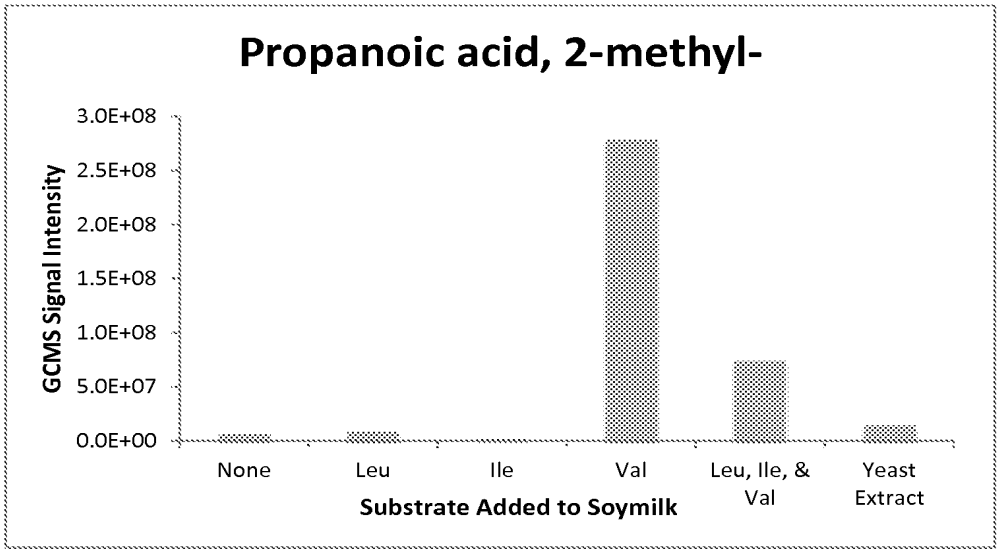


FIGURE 23

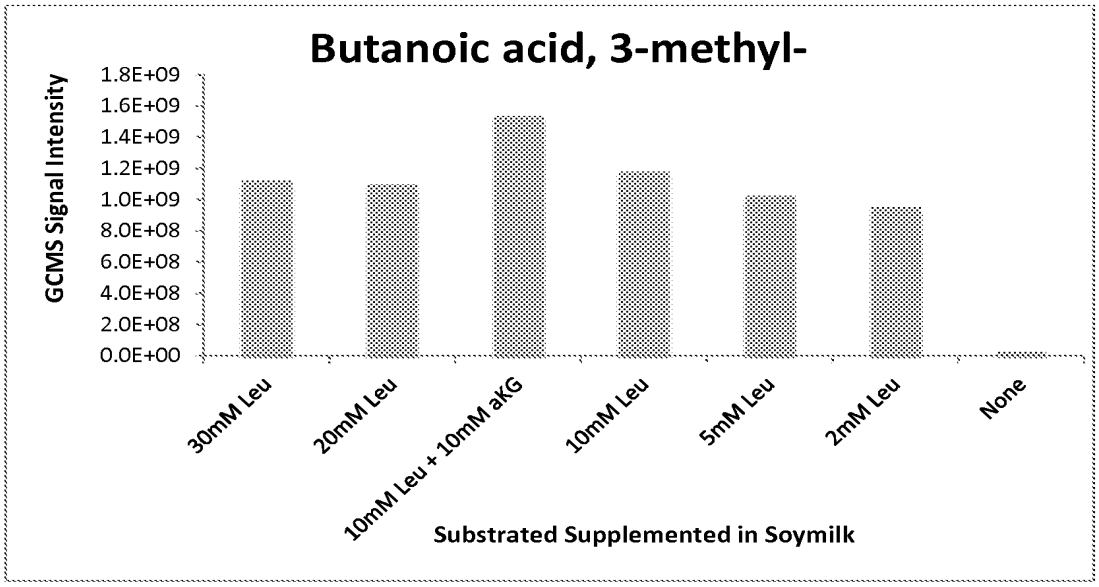


FIGURE 24

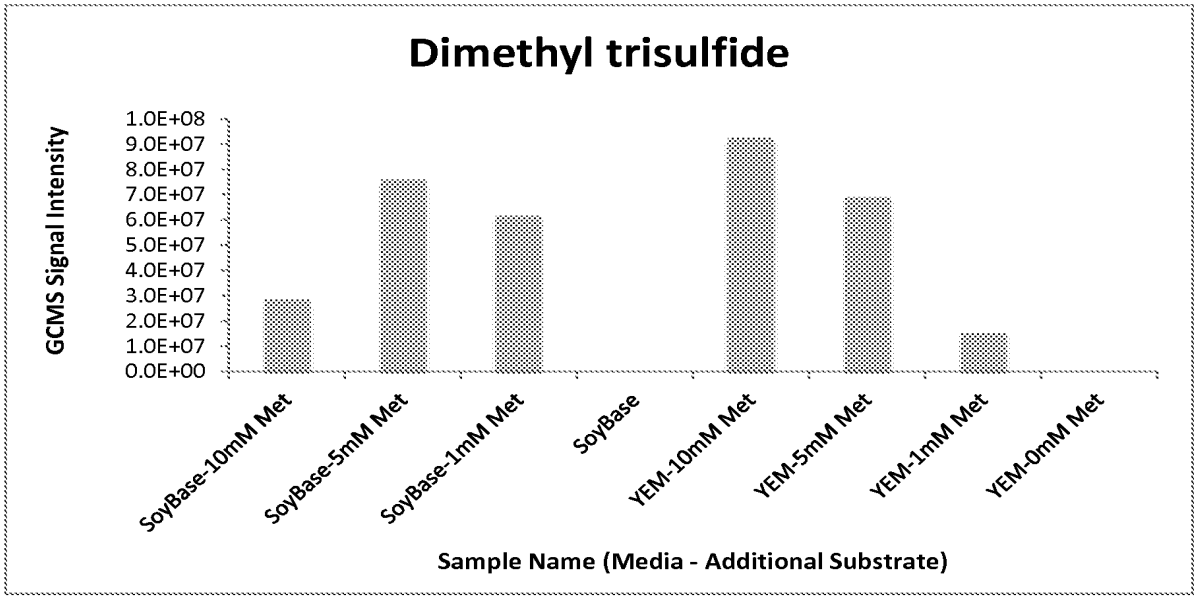


FIGURE 25

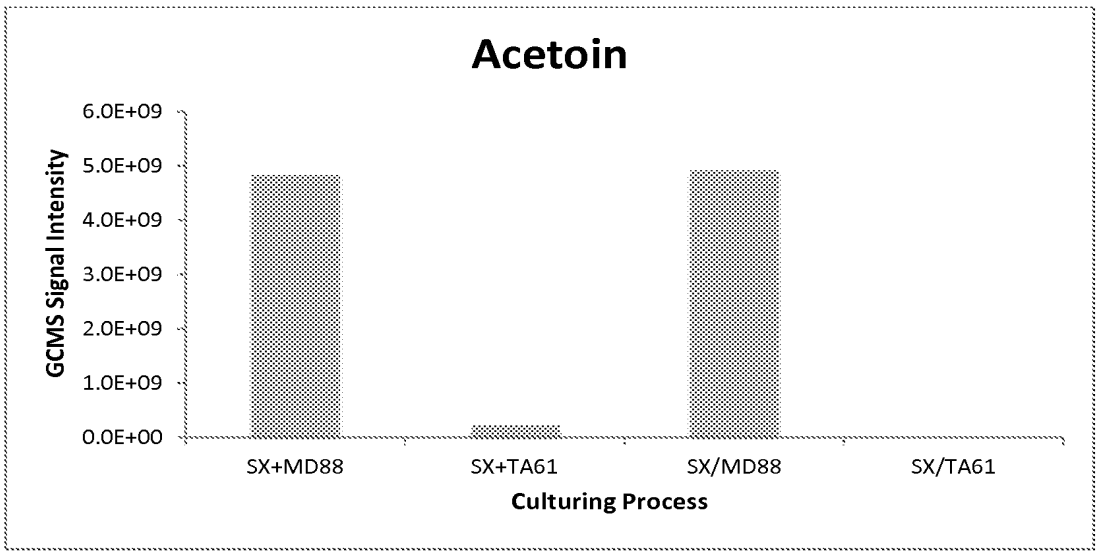


FIGURE 26

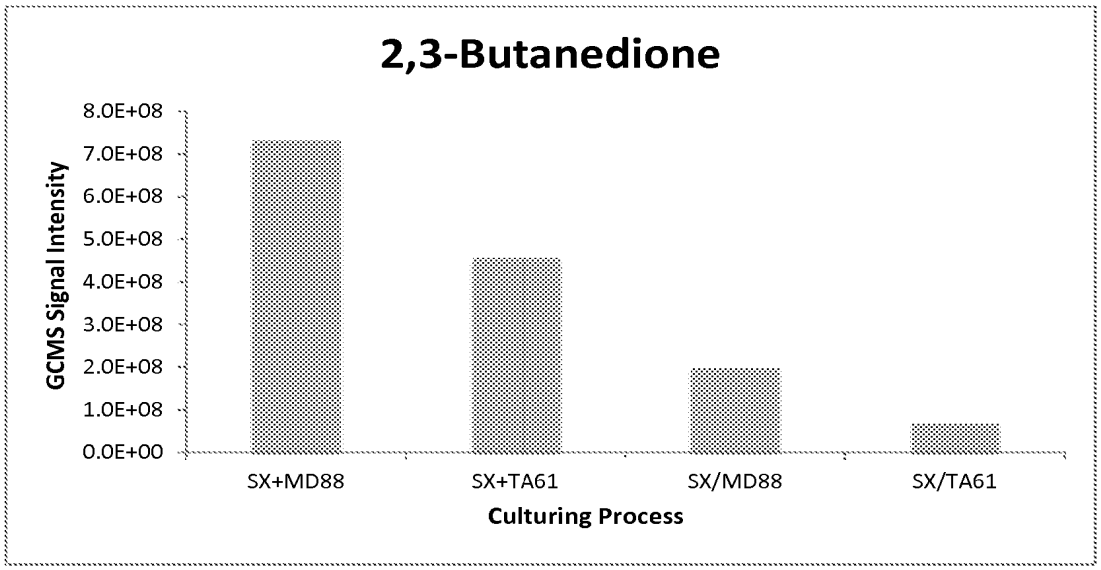
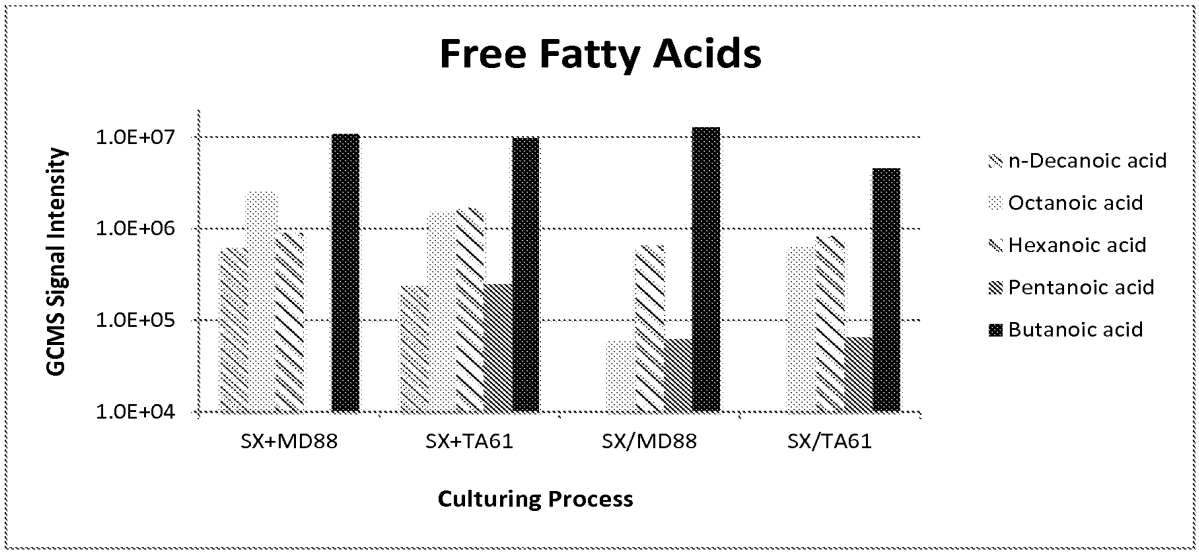


FIGURE 27



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

(71) Applicant: **IMPOSSIBLE FOODS INC.** [US/US]; 525 Chesapeake Drive, Redwood City, California 94063 (US).

(72) Inventors; and
(71) Applicants : **GRZANICH, Stephanie S.** [US/US]; 898 Arcturus Circle, Foster City, California 94404 (US). **KLAPHOLZ, Sue** [US/US]; 76 Peter Coutts Circle, Stanford, California 94305 (US). **VOCCOLA, Lynn S.** [US/US]; 555 Crespi Drive, Apt. N-103, Pacifica, California 94044 (US). **GRIFFITHS, Marjorie Ronan** [US/US]; 704 Bush Street, Apartment 406, San Francisco, California 94108 (US).

(74) Agents: **MCCORMICK GRAHAM, Monica** et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(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 30ºC 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

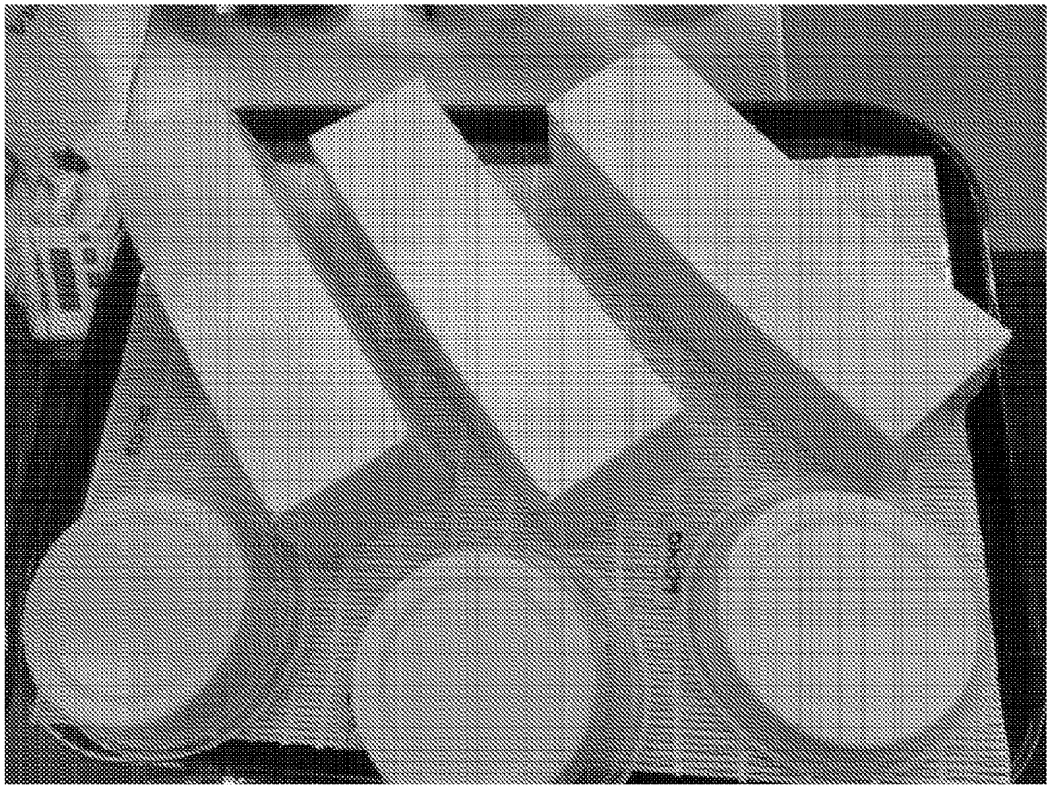


FIGURE 3

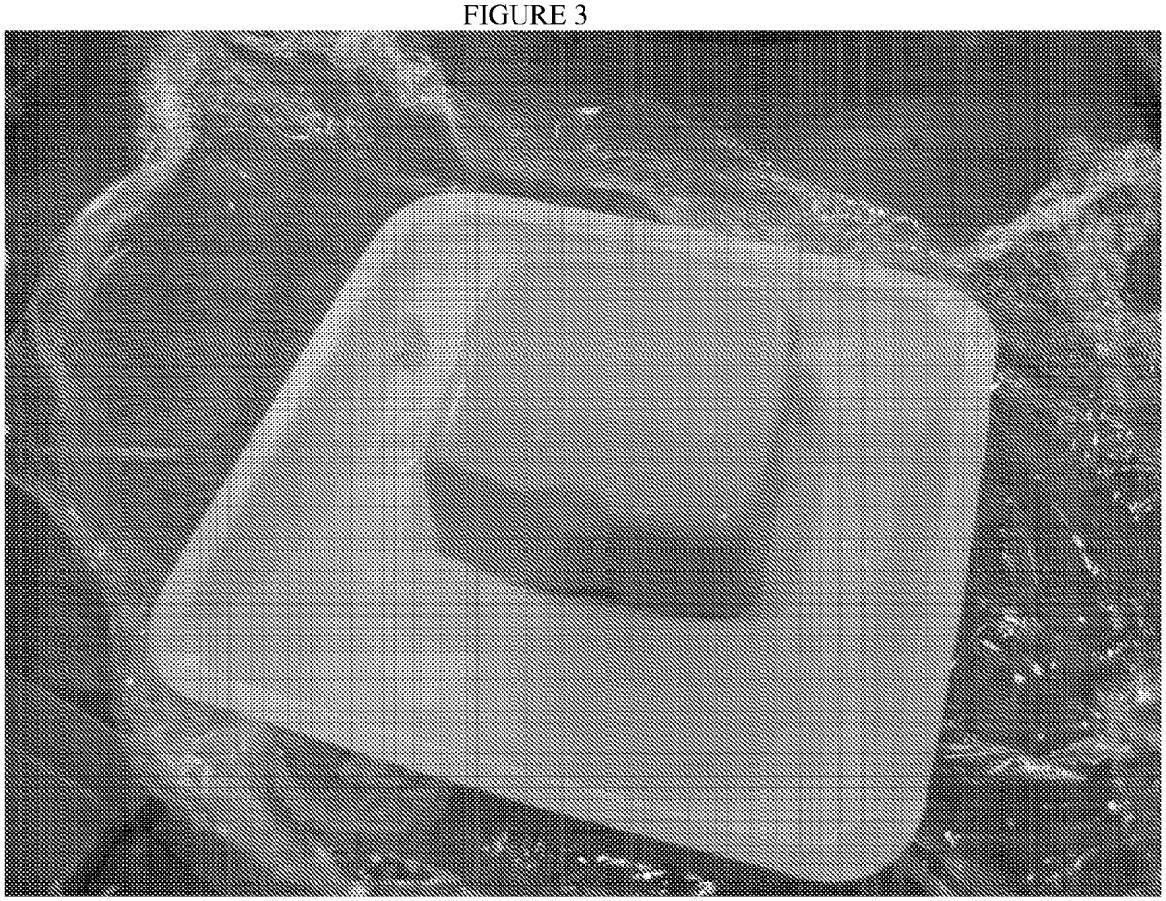


FIGURE 4

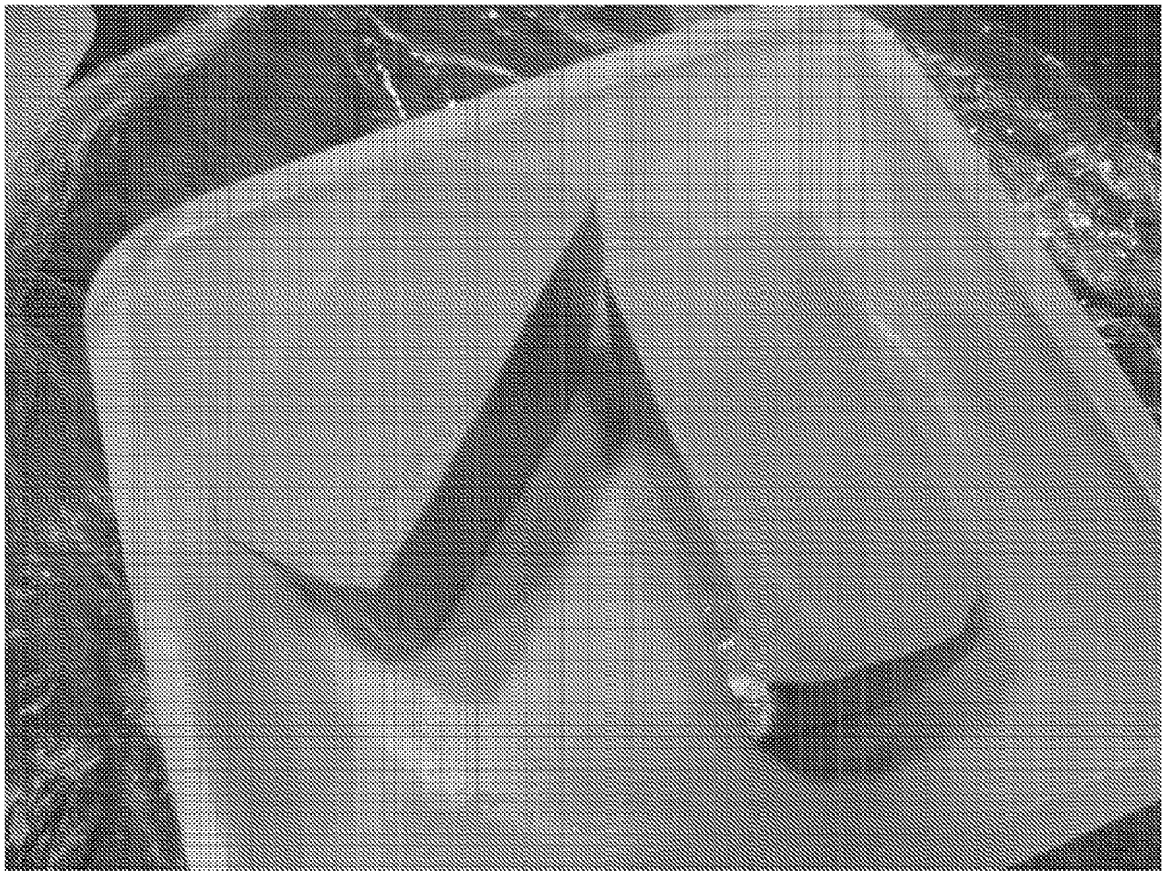


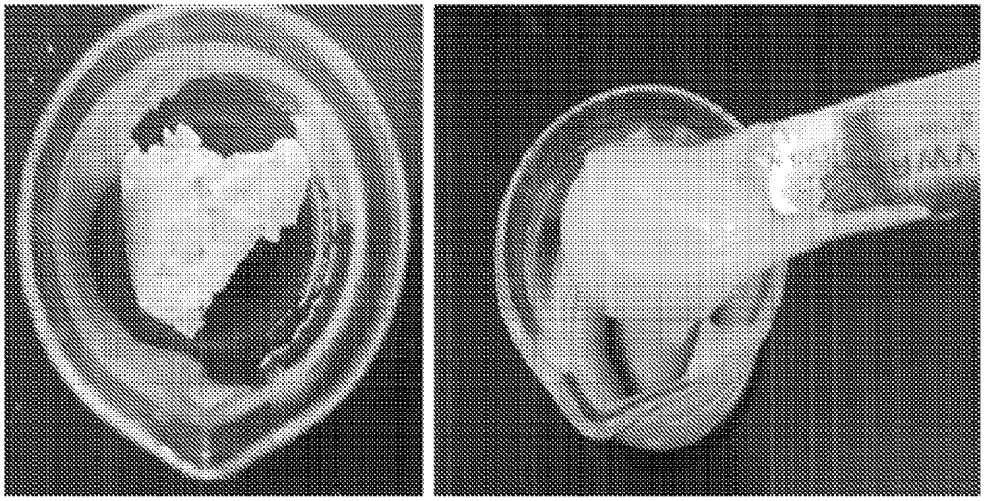
FIGURE 5





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.

Electronic Acknowledgement Receipt

EFS ID:	29788484
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	Ian J.S. Lodovice/Judith Witzel
Filer Authorized By:	Ian J.S. Lodovice
Attorney Docket Number:	38767-0015002
Receipt Date:	14-JUL-2017
Filing Date:	04-JAN-2017
Time Stamp:	15:30:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		RESPONSE.pdf	177107 0a84fe124ed5dcb2145058767f3947c65cb9a75d	yes	21

	Multipart Description/PDF files in .zip description			
	Document Description	**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1	
	Specification	2	6	
	Claims	7	12	
	Applicant Arguments/Remarks Made in an Amendment	13	21	

Warnings:					
Information:					
2	Drawings-only black and white line drawings	ReplacementFig2.pdf	150452 ef514d8e92eeb200035da488d674ee05478b611f	no	1
Warnings:					
Information:					
3	Examination support document	Exhibit1_Holz_SchietingerDeclaration.pdf	5205922 5724ca190d98546751aa10f3f0ed13b2437e9639	no	12
Warnings:					
Information:					
4	Examination support document	Exhibit2_CadwalladerDeclaration.pdf	576745 35fce1c755f88743438a4b462c7ecbd47d7096ad	no	45
Warnings:					
Information:					
5	Examination support document	Exhibit3_PetersonDeclaration.pdf	2443149 cb448c5054d6fe4e422770bf7a8d5a2d8ac24edc	no	20
Warnings:					
Information:					
6	Examination support document	Exhibit4_Lipman_HemeAlignmentDeclaration.pdf	3516702 be45db7bc3c2e0216a1504ea76420a109d6fd02b	no	8
Warnings:					

The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing

Information:					
7	Examination support document	ExhibitB_Needleman.pdf	839222 ce7a9f9ad799167f08f6225de671963c6f4323a4	no	11
Warnings:					
Information:					
8	Examination support document	ExhibitC_NCBIBlastSearch.pdf	181087 41fbeda07df0815065ff2fe9b7c283c728653c64	no	3
Warnings:					
Information:					
9	Examination support document	ExhibitD_HemeProteinPairwiseAlignment.pdf	360738 052dad5f10211c9fba83bd5478a5513864915ac8	no	4
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
10	Examination support document	ExhibitF_Hedges.pdf	4012780 27c04706adca8431a6a531ad82a4262b16470d33	no	11
Warnings:					
Information:					
11	Examination support document	ExhibitA_LipmanCV2017.pdf	159555 155d722972e38903ca8165893a7f0069b845e5e5	no	10
Warnings:					
Information:					
12	Examination support document	ExhibitE_Nei.pdf	269071 1fbd04d933119a15fc2427d662bee63665e63b48	no	9
Warnings:					
Information:					
13	Transmittal Letter	IDS.pdf	69805 2bf48d4b4ab4335acc14cb72b4afbb482298bed5	no	1
Warnings:					

Information:					
14	Information Disclosure Statement (IDS) Form (SB08)	PTO1449.pdf	128307 db279c78c4b0b2fd01ae1fd49ea9d820df60deae	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
15	Non Patent Literature	AU_OA_2012281069_2015_09_15.pdf	2474518 745e3ac4e002a793e2ea08e9d393fd0a7ecf1342	no	5
Warnings:					
Information:					
16	Non Patent Literature	AU_OA_20122281064_2016_01_25.pdf	2298422 f5c940c08ccabd54eb7e4a076bd348e01b70000b	no	5
Warnings:					
Information:					
17	Non Patent Literature	IPRP_PCTUS201411362_2015_07_23.pdf	490028 a56c470c168b211577b6cf6cf2e500ce10d6507e	no	9
Warnings:					
Information:					
18	Non Patent Literature	IPRP_PCTUS2012046552_2014_01_23.pdf	526057 55c85b7cc66158bf86b269334a00761e602ea84f	no	9
Warnings:					
Information:					
19	Non Patent Literature	IPRP_PCTUS2012046560_2014_01_23_.pdf	402189 f67483227baaa2761bac16801bf5b27fa3928ceae	no	9
Warnings:					
Information:					
20	Non Patent Literature	IPRP_PCTUS2014011361_2015_07_14.pdf	846917 6a837b86619ca776c7a61c57c55af084096a7467	no	12
Warnings:					
Information:					

21	Non Patent Literature	ISR_PCTUS201246552_2012_11_19.pdf	702705 45e3f607586fe958a37e2be0b218b463b7a3c467	no	12
Warnings:					
Information:					
22	Non Patent Literature	ISR_PCTUS2012046560_2012_12_14.pdf	590243 a5b4450def1f4c9242d264d7a3ade91697209bfb	no	11
Warnings:					
Information:					
23	Non Patent Literature	ISR_PCTUS2015023679_2015_08_28.pdf	1959616 bb383ccd0c0c4af7ea9fbfdd71363aea8d8dc2b6	no	26
Warnings:					
Information:					
24	Non Patent Literature	ISRWO_PCTUS201411361_2014_06_16.pdf	1259326 148ae7257566c77e21674d4043855dfa73039e08	no	18
Warnings:					
Information:					
25	Non Patent Literature	ISRWO_PCTUS201411362_2014_06_13.pdf	708737 10c89227b10ab501909a5f16cca92801a59fff4e	no	12
Warnings:					
Information:					
26	Non Patent Literature	ISRWO_PCTUS2015017147_2015_05_01.pdf	1512940 5465827ac53f46f2f103335791b24f8911142157	no	15
Warnings:					
Information:					
27	Non Patent Literature	IVNPAYFEES_PCTUS201411361_2014_04_10.pdf	354745 5a85c6ae60e62a8632e0bbf23d2c4456d69e2788	no	4
Warnings:					
Information:					

28	Foreign Reference	WO2013013292.pdf	4121573 e7ac9a87f2100b711b5e8ca0115c91a250d82049	no	50
Warnings:					
Information:					
29	Foreign Reference	WO2014110540.pdf	8038260 ce2af3ac9de162deba524dec5ca9d852cdd5c48b	no	165
Warnings:					
Information:					
30	Foreign Reference	WO2015127388.pdf	3169634 a2a234624d2a3c7ee027ead90440491ceca449b0	no	40
Warnings:					
Information:					
		Total Files Size (in bytes):	47546552		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/398,479	Filing Date 01/04/2017	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **07/14/2017**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
PAUL STANBACK

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

DocCode - SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 15398479 Document Date: 07/14/2017

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions below.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

Attorney Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed. Copies of a Communication from foreign Patent Offices in counterpart applications also are enclosed.

This statement is being filed after a first Office Action on the merits, but before receipt of a Final Office Action or a Notice of Allowance. A submission fee in the amount of $180 as required under 37 CFR § 1.17(p) is being paid with this statement via the Electronic Filing System (EFS). Please apply any other charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: /September 12, 2017/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61561030.doc

Sheet 1 of 1

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No.	Application No.
Information Disclosure Statement by Applicant (Use several sheets if necessary)		38767-0015002	15/398,479
		Applicant: Impossible Foods Inc.	
(37 CFR §1.98(b))		Filing Date: January 4, 2017	Group Art Unit: 1791

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	--	--	--	--			

Foreign Patent Documents or Published Foreign Patent Applications								
Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	1.	CN 101156632	4/9/2008	CHINA			English Abstract	
	2.	CN 101489422	7/22/2009	CHINA			English Abstract	
	3.	CN 101861895	10/20/2010	CHINA			English Abstract	
	4.	CN 101897418	12/1/2010	CHINA			English Abstract	
	5.	CN 102440302	5/9/2012	CHINA			English Abstract	
	6.	CN 102578544	7/18/2012	CHINA			English Abstract	
	7.	CN 102835460	12/26/2012	CHINA			English Abstract	
	8.	JP S53115846	10/9/1978	JAPAN			Machin. Translat.	
	9.	JP H08140627	6/4/1996	JAPAN				
	10.	JP 2012016336	1/26/2012	JAPAN				

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	11.	Naike, "Food Flavor Chemistry," 1st Edition China Light Industry Press, pages 236-243 (1996) (English Translation)
	12.	Office Action in Chinese Application No. 201480014349.9, dated July 24, 2017, 52 pages (English Translation)
	13.	Office Action in Chinese Application No. 201280041713.1, dated July 13, 2017, 28 pages (English Translation)
	14.	Office Action in Chinese Application No. 201480013778.4, dated August 08, 2017, 24 pages (English Translation)
	15.	Xiaoling, "Practical Technology and Quality Management of Deep Processing of Meat Products," China Textile & Apparel Press, pages 9 and 10 (English Translation), 10 pages

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

Espacenet

Bibliographic data: CN101156632 (A) — 2008-04-09

Processing method of remaking soybean cheese combinations

Inventor(s): RUIXIA GU [CN]; XIAOGANG XU [CN]; XU YANG [CN] ± (GU RUIXIA, ; XU XIAOGANG, ; YANG XU)

Applicant(s): UNIV YANGZHOU [CN] ± (YANGZHOU UNIVERSITY)

Classification: **- international:** ***A23C20/02; A23L11/00***
- cooperative:

Application number: CN20071134776 20071019

Priority number (s): CN20071134776 20071019

Also published as: CN101156632 (B)

Abstract of CN101156632 (A)

The invention provides a process method for remade soybean cheese products, belongs to the food processing filed, and relates to improved remade cheese made by soybean and the producing method thereof. Firstly, lactobacillus leaven is inoculated in the bean milk, and through ferment, the clabber is made into; clabber grains are obtained through compression; mucor spore liquid is inoculated, proper metal ion is added, after cultivation, sodium chloride is added in the clabber grains, and through compression, the soybean cheese is formed; the soybean cheese is positioned with 4-8 DEG C for maturing; butterine, butterfat, stabilizing salt, or flavoring are added in the soybean cheese, through thaw, concentration, compression, and figuration, the remade soybean cheese is produced.; The product has high nutrition value with low salt content, is easy to be stored, can satisfy the taste of the Chinese, and is cheap.

[19] 中华人民共和国国家知识产权局

[51] Int. Cl.
A23C 20/02 (2006.01)
A23L 1/20 (2006.01)

[12] 发明专利申请公布说明书

[21] 申请号 200710134776.2

[43] 公开日 2008 年 4 月 9 日

[11] 公开号 CN 101156632A

[22] 申请日 2007.10.19
[21] 申请号 200710134776.2
[71] 申请人 扬州大学
地址 225009 江苏省扬州市大学南路 88 号
[72] 发明人 顾瑞霞 许小刚 杨 旭

[74] 专利代理机构 扬州市锦江专利事务所
代理人 江 平

权利要求书 1 页 说明书 5 页

[54] 发明名称

再制大豆干酪制品的加工方法

[57] 摘要

再制大豆干酪制品的加工方法，属于食品加工领域，是涉及一种改良的利用大豆制备再制干酪及其制作方法。 先在豆乳中接种乳酸菌发酵剂，经发酵，制成凝乳；压榨获得凝乳粒；接种毛霉孢子液，并加入适量金属离子，经培养后，再在凝乳粒中加入氯化钠，经压榨形成大豆干酪；置于 4 ~ 8℃成熟；在大豆干酪中添加人造奶油、乳脂肪、稳定性盐或调味料，经融化、浓缩、压制、成形，制得再制大豆干酪。 产品营养价值高、易贮藏、盐含量低、符合中国人口味、廉价。

1、再制大豆干酪制品的加工方法，其特征在于包括以下步骤：

1）在豆乳中接种乳酸菌发酵剂，经发酵，制成凝乳；

2）将凝乳压榨获得凝乳粒；

3）在每 100g 大豆蛋白凝乳粒接种毛霉孢子液 2ml，置于 25～27℃条件下培养 30～45 小时；

4）再在凝乳粒中加入氯化钠，经压榨形成大豆干酪；

5）将成形的大豆干酪置于 4～8℃成熟 15～30 天；

6）在大豆干酪中添加人造奶油或乳脂肪或稳定性盐或调味料，经融化、浓缩、压制、成形，制得再制大豆干酪。

2、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于在豆乳中添加 0.8%～1.2%的乳清粉或 1.5～2.0%的脱脂乳粉。

3、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于步骤 1）中，当豆乳 pH 值为 4.55～4.62 时停止发酵。

4、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于步骤 2）中先将凝乳切割成小凝乳块，并加热到 50℃，保持 5～10min，然后将小凝乳块压榨，得到凝乳粒。

5、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于在步骤 3)中，还添加 Mg^{2+}0.8～1.2mmol·kg^{-1} 和 Ca^{2+}1.8～2.2mmol·kg^{-1} 和 Mn^{2+}0.8～1.2mmol·kg^{-1}。

6、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于在步骤 3）中，接种毛霉孢子液时凝乳粒酸度为 0.8%～1.1%。

7、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于在步骤 4）中，在每 100g 大豆蛋白凝乳粒加入氯化钠 0.5g。

8、根据权利要求 1 所述再制大豆干酪制品的加工方法，其特征在于在步骤 6）中，融化温度为 105～140℃。

再制大豆干酪制品的加工方法

技术领域

本发明属于食品加工领域，是涉及一种改良的利用大豆制备再制干酪及其制作方法。

技术背景

干酪是一种古老的乳制品，具有悠久的生产历史，含有丰富的蛋白质、脂肪、各种氨基酸，还含有糖类、有机酸、微量元素和矿物质。干酪中的蛋白质在发酵过程中，经过凝乳酶和微生物蛋白酶的作用，逐步被分解为肽、胨、氨基酸及其它小分子物质，这些小分子物质更容易被人体消化吸收，从而使干酪中的蛋白质的消化率达到 96%～98%。所以日常生活中增加干酪的摄取，对增强体质、保持健康极为有利。

目前国内干酪的生产量十分有限，仅有为数不多企业生产干酪产品，如光明已生产出“光明”系列干酪，法国独资的保健然（天津）公司已推出“百吉福”干酪等，市面上其余大多产品为进口品牌，且占据绝大部分的市场份额。目前我国干酪年需求总量仅为 5000～6000t 左右，人均不到 5g，与世界上的乳品消费大国相比有很大的差距。究其原因，一方面是由于现在人们能购买到的干酪大部分是进口产品，价格较高，不属于生活必须品的范畴；另一方面，我国人口大都集中在产粮地区，没有长期食用乳制品的习惯，国人很难接受干酪独特的口味。基于上述原因，国内的乳品工作者参考欧美等国家的先进生产技术，针对中国的市场需求和消费者的嗜好，进行了调整干酪风味和干酪品种开发的研究，以弥补上述空白。

大豆富含蛋白质，不仅具备人体必需氨基酸、维生素，而且不含胆固醇，对动脉硬化、脑溢血、心脏病、肥胖症等有疗效作用。研究表明，人们进食动物性食品过多，是造成导致心血管病、脑血管病、肥胖症、糖尿病等所谓“文明病”增加的原因之一，营养学家认为，增加植物性蛋白，特别是大豆

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

蛋白的食用量，是解决这一问题的一条行之有效的途径。此外大豆蛋白还能解除因重金属盐、生物碱等引起的中毒、降低血液中的低密度脂蛋白、减少身体内钙的损失等重要生理功能。

干酪是欧美人民喜食的食品之一。其生产量较大，而我国的腐乳有“东方干酪”之美称。但由于普通牛奶干酪牛奶消耗较大，价格高。在我国一直未能得到推广。而腐乳由于盐分含量高，仅作为佐餐食品。

由此可见利用大豆开发生产大豆干酪可以满足我国国情的要求。首先，大豆是我国的传统食品原料之一，营养丰富，来源广，产量大，成本低，利用大豆生产大豆干酪原料充裕，且具有成本优势；其二， 大豆食品是我国的传统食品，国人对其早已适应，且有一定嗜好，所以，利用大豆生产的大豆干酪的风味会比较容易被国人接受。

发明内容

本发明的目的是发明一种可廉价、低成本生产的再制大豆干酪制品的加工方法。

本发明包括以下步骤：

1）在豆乳中接种乳酸菌发酵剂，经发酵，制成凝乳；

2）将凝乳压榨获得凝乳粒；

3）在每 100g 大豆蛋白凝乳粒接种毛霉孢子液 2mL，并加入适量金属离子，置于 25～27℃条件下培养 30～45 小时；

4）再在凝乳粒中加入氯化钠，经压榨形成大豆干酪；

5）将成形的大豆干酪置于 4～8℃成熟 15～30 天；

6）在大豆干酪中添加人造奶油、乳脂肪、稳定性盐或调味料，经融化、浓缩、压制、成形，制得再制大豆干酪。

本发明以豆乳为原料，加工方法主要利用乳酸菌发酵豆乳凝乳后，利用霉菌产生的蛋白酶对大分子蛋白进行适度水解，提高其营养价值和贮藏特性，并改善质地和风味后，经加入辅料经融化后而制成的再制大豆干酪产品。经本发明生产方法制成的再制大豆干酪低盐含量、营养丰富、符合中国人口味。

Motif Exhibit 1002, Page 783 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

更主要的是其生产成本低，为实现廉价销售打下良好的基础。

本发明生产特点主要体现在：

1、利用乳酸菌凝乳，替代普通干酪生产用酶法凝乳，可降低生产成本。

2、不用腐乳生产中的凝固剂（如葡糖酸-δ-内酯）凝乳，而用乳酸菌发酵凝乳，可提高加工产品的营养价值和贮藏特性。

3、利用毛霉孢子液发酵凝乳粒，以提高加工产品的营养价值。

4、利用毛霉孢子液发酵及其分泌的蛋白酶成熟，可缩短干酪的成熟时间。

5、经压榨后的凝乳块的理化成分可控制在脂肪 15%～17%，蛋白质 18%～22%，总固形物在 40%～45%。

6、将成熟后的干酪添加人造奶油、乳脂肪、稳定性盐、调味料等，可提高再制大豆干酪制品的营养价值和口感。

因豆乳中不含乳酸菌生长所需的乳糖，为了促进乳酸菌的凝乳速度和效果，本发明还在大豆豆乳中添加 0.8%～1.2%的乳清粉或 1.6%～2%的脱脂乳粉。

为了使大豆蛋白充分凝固，使凝乳过程中蛋白质得到最大化保留，步骤 1）中，当豆乳 pH 值为 4.55～4.62 时停止发酵。

为彻底排出乳清，步骤 2）中先将凝乳切割成小凝乳块，并加热到 50℃，保持 5～10min，然后将小凝乳块压榨，得到大豆蛋白凝乳粒。

为了促进霉菌的生长和蛋白酶的分泌，在凝乳粒中添加 Mg^{2+}0.001mol·kg^{-1} 和 Ca^{2+}0.002mol·kg^{-1} 和 Mn^{2+}0.001mol·kg^{-1}。

控制接种毛霉孢子液时凝乳粒酸度为 0.8%～1.1%。目的是：使毛霉在凝乳粒生长过程中，更适于毛霉的生长与蛋白酶的产生，且蛋白酶活力最高，并控制腐败微生物的生长。

在每 100g 大豆蛋白凝乳粒中加入 0.5g 氯化钠进行压榨形成大豆干酪。可赋予制品良好口感，并具有抑制杂菌生长的作用。

将成熟的大豆干酪加入人造奶油、乳脂肪、稳定性盐及调味料后于 105～140℃下融化成型制得再制大豆干酪。选择合适的融化温度，可使大豆干酪充

Motif Exhibit 1002, Page 784 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

分融化，与辅料更好的融合，并达到进一步杀菌的目的。

具体实施方式

实施例 1

1、将大豆置于 100℃的烘箱中烘 20 分钟后，于三倍体积的 1%的 $NaHCO_3$ 溶液中浸泡 12 小时。将浸泡好的大豆洗净后以豆水重量比 1:7 的比例混合磨浆得到豆乳。

在 100kg 豆乳中加入 0.8～1.2kg 乳清粉或 1.6～2kg 脱脂乳粉。

将豆乳在 100℃杀菌 20 分钟。

2、待豆乳冷却到 40℃时，再在豆乳中加入 2～5kg 乳酸菌发酵剂（如：德氏乳杆菌保加利亚亚种和嗜热链球菌混合发酵剂），于 42℃发酵。经 2.3～3.5h，当豆乳 pH 为 4.55～4.62 时停止发酵。

3、将大豆凝乳切割成小凝乳块，加热到 50℃，保持 5～10min，促使其排出乳清，然后将得到的小凝乳块进行压榨以进一步排出乳清，得到大豆蛋白凝乳粒。

4、于每 100kg 大豆蛋白凝乳粒中接种毛霉孢子液 2 升。将凝乳粒置于 25～27℃条件下培养 30～45 小时。

5、取出发酵好的凝乳粒加入 0.5 kg NaCl，搅拌均匀后进行压榨，得到大豆干酪。将压榨好的大豆干酪放置于 4℃成熟 20 天。

6、加入 200 kg 水和 2.1 kg 柠檬酸钠，然后倒入经切割的干酪、人造奶油，然后加热至 105～140℃，搅拌，待干酪和奶油完全融化后，再徐徐加入奶粉以及其他配料，融化后的物料经胶体磨处理，将处理好的物料进行浓缩，加入柠檬酸，再经压制、成形，然后趁热无菌灌装。

实施例 2

1、按实施例 1 的方法所获得的灭好菌的豆乳。

2、将豆乳冷却到 40℃左右，加入 1%的乳清粉和 3%的乳酸菌发酵剂，在 42℃培养箱中发酵 3 小时，得到大豆凝乳。

3、将大豆凝乳划成小凝乳块，水浴加热到 50℃以促进豆乳凝乳中的乳

Motif Exhibit 1002, Page 785 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

清排除。将得到的小凝乳块进一步压榨得到大豆凝乳粒。

4、在大豆凝乳粒中添加 Mg^{2+}0.001mol·kg^{-1}、Ca^{2+}0.002mol·kg^{-1}、Mn^{2+}0.001mol·kg^{-1}。

可以添加的 Mg^{2+}可以是 $MgSO_4$ 或 $MgCl_2$；可以添加的 Ca^{2+}可以是 $CaCl_2$ 或 $CaCO_3$；可以添加的 Mn^{2+}可以是 $MnSO_4$。

然后于每 100Kg 大豆蛋白凝乳粒中接种毛霉孢子液 2L。将凝乳粒置于 26℃培养箱培养 36 小时。

5、取出发酵好的凝乳粒加入 0.5 kg NaCl，搅拌均匀后进行压榨，得到大豆干酪。将压榨好的大豆干酪放置于 4℃成熟 20 天。

6、加入 200 kg 水和 2.1 kg 柠檬酸钠，然后倒入经切割的干酪、奶油，然后加热至 140℃，搅拌，待干酪和奶油完全融化后，再徐徐加入奶粉以及其他配料（如食盐），融化后的物料经胶体磨处理，将处理好的物料进行浓缩，加入柠檬酸，再经压制、成形，趁热无菌灌装。

实施例 3

将用类似于实施例 2 的方法所获得的大豆凝乳粒，然后按 Mg^{2+}0.001 mol·kg^{-1}、Ca^{2+}0.002 mol·kg^{-1}、Mn^{2+}0.001mol·kg^{-1} 的比例加入 Mg^{2+}、Ca^{2+}和 Mn^{2+}，再按 2%的量接种毛霉孢子液。将凝乳粒置 26℃培养箱培养 36 小时，取出发酵好的凝乳粒加入 0.5%NaCl，搅拌均匀后进行压榨，得到大豆干酪。将压榨好的大豆干酪放置于 4℃成熟 20 天。

将成熟好的大豆干酪切碎，取 50kg 大豆干酪和 16kg 奶油，3kg 明胶，10kg 脱脂奶粉，1.6kg 柠檬酸钠，0.8kg 磷酸氢二钠，3kg 食盐，1kg 蔗糖以及 10kg 胡萝卜汁于融化锅中，加热至 110℃使其融化。将融化好的物料经胶体磨处理，使其乳化。将乳化好的物料进行浓缩，在接近浓缩终点时加入 0.3kg 的柠檬酸，趁热灌装。如此得到风味及口感另人满意的再制大豆干酪。

Motif Exhibit 1002, Page 786 of 1290

Espacenet

Bibliographic data: CN101489422 (A) — 2009-07-22

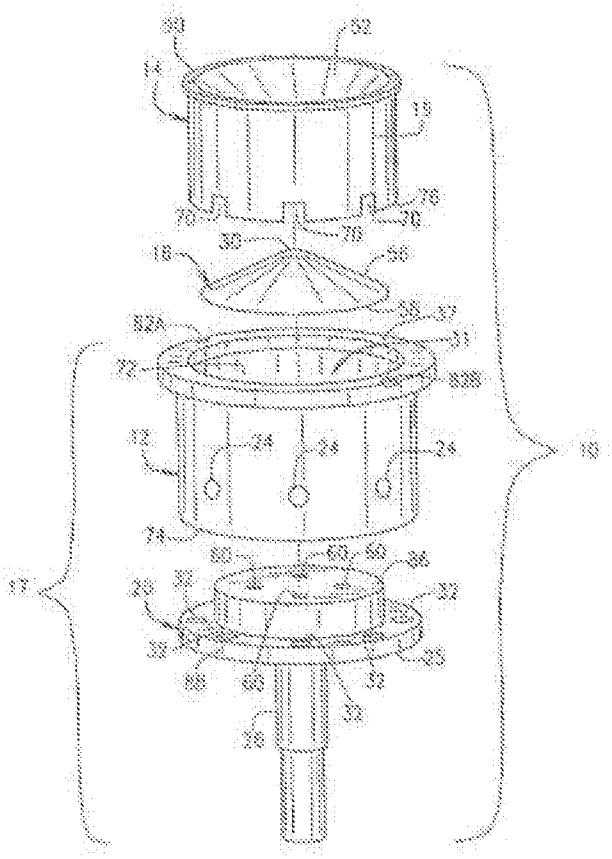
A protein composition and its use in restructured meat and food products

Inventor(s):	EDUARDO GODINEZ [US]; IZUMI MUELLER [US]; MAC ORCUTT [US] ± (GODINEZ EDUARDO, ; MUELLER IZUMI, ; ORCUTT MAC, ; ALTEMUELLER PATRICA A, ; MCMINDES MATTHEW K)
Applicant(s):	SOLAE LLC [US] ± (SOLAE LLC, ; SOLAE, LLC)
Classification:	**- international:** *A23L1/317* **- cooperative:** A23J3/16; A23J3/18; A23J3/227; A23L13/426; A23L13/52; A23L13/67; A23L19/09; A23P30/20
Application number:	CN2007827323 20070521
Priority number (s):	WO2007US69157 20070521 ; US20060437164 20060519 ; US20070749540 20070516
Also published as:	CN101489422 (B) AT490690 (T) BRPI0711209 (A2) CA2652380 (A1) CA2652380 (C) more

Abstract of CN101489422 (A)

The invention provides protein compositions containing structured protein products having protein fibers that are substantially aligned.

Case No. IPR2023-00206
U.S. Patent No. 9,943,096



[19] 中华人民共和国国家知识产权局

[51] Int. Cl.
A23L 1/317 (2006.01)
A23J 3/26 (2006.01)
A23J 3/14 (2006.01)

[12] 发明专利申请公布说明书

[21] 申请号 200780027323.8

[43] 公开日 2009 年 7 月 22 日

[11] 公开号 CN 101489422A

[22] 申请日 2007.5.21
[21] 申请号 200780027323.8
[30] 优先权
[32] 2006.5.19 [33] US [31] 11/437,164
[32] 2007.5.16 [33] US [31] 11/749,540
[86] 国际申请 PCT/US2007/069157 2007.5.21
[87] 国际公布 WO2007/137125 英 2007.11.29
[85] 进入国家阶段日期 2009.1.19
[71] 申请人 索莱有限责任公司
地址 美国密苏里州
[72] 发明人 M·K·麦克明德斯 E·戈迪尼茨
I·米勒 M·奥尔库特
P·A·阿尔特米勒
[74] 专利代理机构 中国专利代理(香港)有限公司
代理人 刘 冬 付 磊

权利要求书 5 页 说明书 48 页 附图 5 页

[54] 发明名称

蛋白组合物及其在重组肉制品和食品中的用途

[57] 摘要

本发明提供包含具有基本上对齐的蛋白纤维的结构化蛋白质制品的蛋白质组合物。

1. 用于制备重组肉组合物的方法，所述方法包括：通过压模装置在提高的温度和压力的条件下挤出植物蛋白材料，以形成具有基本上对齐的蛋白纤维的结构化植物蛋白制品。

2. 权利要求 1 的方法，其中所述压模装置包含：

a 模套，所述模套具有共同确定内室的后面部分和前面部分；

b 模具插入物，所述模具插入物被置于所述内室内，所述模具插入物包括具有前表面和后表面的插入体，所述前表面确定底下部份和具有渐缩流动通道的多个分流器，所述渐缩流动通道被确定在底下部份与多个分流器中的任何两个邻近分流器之间；和

c 模具锥体，所述模具锥体与所述模套配对，其中所述模具锥体和所述流动通道共同确定全渐缩的流动槽。

3. 权利要求 2 的方法，其中所述渐缩的流动通道沿三个侧面渐缩。

4. 权利要求 3 的方法，其中所述全渐缩的流动通道沿四个侧面渐缩。

5. 权利要求 4 的方法，其中所述全渐缩的流动槽在一端与入口和在其另一端与出口相通。

6. 权利要求 5 的方法，其中所述全渐缩的流动槽是从所述入口至所述出口的向内渐缩。

7. 权利要求 6 的方法，所述方法还包括使所述结构化植物蛋白制品与动物肉组合。

8. 权利要求 6 的方法，其中所述结构化植物蛋白制品具有至少 2000 克的平均剪切强度和至少 17%的平均碎片特征。

9. 权利要求 8 的方法，其中所述结构化植物蛋白制品包含以图 1 的显微照相图像所描绘的方式基本上对齐的蛋白纤维。

10. 权利要求 1 的方法，其中所述植物蛋白材料选自：英果、玉

米、豌豆、油菜、向日葵、高粱、水稻、苋菜、马铃薯、木薯粉、竹芋、油菜籽、羽扇豆、芸蓝、小麦、燕麦、黑麦、大麦及其混合物。

11. 权利要求 1 的方法，所述方法还包括在挤出之前使至少一种动物蛋白材料与所述植物蛋白材料组合，以产生包含基本上对齐的蛋白纤维的结构化植物蛋白制品。

12. 权利要求 11 的方法，其中所述动物蛋白材料选自：酪蛋白、酪蛋白酸盐、乳清蛋白、卵清蛋白、卵球蛋白、卵粘蛋白、卵类粘蛋白、卵运铁蛋白、ovovitella、卵黄磷蛋白、清蛋白球蛋白、和卵黄磷蛋白。

13. 权利要求 1 的方法，其中所述植物蛋白材料包括大豆蛋白和小麦蛋白。

14. 权利要求 13 的方法，所述植物蛋白材料还包括乳清蛋白。

15. 权利要求 13 的方法，其中以干物质计，所述植物蛋白材料具有约 40% - 约 75%的蛋白质。

16. 权利要求 15 的方法，其中所述植物蛋白材料包含蛋白质、淀粉、面筋、和纤维。

17. 权利要求 16 的方法，其中所述植物蛋白材料包含:

a 以干物质计，约 45% - 约 65%的大豆蛋白;

b 以干物质计，约 20% - 约 30%的小麦面筋;

c 以干物质计，约 10% - 约 15%的小麦淀粉; 和

d 以干物质计，约 1% - 约 5%的纤维。

18. 权利要求 16 的方法，其中所述植物蛋白材料还包含磷酸二钙和 L-半胱氨酸。

19. 权利要求 1 的方法，其中所述挤出温度为约 90°C - 约 150°C 而压力为约 500 psig - 约 1500 psig。

20. 权利要求 7 的方法，其中所述动物肉选自完整肌肉块、碎肉、机械去骨的肉、及它们的组合。

21. 权利要求 7 的方法，其中所述动物肉得自选自以下的动物:

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

猪、牛、羔羊、家禽、狩猎动物、和鱼。

22. 权利要求 1 的方法，其中所述重组肉组合物还包括一定量的水。

23. 权利要求 6 的方法，所述方法还包括使结构化植物蛋白制品与碎蔬菜组合。

24. 权利要求 6 的方法，所述方法还包括使结构化植物蛋白制品与碎水果组合。

25. 重组肉组合物，所述组合物包含具有基本上对齐的蛋白纤维的结构化蛋白质制品。

26. 权利要求 25 的重组肉组合物，所述组合物还包含动物肉。

27. 权利要求 26 的重组肉组合物，其中所述组合物包含约 40% - 约 60%重量的结构化蛋白质制品，和约 40% - 约 60%重量的肉。

28. 权利要求 27 的重组肉组合物，所述组合物还包含以组合物的重量计，约 10% - 约 20%的脂肪源。

29. 权利要求 28 的重组肉组合物，其中所述结构化蛋白质制品包含以图 1 的显微照相图像所描绘的方式基本上对齐的蛋白纤维。

30. 权利要求 28 的重组肉组合物，其中所述结构化蛋白质制品包含大豆蛋白、淀粉、面筋、和纤维。

31. 权利要求 28 的重组肉组合物，其中所述结构化蛋白质制品包含:

a 以干物质计，约 45% - 约 65%的大豆蛋白;

b 以干物质计，约 20% - 约 30%的小麦面筋;

c 以干物质计，约 10% - 约 15%的小麦淀粉;

d 以干物质计，约 1% - 约 5%的纤维。

32. 权利要求 30 的重组肉组合物，其中所述肉选自牛肉、猪肉、羔羊肉、火鸡肉、和鸡肉。

33. 权利要求 29 的重组肉组合物，所述重组肉组合物还包含着色组合物。

Motif Exhibit 1002, Page 792 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

34. 权利要求 33 的重组肉组合物，其中所述着色组合物包含甜菜、胭脂红、焦糖着色剂、和氨基酸源。

35. 权利要求 34 的重组肉组合物，所述重组肉组合物还包含经分离的大豆蛋白。

36. 权利要求 35 的重组肉组合物，所述重组肉组合物还包含抗氧化剂、水、调料和食用香料。

37. 权利要求 25 的重组肉组合物，所述重组肉组合物还包含碎蔬菜。

38. 权利要求 37 的重组肉组合物，其中所述组合物包含约 40% - 约 60%重量的结构化蛋白质制品，和约 40% - 约 60%重量的碎蔬菜。

39. 权利要求 38 的重组肉组合物，其中所述结构化蛋白质制品包含以图 1 的显微照相图像所描绘的方式基本上对齐的蛋白纤维。

40. 权利要求 38 的重组肉组合物，其中所述结构化蛋白质制品包含大豆蛋白、淀粉、面筋、和纤维。

41. 权利要求 38 的重组肉组合物，其中所述结构化蛋白质制品包含:

a 以干物质计，约 45% - 约 65%的大豆蛋白;

b 以干物质计，约 20% - 约 30%的小麦面筋;

c 以干物质计，约 10% - 约 15%的小麦淀粉;

d 以干物质计，约 1% - 约 5%的纤维。

42. 权利要求 40 的重组肉组合物，其中所述着色组合物包含甜菜、胭脂红、焦糖着色剂、和氨基酸源。

43. 权利要求 42 的重组肉组合物，所述重组肉组合物还包含经分离的大豆蛋白。

44. 权利要求 43 的重组肉组合物，所述重组肉组合物还包含抗氧化剂、水、调料和食用香料。

45. 权利要求 25 的重组肉组合物，所述重组肉组合物还包含碎水果。

Motif Exhibit 1002, Page 793 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

46. 权利要求 45 的重组肉组合物，其中所述组合物包含约 40% - 约 60%重量的结构化蛋白质制品，和约 40% - 约 60%重量的碎水果。

47. 权利要求 46 的重组肉组合物，其中所述结构化蛋白质制品包含以图 1 的显微照相图像所描绘的方式基本上对齐的蛋白纤维。

48. 权利要求 46 的重组肉组合物，其中所述结构化蛋白质制品包含大豆蛋白、淀粉、面筋、和纤维。

49. 权利要求 46 的重组肉组合物，其中所述结构化蛋白质制品包含:

a 以干物质计，约 45% - 约 65%的大豆蛋白;

b 以干物质计，约 20% - 约 30%的小麦面筋;

c 以干物质计，约 10% - 约 15%的小麦淀粉;

d 以干物质计，约 1% - 约 5%的纤维。

50. 权利要求 48 的重组肉组合物，其中所述着色组合物包含甜菜、胭脂红、焦糖着色剂、和氨基酸源。

51. 权利要求 50 的重组肉组合物，所述重组肉组合物还包含大豆分离蛋白。

52. 权利要求 51 的重组肉组合物，所述重组肉组合物还包含抗氧化剂、水、调料和食用香料。

53. 权利要求 25 的重组肉组合物，其中所述结构化蛋白质制品通过压模装置挤出，致使结构化蛋白质制品具有基本上对齐的蛋白纤维。

54. 权利要求 37 的重组肉组合物，其中所述结构化蛋白质制品通过压模装置挤出，致使结构化蛋白质制品具有基本上对齐的蛋白纤维。

55. 权利要求 45 的重组肉组合物，其中所述结构化蛋白质制品通过压模装置挤出，致使结构化蛋白质制品具有基本上对齐的蛋白纤维。

Motif Exhibit 1002, Page 794 of 1290

蛋白组合物及其在重组肉制品和食品中的用途

相关申请的交叉引用

[0001] 这是2006年5月19日提交的美国专利申请序号11/437,164的部分继续专利申请。

发明领域

[0002] 本发明提供蛋白组合物和蛋白质组合物在蔬菜制品、水果制品、和在重组肉制品中的用途。本发明还提供用于制备水合的和切碎的(shredded)蛋白质组合物的方法。

发明背景

[0003] 本发明的重要方面是非结构化蛋白质制品开发成结构化蛋白质制品。详细地讲，在一个实施方案中，本发明提供了制品和方法，其用于赋予非结构化蛋白质制品看不见的颗粒(grain)或质构(texture)并将非结构化蛋白质制品转换成具有与熟肌肉一致的明确形状的结构化蛋白质制品。

[0004] 术语"结构"描述食品的广泛多样的物理性质。产物的可接受结构通常与产物的品质同义。结构已被定义为"由物理性质的组合而引起的并通过触觉包括运动觉和口感、视觉、和听觉而感觉的物质属性"。结构，如International Organization of Standardization所定义的，是"可藉机械的、触觉的和，假如合适的话，视觉的和听觉的感受器而感觉的食品的所有流变学和结构(几何学和表面)属性"。以下术语已用于描述归入"结构"范围的制品特征:

表 I

食物结构形容词的简要列表

粘性的	肉质的	糊状的	柔软的
有弹力的	蓬松的	油滑的	湿润的
脆性的	泡沫状的	糊状的	冒气的(Sparkly)
起泡的	易碎的	塑性的	易裂的
耐嚼的	充实的	多孔的	海棉状的
粘着的	胶粘的	粉状的	多筋的
包衣的	粒状的	膨胀的	胶粘的
粘合的	砂样的	果肉状的	纤维质的
乳脂状的	树胶状的	味浓的	糖浆状的
松脆的	硬的	粗糙的	温和的
易碎的	重的	如橡皮般的	稠的
硬皮的	异类的	松软的	淡的
浓厚的	多汁的	沙质的	缠结的
柔软的	瘦的	不规则的	坚韧的
干的	清淡的	短的	均匀的
弹性的	柔软的	丝状的	粘稠的
脂肪状的	充满块状物的	滑的	水样的
牢固的	湿润的	如银的	蜡状的
片状的	涂嘴的	光滑的	蠕动的

[0005] 人们已经加速对结构的关注，因为结构属于较新的食品内容(substance)，其包括制品和仿制品、成形的肉制品和鱼制品，其中通过加工以复制原始食品或其它天然食品物质的性质而作出非常大的努力。非传统的原料、合成的食用香料、填充剂、伸张剂(stretchers)、膨胀剂的使用均趋向于改变成品的某些质构特征。通常，质构性质的仿制比复制味道、气味、和颜色更具难度。多种操作方法包括挤出结

构化已被开发以模拟天然结构性质。一般发现这些方法为了促进提早的市场接受而谨慎将原始物质的性质复制至技术上和经济上可行的程度。当结构具有与外观有关的属性时，它也具有与触觉有关的属性，并当它与口接触时也具有食物的口感或相互作用。通常，这些涉及咀嚼的感觉可以与期望的或不期望的观感有关。

[0006] 因此，结构术语包括与材料在应力或应变下的行为有关的术语，并包括，例如，以下的：牢固的、硬的、软的、韧的、温和的、耐嚼的、如橡皮的、弹性的、塑性的、粘性的、粘着的、发粘的、易碎的、发嘎吱嘎吱声的、等。第二，结构术语可与材料的结构有关：光滑的、精细的、粉状的、如白垩般的、充满块状物的、碎粉状的、粗糙的、砂样的、等。第三，结构术语可与结构元素的形状和排列有关，例如：片状的、纤维状的、多筋的、果肉状的、蜂窝状的、晶状的、玻璃状的、海棉状的、等。最后，结构术语可与口感特征有关，包括：口感、形体、干的、湿润的、湿的、水样的、蜡状的、粘糊糊的、糊状的、等。

[0007] 当用于本文时，"非结构化的"和"结构化的"描述如表 II 所述的食品特征：

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

表 II

	非结构化特征	结构化特征
在应力或应变下材料的行为	粘性的 胶粘的 塑性的	牢固的 耐嚼的
材料的结构	光滑的	粗糙的
结构元素的形状和排列	凝胶状的 果肉状的 糊状的	纤维状的 硬皮的
口感 糊状的 形体	乳脂状的 干的	湿润的

发明概述

[0008] 本发明的一个方面提供用于制备重组肉组合物的方法。该方法一般包括：通过压模装置在提高的温度和压力条件下挤出植物蛋白材料，以形成具有基本上对齐的(aligned)蛋白纤维的结构化植物蛋白制品。

[0009] 本发明的另一个方面提供重组肉组合物。该重组肉组合物包含具有基本上对齐的蛋白纤维的结构化蛋白质制品。

附图说明

[0010] 图 1 描绘显微照相的摄影图像，该图像显示本发明的具有基本上对齐的蛋白纤维的结构化植物蛋白制品。

Motif Exhibit 1002, Page 798 of 1290

[0011] 图 2 描绘显微照相的摄影图像，该图像显示不是由本发明的方法生产的植物蛋白制品。如本文所述，包含植物蛋白制品的蛋白纤维画有阴影交叉线。

[0012] 图 3 描绘外围压模装置的透视图，该压模装置可用于含材料的蛋白质的挤出方法。

[0013] 图 4 描绘图 3 的外围压模装置的分解图，该分解图显示模具插入物(die insert)、模套(die sleeve)和模具锥体(die cone)。

[0014] 图 5 描绘沿图 3 的线 9-9 的横截面图，该横截面面图显示被模套、模具插入物、和模具锥体排列之间确定的流动槽。

[0015] 图 5A 描绘图 5 的放大的横断面图，该横断面图显示流动通路与模套出口之间的界面。

发明详述

[0016] 本发明提供水合的和切碎的蛋白质组合物和用于生产每一组合物的方法。典型地，该蛋白质组合物包含动物肉和具有基本上对齐的蛋白纤维的结构化蛋白质制品。备选地，该蛋白质组合物包含碎蔬菜或碎水果和具有基本上对齐的蛋白纤维的结构化蛋白质制品。

(I) 结构化蛋白质制品

[0017] 本发明的蛋白质组合物各包含具有基本上对齐的蛋白纤维的结构化蛋白质制品，如下面I(e)中更详细描述。在例示性实施方案中，该结构化蛋白质制品为已经受在以下I(d)中详述的挤出方法的蛋白质材料的挤出物。因为该结构化蛋白质制品以类似于动物肉的方式具有基本上对齐的蛋白纤维，所以本发明的蛋白质组合物一般具有100%动物肉组成的组合物的质构和食用品质特征。

(a) 含蛋白质的材料

[0018] 可在热塑挤出方法中使用包含蛋白质的各种成分以生产适用于本文的蛋白质组合物的结构化蛋白质制品。虽然通常使用包含得自

植物的蛋白质的成分，但也考虑在没有背离本发明领域前提下，利用得自其它源例如动物源的蛋白质。例如，选自酪蛋白、酪蛋白酸盐、乳清蛋白及其混合物的乳酪蛋白可被使用。在例示性实施方案中，该乳酪蛋白(dairy protein)为乳清蛋白。通过另一实施例，可使用选自卵清蛋白、卵球蛋白、卵粘蛋白、卵类粘蛋白、卵运铁蛋白、ovovitella、卵黄磷蛋白(ovovitellin)、清蛋白球蛋白和卵黄磷蛋白(vitellin)的卵蛋白。此外，可包括由胶原、血、器官肉、器官肉(organ meat)、机械分离的肉、部分脱脂的组织和血清蛋白质组成的肉蛋白质或蛋白质成分，作为结构化蛋白质制品的一种或多种成分。

[0019] 所考虑的是，可使用除蛋白质之外的其它成分类型。这样的成分的非限制性实施例包括糖、淀粉、寡糖、大豆纤维和其它膳食纤维。

[0020] 还考虑的是，包含蛋白质的材料可不含面筋。因为面筋通常用于挤出过程期间的纤丝形成，如果使用不含面筋的原材料，可使用食用交联剂以促进纤丝形成。合适的交联剂的非限制性实施例包括Konjac葡甘露聚糖(KGM)面粉、β 1,3葡聚糖(由Takeda-Kirin Foods生产的Pureglucan™或Curdlan)、转谷氨酰胺酶、钙盐和镁盐。即便在不含面筋的实施方案中需要，本领域技术人员可容易地确定必需的交联材料的量。

[0021] 不管其来源或成分分类，在挤出过程中使用的成分通常能够形成具有基本上对齐的蛋白纤维的挤出物。在下面更充分地详述这样的成分的合适的实例。

(i) 植物蛋白材料

[0022] 在例示性实施方案中，将使用至少一种得自植物的成分以形成包含蛋白质的材料。一般而言，该成分包含蛋白质。存在于所使用成分中的蛋白质的量可以和将会根据应用而改变。例如，存在于所使用成分中的蛋白质的量可为约40%－约100%重量。在另一实施方案中，存在于所使用成分中的蛋白质的量可为约50%－约100%重量。在另一

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

实施方案中，存在于所使用成分中的蛋白质的量可为约60%－约100%重量。在又一实施方案中，存在于所使用成分中的蛋白质的量可为约70%－约100%重量。在再一实施方案中，存在于所使用成分中的蛋白质的量可为约80%－约100%重量。在再一实施方案中，存在于所使用成分中的蛋白质的量可为约90%－约100%重量。

[0023] 在挤出物中使用的成分可源自各种合适的植物。通过非限制性实施例，合适的植物包括荚果、玉米、豌豆、油菜、向日葵、高粱、水稻、苋菜、马铃薯、木薯粉、竹芋、油菜籽(canola)、羽扇豆、芸蓝、小麦、燕麦、黑麦、大麦及其混合物。

[0024] 在一个实施方案中，从小麦和大豆中分离该成分。在另一例示性实施方案中，从大豆中分离该成分。合适的得自小麦的包含蛋白质的成分包括小麦面筋、小麦粉及其混合物。可用于本发明的市售小麦面筋的实例包括Gem of the Star Gluten、Vital Wheat Gluten (organic)，每一种都可得自Manildra Milling。合适的得自大豆的包含蛋白质的成分("大豆蛋白材料")包括大豆分离蛋白、大豆浓缩蛋白、大豆粉及其混合物，每一种都在下面详述。合适的得自玉米的包含蛋白质的成分包括玉米面筋粉，例如，玉米醇溶蛋白。在每一个上述实施方案中，大豆材料可与选自淀粉、面粉、面筋、膳食纤维及其混合物中的一种或多种成分结合。

[0025] 在表III中详述分离自各种来源的含蛋白材料的合适实例，其显示各种组合物。

表 III 蛋白质组合物

第一蛋白源	第二成分
大豆	肉
大豆	乳
大豆	蛋
大豆	玉米
大豆	水稻

Motif Exhibit 1002, Page 801 of 1290

大豆	大麦
大豆	高粱
大豆	燕麦
大豆	粟
大豆	黑麦
大豆	黑小麦
大豆	荞麦
大豆	豌豆
大豆	花生
大豆	兵豆
大豆	羽扇豆
大豆	Channa (garbonzo)
大豆	油菜籽(canola)
大豆	木薯
大豆	向日葵
大豆	乳清
大豆	木薯粉
大豆	竹芋
大豆	苋菜
大豆	小麦和乳
大豆	小麦和蛋
大豆	小麦和玉米
大豆	小麦和水稻
大豆	小麦和大麦
大豆	小麦和高粱
大豆	小麦和燕麦
大豆	小麦和粟

大豆	小麦和黑麦
大豆	小麦和黑小麦
大豆	小麦和荞麦
大豆	小麦和豌豆
大豆	小麦和花生
大豆	小麦和兵豆
大豆	小麦和羽扇豆
大豆	小麦和 Channa (garbonzo)
大豆	小麦和油菜籽(canola)
大豆	小麦和木薯
大豆	小麦和向日葵
大豆	小麦和马铃薯
大豆	小麦和木薯粉
大豆	小麦和竹芋
大豆	小麦和苋菜
大豆	玉米和小麦
大豆	玉米和乳
大豆	玉米和蛋
大豆	玉米和水稻
大豆	玉米和大麦
大豆	玉米和高粱
大豆	玉米和燕麦
大豆	玉米和粟
大豆	玉米和黑麦
大豆	玉米和黑小麦
大豆	玉米和荞麦
大豆	玉米和豌豆

大豆	玉米和花生
大豆	玉米和兵豆
大豆	玉米和羽扇豆
大豆	玉米和 Channa (garbonzo)
大豆	玉米和油菜籽(canola)
大豆	玉米和木薯
大豆	玉米和向日葵
大豆	玉米和马铃薯
大豆	玉米和木薯粉
大豆	玉米和竹芋
大豆	玉米和苋菜

[0026] 在表III描述的每一个实施方案中，含蛋白质材料的组合物可与选自淀粉、面粉、面筋、膳食纤维及其混合物的一种或多种成分组合。在一个实施方案中，该含蛋白质材料包含蛋白质、淀粉、面筋、和纤维。在例示性实施方案中，该含蛋白质材料包含以干物质计约45%－约65%的大豆蛋白；以干物质计，约20%－约30%的小麦面筋；以干物质计，约10%－约15%的小麦淀粉；以干物质计，约1%－约5%的纤维。在上述的每一个实施方案中，该含蛋白质材料可包含磷酸二钙、L－半胱氨酸或磷酸二钙和L－半胱氨酸的组合物。

(ii) 大豆蛋白材料

[0027] 在例示性实施方案中，如上面详述，在挤出方法中可使用大豆分离蛋白、大豆浓缩蛋白、大豆粉及其混合物。可依照本领域一般已知的方法从整个大豆(whole soybean)获得这些大豆蛋白材料。整体大豆可为标准大豆(即，非基因修饰大豆)、商品化大豆、基因修饰大豆及其组合物。

[0028] 一般而言，当使用大豆分离物时，分离物优选为非高度水解的大豆分离蛋白。然而，在某些实施方案中，高度水解的大豆分离蛋白

可以与其他大豆分离蛋白结合使用，前提是前者在结合后的大豆分离蛋白中的含量一般少于约40%重量。另外，所使用的大豆分离蛋白优选具有足够的乳化强度和凝胶强度以使分离物中的蛋白质能够形成在挤出时基本上对齐的纤维。用于本发明的大豆分离蛋白的实例可市购自，例如，Solae, LLC (St. Louis, Mo.)，并包括SUPRO® 500E、SUPRO® EX 33、SUPRO® 620、SUPRO® 630、和SUPRO® 545。在例示性实施方案中，使用SUPRO® 620的形式，如实施例3所详述。

[0029] 备选地，可将大豆浓缩蛋白与大豆分离蛋白共混以代替部分的大豆分离蛋白，作为大豆蛋白材料来源。一般来说，如果用大豆浓缩蛋白代替部分的大豆分离蛋白，那么用大豆浓缩蛋白代替至多达约40%重量的大豆分离蛋白，更有选地代替达约30%重量的大豆分离蛋白。用于本发明的合适的大豆浓缩蛋白的实例包括市购自Solae, LLC (St. Louis, Mo.)的Promine DSPC、Procon、Alpha 12和Alpha 5800。

[0030] 可任选地使用大豆子叶纤维作为纤维源。一般来说，当共挤出大豆蛋白和大豆子叶纤维的混合物时，合适的大豆子叶纤维一般有效地与水结合。本文中，"有效地与水结合"一般指大豆子叶纤维具有至少 5.0 至约 8.0 克水/克大豆子叶纤维的持水能力，优选地大豆子叶纤维具有至少约 6.0 至约 8.0 克水/克大豆子叶纤维的持水能力。大豆子叶纤维存在于大豆蛋白材料中的量一般可为以无水重量计约 1% - 约 20%，优选约 1.5% - 约 20%，最优选约 2% - 约 5%。可市购合适的大豆子叶纤维。例如，FIBRIM® 1260 和 FIBRIM® 2000 为市购自 Solae, LLC (St. Louis, Mo.)的大豆子叶纤维材料。

(b)添加的成份

[0031] 各种添加成分在不背离本发明的范围的情况下可添加至上面详述的任何含蛋白质材料。例如，可包括抗氧化剂、抗微生物剂及其组合物。 抗氧化添加剂包括BHA、BHT、TBHQ、维生素A、C和E及其衍生物。另外，可包括各种植物提取物例如包含具有抗氧化剂性

质的类胡萝卜素、生育酚或黄酮类化合物，以增加贮藏期限或增加该蛋白质组合物营养。抗氧化剂和抗微生物剂可以以含蛋白质材料的0.01%－约10%重量，优选约0.05%－约5%重量，更优选约0.1%－约2%重量水平的组合形式存在。

(c) 含水量

[0032] 如本领域技术人员所理解，含蛋白质材料和任选的附加成分的含水量可以并将会改变。水的目的为使蛋白质组合物的成分水合。一般而言，含水量可为约1%－约80%重量。在低水分的挤出应用中，含蛋白质材料的含水量可为约1%－约35%重量。备选地，在高水分的挤出应用中，含蛋白质材料的含水量可为约35%－约80%重量。在例示性实施方案中，用来形成挤出物的挤出应用为低水分的。在I(d)和实施例3中详述了产生挤出物的低水分挤出方法的例示性实例，该挤出物具有含基本上对齐的纤维的蛋白质。

(d) 含蛋白质材料的挤出

[0033] 用于制备结构化蛋白质制品的合适的挤出过程包括将蛋白质材料和其它成分引入混合器(即，成分搅拌器)，以使这些成分结合并形成干的共混蛋白质材料预混物。可将干的共混蛋白质材料预混物传输至料斗(hopper)，将干的共混成分从该料斗与水分一起引入预调料槽(pre－conditioner)以形成经调匀的蛋白质材料混合物。 然后将经调匀的材料装入挤压机，在挤压机的螺杆(screw)产生的机械压力下加热混合物以形成熔体挤出块。备选地，可将干的共混蛋白质材料预混物直接装入挤压机，其中引入水分并加热以形成熔体挤出块。熔体挤出块穿过挤出压模装置离开挤压机，形成包含具有基本上对齐的蛋白纤维的结构化蛋白质制品的挤出物。

(i) 挤出过程的条件

[0034] 用于本发明实践的合适的挤出设备为双筒、双螺杆挤压机，例

如，如美国专利第4,600,311号所详述。合适的市售挤出设备的更多实例包括Clextral，Inc. (Tampa, Florida)生产的CLEXTRAL Model BC-72挤压机；全部由Wenger Manufacturing，Inc. (Sabetha, Kansas)生产的WENGER Model TX-57挤压机、WENGER Model TX-168挤压机、和WENGER Model TX-52挤压机。例如，在美国专利第4,763,569、4,118,164和3,117,006号中描述适用于本发明的其它常规挤压机，该专利通过整体引用结合至本文中。

[0035] 双螺杆挤压机的螺杆可以相同或相反的方向在筒内旋转。以相同方向的螺杆的旋转称为单流，而以相反方向的螺杆的旋转称为双流。挤压机的单螺杆或双螺杆的速度可根据特定的设备改变；然而，它通常为约250－约400转/分(rpm)。一般地，当螺杆速度增加时，挤出物的密度减少。挤出设备包含由轴和蜗杆部分组装的螺杆、以及如挤出设备制造商所推荐用于挤出植物蛋白材料的混合叶片(mixing lobe)和环型剪切锁死(shearlock)元件。

[0036] 挤出设备一般包含多个温度控制区，在通过挤出压模装置离开挤出设备之前，在机械压力下将蛋白质混合物传输经过这些温度控制区。每一连续加热区中的温度一般比前一加热区的温度高约10°C和约70°C。在一个实施方案中，将经调匀的预混物传输通过挤出设备内的四个加热区，使蛋白质混合物加热至约100°C－约150°C的温度，致使熔体挤出块在约100°C－约150°C的温度下进入挤出压模装置。

[0037] 筒压力取决于许多因素，这些因素包括，例如，挤压机螺杆速度、筒的混合物进料速度、筒的水进料速度和筒内熔体的粘度。

[0038] 可将水注入至挤压机筒以使植物蛋白材料混合物水合并促进蛋白质的质构化。作为形成熔体挤出块的辅助，水可充当增塑剂。可通过与加热区相通的一个或多个注射喷嘴将水引入挤压机筒。一般来说，筒中的混合物包含约1%－约30%重量的水。在一个实施方案中，筒中的混合物包含约5%－约20%重量的水。一般地，控制将水引入至任一加热区的速率以促进具有所需特征的挤出物的产生。已经注意

到，随着水引入筒的速率减少，挤出物的密度减少。

(ii) 任选的预调匀

[0039] 在预调料器中，将含蛋白质材料和任选的添加成分(包含蛋白质的混合物)预热，与水分接触，在温度和压力条件下保持，以允许水分渗入和软化单一颗粒。预调料步骤增加微粒纤维状材料混合物的体积密度，改善它的流动特性。预调料器包含一个或多个桨以促进蛋白质的均匀混合和蛋白质混合物通过预调料器的传输。桨的构型和转速广泛地改变，视预调料器的容量、挤压机吞吐量和/或预调料器或混合物在挤压机筒中的期望滞留时间而定。一般而言，桨的速度为约500－约1300转/分(rpm)。

[0040] 通常来说，在引入挤出设备之前通过将预混物与水分(即，蒸汽和/或水)接触来预调匀包含蛋白质的混合物。优选地，在预调料器中将包含蛋白质的混合物加热至约20°C－约60°C的温度，更优选地约30°C－约45°C。

[0041] 典型地，将包含蛋白质的预混物进行约0.5分钟－约10.0分钟时间的调料，视预调料器的速度和尺寸而定。在示范性实施方案中，将包含蛋白质的预混物进行约3.0分钟－约5.0分钟时间的调料。在预调料器中一般以恒定蒸汽流使预混物与蒸汽和/或水接触并加热，以达到所需温度。在引入至将蛋白质质构化的挤压机筒之前，水和/或蒸汽调匀了(即，水合)预混物，增加它的密度，无干扰地促进干混合物的流动性。如果需要低水分的预混物，那么经调匀的预混物可包含约1%－约35%(重量)的水。如果需要高水分的预混物，那么经调匀的预混物可包含约35%－约80%(重量)的水。

[0042] 经调匀的预混物的体积密度通常为约0.25 g/cm^3－约0.60 g/cm^3。一般来说，随着预调料的蛋白质混合物的体积密度在该范围内增加，蛋白质混合物则更容易加工。目前认为这归因于这样的混合物占据挤压机的螺杆之间的全部或大部分空间，从而促进挤出块传输通

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

过筒。

(iii) 挤出方法

[0043] 然后将干预混物或经调匀的预混物装入挤压机以加热，剪切，最终增塑混合物。挤压机可选自任何市售挤压机，可为单螺杆挤压机或优选地双螺杆挤压机，该挤压机用螺杆元件机械地剪切混合物。

[0044] 预混物一般引入挤出设备的速率随特定设备而改变。一般来说，以至多约25千克/分钟的速率引入预混物。一般来说，已注意到，随着预混物进料至挤压机速率的增加，挤出物的密度减少。

[0045] 预混物经受挤压机的剪切和压力以增塑该混合物。在挤压机中，挤压机的螺杆元件通过迫使混合物向前通过挤压机和通过压模装置来剪切混合物同时产生压力。螺杆马达速度决定螺杆施加至混合物的剪切力和压力的量。优选地，螺杆马达速度设定至约200 rpm－约500 rpm，更优选约300 rpm－约400 rpm的速度，该速度以至少约20千克/小时和更优选至少约40千克/小时的速率推动混合物通过挤压机。优选地，挤压机产生约500－约1500 psig的挤压机筒出口压力，更优选地产生约600－约1000 psig的挤压机筒出口压力。

[0046] 当混合物经过挤压机时，挤压机加热混合物使混合物中的蛋白质变性。挤压机包括用于加热和/或冷却混合物至约100°C－约180°C的温度的装置。优选地，用于加热或冷却挤压机中的混合物的设备包含挤压机筒外壳，可将加热或冷却介质如蒸汽或水引入该外壳以控制经过挤压机的混合物的温度。挤压机也可包括用于将蒸汽直接注射进挤压机内的混合物中的蒸汽注射口。挤压机优选地包括可受控至独立温度的多个加热区，其中加热区的温度优选地设定至随着混合物经过挤压机前进而增加混合物的温度。在一个实施方案中，可以四个温度区排列设定挤压机，其中第一区域(邻近挤压机入口)设定至约80°C－约100°C的温度，第二区域设定至约100°C－ 135°C的温度，第三区域设定至135°C－约150°C的温度，第四区域(邻近挤压机出口)设定至

Motif Exhibit 1002, Page 809 of 1290

150°C－180°C的温度。可根据需要以其它温度区排列设定挤压机。在另一实施方案中，可以五个温度区排列设定挤压机，其中第一区域设定至约25°C的温度，第二区域设定至约50°C的温度，第三区域设定至约95°C的温度，第四区域设定至约130°C的温度，第五区域设定至约150°C的温度。在又一实施方案中，可以六个温度区排列设定挤压机，其中第一区域设定至约90°C的温度，第二区域设定至约100°C的温度，第三区域设定至105°C的温度，第四区域设定至约100°C的温度，第五区域设定至约120°C的温度，第六区域设定至约130°C的温度。

[0047] 混合物在挤压机中形成熔化增塑块。压模装置以一定的排列连接至挤压机，该排列允许增塑混合物从挤压机出口流入压模装置，并当增塑混合物流过压模装置时在经增塑的混合物内产生基本上对齐的蛋白纤维。压模装置可包括面板模具或外围模具。刀速设定至使碎片的尺寸至多5%通过16目筛，至多65%在1/2 -英寸筛上。

[0048] 一个实施方案包括外围压模装置，如图3－5中阐述，一般标记为10。

[0049] 如图3和图4所示，外围压模装置10可包括具有圆柱形两部分外壳模体17的模套12。外壳模体17可包括与前面部分20连接的后面部分18，共同定义与相对的开口72、74相通的内室31。模套12可适于容纳模具插入物14和模具锥体16，用于提供必要的结构元件以在挤出过程期间促进塑化混合物的层流通过外围压模装置10。

[0050] 另外，在外围压模装置10的组装期间，当使前面部分20固定至模套12的后面部分18时，可使模套12的前面部分20固定至适于与具嵌入物14面接的模具锥体16。如进一步显示，模套12的后面部分18确定沿外壳体17的多个圆形出口24，该出口适于在挤出过程期间为挤出物从外围压模装置10挤出提供导管。备选地，多个出口24可具有不同的构型，如正方形、长方形、扇形或不规则的。如进一步显示，模套12的后面部分18可包括环绕开口72的圆形凸缘37，该圆形凸缘确定一对相对的槽82A和82B，当将模套12接合至挤压机时，这些槽用于正确地

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

使模套12对准。

[0051] 如图5所示，当完全组装外围压模装置10时，模具插入物14置于模套12的后面部分18内，后面部分18固定至模套12的前面部分20，致使模具锥体16的锥形侧面56面向室31并和包在后面部分18和前面20之间。在该方向上，锥形侧面56可操作地联合模具插入物14的前表面27。因此，每一个相邻分流器38的对侧墙50、模具插入物14的底部64、和模具锥体16的锥形侧面56共同确定与相应出口24相通的单独流动槽40。如上所述在模套12、模具插入物14和模具锥体16之间定义的流动槽40在其全部四个侧面上都可为锥形的。因此，从每一流动槽40的入口84至出口24，流动槽40在全部四个侧面上逐渐地向内形成锥体。

[0052] 提及图5A，该图显示阐述通过流动槽40的流动途径"A"的放大图。具体地说，流动槽40通过模具插入物14所定义的开口70与出口24连通。

[0053] 在挤出过程期间，外围压模装置10可操作地与挤压机接合，产生增塑混合物，该混合物接触由模具插入物14的后表面29所确定的凹井52，流入狭口34，进入内部空间开口36，如流动途径"A"所标明。增塑混合物可进入由模具插入物14定义的内部空间44，并进入每一渐缩流动槽42的入口84。然后增塑混合物以以下方式流过每一流动槽42，从各个出口24流出，该方式在外围压模装置10产生的挤出物中形成基本上对齐的蛋白纤维。

[0054] 选择出口24的宽度和高度尺寸，在混合物的挤出之前设定以提供具有所需尺寸的纤维状的材料挤出物。可设定出口24的宽度，致使挤出物类似于从立方肉块到肉片，其中加大出口24的宽度减少挤出物的方块状性质且增加挤出物的片状性质。在示范性实施方案中，出口24的宽度可设定至约10毫米－约40毫米的宽度。

[0055] 可设定出口24的高度尺寸以提供所需挤出物厚度。可设定出口24的高度以提供很薄的挤出物或厚的挤出物。例如，可将出口24的高

Motif Exhibit 1002, Page 811 of 1290

度设定至约1毫米－约30毫米。在示范性实施方案中，可将出口24的高度设定至约8毫米－约16毫米。

[0056] 还考虑出口24可为圆的。可设定出口24的直径(diameter)以提供期望的挤出物厚度。可设定出口24的直径以提供很薄的挤出物或厚的挤出物。例如，可将出口24的直径设定至约1毫米－约30毫米。在示范性实施方案中，可将出口24的直径设定至约8毫米－约16毫米。

[0057] 在美国专利申请第60/882,662号中描述适用于本发明的其它外围模具装置，该专利通过整体引用结合到本文中。

[0058] 在离开压模装置之后切割挤出物。用于切割挤出物的合适的装置包括由Wenger Manufacturing, Inc.(Sabetha, Kansas)和Clextral, Inc.(Tampa, Florida)生产的柔韧的刀。典型地，切割装置的速度为约1000 rpm－约2500 rpm。在示范性实施方案中，切割装置的速度为约1600 rpm。

[0059] 干燥器，如果使用一个，通常包含多个干燥区，其中空气温度可改变。一般来说，一个或多个区域内的空气温度为约100°C－约185°C。典型地，挤出物在干燥器中存在足够的时间以提供具有所需湿度的挤出物。通常将挤出物干燥至少约5分钟，更通常至少约10分钟。备选地，可将挤出物在较低温度如约70°C下干燥较长时间。合适的干燥器包括Wolverine Proctor & Schwartz (Merrimac, Mass)、National Drying Machinery Co. (Philadelphia, Pa.)、Wenger (Sabetha, Kans.)、Clextral (Tampa, FIa.)和Buehler (Lake Bluff, I11.) 制造的那些干燥器。

[0060] 所需的水分可根据挤出物的预定应用而广泛改变。一般而言，挤出材料具有约6%－约13%重量的水分，如果干燥，该挤出材料需要在水中水合直至水被吸收和纤维被分离。如果蛋白质材料不干燥或不完全干燥，那么它的含水量较高，一般为约16%－约30%重量。

[0061] 还可将干燥后的挤出物进一步捣碎以降低挤出物的平均粒度。合适的磨具包括锤式粉碎机如Hosokawa Micron Ltd.(英国)制造的Mikro Hammer Mills。

(e) 结构化蛋白质制品的特征

[0062] 在I(d)中产生的挤出物典型地包含具有基本上对齐的蛋白纤维的结构化蛋白质制品。在本发明的上下文中，"基本上对齐的"一般指蛋白纤维的排列，致使当水平面观察时，非常高百分比的形成结构化蛋白质制品的蛋白纤维以小于约45°角彼此邻近。典型地，平均至少55%的包含结构化蛋白质制品的蛋白纤维为基本上对齐的。在另一实施方案中，平均至少60%的包含结构化蛋白质制品的蛋白纤维为基本上对齐的。在另一实施方案中，平均至少70%的包含结构化蛋白质制品的蛋白纤维为基本上对齐的。在另一实施方案中，平均至少80%的包含结构化蛋白质制品的蛋白纤维为基本上对齐的。在还另一实施方案中，平均至少90%的包含结构化蛋白质制品的蛋白纤维为基本上对齐的。用于测定蛋白纤维对齐程度的方法为本领域已知，该方法包括基于显微照相图像的目测。

[0063] 通过实施例，**图1和图2**描述的显微照相图像阐述具有基本上对齐的蛋白纤维的结构化蛋白质制品与具有显著的交叉影线的蛋白纤维的蛋白质产物相比之间的差异。**图1**描述依照I(a) - I(d)制备的具有基本上对齐的蛋白纤维的结构化蛋白质制品。相比之下，**图2**描述包含有显著交叉影线而没有基本上对齐的蛋白纤维的蛋白质产物。因为蛋白纤维为基本上对齐的，如**图1**所示，所以用于本发明的结构化蛋白质制品一般具有熟肌肉的质构和一致性。相反，具有随机取向或交叉影线的蛋白纤维的挤出物一般具有软的或海棉状的质构。

[0064] 除具有基本上对齐的蛋白纤维之外，该结构化蛋白质制品通常还具有基本上类似于整块肌肉的剪切强度。在本发明的上下文中，术语"剪切强度"提供量化充分纤维状的网络的形成的一种方法，该网络的形成赋予结构化蛋白质制品整体-肌肉样质构和外观。剪切强度为切削给定样品所需要的以克计算的最大力。在实施例1中描述用于测定剪切强度的方法。

[0065] 一般而言，本发明的结构化蛋白质制品具有至少 1400 克的平均剪切强度。在另一实施方案中，所述结构化蛋白质制品具有约 1500 - 约 1800 克的平均剪切强度。在又一实施方案中，结构化蛋白质制品具有约 1800 - 约 2000 克的平均剪切强度。在再一实施方案中，结构化蛋白质制品具有约 2000 - 约 2600 克的平均剪切强度。在再一实施方案中，结构化蛋白质制品具有至少 2200 克的平均剪切强度。在再一实施方案中，结构化蛋白质制品具有至少 2300 克的平均剪切强度。在又另一实施方案中，结构化蛋白质制品具有至少 2400 克的平均剪切强度。在还再一实施方案中，结构化蛋白质制品具有至少 2500 克的平均剪切强度。在再一实施方案中，结构化蛋白质制品具有至少 2600 克的平均剪切强度。

[0066] 可通过碎片特征试验进行量化在结构化蛋白质制品中形成的蛋白纤维尺寸的方法。碎片特征为一般确定在结构化蛋白质制品中形成的大碎片的百分比的试验。碎片特征的百分比以间接的方式提供附加方法以量化在结构化蛋白质制品中的蛋白纤维对齐程度。一般而言，当大碎片的百分比增加时，在结构化蛋白质制品内的蛋白纤维的对齐程度通常也增加。相反地，当大碎片的百分比减少时，在结构化蛋白质制品内的蛋白纤维的对齐程度也典型地减少。

[0067] 在实施例2中详述用于确定碎片特征的方法。本发明的结构化蛋白质制品通常具有至少10%重量大碎片的平均碎片特征。在又一实施方案中，该结构化蛋白质制品具有约10% - 约15%重量大碎片的平均碎片特征。在另一实施方案中，该结构化蛋白质制品具有约15% - 约20%重量大碎片的平均碎片特征。在又一实施方案中，该结构化蛋白质制品具有约20% - 约25%重量大碎片的平均碎片特征。在另一实施方案中，平均碎片特征为至少20%重量，至少21%重量，至少22%重量，至少23%重量，至少24%重量，至少25%重量，或至少26%重量大碎片。

[0068] 本发明的合适的结构化蛋白质制品通常具有基本上对齐的蛋

白纤维，具有至少1400克的平均剪切强度，和具有至少10%重量大碎片的平均碎片特征。更典型地，该结构化蛋白质制品具有至少55%对齐的蛋白纤维，具有至少1800克的平均剪切强度，和具有至少15%重量大碎片的平均碎片特征。在示范性实施方案中，该结构化蛋白质制品具有至少55%对齐的蛋白纤维，具有至少2000克的平均剪切强度，和具有以大碎片重量计至少17%的平均碎片特征。在另一示范性实施方案中，该结构化蛋白质制品具有至少55%对齐的蛋白纤维，具有至少2200克的平均剪切强度，和具有以大碎片重量计至少20%的平均碎片特征。

(II) 重组肉组合物

[0069] 结构化蛋白质制品在本发明中用作重组肉组合物的成分。重组肉组合物可包含动物肉和结构化蛋白质制品的混合物，或它可不包含肉而主要包含结构化蛋白质制品。用于产生重组肉组合物的方法一般任选地包括将其与动物肉混合、染色和水合结构化蛋白质制品，减少其粒度，将组合物进一步处理成包含肉的食品。

(a)任选与动物肉共混

[0070] 在用下面详述的着色组合物接触结构化蛋白质制品之前或之后，该结构化蛋白质制品可任选地与动物肉共混以产生动物肉组合物。一般来说，该结构化蛋白质制品与具有类似粒度的动物肉共混。

[0071] 本领域已知使用高压机器来产生机械去骨的或分离的生肉，所述高压机器通过首先将骨压碎，使动物组织粘连，然后迫使动物组织，而不是骨，通过筛或类似筛选装置来将骨从动物组织分离。在本发明中，动物组织包含肌肉组织、器官组织、结缔组织和皮肤。该方法形成具有面糊般(batter like)稠度的非结构化、糊状软动物组织共混物，通常称为机械去骨肉或MDM。该糊状共混物的粒度为约0.25－约15毫米，优选达约5毫米，最优选达约3毫米。

[0072] 虽然优选以至少基本上冷冻形式提供动物组织，亦称生肉，以避免在加工之前的微生物腐败，但是一旦将肉磨碎，就不需要将其冻结以提供形成单一条或片的可切性。与肉碎不同，生肉具有天然的高水分含量，蛋白质对水分的比例为约1:3.6－1:3.7。

[0073] 用于本发明的生肉可为适于食用的任何食用肉。该肉可为非炼制的、非干燥的、生肉、生肉制品、生肉副制品及其混合物。所述肉或肉制品为捣碎的，一般以完全冻结或至少基本上冻结的状况每日供应因此避免微生物腐败。碎肉的温度一般低于约40°C(104°F)，优选低于约10°C(50°F)，更优选约－4°C(25°F)－约6°C(43°F)，最优选约－2°C(28°F)－约2°C(36°F)。若使用冷藏或冷冻肉，在工厂地点长时期地贮存大量解冻肉一般是不切实际的。冻结制品比冷藏或冷却制品提供更长的放置时间(lay time)。

[0074] 为代替冻结的碎肉，可新鲜制备用于制备重组肉制品的碎肉，只要新鲜制备的碎肉满足不超过约40°C (104°F)的温度条件。

[0075] 基于生肉的重量，生的冻结或解冻肉的含水量一般为至少约50%重量，最经常为约60%重量－约75%重量。在本发明的一个实施方案中，生的冻结或解冻肉的脂肪含量为至少2%重量。生的冻结或解冻肉的脂肪含量一般为约3%重量－约95%重量。在另一实施方案中，生的冻结或解冻肉的脂肪含量为约20%重量－约95%重量。在本发明的其它实施方案中，可组合肉制品以产生脂肪含量为约15%重量－约30%重量的肉组合物。在另一实施方案中，该肉组合物的脂肪含量可小于约10%重量，可使用脱脂肉制品。

[0076] 可在约－18°C(.4°F)－约0°C(32°F)的温度下贮存冻结或冷冻肉。一般以20千克块供应。使用时，允许肉块融化直至约10°C(50°F)，即，解冻，但是在温和(tempered)的环境中。因此，肉块的外层，例如直至约1/4"的深度，可解冻或融化但仍处于约0°C(32°F)的温度，而剩下的肉块内部，虽然还冻结，但继续融化，因此保持外部低于约10°C(50°F)。

[0077] 各种动物肉适用于重组肉组合物。例如，该肉可为选自绵羊、牛、山羊、猪、野牛(bison)和马的家畜。动物肉可来自家禽，例如鸡、鸭、鹅或火鸡。备选地，动物肉可来自狩猎动物。合适的狩猎动物的非限制性实施例包括野牛(buffalo)、鹿、麋鹿、驼鹿、驯鹿、北美驯鹿、羚羊、兔、松鼠、河狸、麝鼠、负鼠、浣熊、犰狳、豪猪、鳄鱼和蛇。在又一实施方案中，该动物肉可来自鱼或贝。合适的鱼或鱼制品的非限制性实施例包括海水鱼和淡水鱼，例如，鲶鱼、鲔鱼、鲑鱼、鲈鱼、鲭、绿鳕、狗鳕、罗非鱼、鳕鱼、石斑鱼、白鲑、弓鳍鱼、雀鳝、白鲟、鲟鱼、鳊鱼、鲤鱼、鳟鱼、鱼糜、walleye、黑鱼和鲨鱼。在示范性实施方案中，动物肉来自牛肉、猪肉或火鸡肉。

[0078] 肉包括骨骼的横纹肌或例如在舌、膈膜、心脏或食道发现的横纹肌，有或没有伴随覆盖的脂肪和通常伴随肉的部分皮肤、腱、神经和血管。肉副制品的实例为器官和组织如肺、脾、肾、脑、肝、血、骨、部分脱脂低温脂肪组织、不含其内容物的胃、肠、等。

[0079] 术语"肉副制品"意指屠宰动物(包括但不限于哺乳动物、家禽等)的胴体的非炼制部分，并包括如在Association of American Feed Control Officials, Incorporated出版的Definitions of Feed Ingredients中的术语"肉副制品"所包含的这样的成分。

[0080] 典型地，与动物肉组合物中的动物肉的量有关的结构化蛋白质制品的量可以并将会根据组合物的预定用途改变。通过实施例，当期望具有较小程度的动物风味的显著素食组合物时，在重组肉组合物中动物肉浓度可为约45%、40%、35%、30%、25%、20%、15%、10%、5%、2%、或0%重量。备选地，当期望具有较高程度的动物风味的重组肉组合物时，在重组肉组合物中动物肉浓度可为约50%、55%、60%、65%、70%、75%、80%、85%、90%或95%重量。结果，在重组肉组合物中水合结构化植物蛋白制品的浓度可为约5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、或99%重量。在示范性实施方案中，重组肉

组合物一般具有约40% - 约60%重量的水合结构化蛋白质制品和约40% - 约60%重量的动物肉。

[0081] 还考虑在本发明中可使用各种肉质。例如，可使用碎的或块或肉片形式的整块肌肉。该肉的脂肪含量可广泛改变。

(b) 任选与碎蔬菜或碎水果共混

[0082] 在用下面详述的着色组合物接触结构化蛋白质制品之前或之后，该结构化蛋白质制品可任选地与碎蔬菜或碎水果共混以产生重组肉组合物。一般来说，结构化蛋白质制品与具有类似粒度的碎蔬菜或碎水果共混。

[0083] 各种植物或水果适用于重组肉组合物。典型地，与重组肉组合物中的碎蔬菜或碎水果的量有关的结构化蛋白质制品的量可以和将会取决于组合物的预定用途改变。通过实施例，在重组肉组合物中的碎蔬菜或碎水果的浓度可为约95%、90%、85%、80%、75%、70%、65%、60%、55%、50%、45%、40%、35%、30%、25%、20%、15%、10%、5%、2%或0%重量。因此，在重组肉组合物中的水合结构化植物蛋白制品的浓度可为约5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、或99%重量。在示范性实施方案中，重组肉组合物一般具有约40% - 约60%重量的水合结构化蛋白质制品和约40% - 约60%重量的碎蔬菜或碎水果。

(c)使结构化蛋白质制品水合和着色

[0084] 一般用着色组合物将结构化蛋白质制品染色，因此与生肉和/或熟肉相似。 本发明的着色组合物可包含热不稳定的色素、热稳定的色素和褐变剂。色素类型的选择和存在于着色组合物中的量可以并将会根据所需重组肉组合物颜色改变。当重组肉组合物模拟"预熟制品"时，结构化植物产物通常与褐变剂和/或热稳定的色素接触。 备选地，

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

当重组肉组合物模拟生肉时，结构化蛋白质制品一般与热不稳定的红色素，也与褐变剂和/或热稳定的色素接触，致使当煮熟该重组肉组合物时它的外观从生肉颜色变到全熟的颜色。合适的热不稳定的红色素、热稳定的色素和褐变剂描述如下。

[0085] 可在装入挤压机之前将着色剂与含蛋白质材料和其它成分混合。备选地，可在装入挤压机之后将着色剂与含蛋白质材料和其它成分结合。在挤出过程期间所使用的热或热和压力的存在下，一些着色剂和含蛋白质材料的组合物产生不必要的颜色。作为实例，当在挤出过程期间使洋红(或紫胶)与含蛋白质材料接触时，颜色从红色变到紫罗兰色/紫色。

[0086] 该着色剂可为天然着色剂、天然着色剂的组合物、人造着色剂、人造着色剂的组合物、或天然着色剂和人造着色剂的组合物。合适的批准用于食品的天然着色剂的实例包括胭脂树红(微红－橙色)、花色苷(红色到蓝色，取决于pH)、甜菜汁、β－胡萝卜素(橙色)、β－APO 8胡萝卜醛(橙色)、黑加仑、焦化糖；斑蝥黄质(粉红色－红色)、焦糖、洋红/胭脂红酸(鲜红)、胭脂虫提取物(红色)、姜黄素(黄色－橙色)；紫胶(猩红)、叶黄素(红色－橙色)；番茄红素(橙色－红色)、混合类胡萝卜素(橙色)、红曲霉(红色－紫色，得自发酵的红米)、红辣椒、红甘蓝汁、核黄素(黄色)、藏红花、二氧化钛(白色)和姜黄(黄色－橙色)。合适的在美国批准用于食品用途的人造着色剂包括FD&C Red No. 3 (藻红)、FD&C Red No. 40 (诱惑红)、FD&C Yellow No. 5 (柠檬黄)、FD&C Yellow No. 6 (日落黄FCF)、FD&C Blue No. 1 (亮蓝)、FD&C Blue No. 2 (靛蓝)。可用于其它国家的人造着色剂包括Cl Food Red 3 (淡红)、Cl Food Red 7 (胭脂红)、Cl Food Red 9 (苋菜红)、Cl Food Yellow 13 (喹啉黄)和Cl Food Blue 5 (专利蓝V)。食品着色剂可为染料，这些染料为可溶于水的粉末、颗粒或液体。备选地，天然和人造食品着色剂可为色淀染料，该色淀染料为染料和不可溶材料的组合物。色淀染料为非油溶性的，但是为油可分散的；它们通过分散染色。

Motif Exhibit 1002, Page 819 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0087] 合适的着色剂可以各种形式与含蛋白质材料组合。非限制性实例包括固体、半固体、粉末、液体和明胶。所使用的着色剂的类型和浓度可根据使用的含蛋白质材料和染色的结构化蛋白质制品的所需颜色而改变。一般来说，着色剂的浓度可为约0.001%－约5.0%重量。在一个实施方案中，着色剂的浓度可为约0.01%－约4.0%重量。在另一实施方案中，着色剂的浓度可为约0.05%－约3.0%重量。在又一实施方案中，着色剂的浓度可为约0.1%－约3.0%重量。在又一实施方案中，着色剂的浓度可为约0.5%－约2.0%重量。在另一实施方案中，着色剂的浓度可为约0.75%－约1.0%重量。

[0088] 热不稳定的色素可用于着色组合物以提供生的未煮熟肉的红颜色。热不稳定的色素通常为具有与未熟状态的褐色肉(即，生肉)的红颜色相似的红色的食品着色染料或粉末。一般而言，热不稳定的色素为具有当暴露于有效煮熟结构化蛋白质制品的温度时降解的结构的着色染料或粉末。因此，该色素热降解，因此当煮熟结构化蛋白质制品时不能给结构化蛋白质制品提供实质的颜色。该热不稳定的色素典型地在约100°C或更高的温度降解，更优选在约75°C或更高的温度，最典型地在约50°C或更高的温度。在一个实施方案中，热不稳定的色素为甜菜苷，具有低的热稳定性的红色食品着色染料或粉末。甜菜苷源自红色甜菜，典型地制备自红色甜菜汁或甜菜粉末。热不稳定的色素可以着色组合物干重的约0.005%－约30%存在于着色组合物中。当热不稳定的色素为甜菜苷时，甜菜苷优选地构成约0.005%－约0.5%干重的着色组合物，更优选地构成约0.01%－约0.05%干重的着色组合物。备选地，包含甜菜苷的甜菜粉或甜菜提取制备物可以着色组合物干重的约5%－约30%、更优选约10%－约25%存在于着色组合物中。作为实例，着色组合物可以由0.0087%胭脂红、21.68%甜菜粉、52.81%葡萄糖、和25.43% NFE (所有百分比均以重量计)组成。

[0089] 由一种或多种热稳定的食品着色染料组成的热稳定的色素可用于该着色组合物。合适的热稳定的色素包括给结构化蛋白质制品有

Motif Exhibit 1002, Page 820 of 1290

效提供与未熟状态和熟状态下的褐变肉相似的颜色的那些色素。合适的热稳定的色素包括焦糖食品着色材料，和黄色、褐色、和/或橙色食品着色剂。多种焦糖食品着色剂可用于本发明，并以粉末形式或液体形式市售，该着色剂包括Caramel Color No. 602 (可得自Williamson Company，Louisville，Kentucky)和5440 Caramel Powder D. S. (可得自Sensient Colors，Inc.，St. Louis，Missouri)。

[0090] 几种类型的市售黄色/橙色食品着色剂可用于热稳定的色素。合适的黄色/橙色食品着色剂包括胭脂红、姜黄和人造黄色染料如FD&C Yellow #5。存在于着色组合物中的热稳定色素的量为着色组合物干重的约0%－约7%、更优选约0.1%－约3%干重的着色组合物。黄色/橙色食品着色剂材料，优选胭脂红，可占着色组合物的干重的约0%－约2%、优选约0.1%－约1%。焦糖食品着色剂材料典型地占着色组合物干重的约0%－约5%，优选约0.5%－约3%。

[0091] 着色组合物可包括褐变剂，该褐变剂包含胺源和还原糖。如上详述，当煮熟蛋白质材料时，褐变剂一般引起混合了着色组合物的含蛋白质材料变成与熟的褐色肉类似的褐色。在备选的实施方案中，着色组合物的褐变剂也可包括胺源。胺化合物与还原糖反应以引起褐变。合适的胺源包括多肽材料、水解的蛋白质材料、或氨基酸材料。在没有任何特定理论的约束下，优选包括多肽材料、水解的蛋白质和/或氨基酸材料作为褐变剂中的胺源以增强所需的褐变。在示范性实施方案中，该分离的大豆蛋白为褐变剂中的胺基源。当包括进着色组合物中时，存在于着色组合物中的胺源一般为着色组合物干重的约20%－约55%。例示性褐变剂为还原糖。当煮熟含蛋白质材料时，在存在包含胺基的化合物时，合适的还原糖通常能够经过Maillard褐变反应以提供所需的褐变。合适的还原糖的代表性实施例包括木糖、阿拉伯糖、半乳糖、果糖、甘油醛、甘露糖、葡萄糖、乳糖和麦芽糖。在示范性实施方案中，还原糖为葡萄糖。还原糖可以着色组合物干重的约25%－约95%、优选约30%－约60%存在于着色组合物中。

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0092] 在示范性实施方案中，着色组合物包含甜菜色素、胭脂红、焦糖着色剂、还原糖、氨基酸源。在本实施方案的一个备选方案中，氨基酸源包含由氨基酸和二级氨基酸组成的肽。在另一备选实施方案中，氨基酸源为分离的大豆蛋白。

[0093] 着色组合物还可包含酸度调节剂以维持pH在着色剂的最优范围。 该酸度调节剂可为酸化剂。可添加至食物的酸化剂的实例包括柠檬酸、乙酸(醋酸)、酒石酸、苹果酸、富马酸、乳酸、葡糖酸、磷酸、山梨酸、盐酸、丙酸和苯甲酸。着色组合物中的酸化剂的最终浓度可为以着色组合物的重量计约0.001%－约5%。酸性调节剂也可为pH－提高剂(raising agent)，例如磷酸氢二钠、碳酸钠、碳酸氢钠、氢氧化钠和氢氧化钾。

[0094] 可通过使用本领域普通技术人员已知的方法和过程将成分组合来制备本发明的着色组合物。典型地，该成分可以液体形式或粉末形式、并且经常以两种形式有效。可直接混合这些成分以形成着色组合物，但优选地在水溶液中组合这些着色组合物的成分，总浓度为约10%－约25%重量，其中着色剂水溶液可方便地添加至大量水，用于混合和染色结构化蛋白质制品。

(d)添加任选的成分

[0095] 重组肉组合物可任选地包括各种香料、调料、抗氧化剂、或其它成分以赋予所需的风味或质构或增加最终食品营养。如技术人员所理解，添加至重组肉组合物的成分的选择可以并将会取决于要生产的食品。

[0096] 重组肉组合物可包含约1%－约30%重量的脂肪源以赋予风味。一般来说，该脂肪源为动物脂肪。合适的动物脂肪包括牛肉脂肪、猪肉脂肪、家禽脂肪和羔羊肉脂肪。在示范性实施方案中，重组肉组合物包含约10%－约20%重量的脂肪源。在另一实施方案中，可使用植物来源的脂肪源，非限制性实例包括油例如芥花油、棉子油、葡萄油、

Motif Exhibit 1002, Page 822 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

橄榄油、花生油、棕榈油、大豆油、向日葵油、植物油、及其组合。植物来源的脂肪源的重量百分比可为约10%－20%。

[0097] 重组肉组合物还可包含抗氧化剂。抗氧化剂可防止动物肉中的多不饱和脂肪酸(例如，ω-3 脂肪酸)的氧化，抗氧化剂也可防止重组肉组合物中的氧化变色。抗氧化剂可为天然的或合成的。 合适的抗氧化剂包括，但不限于，抗坏血酸及其盐、抗坏血酸棕榈酸酯、抗坏血酸硬脂酸酯、阿诺克索牟(anoxomer)、N-乙酰基半胱氨酸、异硫氰酸苄酯、间氨基苯甲酸、邻氨基苯甲酸、对氨基苯甲酸(PABA)、叔丁对甲氧酚(BHA)、丁基羟基甲苯(BHT)、咖啡酸、斑蝥黄质、α-胡萝卜素、β-胡萝卜素、β-caraotene、β-阿朴-胡萝卜素酸、鼠尾草酚、香芹酚、儿茶酚、没食子酸十六酯、绿原酸、柠檬酸及其盐、丁香提取物、咖啡豆提取物、p-香豆酸、3,4-二羟基苯甲酸、N,N'-二苯对苯二胺(DPPD)、硫代二丙酸二月桂酯、硫代二丙酸二硬脂酰酯、2,6-二-叔-丁基苯酚、没食子酸十二酯、依地酸、鞣花酸、异抗坏血酸、异抗坏血酸钠、 七叶亭、七叶苷、6-乙氧基-1,2-二氢-2,2,4-三甲基喹啉、没食子酸乙酯、乙基麦芽酚、乙二胺四乙酸(EDTA)、桉树提取物、丁香酚、阿魏酸、类黄酮(例如，儿茶素、表儿茶精、没食子酸表儿茶精、表没食子儿茶精(EGC)、没食子酸表儿茶精(EGCG)、多酚表儿茶素-3-没食子酸酯)、黄酮(例如，芹菜素、白杨素、木犀草素)、黄酮醇(例如，橡精、杨梅酮、daemfero)、黄烷酮、白蜡树亭、富马酸、没食子酸、龙胆提取物、葡糖酸、甘氨酸、愈创木脂、橙皮素、α-羟苄基次膦酸、羟基桂皮酸、羟基戊二酸、对苯二酚、N-羟基丁二酸、羟基酪醇、羟脲、米糠提取物、乳酸及其盐、卵磷脂、柠檬酸卵磷脂； R-α-硫辛酸、叶黄素、番茄红素、苹果酸、麦芽酚、5-甲氧色胺、没食子酸甲酯、 柠檬酸一甘油酯; 柠檬酸单异丙酯; 桑黄素、β-萘黄酮、去甲二氢愈创木酸(NDGA)、没食子酸辛酯、草酸、柠檬酸棕榈酯、吩噻嗪、磷脂酰胆碱、磷酸、磷酸酯、植酸、phytylubichromel、甘椒提取物、没食子酸丙酯、多磷酸、栎皮酮、反-白藜芦醇、迷迭香提取物、迷迭香酸、鼠尾草提取物、

Motif Exhibit 1002, Page 823 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

芝麻酚、水飞蓟素、芥子酸、琥珀酸、柠檬酸十八酯、丁香酸、酒石酸、麝香草酚、生育酚(即，α-、β-、γ-和Δ-生育酚)、生育三烯酚(即，α-、β-、γ-和Δ-生育三烯酚)、酪醇、香草酸、2,6-二-叔丁基-4-羟甲基苯酚(即，Ionox 100)、2,4-(三-3',5'-二-叔丁基-4'-羟基苄基)-均三甲苯(即，Ionox 330)、2,4,5-三羟基丙基苯基酮、泛醌、叔丁基氢醌(TBHQ)、硫代二丙酸、三羟基丙基苯基酮、色胺、酪胺、尿酸、维生素K及衍生物、维生素Q10、小麦胚芽油、玉米黄素、或它们的组合。

[0098] 在重组肉组合物中抗氧化剂的浓度可为约0.0001%－约20%重量。在另一实施方案中，在重组肉组合物中抗氧化剂的浓度可为约0.001%－约5%重量。在又一实施方案中，在重组肉组合物中抗氧化剂的浓度可为约0.01%－约1%重量。

[0099] 在另一实施方案中，重组肉组合物还可包含至少一种调味调味剂。所述调味剂可为天然的，或调味剂可为人造的。所述调味剂可模仿或替换在瘦肉或脂肪组织中发现的成分，例如，血清蛋白质，肌肉蛋白质，水解的动物蛋白质，牛油、脂肪酸、等。调味剂可提供动物肉风味、烤肉风味、半熟牛肉风味等。调味剂可为动物肉油、香料提取物的油树脂或aquaresin、香料油、天然熏液、天然烟提取物(natural smoke extract)、酵母抽提物、或香菇提取物。 其它调味剂可包括洋葱香料、大蒜香料或草药香料。重组肉组合物还可包含增味剂。可使用的增味剂的实施例包括盐(氯化钠)、谷氨酸盐(例如，谷氨酸单钠)、甘氨酸盐、鸟苷酸盐、肌苷酸盐、5'-核苷酸盐、水解的动物蛋白、和水解的植物蛋白。

[00100] 重组肉组合物可任选包括多种食用香料。合适的调味剂包括动物肉香料、动物肉油、香料提取物、香辛料油、天然熏液、天然烟提取物、酵母抽提物、雪利酒、薄荷、红糖、蜂蜜。食用香料和药料也可以含油树脂和乙二醇硼酸脂的形式生效。其它调味剂可包括洋葱香料、大蒜香料或草药香料。在备选的实施方案中，调味剂可为坚果味的、甜的、或水果味的。 合适的水果食用香料的非限制性实例包

Motif Exhibit 1002, Page 824 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

括苹果、杏、鳄梨、香蕉、黑莓、黑樱桃、蓝莓、博伊森莓、网纹甜瓜、樱桃、椰子、蓝莓(cranberry)、无花果、葡萄、葡萄柚、青苹果、蜜莓果、猕猴桃、柠檬、来檬、芒果、野莓(mixed berry)、橙子、桃、柿子、菠萝、木莓、草莓和西瓜。重组肉组合物还可包括增味剂。合适的增味剂的非限制性实例包括氯化钠盐、谷氨酸盐、甘氨酸盐、鸟苷酸盐、肌苷酸盐和5-核苷酸盐、酵母抽提物、香菇提取物、干鲣提取物、和海藻提取物。重组肉组合物也可使用各种调味汁和醋渍汁，所述调味汁和醋渍汁可通过发酵或共混食用香料、调味料、油剂、水、增味剂、抗氧化剂、酸化剂、防腐剂和甜味剂制备。

[00101] 在其它实施方案中，重组肉组合物还可包含增稠剂或胶凝剂，例如海藻酸及其盐、琼脂、角叉菜胶及其盐、加工过的麒麟菜(Eucheuma)海藻、树胶(阿拉伯胶、豆角胶、槐豆胶、瓜尔豆胶、西黄耆胶和黄原胶)、果胶、羧甲基纤维素钠、甲基纤维素和改性淀粉。

[00102] 在又一实施方案中，重组肉组合物还可包含营养素例如维生素、矿物质、抗氧化剂、ω-3脂肪酸、或草药。合适的维生素包括维生素A、C、和E，这些也是抗氧化剂，和维生素B和D。可添加的矿物质的实例包括铝、铵、钙、镁、铁和钾的盐。合适的ω-3脂肪酸包括二十二碳六烯酸(DHA)、EPA(二十碳五烯酸)和ALA(α-亚麻酸)。 可添加的草药包括月桂叶、罗勒、芹菜叶、香叶芹、细香葱、芫荽叶、胡荽、小茴香、莳萝、姜、肉豆蔻、马珠草、胡椒、姜黄、欧芹、牛至、龙蒿和百里香。

(III)食品

[00103] 可将重组肉组合物加工成具有各种形状的各种食品。当蛋白质组合物还包含选自以下的至少一种成分时：凝胶蛋白质、动物脂肪、氯化钠、磷酸盐(三聚磷酸钠、酸式焦磷酸钠、六偏磷酸盐等)、着色剂、固化剂、抗氧化剂、抗微生物剂、风味剂、或其混合物，该制品和过程以只使用结构化蛋白质制品、动物肉和水的制品和过程类似的

Motif Exhibit 1002, Page 825 of 1290

方法来实现。 首先用水将蛋白质组合物水合和破碎以暴露并分离纤维。当完成水合和破碎时，添加着色剂。添加动物肉和水，将内容物混合直至获得均质体。然后添加动物脂肪，风味剂、氯化钠、磷酸盐和凝胶蛋白质。在另一实施方案中，可与盐和磷酸盐一起添加硝酸钠。

[00104] 可通过手工或通过机器将产生的均质重组肉制品形成肉条、肉排、肉片、肉饼、或一般的用于烤肉块的立方－形状。重组肉制品可形成为肉棒。也可将重组肉制品塞入可渗透的或不可渗透的肠衣以形成香肠。

[00105] 一旦形成，将重组肉制品煮熟、部分煮熟用于随后完成，或在未煮熟状态、部分煮熟状态或煮熟状态冻结。煮熟包括炸或指嫩煎或油炸、烘焙、烟熏和碰撞烹调(impingement cooking)和蒸。还可将完全煮熟的重组肉制品切片、破碎或研磨。

[00106] 此外、重组肉制品可经受发酵。通过调整肉制品的pH至约4.0－约5.2将肉制品发酵。发酵通过添加乳酸培养液(lactic acid culture)来完成。也可通过使用柠檬酸、乳酸、葡糖酸δ内酯及其混合物的直接酸化进行酸化。

[00107] 可不作加工包装重组肉制品(在正在干燥、已部分干燥、已干燥、已煮熟或未煮熟之前)。重组肉制品的进一步加工(在正在干燥、已部分干燥、已干燥、已煮熟或未煮熟之前)可为例如在冷冻通道中瞬时冷冻，和随后在合适类型的容器如塑料小袋等中自动分配包装。如果制品预定用于快餐市场或用于饮食服务应用，其中在消费之前通常将该制品油炸或烘焙，所述类型的进一步加工和包装是合适的。

[00108] 备选地，在重组肉制品(在正在干燥、已部分干燥、已干燥、已煮熟或未煮熟之前)形成之后，也可能用糖溶液或相关物质喷洒制品表面以在油炸或烘焙期间获得均匀的褐变。然后，现在可以将制品瞬时冷冻，以分配包装出售(即在小袋中)。也可由消费者在对流烘箱中烘焙或处理重组肉制品，而不是油炸。此外，也可在煮熟之前或之后将重组肉制品夹上面包，或覆盖另一类型的覆盖物。另外，可在压力

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

锅中蒸煮重组肉制品以杀死可能存在的任何微生物。

[00109] 也可以以常规方式并使用常规密封方法将已煮熟或未煮熟的重组肉制品包装并密封在罐中。通常，该阶段的罐维持在65°C－77°C的温度下，尽快运送至压力锅或煮熟阶段以防止在此阶段的装罐和灭菌之间的时期内存在任何微生物变质的风险。填在设计用于压力锅煮熟的不可渗透肠衣中的重组肉可在压力锅炊具中煮熟，以制备耐贮存的香肠。

[00110] 为了保证重组肉制品经一旦形成具有完整的肌肉质构，有必要至少约75%重量的蛋白质组合物包含至少约15%重量的由以下组成的大碎片：至少约4厘米长的植物蛋白(vegetable protein)纤维、至少约3厘米长的植物蛋白质丝条、和至少约2厘米长的植物蛋白质块；并且有必要至少约75%重量的蛋白质组合物具有至少约1400克的剪切强度。

[00111] 可通过以下方法制备蔬菜制品(vegetable product)：将蛋白质组合物，优选水合的和破碎的大豆蛋白组合物组合；其中约75%重量的蛋白质组合物由至少约15%重量的碎片组成：所述碎片由至少约4厘米长的蛋白纤维、至少约3厘米长的蛋白质丝条、和至少约2厘米长的蛋白质块组成；且其中至少约75%重量的蛋白质组合物具有至少约1400克的剪切强度；与

碎蔬菜组合(combine)；和

将优选的水合的和破碎的大豆蛋白组合物与碎蔬菜混合以产生均质的、纤维状的和结构化蔬菜制品，该蔬菜制品具有基本上对齐的蛋白纤维。

[00112] 由上述方法制备的蔬菜制品的实例为素食食品，该素食食品包括素食小馅饼、素食热狗、素食香肠和素食脆饼(vegetarian crumble)。素食食品的另一实例为搀入了水合的和破碎的蛋白质组合物的奶酪。

[00113] 可通过以下方法制备水果制品(fruit product)：将蛋白质组合物，优选水合的和破碎的大豆蛋白组合物与碎水果组合；其中约75%

Motif Exhibit 1002, Page 827 of 1290

重量的蛋白质组合物由至少约15%重量的碎片组成：所述碎片由至少约4厘米长的蛋白纤维、至少约3厘米长的蛋白质丝条、和至少约2厘米长的蛋白质块组成；且其中至少约75%重量的蛋白质组合物具有至少约1400克的剪切强度，随后将优选的水合的和破碎的大豆蛋白组合物与碎水果混合以产生均质的、纤维状的和结构化水果制品，该水果制品具有基本上对齐的蛋白纤维。

[00114] 由上述方法制备的水果制品的实例为休闲食品，该休闲食品包括水果卷、谷类食品和水果脆饼(fruit crumble)。

定义

[00115] 当用于本文时，术语"动物肉"指得自动物的肉、整块肌肉、或其部分。

[00116] 当用于本文时，术语"碎水果"指单个水果的果泥或多于一种水果的果泥混合物。

[00117] 当用于本文时，术语"碎肉"指从动物胴体重新获得(recover)的肉糊。带或不带骨的肉被强迫通过去骨装置，致使肉与骨分离并减小尺寸。

[00118] 当用于本文时，术语"碎蔬菜"指指单个蔬菜的菜泥或多于一种蔬菜的菜泥混合物。

[00119] 当用于本文时，术语"挤出物"指挤出的产物。在该上下文中，包含基本上对齐的蛋白纤维的植物蛋白制品在一些实施方案中可被挤出。

[00120] 当用于本文时，术语"纤维"指在进行实施例 2 详述的碎片特征试验之后长约 4 厘米和宽 0.2 厘米的植物蛋白制品。在该上下文中，术语"纤维"不包括营养类的纤维，所述营养类纤维例如大豆子叶纤维，并也不是指包含植物蛋白制品的基本上对齐的蛋白纤维的结构构成。

[00121] 当用于本文时，术语"面筋"指具有高含量蛋白质以及独特结构化质和粘附性质的在谷类粉末例如小麦中的蛋白部分。

[00122] 当用于本文时，术语"无面筋的淀粉"指各种淀粉制品例如改性的木薯淀粉。无面筋或基本上无面筋的淀粉由小麦、玉米、和木薯基淀粉制成。它们无面筋，因为它们不包含来自小麦、燕麦、黑麦或大麦的面筋。

[00123] 当用于本文时，术语"水合试验"测量水合已知量的蛋白质组合物所必需的分钟数。

[00124] 当用于本文时，术语"大碎片"是表征有色或无色的结构化植物蛋白制品的碎片百分比的方式。在实施例 2 中详述了碎片特征的测定。

[00125] 当用于本文时，术语"机械去骨的肉(MDM)"指使用可市购的设备从牛肉、猪肉和鸡肉骨重新获得的肉糊。MDM 是没有在完整肌肉中发现的天然纤维状质构的捣碎的产物。

[00126] 当用于本文时，术语"含水量"指在材料中的水分的量。材料的含水量可以通过 A. O. C. S. (American Oil Chemists Society)方法 Ba 2a-38 (1997)测定，该方法通过整体引用结合到本文中。

[00127] 本文所使用的术语"蛋白质含量"，例如，如大豆蛋白含量，指如通过以下方法所确定的材料的相对蛋白质含量：A.O.C.S. (American Oil Chemists Society)法定方法 Bc 4-91(1997)、Aa 5-91(1997)、或 Ba 4d-90(1997)，各方法通过整体引用结合到本文中，该方法测定作为氨的材料样品的总氮含量，而蛋白质含量为样品总氮含量 6.25 倍。

[00128] 当用于本文时，术语"蛋白质纤维"指共同定义本发明植物蛋白制品结构的不同长度的单个连续的丝或不连续的长形碎片。另外，因为本发明的有色的和无色的结构化植物蛋白制品具有基本上对齐的蛋白纤维，所以蛋白纤维的排列将整块肌肉的质构赋予有色的和无色的结构化植物蛋白制品。

[00129] 当用于本文时，术语"剪切强度"测量质构化蛋白质形成具有足够高的强度以将类似肉的质构和外观赋予所形成的制品的纤维状网络的能力。剪切强度的测量单位为克。

[00130] 当用于本文时，术语"模拟的"指不含动物肉的动物肉组合物。

[00131] 当用于本文时，术语"大豆子叶纤维"指包含至少约 70%膳食纤维的大豆子叶的多糖部分。大豆子叶纤维通常包含一些较少量的大豆蛋白，但也可能是 100%的纤维。当用于本文时，大豆子叶纤维不指或不包括大豆外壳纤维。一般，大豆子叶纤维通过以下步骤形成：去除大豆的外壳和胚芽，压片(flake)或磨碎子叶并从压片或磨碎的子叶中去除油，和使大豆子叶纤维与大豆材料和子叶的糖类分离。

[00132] 当用于本文时，术语"大豆浓缩蛋白"是以不含水的大豆蛋白计，蛋白质含量为约 65%－小于约 90%的大豆材料。大豆浓缩蛋白也包含大豆子叶纤维，通常含有以无水重量来计算约 3.5%直至约 20%的大豆子叶纤维。大豆浓缩蛋白通过以下步骤由大豆形成：去除大豆的外壳和胚芽，压片或磨碎子叶并从压片或磨碎的子叶中去除油，和使大豆蛋白和大豆子叶纤维与子叶的可溶性糖类分离。

[00133] 当用于本文时，术语"大豆粉"指优选包含少于约 1%油的脱脂大豆材料的捣碎形式，由粒径能使颗粒可以通过NO. 100 目(美国标准)筛的颗粒组成。使用常规的大豆研磨方法将大豆饼、薄片、压片、粗粉、或这些材料的混合物捣碎成大豆粉。以无水计算，大豆粉的大豆蛋白含量为约 49%－约 65%。优选粉末磨得非常细，最优选致使少于约 1%的粉末被截留在 300 目(美国标准)筛上。

[00134] 当用于本文时，术语"大豆分离蛋白"是以无水计算，蛋白质含量为至少约 90%大豆蛋白的大豆材料。通过以下步骤由大豆形成大豆分离蛋白：从子叶去除大豆的外壳和胚芽，压片或磨碎子叶并从压片或磨碎的子叶去油，使子叶的大豆蛋白和糖类与子叶纤维分离，接着使大豆蛋白与糖类分离。

[00135] 当用于本文时，术语"丝条"指在进行实施例 2 详述的碎片特征试验之后，尺寸为长约 2.5 - 约 4 厘米和宽大于约 0.2 厘米的植物蛋白制品。

[00136] 当用于本文时，术语"淀粉"指得自任何天然来源的淀粉。典型的淀粉源为谷类、根茎类、根类、荚果类、和水果类。

[00137] 当用于本文时，术语"以无水计算的重量"指干燥至完全去除所有水分(例如材料的水分含量为 0%)之后材料的重量。具体地讲，材料的以无水计算的重量可以通过将材料置于 45°C 烘箱直至材料达到恒重之后称重材料而获得。

[00138] 当用于本文时，术语"小麦粉"指由碾碎小麦而获得的粉末。一般而言，小麦粉的粒度为约 14 - 约 120 μm。

[00139] 包括以下实施例以阐述本发明的优选实施方案。本领域的技术人员应该理解的是：在以下实施例中所公开的技术代表由发明者揭示的、目的在于良好地开展本发明的实践的技术。然而，依照本公开，本领域的技术人员应该理解：在不脱离本发明的精神和范围下，在所公开的具体实施方案中可作出许多改变，并且还获得相似的或类似的结果，因此在附图中阐述或显示的所有事物被解释为说明性而不是限制性的。

实施例

[00140] 实施例 1-105 举例说明本发明的各种实施方案。

实施例 1 剪切强度的测定

[00141] 样品剪切强度以克为单位进行测量并可通过以下过程测定。称重结构化蛋白质制品样品并将它置于隔热的小袋中，用约 3 倍样品重量的室温自来水水合样品。将小袋抽真空至约 0.01 Bar 的压力，并密封小袋。允许样品水合约 12 - 约 24 小时。取出水合的样品并将它置于定向的质构分析仪基板上，致使质构分析仪的刀将切穿样品的直

径。进一步地，样品应该在质构分析仪的刀下定向，致使刀口垂直于质构片的长轴。用于切割挤出物的合适的刀为 TA-45 型，由 Texture Technologies (美国)制造的切刀片。进行该试验的合适的质构分析仪为 TA 型，由 Stable Micro Systems Ltd. (英国)制造的配有 25、50、或 100 千克测力计的 TXT2。在该试验的上下文中，剪切强度为刺穿(puncture through)样品所需的以克计算的最大的力。

实施例 2 碎片特征的测定

[00142] 用于测定碎片特征的方法可如下执行。仅使用整块碎片称重约 150 克的结构化蛋白质制品。将样品置入隔热的塑料袋中并添加约 450 克 25°C 的水。在约 150 mm Hg 下真空密封该袋，并允许内含物水合约 60 分钟。将水合的样品置入配有单个刀浆的 KM 14G0 型 Kitchen Aid 混合器的钵内，并在 130 rpm 下混合内含物两分钟。刮浆和钵的侧面，将刮下物放回钵的底部。重复混合和刮两次。从钵中取出约 200 g 的混合物。分离该混合物致使所有长于 2.5 cm 的纤维或长丝条与切碎混合物分离。称重从切碎混合物分选的纤维群，该重量除以起始重量(例如约 200 g)，并将该值乘以 100。这确定样品中大碎片的百分比。假如结果值低于 15%或高于 20%，那么结束试验。假如值为 15% - 20%，然后从钵中称出另一约 200 g，使长于 2.5 cm 的纤维或长丝条从切碎混合物分离，再进行计算。

实施例 3 植物蛋白制品的生产

[00143] 以下挤出方法可用于制备本发明的有色的结构化植物蛋白制品。将以下添加至干共混混合容器：1000 千克(kg)Supro 620 (大豆分离物)、440 kg 小麦面筋、236 kg 小麦淀粉、34 kg 大豆子叶纤维、8 kg 磷酸二钙、和 2 kg L-半胱氨酸。混合内含物以形成干混的大豆蛋白混合物。然后将干混物转移至加料斗，干混物连同 480 kg 水一起从该加料斗引入预调料器，以形成调湿的大豆蛋白预混合物。然后按不大于

25 kg/分钟的速率将有调湿的大豆蛋白预混合物给料至双螺杆的挤出装置。挤出装置包含五个温度控制区，蛋白质混合物在第一区被控制至约 25°C，在第二区约 50°C，在第三区约 95°C，在第四区约 130°C，和在第五区约 150°C。挤出物受在第一区至少约 400 psig 直至在第五区约 1500 psig 的压力。经由与加热区相通的一个或多个注射口将 60 kg 水注入挤压机的筒中。

[00144] 将压模装置连接至挤压机，以以下方式布置：允许增塑的混合物从挤压机出口排入压模装置并随增塑的混合物内流过压模装置在它之中产生基本上对齐的蛋白纤维。

[00145] 当包含基本上对齐的蛋白纤维的挤出物挤出压模装置时，用刀切下并然后将切块干燥至约 10%重量的含水量。一经干燥，部分切块分成小碎片和较大碎片。共获得各尺寸的 25 个碎片。然后测量这些碎片并列于表 IV 中。

表 IV

样品编号	小碎片				大碎片	
	长(mm)	宽(mm)			长(mm)	宽(mm)
1	11	10			16	11
2	10	6			16	13
3	8	8			22	11
4	11	8			19	11
5	14	9			17	11
6	10	8			20	13
7	14	4			15	10
8	8	6			21	12
9	10	8			19	12
10	11	8			12	12
11	13	8			15	10
12	19	9			17	9

13	12	9			11	10
14	14	6.5			14	10
15	10	7			14	10
16	10	7			17	12
17	10	6			15	13
18	12	8			14	14
19	10	7			19	12
20	9	6			18	10
21	10	8			14	12
22	9	5			19	12
23	10	7			12	11
24	11	9			16	10
25	9	8			16	9

平均值	11.0	7.4		平均值	16.3	11.2
标准差	2.3	1.4		标准差	2.8	1.3
最大值	19.0	10.0		最大值	22.0	14.0
最小值	8.0	4.0		最小值	11.0	9.0

实施例 4－94 植物蛋白制品的生产

[00146] 实施例 4-94 是实施例 3 的重复。下面的表 V 描绘实施例 3-94 的分析。

表 V

实施例编号 #	较大的碎片%	剪切强度 (g)	水合	密度(g/cc)
3	30.2	2150	80	0.27
4	24.2	2366	80	0.24
5	29.4	2341	60	0.30
6	26.0	2142	70	0.29
7	27.1	2291	70	0.28
8	32.7	2442	70	0.23
9	17.4	2668	70	0.27
10	26.1	2511	90	0.26

实施例编号 #	较大的碎片%	剪切强度 (g)	水合	密度(g/cc)
11	21.1	2260	80	0.28
12	22.3	2421	80	0.24
13	21.9	2490	75	0.28
14	22.4	2438	104	0.28
15	7.8	59	81	0.30
16	7.3	675	83	0.28
17	9.3	553	100	0.24
18	7.3	226	90	0.23
19	3.5	412	72	0.24
20	0.0	055	100	0.23
21	2.6	511	75	0.25
22	2.7	168	100	0.25
23	2.0	207	102	0.25
24	7.7	247	62	0.29
25	1.2	51	73	0.28
26	0.2	164	63	0.27
27	6.6	966	68	0.28
28	4.9	164	50	0.31
29	5.0	812	58	0.28
30	9.6	108	60	0.31
31	5.8	864	70	0.27
32	6.5	473	58	0.25
33	0.7	879	65	0.28
34	5.4	688	70	0.29
35	0.3	038	74	0.26
36	9.3	074	73	0.28
37	1.5	937	70	0.39
38	2.5	462	77	0.40

实施例编号 #	较大的碎片%	剪切强度 (g)	水合	密度(g/cc)
39	0.1	051	66	0.28
40	7.9	384	54	0.31
41	8.1	064	58	0.28
42	9.2	158	60	0.27
43	0.0	834	58	0.28
44	6.8	202	58	0.28
45	2.8	363	57	0.26
46	3.9	361	57	0.28
47	6.9	293	103	0.25
48	6.3	205	73	0.28
49	9.0	286	53	0.29
50	2.6	206	63	0.25
51	0.5	125	63	0.31
52	5.5	290	55	0.29
53	8.2	274	55	0.26
54	1.5	205	42	0.33
55	1.3	185	55	0.31
56	1.8	969	40	0.30
57	9.1	028	55	0.31
58	7.2	598	63	0.37
59	8.3	869	60	0.31
60	9.7	044	50	0.29
61	7.6	216	52	0.28
62	5.0	001	53	0.28
63	8.1	096	45	0.27
64	9.0	796	53	0.27
65	0.0	924	51	0.27
66	3.7	295	51	0.28

实施例编号 #	较大的碎片%	剪切强度 (g)	水合	密度(g/cc)
67	7.4	259	50	0.29
68	9.2	204	43	0.28
69	5.3	059	38	0.31
70	6.1	284	70	0.32
71	3.6	085	70	0.30
72	5.6	279	44	0.28
73	3.7	170	44	0.32
74	1.2	128	49	0.29
75	2.4	068	50	0.29
76	0.1	939	40	0.30
77	8.7	592	50	0.30
78	9.6	812	68	0.28
79	5.2	848	64	0.28
80	3.6	973	70	0.30
81	3.7	078	66	0.36
82	5.6	940	44	0.31
83	8.5	339	33	0.29
84	0.2	366	50	0.24
85	8.1	425	40	0.29
86	9.6	122	59	0.27
87	7.5	193	56	0.16
88	1.1	186	56	0.28
89	2.4	061	56	0.27
90	1.3	143	50	0.27
91	4.4	108	54	0.26
92	9.9	101	53	0.30
93	2.3	551	55	0.25
94	4.3	164	57	0.28

实施例编号 #	较大的碎片%	剪切强度(g)	水合	密度(g/cc)
第 1 四分位数	2.6	045	53	0.27
中值	6.5	164	60	0.28
第 3 四分位数	0.2	291	70	0.30
平均值	6.6	156	63	0.28

实施例 95 蛋白质组合物的生产

[00147] 将约 10°C (50°F)的 3625 克自来水添加至混合容器，搅拌时添加 1160 克干燥、低水分(约 7% - 约 12%)大豆蛋白组合物(标识为 FXP MO339，得自 Solae, LLC, St. Louis, MO)直至大豆蛋白组合物被水合且纤维被分离，所述大豆蛋白组合物包含大豆分离蛋白、大豆子叶纤维、小麦面筋和淀粉。将 5216 克含水量为至少约 50%的机械去骨的碎鸡肉添加至混合器。使机械去骨的鸡肉处于约 2°C (36°F) - 约 4°C (39°F)的温度。混合内含物直至获得均质的重组肉制品。将重组肉制品转移至 Hollymatic 成形机器，在其中使重组肉制品成形，变成肉排或肉片，然后冷冻。

[00148] 重复实施例 3 的操作，不同之处为：用 3175 克水水合含大豆分离蛋白、大豆子叶纤维、小麦面筋和淀粉的 1500 克非干燥低含水量(约 28 - 约 35%)的大豆蛋白质组合物。将重组肉制品转移至填料机器，在其中重组肉制品被填入不能渗透的肠衣内，然后冷冻。填料机器可得自多个制造商，包括但不限于位于 Elk Grove Village, I11.的 HITEC Food Equipment, Inc.、位于 Des Moines, Iowa 的 Townsend Engineering Co.、位于 Canton, Mass.的 Robert Reiser & Co., Inc.、和位于 Buffalo Grove, I11.的 Handtmann, Inc.。

实施例 97

[00149] 将约 12°C (54°F)的 2127 克自来水加至第一混合容器，搅拌时加入 1000 克干燥、低含水份(约 7% - 约 12%)的大豆蛋白组合物，直至大豆蛋白组合物被水合且纤维被分离。然后将 43 克焦糖着色剂添加至水合的大豆蛋白组合物。在约 2°C(36°F)下，添加 4500 克含水量为约 50%的机械去骨的碎鸡肉。然后添加 100 克氯化钠和 30 克三聚磷酸钠以提取/溶解用于结合的经捣碎肉中的肌原纤维蛋白质。当继续混合时，添加 500 克牛肉脂肪和 100 克牛肉香料并继续混合。在第二混合容器中，使 Supro® 620 的 600 克凝胶蛋白在 1000 克水中水合，并添加至第一混合容器。混合内含物直至获得同质的重组肉制品。将重组肉制品转移至 Hollymatic (Hollymatic Corp., Park Forest IL)成形机器，在其中使重组肉制品成形为肉饼，然后冷冻。

实施例 98

[00150] 将约 10°C (50°F)的 3000 克自来水添加至混合容器，搅拌时添加 1500 克由 Supro® 620 制备的大豆蛋白挤出物，直至大豆蛋白挤出物被水合，并通过切碎而分离纤维。将 5000 克含水量为至少约 50%的机械去骨的碎鸡肉加至混合器。使机械去骨的鸡肉处于约 2°C (36°F) - 约 4°C (39°F)的温度。混合内含物直至获得同质的重组肉制品。将重组肉制品转移至 Hollymatic 成形机器，在其中使重组肉制品成形为肉排或肉片，然后冷冻。

实施例 99

[00151] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白、大米粉、和无面筋的淀粉。

实施例 100

[00152] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白和大米粉。

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

实施例 101

[00153] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白和无面筋的淀粉。

实施例 102

[00154] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白、小麦粉和淀粉。

实施例 103

[00155] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白和大豆子叶纤维。

实施例 104

[00156] 重复实施例 98 的操作，不同之处为：水合并切碎的大豆蛋白组合物包含大豆分离蛋白、大豆子叶纤维和小麦面筋。

实施例 105

[00157] 将约 10°C (50°F)的 3383 克自来水添加至混合容器，搅拌时添加 1208 克干燥、低水分(约 7% - 约 12%)的标识为 SUPROMAX® 5050 的大豆蛋白挤出物，直至大豆蛋白挤出物被水合，并通过切碎而分离纤维。将 3340 克含水量为至少约 50%的机械去骨的捣碎鸡肉与 3383 克脂肪含量为约 10%的 1/2 英寸绞碎的牛肉添加至混合器。在约 2°C (36°F) - 约 4°C (39°F)下绞碎机械去骨的鸡肉和牛肉。也添加各种着色剂和风味剂：盐、异抗坏血酸盐、亚硝酸钠、葡萄糖、破碎的黑胡椒、肉豆蔻、肉豆蔻皮、蒜粒、芫荽、红辣椒、和再水合的 LHP 起子培养物。混合内含物直至获得同质的重组肉制品。然后将重组肉制品成形为肉棒。

[00158] 虽然以与本发明有关的优选实施方案解释了本发明，但应理解：本领域的技术人员通过阅读本说明书将明白其各种修改。因此，

Motif Exhibit 1002, Page 841 of 1290

应理解：本文所公开的发明有意涵盖落入所附权利要求范围内的修改。



图 1



图 2

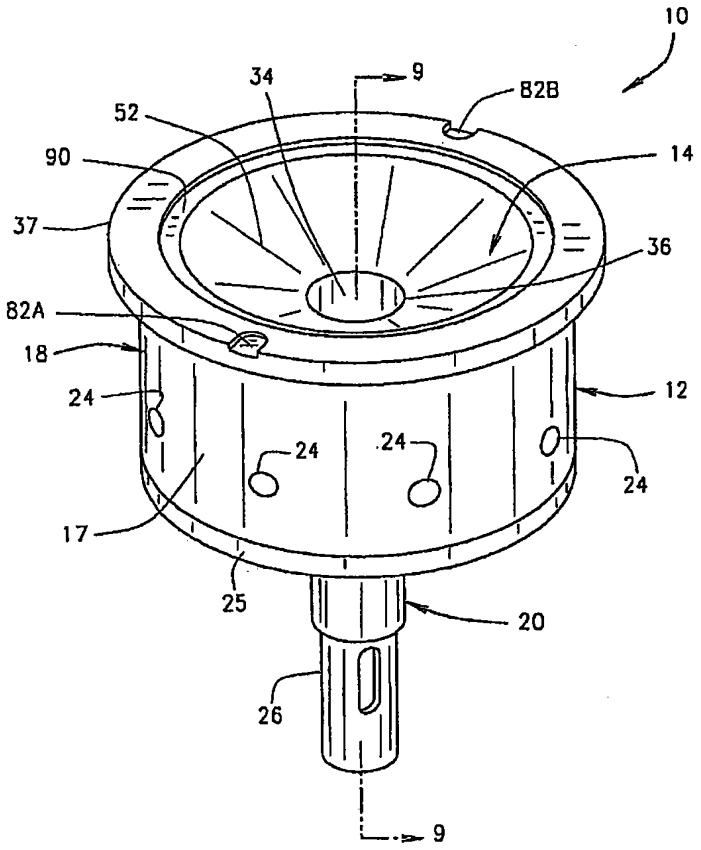


图 3

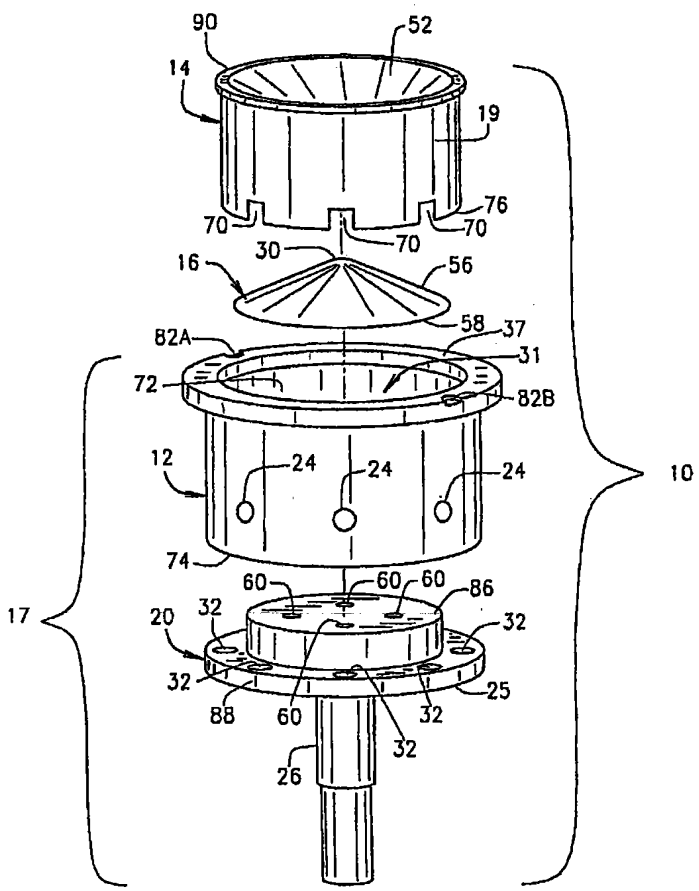

90
52
14
19
76
70
70
70
30
16
56
58
37
82A
31
72
82B
12
24
24
24
10
74
60
60
60
86
17
20
32
32
32
32
88
60
32
25
32
26

图 4

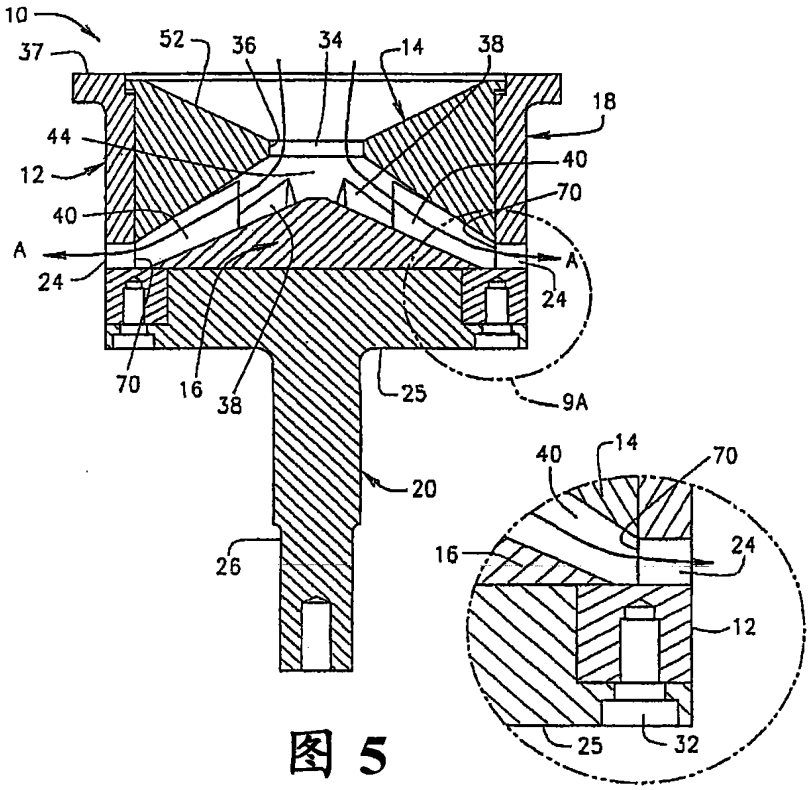

10
37
52
36
34
14
38
18
44
12
40
70
40
A
A
24
24
70
16
38
25
9A
20
26
40
14
70
24
16
12
25
32

图 5

图 5A

Espacenet

Bibliographic data: CN101861895 (A) — 2010-10-20

Method for preparing novel fermented soybean milk products

Inventor(s): RUIXIA GU; CAIPING LIU; YIN XU ± (GU RUIXIA, ; LIU CAIPING, ; XU YIN)

Applicant(s): UNIV YANGZHOU ± (YANGZHOU UNIVERSITY)

Classification: **- international:** ***A23C11/10; A23C20/02; C12N1/20;*** *C12R1/225*
- cooperative:

Application number: CN20101139465 20100406 Global Dossier

Priority number(s): CN20101139465 20100406

Also published as: CN101861895 (B)

Abstract of CN101861895 (A)

The invention relates to a method for preparing novel fermented soybean milk products, such as fermented soybean milk, fermented mixed soybean milk, fermented dried tofu and the like, which prepares the produces such as soybean milk, fermented mixed soybean milk, dried tofu or dried tofu paste and the like by obtaining lactic acid bacteria by using vitality of alpha acetolacetate decearboxylase (ALDC) as an index and fermenting soybean milk or mixed milk containing the soybean milk by using one ore more lactic acid bacteria. The prepared products have a good flavor and are easy to be accepted by consumers.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 101861895 A
(43)申请公布日 2010.10.20

(21)申请号 201010139465.7

(22)申请日 2010.04.06

(83)生物保藏信息
CGMCC NO:3622 2010.02.01

(71)申请人 扬州大学
地址 225009 江苏省扬州市大学南路 88 号

(72)发明人 顾瑞霞 徐寅 刘彩平

(74)专利代理机构 南京知识律师事务所 32207
代理人 卢亚丽

(51)Int.Cl.
A23C 11/10(2006.01)
A23C 20/02(2006.01)
C12N 1/20(2006.01)
C12R 1/225(2006.01)

权利要求书 1 页 说明书 10 页

(54)发明名称

一种新型发酵豆乳制品的制备方法

(57)摘要

本发明涉及一种新型发酵豆乳制品，如发酵豆乳、发酵混合豆乳、发酵豆干等制备方法，即利用 α 乙酰乳酸脱羧酶(ALDC)活力作为指标，获得乳酸菌以一种或多种乳酸菌作为发酵剂发酵豆乳或含豆乳的混合乳，制备成发酵豆乳、发酵混合豆乳、豆干或豆干酪等产品，风味良好，易为消费者接受。

CN 101861895 A

1. 一种新型发酵豆乳制品的制备方法，其特征在于，对蛋白质重量百分含量在1.5% -6.0%的豆乳基质在95℃进行20min的热处理后，用α-乙酰乳酸脱羧酶的活力大于0.1000μmol/mL的乳酸菌进行发酵，使其凝固，制备发酵豆乳。

2. 根据权利要求1所述的新型发酵豆乳制品的制备方法，其特征在于，是将对凝乳进行处理，加工成豆干或豆干酪。

3. 根据权利要求1或2所述的新型发酵豆乳制品的制备方法，其特征在于，豆乳基质中大豆蛋白含量占豆乳基质总蛋白质的15% -90%，且至少添加10% -85%的牛奶，或1% -2%的奶粉，或添加0.5% -2%的乳清粉。

4. 根据权利要求3所述的新型发酵豆乳制品的制备方法，其特征在于在豆乳基质或发酵豆乳中添加糖、盐、奶油或人造油，制备成发酵豆乳、豆干或豆干酪等发酵豆制品。

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

一种新型发酵豆乳制品的制备方法

技术领域

[0001] 本发明属于食品加工领域，具体涉及一种新型发酵豆乳制品的制备方法。

技术背景

[0002] 随着人们生活水平的提高，普通的日常饮食并不能满足人们的需求，各种功能保健食品倍受人们的关注。大豆蛋白是一种优质的完全蛋白质。由于大豆蛋白的营养性和功能性，以豆乳全部或部分替代牛乳生产各种豆乳制品，为广大消费者所接受。

[0003] 大豆蛋白含有人体所必需的各类氨基酸，尤其是人体不能合成的赖氨酸、色氨酸等，除含硫氨基酸（半胱氨酸和蛋氨酸）外，其它营养成分均为均衡的，在氨基酸组成平衡胜过其它豆类、油料作物、谷类作物和棉籽；其生物学价值平均含有 96 个标准单位，同肉、蛋和奶的蛋白质相似，是植物中唯一类似于动物蛋白的“完全”蛋白质，因此极易被人所体收，且没有动物性蛋白质的副作用，如引发肥胖症、心血管病和高胆固醇症等。研究表明，人们进食动物性食品过多，是造成导致心血管病、脑血管病、肥胖症、糖尿病等所谓“文明病”增加的原因之一，营养学家认为，增加植物性蛋白，特别是大豆蛋白的食用量，是解决这一问题的一条行之有效的途径。此外大豆蛋白还能解除因重金属盐、生物碱等引起的中毒、降低血液中的低密度脂蛋白、减少身体内钙的损失等重要生理功能。

[0004] 双乙酰是由柠檬酸利用微生物包括乳酸乳球菌属中双乙酰亚种、明串珠菌、乳杆菌在发酵中形成的。在通常条件下，柠檬酸发酵和双乙酰形成仅出现在含有编码运输和代谢柠檬酸的乳酸菌中。在乳制品的乳酸菌中，柠檬酸利用最常与若干种明串珠菌和筛选的乳球菌亚种联系在一起，已发现在发酵柠檬酸的菌中含有编码运输柠檬酸基因的质粒。双乙酰是酸豆乳发酵过程中乳酸菌柠檬酸代谢过程里的一种重要副产物。当其超过一定风味阈值时，就会给酸豆乳带来不良口味，从而影响酸豆乳的质量。α－乙酰乳酸能从乳酸菌细胞中渗出，在细胞外经非酶促氧化脱羧产生双乙酰，双乙酰具有令人不愉快的饭嗖味，影响酸豆乳风味。双乙酰经过双乙酰还原酶将其还原为乙偶姻 (acetoin，3-羟基 -2- 丁酮），再进一步由 3- 羟基 -2- 丁酮还原酶还原为 2，3- 丁二醇。a- 乙酰乳酸脱羧酶 (a-acetolactatedecarboxylase，ALDC) 催化 a- 乙酰乳酸脱羧酶脱羧形成乙偶姻和二氧化碳，不经过双乙酰形成途径。在双乙酰形成与消除这两步反应中，前体物质 α－乙酰乳酸的氧化脱羧反应速率比双乙酰的还原反应速率慢 10 倍之多。这样 α－乙酰乳酸的去除反而成了双乙酰去除速率的限制步骤。因此如何控制好的酸豆乳中双乙酰的含量，就要控制好乳酸菌柠檬酸的代谢。α－乙酰乳酸脱羧酶能催化 α－乙酰乳酸直接脱羧生成乙偶姻和二氧化碳，有利于改善酸豆乳的风味，从而改善酸豆乳的品质。

[0005] 酸豆奶是以大豆和新鲜牛奶（或奶粉）为主要原料，经乳酸菌发酵制成含活性乳酸菌的保健食品，其将动物蛋白和植物蛋有效地结合，提高了蛋白质的消化利用率。酸豆奶将豆奶的营养与乳酸菌的保健集于一体，克服了豆奶酸味不足，品种单一的缺点，同时由于乳酸菌的作用，使产品风味更加柔和。酸豆奶不含胆固醇和乳糖，与酸牛奶相比更适合老年人和儿童饮用，是一种优质植物动物复合蛋白乳酸发酵食品。

Motif Exhibit 1002, Page 851 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0006] 因此利用高产 ALDC 的乳酸菌生产一种大众普遍接受的酸豆奶及其相关产品具有很好的应用价值。本发明以高 ALDC 活性的乳酸菌对豆乳进行发酵，生产出一种营养丰富、风味良好、易为消费者接受的酸豆乳。

发明内容

[0007] 为了克服现有技术的不足，本发明提供了一种新型发酵豆乳制品的制备方法。利用人们尚未发现的具有较高 ALDC 活性的乳酸菌单一或混合发酵来制备口感好的含豆乳发酵制品，可以消除发酵豆乳中表现出来的饭馊味。

[0008] 本发明方法是全部使用具有较高 ALDC 活力的乳酸菌进行发酵，发酵豆乳风味良好，易为消费者接受。具体的操作是：对蛋白质重量百分含量在 1.5％ -6％的豆乳基质进行 95℃杀菌 20min 的热处理后，用 α 乙酰乳酸脱羧酶的活力大于 0.1000μmol/mL 的乳酸菌进行发酵，使其凝固，制备发酵豆乳；或是将对凝乳进行处理，加工成豆干或豆干酪。

[0009] 本发明还提供一种筛选能用于本发明的具有高产 ALDC 的乳酸菌乳酸菌的方法，具体而言，它采用下述步骤制得：(1) 从传统乳制品及天然发酵食品中分离获得乳酸菌；(2) 对分离获得的乳酸菌比较其 ALDC 活力，选择 ALDC 活力大于 0.1000μmol/mL 的乳酸菌用于本发明的发酵。

[0010] 经热处理的豆乳或含豆乳的混合乳，利用乳酸脱氢酶活力较高的乳酸菌作为发酵剂发酵后，添加或不添加其它食品添加物，直接制成发酵豆乳或经过适当加工处理后制成豆干或豆干酪等产品，其产品特征在于，风味良好，易为消费者接受。

具体实施方式

[0011] 本发明提供具有较高 ALDC 活性的乳酸菌作为发酵剂，用于豆乳基质发酵，具有消除发酵豆乳中的饭馊味的功能，使制备的发酵豆乳风味良好，易为消费者接受。

[0012] 具体而言，包括以下几个方面：

[0013] 1. 样品采集

[0014] 本发明样品来源主要是新疆、云南、四川、江苏等地民族传统乳制品及天然发酵食品，如酸奶酒、酸马奶酒、泡菜等，将样品采集后放入事先准备好的缓冲溶液中，冷藏，2 天之内对样品进行分离。

[0015] 2. 乳酸菌分离与鉴定

[0016] 将新疆、云南、四川、江苏等地民族传统乳制品及天然发酵食品采用 PTYG 培养物于 37℃培养 24 小时后。将该培养物涂布在 PTYG 琼脂平板上，然后在 37℃培养 3 天，挑取单菌落，并进一步纯化，用其检测细菌特征，如下文所述的革兰氏染色，在生理生化基础上进一步进行 API 鉴定及 16SrDNA 序列测定结果鉴别出上述细菌是乳酸菌。

[0017] 将分离获得的菌株用含 11％脱脂乳的培养物培养后，加入保护剂，冻干、保存在 -18℃，然后用于各种检测细菌特征的试验。

[0018] 3. ALDC 活力的测定

[0019] 标准曲线的绘制：分别吸取 800μM 的 3- 羟基 -2- 丁酮（乙偶姻）0、20、40、60、80、100、120、140μL，加水补足至 1000μL，加入 200μl 5％ α - 萘酚（溶于 2.5mol/L 的 NaOH 中）和 200μL 0.5％的肌酸，室温显色 1h，522nm 比色测 OD 值。其数据回归处理，以乙偶姻

Motif Exhibit 1002, Page 852 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

的量为横坐标，OD 值为纵坐标，绘制标准曲线。

[0020] 菌株的活化：将分离到的菌株接种于新配制灭菌后的 12％脱脂乳培养基中，混匀后置 42℃培养箱中培养，活化 3 代。

[0021] 发酵液制备：各取活化后的菌株 3％接种到 10mLMRS 试管中培养 3 代，将第 3 代乳酸菌菌种按 3％的量接入装有 50mLMRS 培养基的三角瓶中培养。

[0022] 粗酶液制备：将上述发酵液于 4℃，5000r/min 离心 20min 收集菌体，用 0.85％的 NaCl 溶液洗涤，并离心。所得菌体用 pH6.0，0.03mol/L 柠檬酸－磷酸盐缓冲液悬浮，在冰浴中用超声波（200w）间断处理 7min，至绝大多数菌体破碎为止，离心除去细胞碎片，所得上清液为粗酶液。

[0023] 测定方法：反应总体积 400μL，其中 200μL 为新配制底物 α－乙酰氧基－α－甲基－乙酰乙酸乙酯，用 200μLpH6.0 的 0.03mol/L 柠檬酸－磷酸盐缓冲液适当稀释的酶样，置于 30℃水浴 20min；加入 4.6mL 显色剂（0.1％肌酸，1％ α－萘酚，溶 1mol/LNaOH 溶液中，临用前配），于 15 ～ 20℃下显色 50min，在 721 型分光光度计上于 522nm 处测吸光度，根据吸光度计算酶活单位。一个酶活单位定义为：在 30℃，pH6.2 条件下，由 α－乙酰乳酸脱羧酶脱羧，每分钟产生 1μmol 乙偶姻的酶量。

[0024] 酶活的计算公式

[0025] U ＝ OD×0.00113494×F×T/H

[0026] OD：样品在 522nm 处测得的值；

[0027] 0.00113494：将结果换算成 μmol/min 的常数；

[0028] F：样品被稀释的倍数；

[0029] T：样品的总体积；

[0030] H：标准曲线斜率

[0031] 从新疆、云南、四川、江苏等地采集的民族传统乳制品及天然发酵食品，如酸奶酒、酸马奶酒、泡菜等分离获得的 106 株乳酸菌，ALDC 活性大于 0.1000μmol/mL 的占 13.19％，ALDC 活性在 0.0065-0.1000μmol/mL 之间的占 56.43％，ALDC 活性小于 0.0065μmol/mL 的占 30.38％。

[0032] 4. 发酵豆乳试验

[0033] 选取 9 株酶活大于 0.1000μmol/mL、3 株酶活在 0.0065-0.1000μmol/mL 之间、3 株酶活小于 0.0065μmol/mL 乳酸菌共 15 株菌进行发酵豆乳试验。结果如表 1。

[0034] grx90 为本实验室分离的菌株，于 2010 年 2 月 1 日保藏于中国微生物菌种保藏管理委员会普通微生物中心，地址：北京市朝阳区大屯路，中国科学院微生物研究所；分类命名为：嗜热链球菌（Streptococcus thermophilus），保藏号为 CGMCCNo：3622。表 1 中的其他乳酸菌可从申请人实验室获取。XN53、AM1、CM1、AP3、BM5、ST、qy（ST-日）3 球见杨旭（乳酸菌 rep-PCR 分子鉴别及其与体外细胞的相互作用，扬州大学硕士研究生论文，2009）；白杆见王慧晶（对酒精肝损伤具有保护作用的乳酸菌筛选，扬州大学硕士研究生论文，2008）；BB-16、P971P11、XPL-1、BP2、西草 2 杆、西原 1 球见刘彩平（酸豆乳专用乳酸菌的筛选及应用研究，扬州大学硕士研究生论文，2010）。

[0035] 表 1 单菌株发酵豆乳试验

[0036]

Motif Exhibit 1002, Page 853 of 1290

菌株	ALDC/ μmol/mL	凝乳时间 /h	酸度 /° T	Cfu/mL	感官评价
grx90	0.1542	4.5	64	1.7×10^{8}	色乳白,香气自然,无乳清,口感细腻
$XN_5$3	0.1324	5.1	63	1.06×10^{9}	色微暗,弱豆味,少量乳清,口感细腻
BB-16	0.1123	17	60	5.3×10^{8}	色暗红,弱豆味,少量乳清,口感细腻
AM1	0.1121	5.1	54	3.2×10^{8}	色暗红,弱豆味,少量乳清,口感细腻
CM1	0.1121	5.5	57	1.9×10^{8}	色微暗,弱豆味,无乳清,口感细腻
BM5	0.1070	5.5	60	7.1×10^{8}	色暗红,弱豆味,少乳清
白杆	0.1043	14	63	4.5×10^{8}	色微暗,弱豆味,少乳清
AP3	0.1020	5.5	51	5.3×10^{8}	色暗红,微涩,无乳清,无异味
P971P11	0.1000	4.6	52	1.01×10^{9}	色乳白,微涩,无乳清,弱豆味
ST	0.0934	5.5	45	1.58×10^{8}	色乳白,弱豆腥味,微涩
西草 2 杆	0.0087	13	47	3.1×10^{6}	色微暗,凝乳不均匀,豆腥味,少乳清
XPL-1	0.0069	7.6	35	7.7×10^{7}	色暗,豆腥味,少量乳清,微涩

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096
Motif Exhibit 1002, Page 854 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

菌株	ALDC/ μmol/mL	凝乳时间 /h	酸度 /° T	Cfu/mL	感官评价
BP2	0.0046	10	30	1.3×10^{7}	色暗，乳清析出，豆腥味，气味酸
西原 1 球	0.0005	3.8	27	1.9×10^{7}	凝乳不均与，色暗，有异味
qy(ST-日)3 球	0.0003	4.1	20	1.21×10^{7}	色暗，凝乳不均匀，重豆腥味，涩口

[0037] 从上表可以看出不同酶活的乳酸菌发酵的酸豆乳在滴定酸度和感官方面有很大的差异。酶活大于 0.1000 μmol/mL 的菌株发酵的酸豆乳在色泽，气味，表观和口感方面都优于酶活低于 0.1000 μmol/mL 的菌株，特别是酶活小于 0.0065 μmol/mL 的菌株发酵的酸豆乳不仅表观差，还具有令人难以接受的异味。酶活大于 0.1000 μmol/mL 的菌株适合做发酵豆乳的发酵剂。

Motif Exhibit 1002, Page 855 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0038] 下述实施例阐述了本发明的实践和目前优选的实施方式。

[0039] 但是，应理解，本领域熟练的技术人员在考虑到本公开内容之后可在本发明的精神和范围内作出改动和改进。

[0040] 实施例 1、高产 ALDC 乳酸菌的筛选及鉴定

[0041] 将分离的乳酸菌按 3％的接种量接入 50mL MRS 培养基中培养→发酵液于 4℃，5000r/min 离心 20min 收集菌体→ 0.85％的 NaCl 溶液洗涤，并离心→ Ph6.0 柠檬酸－磷酸盐缓冲液悬浮进行超声波细胞破碎→离心得上清为所要酶液→ 200μL 新配制底物 α－乙酰氧基－α－甲基－乙酰乙酸乙酯 +200μL 酶液 30℃水浴 20 分钟后加入显色剂→ 15-20℃显色 50 分钟，于 522nm 处测吸光度。对所获得的高产 ALDC 活性的乳酸菌进行 API 鉴定。表 1 中 15 株乳酸菌（grx90、$XN_5$3、BB-16、AM1、CM1、BM5、白杆、AP3、P971P11、ST、西草 2 杆、XPL-1、BP2、西原 1 球、qy(ST-日)3 球）的 API 鉴定见表 2 和 3。鉴定结果为：grx90、CM1 为嗜热链球菌；$XN_5$3、ST、AM1、BM5、P971P11、BP2 屎肠球菌；AP3、西原 1 球、qy(ST-日)3 为巴黎链球菌；BB-16、西草 2 杆为瑞士乳杆菌；白杆、XPL-1 为鼠李糖乳杆菌。

[0042] 表 2 乳酸菌分离株的 API20ATREP 鉴定结果

[0043]

编号	发酵管	分离株										
		grx90	$XN_5$3	AM1	CM1	BM5	AP3	P971P11	ST	BP2	西原 1 球	qy(ST-日)3 球
1	VP	+	+	+	+	+	+	+	+	+	+	+
2	HIP	+	+	-	+	-	-	-	-	-	-	-
3	ESC	-	+	+	-	+	+	+	+	+	+	+
4	PYRA	-	+	+	-	+	-	+	+	+	-	-
5	αGAL	-	+	+	-	+	+	+	+	+	+	+
6	GUR	-	-	-	-	-	-	-	-	-	-	-
7	βGAL	-	+	+	-	+	-	+	+	+	-	-
8	PAL	-	-	-	-	-	-	-	_	-	-	-
9	LAP	+	+	+	+	+	+	+	+	+	+	+
10	ADH	-	+	+	-	+	-	+	+	+	-	-
11	RIB	-	+	+	-	+	-	+	+	+	-	-
12	ARA	-	+	+	-	-	-	-	+	-	-	-
13	MAN	-	+	+	-	+	-	-	+	+	+	-
14	SOR	-	-	-	-	-	-	-	-	-	-	-
15	LAC	+	+	+	+	+	+	+	+	+	+	+
16	TRE	-	+	+	-	+	-	+	+	+	+	-
17	INU	-	-	-	-	-	+	-	-	-	+	+
18	RAE	-	-	-	-	-	+	-	-	-	+	+
19	ADM	-	-	+	-	+	+	+	+	+	+	+
20	GLGY	-	-	-	-	-	+	-	-	-	+	+
21	HEM	-	-	-	-	-	-	-	-	-	-	-
ID%		98.5	99.3	98.8	99.3	99.2	99.2	99.8	99.2	99.2	98.9	99.9

[0044] 表 3 乳酸菌分离株的 API50HCL 鉴定结果

[0045]

Motif Exhibit 1002, Page 856 of 1290

编号	发酵管	分离株			
		BB-16	白杆	西草 2 杆	XPL-1
1	CTRL	-	-	-	-
2	GLY	-	-	-	-
3	ERY	-	-	-	-
4	DARA	-	+	-	-
5	LARA	-	-	+	+
6	RIB	-	+	+	+
7	OXYL	-	-	-	+
8	LXYL	-	-	-	-
9	ADD	-	-	-	-
10	MDX	-	-	-	-
11	GAL	+	+	+	+
12	GLU	+	+	+	+
13	FRU	+	+	+	+
14	MNE	+	+	+	+
15	BBE	-	+	+	+
16	RHA	+	+	+	+
17	DUL	-	-	-	-
18	INO	-	+	-	+
19	MAN	-	+	+	+
20	SOR	-	+	+	+
21	MDM	+	+	+	+
22	MDO	-	-	-	-
23	NAO	+	+	-	+
24	AMY	+	+	+	+
25	ARB	-	+	+	+
26	ESC	-	+	+	+
27	SAL	+	+	-	+
28	CEL	+	+	+	+
29	MAL	+	+	+	+
30	LAC	+	+	-	+
31	MEL	+	-	+	+
32	BAC	+	+	+	+
33	TRE	+	+	+	+
34	INU	-	-	-	-
35	MLZ	-	+	+	+
36	RAF	-	-	+	-
37	ADM	-	-	+	+

[0046]

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

38	GLYD	-	-	+	+
39	XLT	-	-	-	-
40	GEN	+	+	-	+
41	TUR	-	-	+	-
42	LYX	-	-	+	-
43	TAG	-	+	+	+
44	DFUC	-	-	+	-
45	LFUC	-	+	+	-
46	DARA	-	-	-	-
47	LARL	-	-	+	-
48	ONT	-	+	+	-
49	2KG	-	-	-	-
50	5KG	-	-	-	-
ID%		98.8	99.9	99.9	98.9

[0047] 实施例 2、混合菌株发酵豆乳试验

[0048] 选取 9 株酶活大于 0.1000 μmol/mL 的乳酸菌经过组合后进行发酵豆乳试验。结果如下表：

[0049] 表 4 混合菌株发酵豆乳试验

[0050]

编号	ALDC/ μmol/mL	凝乳时间 /h	酸度 /° T	Cfu/mL	感官评价
H1	0.1572	4.5	69	7.1×10^{8}	色乳白，香气自然，无乳清，口感细腻
H2	0.1394	4.5	69	5.9×10^{8}	色微暗，弱豆乳香，无乳清，口感细腻
H3	0.1223	5.7	68	5.3×10^{8}	色白，弱豆味，无量乳清，口感细腻
H4	0.13181	4.5	64	3.8×10^{8}	色微红，豆香，少量乳清，口感细腻
H5	0.1221	5.1	65	2.5×10^{8}	色微暗，豆香，无乳清，口感细腻
H6	0.1470	4.5	69	1.1×10^{9}	色暗红，香气自然，少乳清，口感细腻
H7	0.1143	6.5	62	4.8×10^{8}	色微暗，弱豆香，少乳清，口感细腻
H8	0.1080	5.5	61	5.6×10^{8}	色暗红，弱豆香，无乳清，口感细腻
H9	0.1233	4.3	50	9.2×10^{8}	色乳白，无乳清，弱豆味
H10	0.1134	4.5	55	3.4×10^{8}	色乳白，弱豆腥味、无乳清

Motif Exhibit 1002, Page 858 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

编号	ALDC/ μmol/mL	凝乳时间 /h	酸度 /° T	Cfu/mL	感官评价
H11	0.1087	5.8	57	2.1×10^8	色微暗，弱豆腥味，少乳清
H12	0.1069	5.6	59	3.7×10^9	色微暗，弱豆腥味，少量乳清
H13	0.1106	5.5	56	3.3×10^7	色微暗，少乳清析出，弱豆腥味
H14	0.1109	4.0	52	2.3×10^7	色微暗，凝乳均匀，弱豆味、微涩口
H15	0.1183	4.1	52	1.25×10^7	色微暗，凝乳均匀，弱豆腥味，微涩口

[0051] 注 :H1-H8 分别为 grx90 与 $XN_5$3、BB-16、AM1、CM1、BM5、白杆、AP3、P971P11 混合发酵的酸豆乳 ;H9-H12 分别为 $XN_5$3 与 CM1、BM5、白杆、P971P11 混合发酵的酸豆乳 ;H13-H15 分别为 AM1 与白杆、AP3、P971P11 混合发酵的酸豆乳。

[0052] 从表 1 可以看出不同酶活的乳酸菌发酵的酸豆乳在滴定酸度和感官方面有很大的差异。酶活大于 0.1000 μmol/mL 的菌株发酵的酸豆乳在色泽，气味，表观和口感方面都优于酶活低于 0.1000 μmol/mL 的菌株，特别是酶活小于 0.0065 μmol/mL 的菌株发酵的酸豆乳不仅表观差，还具有令人难以接受的异味。酶活大于 0.1000 μmol/mL 的菌株适合做发酵豆乳的发酵剂。

[0053] 从表 4 和表 1 可以看出混合菌株发酵的酸豆乳的凝乳时间较单菌株发酵的酸豆乳有所减短，并且混合菌株发酵的酸豆乳在色泽、气味、口感方面都优于单菌株发酵的酸豆乳。

[0054] 实施例 3、酸豆乳的制作方法

[0055] 精选黄豆→ 0.25％的 $NAaHCO_3$ 浸泡 14h →清水洗两次→ 1 ： 8 的比例用热水 (85℃左右) 磨浆→煮浆→加入 8％的白砂糖和 3％的全脂乳粉→均质 (15-20MPa) →灭菌 (90-95℃，15 分钟) →冷却 42℃左右接入 3％酶活大于 0.1000 μmol/mL 乳酸菌→ 42℃恒温发酵 4h 至 pH 值为 4.3 ～ 4.6 →放入 4℃冰箱过夜即制成凝固型酸豆乳。发酵结束后经搅拌，添加果酱果汁等，冷藏，即制成搅拌型酸豆乳。

[0056] 实施例 4、乳酸菌饮料的制作方法

[0057] 按应用实施例 1 制备好酸乳，将酸乳用杀菌并冷却的水、糖浆、乳化稳定剂等混合物稀释 3-4 倍左右，制成可直接饮用的酸豆乳饮料。

[0058] 实施例 5、发酵豆干的制作方法

[0059] 按实施例 2 制备好酸豆乳，切割成小凝乳块，阶段性加热，加热到 45-50℃促使其排出乳清，压力为 0.06 ～ 0.09kg/cm^2 之间压榨到水分含量为 40-43％，制成发酵豆干。

[0060] 实施例 6、大豆再制干酪制作

[0061] 按实施例 2、4 制备好发酵，接种毛霉 26℃培养 36h，8℃成熟 12 天制成大豆干酪。加入 14％胡萝卜汁、2.3％盐、1％糖、6.7％人造奶油、6.7％全脂奶粉、2％乳化稳定剂，85℃融化后进行包装。

[0062] 以上本发明提供的菌株发酵的酸豆乳、大豆豆干、再制豆干酪几种产品风味良好，

Motif Exhibit 1002, Page 859 of 1290

易为消费者接受。

Espacenet

Bibliographic data: CN101897418 (A) — 2010-12-01

Body taste improver comprising long-chain highly unsaturated fatty acid and/or ester thereof

Inventor(s): SUSUMU YAMAGUCHI; NARIHIDE MATSUZAKI; KEIKO BABA; IKUKAZU TASHIMA; OSAMU MORI ± (YAMAGUCHI SUSUMU, ; MATSUZAKI NARIHIDE, ; BABA KEIKO, ; TASHIMA IKUKAZU, ; MORI OSAMU)

Applicant(s): J OIL MILLS INC ± (J. OIL MILLS INC)

Classification: - **international:** ***A23D9/00; A23D9/013; A23D9/06; A23L1/221; A23L1/226; A23L1/30***
- **cooperative:** **A23D9/00**; **A23D9/06**; **A23L27/202**; **A23L33/12**; A23V2002/00 more

Application number: CN20101240821 20041109

Priority number (s): JP20030382729 20031112

Also published as: CN101897418 (B) AU2004288674 (A1) AU2004288674 (B2) AU2009202076 (A1) AU2009202076 (B2) more

Abstract of CN101897418 (A)

A body taste improver comprises a long-chain highly unsaturated fatty acid and/or ester thereof and is derived from a specific vegetable oil; a vegetable fat composition containing a long-chain highly unsaturated fatty acid and/or ester thereof, characterized by having an alpha-linolenic acid content of 5% or lower; various foods each containing any of the body taste improvers and vegetable fat composition; and a method of improving the body taste of a food, which comprises incorporating any of the body taste improvers and vegetable fat composition into the food; and a method of improving thebody taste of a food, which comprises respectively incorporating a long-chain highly unsaturated fatty acid and/or ester thereof and body taste reinforcement composition into the food.

(19)中华人民共和国国家知识产权局

(12)发明专利

(10)授权公告号 CN 101897418 B
(45)授权公告日 2013.08.14

(21)申请号 201010240821.4
(22)申请日 2004.11.09
(30)优先权数据
2003-382729 2003.11.12 JP
(62)分案原申请数据
200480033206.9 2004.11.09
(73)专利权人 J-制油株式会社
地址 日本东京都
(72)发明人 山口进 马场启子 森修
田岛郁一 松崎成秀
(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 付磊 李炳爱
(51)Int.Cl.
A23L 1/221(2006.01)
(56)对比文件
CN 1090318 A,1994.08.03, 全文.
CN 1145729 A,1997.03.26, 全文.
CN 1389126 A,2003.01.08, 说明书第1页所属技术领域和第6页实施例2.
审查员 张敏

权利要求书1页 说明书14页

(54)发明名称

含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂

(57)摘要

本发明涉及醇香味改良剂,其含有来源于植物油的长链高不饱和脂肪酸和/或其酯;含有长链高不饱和脂肪酸和/或其酯的植物油脂组合物,其中α-亚麻酸含量是5%或更少;具有改善的醇香味的食品,所述食品含有所述植物的油脂组合物;改善食品醇香味的方法,包括向所述食品中添加植物油脂组合物;以及改善食品醇香味的方法,包括向所述食品分开添加长链高不饱和脂肪酸和/或其酯以及醇香味增强组分。

CN 101897418 B

1. 含有具有 18 个或更多碳原子和 3 个或更多双键的 n-6 长链高不饱和脂肪酸和 / 或其酯的植物油脂组合物作为醇香味改良剂的用途，其中 α - 亚麻酸的含量是植物油脂组合物的 5％或更少；所述长链高不饱和脂肪酸和 / 或其酯的含量是 10 ～ 100,000ppm，以所述长链高不饱和脂肪酸的含量表示。

2. 权利要求 1 所述的植物油脂组合物的用途，其中油酸的含量是植物油脂组合物的 20 ～ 86％，并且 / 或者亚油酸的含量是植物油脂组合物的 3 ～ 25％。

3. 权利要求 1 或 2 所述的植物油脂组合物的用途，其中所述长链高不饱和脂肪酸是花生四烯酸。

4. 权利要求 3 所述的植物油脂组合物的用途，其中花生四烯酸得自微生物。

5. 一种烹调食品的方法，包括向食品中添加权利要求 1-4 中任一项所述的植物油脂组合物并加热它们。

6. 一种改善食品醇香味的方法，包括向所述食品中添加权利要求 1-4 中任一项所述的植物油脂组合物。

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂

[0001] 本申请是以下申请的分案申请：申请日2004年11月9日，申请号200480033206.9(PCT/JP2004/016557)，标题“含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂”。

技术领域

[0002] 本发明涉及一种含有作为主要成分的长链高不饱和脂肪酸和/或其酯和醇香味增强组分的醇香味（或“kokumi”味道）改良剂（或增香剂）；一种增强含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂的效果的方法，包括向醇香味改良剂中添加醇香味增强组分；一种植物油脂组合物，其含有具有低含量α-亚麻酸的植物油脂和一种含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂；一种含有上述成份并且具有改善的醇香味的食品；和一种改善食品醇香味的方法，包括向所述食品中添加醇香味改良剂或所述植物油脂组合物。

[0003] 发明技术

[0004] 花生四烯酸（顺式-5,8,11,14-二十碳四烯酸）属于长链高不饱和（多不饱和）脂肪酸，并且存在于获自动物器官或组织的磷脂中。该脂肪酸是必需的，并且作为合成前列腺素、促血栓素、白细胞三烯等的前体非常重要。

[0005] 已经试图添加例如花生四烯酸及其酯的长链高不饱和脂肪酸用于营养富集，并且由于花生四烯酸的上述显著功能而提供各种生理功能。

[0006] 已公开的日本专利申请Hei 10(1998)-99048描述的富集成分包括：以重量计0.1～10％的花生四烯酸，其作为一种添加组分以获得类似于母乳的组合物。

[0007] 作为上述酯的例子，已公开日本专利申请Hei 4(1992)-197134公开了用于油炸的油脂组合物，其防止蒸发潜热导致的原料内部温度下降。上述组合物包括以重量计20～60％的组成脂肪酸不饱和脂肪酸，例如花生四烯酸。

[0008] 已公开的日本专利申请Hei 9(1997)-13075公开了由包含长链高不饱和脂肪酸例如花生四烯酸的甘油酯组成的油脂，并且其具有降低血液中油脂浓度的功能。通过转酯作用获得上述甘油酯。其具有与天然甘油酯不同的结构，其中小于40mol％的总长链高不饱和脂肪酸连接于甘油酯的2位。

[0009] 已公开的日本专利申请Hei 9(1997)-13076公开了与上述具有相同组成的油脂，其具有抑制血小板聚集的功能。

[0010] 已公开的日本专利申请Hei 11(1999)-89513公开了一种类似于人乳油脂的合成油脂组合物，其中n-6长链高不饱和脂肪酸例如花生四烯酸被用作一种甘油三酯的组成脂肪酸。

[0011] 此外，已公开的日本专利申请Hei 10(1998)-70992和已公开的日本专利申请Hei 10(1998)-191886公开了来源于微生物的食用油，其具有大量甘油三酯形式的花生四烯酸。其优选的应用包括用于早产婴儿或婴儿的改变成分的牛乳、婴儿食品和孕妇食品。

[0012] 然而，没有披露利用长链高不饱和脂肪酸以便改善口味，例如食品和植物油脂的

Motif Exhibit 1002, Page 864 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

醇香味的技术，或没有记载上述可能性的建议。

[0013] 存在一个问题，向食品中添加长链高不饱和脂肪酸可能恶化其味道，这是由于来源于脂肪酸氧化分解产生的臭味回复味。已经试图使用多种手段解决上述问题。

[0014] 已公开的日本专利申请 Sho 63(1988)-44843 公开了其中一种方法，其中高不饱和脂肪酸被包含于油包水包油型乳剂组合物的内部油相中。已公开的日本专利申请 Hei 6(1994)-172782 公开了粉碎包含高不饱和脂肪酸的油脂的技术。已公开的日本专利申请 Hei9(1997)-176679 公开了将抗氧化剂粉末和粉碎的不饱和脂肪酸混合的技术。已公开的日本专利申请 Hei 9(1997)-263784 公开了将 δ-生育酚和包含多不饱和脂肪酸的油脂混合的技术。已公开的日本专利申请 Hei 11(1999)-12592 公开了将大豆源添加至包含高不饱和脂肪酸的鱼油中的技术。

[0015] 已公开的日本专利申请 2001-78702 公开了一种通过将油脂与提取物混合成水包油型乳剂而制备的具有增强的清淡味道、味道和回味的调味品，其作为在食品领域中应用高不饱和脂肪酸的例子。作为上述油脂的例子公开了鱼油或含有鱼油的油脂，包括以重量计 10％或更多所述鱼油是由 n-3(ω-3) 高不饱和脂肪酸组成的。

[0016] 优选使用聚甘油的脂肪酸酯作为乳化剂，并且使用含有抗氧化剂例如肌肽和鹅肌肽的提取物以防止油脂的氧化。在制备所述调味品的方法中不进行氧化处理例如不进行热处理。所述调味品对其具有显著效果的食品的例子包括切碎的产品、鱼类和加工鱼类产品。

[0017] 日本专利 No. 3220155 公开了一种调味品组合物，其是通过氧化除乳脂之外的脂肪酸而制备的，并且其特征在于含有至少一种以重量计大于 0.01％的具有 n-3 非共轭双键的多不饱和脂肪酸。该调味品组合物含有在黄油样香味中显著分辨的甜的奶油味的特征。为了获得上述特征，所述油脂需要进行氧化处理，其应该在可以轻微延迟氧化的抗氧化剂存在的过程中在可控的条件下进行。由于氧化处理过程中产生的香味含有挥发性组分，优选在封闭系统中进行氧化处理。实际上，在一个实施例中通过使用回流冷凝器进行氧化处理。据描述，所述调味品组合物特别适用于向希望具有黄油香味的食品中添加香味。

[0018] 美国专利 US 3,689,289 公开了一种通过在特定条件下热处理还原糖、氨基酸和花生四烯酸或其甲酯来生产人造鸡肉香味的方法。进一步，国际公开的 WO 03/051139 小册子公开了一种通过在特定条件下热处理还原糖、氨基酸和花生四烯酸来生产人造鸡肉香味的方法，其中通过使用甘油酯形式的花生四烯酸来获得所述人造鸡肉香味的耐热性和持久性。

[0019] 虽然在上述方法中通过加热三种化合物糖、氨基酸和花生四烯酸而获得的产品可以产生人造鸡肉香味，没有关于通过利用长链高不饱和脂肪酸例如单独使用花生四烯酸，或与氨基酸或糖联合使用以增加食品“umami”味道的描述。

[0020] 已公开的日本专利申请 2002-95439 公开了含有长链高不饱和脂肪酸甘油酯的调味品，允许人们在食用各种食品的同时摄入长链高不饱和脂肪酸。本发明的目的是增加长链高不饱和脂肪酸对氧化所致恶化敏感的氧化稳定性。因此，其特征在于向通过发酵大豆或鱼类和贝壳类而制备的加工食品或向主要由番茄成份组成的调味品中添加长链高不饱和脂肪酸甘油酯。因此，没有披露或教导氧化处理长链高不饱和脂肪酸，或通过相同的酸本身增加或提供食品的醇香味。

[0021] [专利文献 1] 日本专利 3220155

Motif Exhibit 1002, Page 865 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0022] [专利文献 2] 美国专利 US 3,689,289

[0023] [专利文献 3] 国际公开 WO 03/051139 小册子

[0024] [专利文献 4] 已公开的日本专利申请 2002-95439

发明内容

[0025] 在食品领域，存在某些种类的需要“醇香味”和“浓厚的或浓重的味道”的食品，例如油炸食品，包括炸猪排，含有油脂的食品，包括咖喱和“饺子”（中国式猪肉饺子）。通常，加入香料，或者单独使用动物油脂或与植物油脂联合使用来为食品提供上述味道。

[0026] 然而，存在一个问题，所添加的香料在热处理期间挥发以致无法维持所产生的醇香味。还有一些担心，动物油脂所含的胆固醇或饱和脂肪酸可能对健康不利。另一方面，存在一个问题，由于植物油脂含有少量胆固醇或饱和脂肪酸，使用植物油脂烹调的食品的味道单一或平淡，致使需要醇香味的食品不能令人满意。

[0027] 因此希望提供不含胆固醇并具有低含量的饱和脂肪酸然而具有醇香味的油脂。

[0028] 长期以来，认为长链高不饱和脂肪酸例如花生四烯酸和/或其酯是肉类腐败气味和类似异味的原因。然而，据发现当单独将食品与长链高不饱和脂肪酸和/或其酯混合，或进行氧化处理例如与含有预先确定量的长链高不饱和脂肪酸和/或其酯的植物油脂一同加热时，食品的醇香味增强并且食品的最初味道增加 (PCT/JP03/00182)。

[0029] 本发明人进行了研究以力图攻克上述问题，并且最终发现可以通过向含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂中添加醇香味增强组分例如 α-生育酚和/或含铁组分，以进一步增强醇香味改善效果。本发明人同时发现，通过向具有低 α-亚麻酸和/或亚油酸含量的植物油脂组合物中添加长链高不饱和脂肪酸和/或其酯，可以大大改善所述植物油脂组合物的醇香味。基于上述发现完成了本发明。

[0030] 因此，本发明涉及一种含有作为主要成分的长链高不饱和脂肪酸和/或其酯和醇香味增强组分的醇香味改良剂；一种含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂，所述长链高不饱和脂肪酸和/或其酯来源于具有大量 γ-亚麻酸的特定植物油；一种增强含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂的效果的方法，包括向醇香味改良剂中添加醇香味增强组分；一种含有长链高不饱和脂肪酸和/或其酯的具有 5％或更少 α-亚麻酸含量的植物油脂组合物；一种具有改善的醇香味的的食品，所述食品含有醇香味改良剂或所述植物油脂组合物；一种改善食品醇香味的方法，包括向所述食品中添加醇香味改良剂或植物油脂组合物；以及一种改善食品醇香味的方法，包括向所述食品中分开添加长链高不饱和脂肪酸和/或其酯以及醇香味增强组分。

[0031] 本说明书中的“醇香味改良剂”意指一种实现本发明优越性的试剂，即一种添加于食品中，为食品重新提供显著的“醇香味”或“浓厚的或浓重的味道”或使其改善的试剂。本说明书的实施例中描述了通过感官测试对“醇香味”进行评价。

[0032] 本说明书中的“醇香味增强组分”意指一种可以通过长链高不饱和脂肪酸和/或其酯额外地或协同地增强上述优越性的成分。

[0033] 可以通过向含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂中添加醇香味增强组份例如 α-生育酚和/或铁组份，使醇香味改善效果得到进一步增强。通过向具有低 α-亚麻酸和/或亚油酸含量的植物油脂组合物中添加长链高不饱和脂肪酸和/或其酯，可

Motif Exhibit 1002, Page 866 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

以大大改善所述植物油脂组合物的醇香味。

[0034] 对醇香味增强成分的种类或结构没有限制，包括作为典型例子的 α－生育酚以及铁组份。虽然不应该视为以任何方式限制本发明，但是已知 α－生育酚和铁组份促进油脂的氧化。

[0035] 可以从本领域技术人员已知的任何市场上可买到的含铁原料提供所述铁组份，包括离子盐例如硫酸亚铁、柠檬酸亚铁、柠檬酸铁、葡萄糖酸亚铁、氯化铁和乳酸亚铁；以及血红素铁；血浆粉末；和血细胞粉末。存在 3 种形式的铁，即铁原子、亚铁盐以及铁盐，优选亚铁盐。可以通过任何已知的方法例如溶解、分散和乳化，以使所述铁组份与长链高不饱和脂肪酸和／或其酯混合。

[0036] 也可以从任何本领域技术人员已知的商品原料提供 α－生育酚。一种市场上可买到的含有 α－生育酚作为最主要的生育酚成分，更具体地说，α－生育酚占总生育酚的 50％或更多的生育酚配制品，可以用于提供本发明的 α－生育酚。还可以使用含有高含量 α－生育酚的植物油脂来提供本发明的 α－生育酚。另外，可以从任何市场上可买到的生育酚配制品中去除 β－，γ－，δ－生育酚，以得到用于本发明的具有富集的 α－生育酚的生育酚配制品。

[0037] 对醇香味改良剂中的醇香味增强组分的含量没有限制，考虑优势和成本，对于 α－生育酚，优选 50 ～ 15,000ppm，更优选 50 ～ 5,000ppm，更优选 100 ～ 2,000ppm，以及对于铁组份，以 Fe 表示的含量为 0.1 ～ 200ppm，优选 0.5 ～ 100ppm。

[0038] 在本说明书中的“长链高不饱和脂肪酸”在 n-3 长链高不饱和脂肪酸的情况下，意指一种具有 20 或更多碳原子和 3 个或更多双键的脂肪酸，和在 n-6 长链高不饱和脂肪酸的情况下，意指具有 18 个或更多碳原子和 3 个或更多双键的脂肪酸。在两种情况中优选具有 20-24 个碳原子和 4-6 个双键的长链高不饱和脂肪酸。n-6 长链高不饱和脂肪酸的例子包括 γ－亚麻酸、花生四烯酸 (AA) 和二十二碳四烯酸 (DTA)，优选花生四烯酸。n-3 长链高不饱和脂肪酸的例子包括二十二碳六烯酸 (DHA) 和二十碳五烯酸 (EPA)。

[0039] 对用于本发明的长链高不饱和脂肪酸，例如花生四烯酸和其酯的来源没有限制。本领域技术人员可以选择性地使用市场上可买到的来源于各种动物和蔬菜、微生物和藻类的长链不饱和脂肪酸。

[0040] 例如，已公开的日本专利申请 Hei 10(1998)-70992 和 Hei10(1998)-191886 公开了来源于细菌的含有大量甘油三酯形式的花生四烯酸的食用油脂。因此可以从所述食用油脂得到花生四烯酸。

[0041] 还可以混合和使用两种或更多种长链高不饱和脂肪酸或具有不同来源但属于相同种类的长链高不饱和脂肪酸。

[0042] 对于所述长链高不饱和脂肪酸酯的结构和制备没有限制，并且一元醇和多元醇可以用作构成上述酯的醇。考虑到安全和成本，甘油是一种优选的多元醇。所述甘油可以构成甘油三酯、甘油二酯或甘油一酯。除了所述长链高不饱和脂肪酸之外还可以含有其它脂肪酸，作为构成本发明所述酯的脂肪酸。

[0043] 植物油例如豆油、玉米油、橄榄油、HOS 和芸苔油不含 γ－亚麻酸。另一方面，琉璃苣籽油、月见草油、蔷薇果油和黑色穗醋栗油含有约 3 ～ 22％的 γ－亚麻酸。特别是，由于琉璃苣籽油含有约 22％的 γ－亚麻酸，可以优选用作本发明长链高不饱和脂肪酸的原料。

Motif Exhibit 1002, Page 867 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0044] 因此，本发明涉及一种醇香味改良剂，其含有来源于植物油的长链高不饱和脂肪酸和/或其酯，所述植物油选自琉璃苣籽油、月见草油、蔷薇果油和黑色穗醋栗油，特别是来源于琉璃苣籽油。

[0045] 对本发明醇香味改良剂中含有的长链高不饱和脂肪酸和其酯的含量没有限制。然而，如果含量过低，需要更多量的醇香味改良剂，由此可能由于其含有的其它组分而导致不利的影响。本发明的醇香味改良剂含有长链高不饱和脂肪酸和其酯的量优选为以重量计10％或更多，更优选以重量计30％或更多。

[0046] 本发明的醇香味改良剂可以额外含有本领域技术人员已知的其它任选组分，例如乳化剂；生育酚；甾醇；磷脂及其他脂肪酸；含有其它脂肪酸的甘油三酯、甘油二酯和甘油一酯。

[0047] 优选将n-3长链高不饱和脂肪酸和/或其酯进行氧化处理，以充分实现本发明的优越性。对所述氧化处理的方法没有限制，例如包括热处理。对热处理的方法也没有限制。存在抗氧化剂或在封闭系统中时不必进行氧化处理。因此所述加热通常在40℃～200℃进行0.1～240小时，优选在80℃～180℃进行0.5～72小时。虽然不必对n-6长链高不饱和脂肪酸和/或其酯进行氧化处理以实现其作为醇香味改良剂的优越性，应该进一步通过氧化处理使其优越性得到增强。

[0048] 例如，可以通过加热含有长链高不饱和脂肪酸和/或其酯的醇香味改良剂，或对含有醇香味改良剂的食品进行氧化处理。

[0049] 长链高不饱和脂肪酸比其酯更具挥发性，特别是甘油酯，甚至较少量的所述酸就可以实现本发明的优点。另一方面，酯相对较少挥发性但是持续有效，其以相对较大的量包含于组合物中。

[0050] 本发明进一步涉及含有醇香味改良剂的植物油脂组合物。可以将任何本领域技术人员已知的植物油脂用于本发明的基础油脂，包括大豆油、菜子油、玉米油、向日葵油、HOS、米糠油、芝麻油、橄榄油和棕榈油。其中，可以通过向主要用于加热烹调例如油炸和炒制的豆油、菜子油、玉米油和棕榈油中添加醇香味改良剂以有效地实现本发明的优点。可以任选地混合并使用任何两种或多种所述植物油脂。

[0051] 取决于长链高不饱和脂肪酸的种类和应用，本领域技术人员可以选择性地确定所述植物油脂组合物中的长链高不饱和脂肪酸和/或其酯含量，通常是10～100,000ppm，优选10～20,000ppm，更优选10～5,000ppm，更优选20～1,000ppm，以长链高不饱和脂肪酸的含量表示。特别是，当含有来源于植物油的长链高不饱和脂肪酸例如γ-亚麻酸的植物油脂组合物被用作油类烹调例如油炸所用油脂的基础油脂，或用作供给香气或香味的油类的基础油脂时，植物油脂组合物中的长链高不饱和脂肪酸例如γ-亚麻酸的含量通常是0.05～10％，优选0.1～5％。

[0052] 本说明书已经提及，琉璃苣籽油、月见草油、蔷薇果油和黑色穗醋果油含有的γ-亚麻酸的含量为约3～22％。因此，来源于蔬菜的油可以有益地用作本发明的植物油脂，不用更进一步添加长链高不饱和脂肪酸和/或其酯。

[0053] 此外，本发明人发现，如果根据本发明特定条件混合所述植物油脂的脂肪酸和长链高不饱和脂肪酸和/或其酯，可以更加有效地增加醇香味。

[0054] 由此，优选的植物油脂组合物的α-亚麻酸含量为5％或更少，例如0.01～5％，

Motif Exhibit 1002, Page 868 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

优选 0.05 ～ 3%。进一步，更优选地，植物油脂组合物的油酸含量为 20 ～ 86%并且 / 或亚油酸的含量为 3 ～ 25%。

[0055] 如上所述，所述植物油脂组合物含有的长链高不饱和脂肪酸和 / 或其酯的量通常为 10 ～ 100,000ppm，优选 10 ～ 20,000ppm，更优选 10 ～ 5,000ppm，更优选 20 ～ 1,000ppm。所述植物油脂组合物不必含有醇香味增强组分。

[0056] 由于琉璃苣籽油和黑色穗醋栗油基本上不含 α - 亚麻酸，但是含有上述含量的油酸，由此观点，它们优选作为植物油脂组合物。

[0057] 根据本发明得到的植物油脂组合物可以用于各种烹饪方法，例如加热烹饪方法，优选在 80℃～ 300℃，更优选在 110℃～ 300℃下的加热烹饪方法。在上述温度范围的热处理可以增加用植物油脂组合物烹调过的食物的醇香味并增强其最初的味道。

[0058] 最后，本发明涉及一种具有增加的醇香味的食物，其含有醇香味改良剂或本发明所述植物油脂组合物。对食物的种类没有限制，包括用油烹调过的食物例如各种油炸和炒制的食物；以及含有脂肪的食物例如咖喱、中国式猪肉饺子和中国式猪肉蒸饺。本发明所述食物通过其中含有的醇香味改良剂或植物油脂组合物而具有显著的醇香味以及浓厚的或醇厚的味道。可以使用热处理进一步增加上述食物的醇香味。

[0059] 因此，本发明涉及一种增加食物醇香味的方法，包括添加醇香味改良剂或所述植物油脂组合物。可以向食物中分开添加醇香味改良剂中含有的长链高不饱和脂肪酸和 / 或其酯以及醇香味增强组分，以便得到相同的优越性。因此，本发明还涉及一种增加食物醇香味的方法，包括分开添加长链高不饱和脂肪酸和 / 或其酯和醇香味增强组分至所述食物中。在分开添加上述组分情况下，优选获得最终含有长链高不饱和脂肪酸和 / 或其酯和醇香味增强组分的食品，其用量与已经添加有醇香味改良剂的食物中的用量相同。本领域技术人员可以任选地选择添加上述组分的时机和顺序。

[0060] 对食品中醇香味改良剂或植物油脂组合物的量没有限制。通常，可以在食用时以 0.1 ～ 100,000ppm，优选 0.1 ～ 0,000ppm 的量使用长链高不饱和脂肪酸和 / 或其酯来获得醇香味增强效果。例如，对于液态食品例如大豆源和汤，可以在食用时以 1 ～ 1,200ppm，优选 1 ～ 500ppm 的量使用长链高不饱和脂肪酸和 / 或其酯来获得醇香味增强效果。对于干燥的食品例如汉堡，可以以优选 20 ～ 2,500ppm，优选 20 ～ 1,000ppm 的量使用长链高不饱和脂肪酸和 / 或其酯来获得醇香味增强效果。

具体实施方式

[0061] 下面将参照实施例更详细地描述本发明，其不应该理解为对本发明技术范围的限制。除非另有说明，下文实施例中的术语 " %" 意指 " 以重量计的%" 。

[0062] 通过用于分析脂肪、油类和相关原料的标准方法 2.4.10-1996(Japan Oil Chemistry Association) 来确定生育酚含量。通过用于分析脂肪、油类和相关原料的标准方法 2.4.2.2-1996(Japan Oil ChemistryAssociation) 来确定脂肪酸的含量。

[0063] 感官测试：

[0064] 在感官测试中，所述改善醇香味是指增加“香气强度”、“香味强度”、“味道强度”和“回味强度”，而没有使相应的“香气令人满意的程度”、“香味令人满意的程度”、“味道令人满意的程度”和“回味令人满意的程度”恶化。

Motif Exhibit 1002, Page 869 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0065] 评味小组 :n = 7

[0066] 下表所用表示评价结果的符号的含义如下 :

[0067] “×”:弱于或劣于对照 ;

[0068] “△”:与对照相同的程度 ;

[0069] “○”:强于或优于对照 ;

[0070] “◎”:大大强于或优于对照。

[0071] 实施例

[0072] 实施例中所用的长链高不饱和脂肪酸 :

[0073] 含有 AA 的甘油三酯 (AATG) :AA 含量 40-45％,由 Nakarai Tesk Ltd. 经销,由 Suntory Ltd

[0074] 生产 ;

[0075] 琉璃苣籽油 :γ- 亚麻酸含量 20％,由 Statfold Co. 生产 ;

[0076] 低亚麻酸芸苔油 :Ajinomoto Oil Mills Co. lnc. 生产的″ LLC″

[0077] 菜籽油 :Ajinomoto Oil Mills Co. Inc. 生产的″ CO″

[0078] 高油红花油 :Ajinomoto Oil Mills Go. Inc 生产的″ HOS″

[0079] [表 1]

[0080] 脂肪酸组成 (％)

[0081]

	C16	C18:0	C18:1	C18:2	C18:3(n-3)
CO	3.9	1.9	62.5	19.5	8.8
HOS	4.6	1.9	76.3	14.2	0.2
LLC	3.3	1.7	73.5	15.4	2.4

[0082] [表 2]

[0083] 生育酚制备及其组成

[0084] 由 ADM Co. 生产的“维生素 E5-67”

[0085]

	α	β	γ	δ
含量 (mg/g)	707	16	4	3

[0086] < α- 生育酚作为醇香味增强组分的效果 >

[0087] 将下列长链高不饱和脂肪酸和 / 或其酯和 α- 生育酚制品与所述植物油脂组合物混合以得到下列配制油。

[0088] (1)LLC

[0089] (2)1％ AAATG/LLC(AA 含量 0.4％)

[0090] (3)0.5％ AATG/LLC(AA 含量 0.2％)

[0091] (4)1％ AATG+0.002％ α- 生育酚制品 /LLC(AA 含量 0.4％, α- 生育酚含量

Motif Exhibit 1002, Page 870 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

14.1ppm)

[0092] (5)1％ AATG+0.01％ α-生育酚制品/LLC(AA含量0.4％，α-生育酚含量70.7ppm)

[0093] (6)1％AATG+0.02％ α-生育酚制品/LLC(AA含量0.4％，α-生育酚含量141ppm)

[0094] (7)1％ AATG+0.2％ α-生育酚制品/LLC(AA含量0.4％，α-生育酚含量1,410ppm)

[0095] (8)1％AATG+2％ α-生育酚制品/LLC(AA含量0.4％，α-生育酚含量14,100ppm)

[0096] (9)0.5％AATG+0.02％ α-生育酚制品/LLC(AA含量0.2％，α-生育酚含量141ppm)

[0097] 将每种配制油(15g)与面筋含量低的面粉(15g)混合，在120℃加热30分钟，然后与3g咖喱粉(S&B Food Inc.生产的“Tokusei SB Curry”)混合，并在120℃再一次加热10分钟以制备咖喱酱。添加500ml清炖肉汤(Ajinomoto Co.Inc.生产的Consomme，1.7％溶液)并加热。用清炖肉汤稀释咖喱酱以制备咖喱汤。

[0098] [表3]

[0099]

配制油(醇香味改良剂)	(1)	(2)
食用时的AA浓度(ppm)	0	112.5
香气强度	-	◎
香气令人满意的程度	-	◎
香味强度	-	◎
香味令人满意的程度	-	◎
味道强度	-	◎
味道令人满意的程度	-	◎
回味强度	-	◎
回味令人满意的程度	-	◎

[0100] [表4]

[0101] (2)对照和(3)-(9)之间的比较

[0102]

Motif Exhibit 1002, Page 871 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

配制油(醇香味改良剂)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
油中的α-生育酚浓度(ppm)	0	0	14	71	141	1410	14100	141
食用时 AA 的浓度(ppm)	112.5	56.3	112.5	112.5	112.5	112.5	112.5	56.3
香气强度	-	×	△	○	○	◎	◎	◎
香气令人满意的程度	-	×	△	◎	◎	◎	◎	◎
香味强度	-	×	△	○	○	◎	◎	◎
香味令人满意的程度	-	×	△	○	◎	◎	◎	◎
味道强度	-	×	△	◎	◎	◎	◎	◎
味道令人满意的程度	-	×	△	◎	◎	◎	○	◎
回味强度	-	×	△	◎	◎	◎	◎	◎
回味令人满意的程度	-	×	△	○	○	○	○	◎

[0103] 表 4 中的结果显示,与配制油 (2)(对照:不添加 α-生育酚)相比,每一数量的配制油 (5)-(8) 中存在的 α-生育酚增强了醇香味改善效果,即使他们具有相同的 AATG 浓度。进一步,观察到,虽然含有比配制油 (2) 较低 AATG 浓度的配制油 (3) 在醇香味改善效果方面次于配制油 (2),但是与 α-生育酚制品混合的配制油 (9) 显示比配制油 (2) 更强的醇香味改善效果,尽管其具有与配制油 (3) 相同的 AATG 浓度。

[0104] <铁组分作为醇香味增强组分的效果>

[0105] 将 $FeSO_4.7H_2O$ 以 0.00005 ～ 0.1% (铁:0.1 ～ 200ppm) 的浓度添加至 2.5% 的 AATG/LLC 中,并均匀分散。将得到的配制油添加至 0.8% 盐水中至最终浓度为 0.1%。

[0106] (1) 2.5% AATG/LC

[0107] (2) $FeSO_4.7H_2O$ 0.00005% (铁:0.1ppm)/2.5% AATG/LLC

[0108] (3) $FeSO_4.7H_2O$ 0.00025% (铁:0.5ppm)/2.5% AATG/LLC

[0109] (4) $FeSO_4.7H_2O$ 0.005% (铁:10ppm)/2.5% AATG/LLC

[0110] (5) $FeSO_4.7H_2O$ 0.05% (铁:100ppm)/2.5% AATG/LLC

[0111] (6) $FeSO_4.7H_2O$ 0.1% (铁:200ppm)/2.5% AATG/LLC

[0112] [表 5]

Motif Exhibit 1002, Page 872 of 1290

[0113]

配制油（醇香味改良剂）	(1)	(2)	(3)	(4)	(5)	(6)
食用时 AA 的浓度 (ppm)	10	10	10	10	10	10
油中铁的浓度 (ppm)	0	0.1	0.5	10	100	200
香气强度	-	△	○	◎	◎	◎
香气令人满意的程度	-	○	○	◎	○	△
香味强度	-	△	○	◎	◎	◎
香味令人满意的程度	-	○	○	○	○	△
味道强度	-	△	○	◎	◎	◎
味道令人满意的程度	-	○	○	◎	○	△

[0114] 表 5 中的结果显示，与配制油 (1) 对照相比，配制油 (2)-(6) 的香气、香味和味道强度均由于其中存在含铁组分而得到增强，即使两者盐水中 AATG 的浓度相同。结果还显示，与配制油 (1) 对照相比，更优选配制油 (2)-(5) 的香气、香味和味道，但是与配制油 (3)、(4) 和 (5) 相比，大概由于其来源于铁，较少地偏爱配制油 (6) 的香气、香味和味道。

[0115] 以下列浓度向方便面汤中添加 $FeSO_4.7H_2O$ 和 5% AATG/LLC（用 FAITAN Tonkostu 酱油进行 9 倍稀释，Ajinomoto Go. Inc 生产），并评价香气、香味和味道。

[0116] (1) 5% AATG/LLC，1g/1,000g 面汤

[0117] (2) (1)+$FeSO_4.7H_2O$ 0.001mM/1,000g 面汤（铁 :0.06ppm）

[0118] (3) (1)+$FeSO_4.7H_2O$ 0.01mM/1,000g 面汤（铁 :0.56ppm）

[0119] (4) (1)+$FeSO_4.7H_2O$ 0.1mM/1,000g 面汤（铁 :5.6ppm）

[0120] (5) (1)+$FeSO_4.7H_2O$ 1.0mM/1,000g 面汤（铁 :56ppm）

[0121] (6) (1)+$FeSO_4.7H_2O$ 5.0mM/1,000g 面汤（铁 :280ppm）

[0122] [表 6]

[0123]

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

配制油（醇香味改良剂）	（1）	（2）	（3）	（4）	（5）	（6）
食用时 AA 的浓度（ppm）	20	20	20	20	20	20
铁的浓度（ppm）	0	0.06	0.56	5.6	56	280
香气强度	-	△	○	◎	◎	◎
香气令人满意的程度	-	○	○	◎	○	△
香味强度	-	△	○	◎	◎	◎
香味令人满意的程度	-	○	○	○	○	△
味道强度	-	△	○	◎	◎	◎
味道令人满意的程度	-	○	◎	○	○	△

[0124] 表 6 中的结果显示，与配制油 (1) 对照相比，配制油 (2)-(6) 的香气、香味和味道强度均由于其中存在铁组分而得到增强，即使两者方便面汤中 AATG 浓度相同。结果还显示，与配制油 (1) 对照相比，更优选配制油 (2)-(5) 的香气、香味和味道，但是与配制油 (3)、(4) 和 (5) 相比，大概由于其来源于铁，较少地偏爱配制油 (6) 的香气、香味和味道。

[0125] < 琉璃苣籽油作为醇香味改良剂的效果 >

[0126] 在 3kg 每种上述植物油脂组合物中油炸 150g 马铃薯圈 (Potato shoe strings) (Ajinomoto Frozen Foods Co., Inc. 生产的“马铃薯圈”)，在 180℃下油炸 3 分钟。

[0127] [表 7]

[0128]

Motif Exhibit 1002, Page 874 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

琉璃苣籽油含量（%）	0	0.1	0.5	1	5	10	20	30	50	100
γ-亚麻酸含量（%）	0	0.02	0.1	0.2	1	2	4	6	10	20
香气强度	-	△	○	◎	◎	◎	◎	◎	◎	◎
香气令人满意的程度	-	△	○	◎	◎	◎	○	○	○	×
香味强度	-	△	○	○	◎	◎	◎	◎	◎	○
香味令人满意的程度	-	△	○	○	◎	◎	◎	○	○	×
味道强度	-	△	○	○	◎	◎	◎	◎	◎	◎
味道令人满意的程度	-	△	○	○	◎	◎	◎	○	○	×
回味强度	-	△	○	○	◎	◎	◎	◎	◎	◎
回味令人满意的程度	-	△	○	○	◎	◎	◎	○	○	×

[0129] 在含有 γ-亚麻酸作为长链高不饱和脂肪酸的琉璃苣籽油中油炸所述马铃薯圈来为其提供醇香味。另一方面，油中过量的 γ-亚麻酸将会恶化香气、香味和味道。

[0130] 通过使用下列配制油来烹调调味的蒸肉饭。

[0131] (1)LLC

[0132] (2)3%琉璃苣籽油 /LLC(γ-亚麻酸含量 0.6%)

[0133] (3)30%琉璃苣籽油 /LLC(γ-亚麻酸含量 6%)

[0134] (4) 琉璃苣籽油 (γ-亚麻酸含量 20%)

[0135] 通过向未洗的大米 (200g) 中加水 (300g)、Ajinomoto Co. Inc. 生产的“炒米饭用烹调米”(10g) 和每种配制油，然后在电饭锅中煮沸以烹调肉饭。表 8 的结果显示，与含有适当量的 γ-亚麻酸的琉璃苣籽油一同烹调可以为肉饭提供醇香味。

[0136] [表 8]

[0137]

Motif Exhibit 1002, Page 875 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

配制油（醇香味改良剂）	（1）	（2）	（3）	（4）
添加量（g）	5	5	5	5
添加的γ-亚麻酸的浓度（ppm）	0	58	580	1,942
香气强度	-	○	◎	◎
香气令人满意的程度	-	○	◎	◎
香味强度	-	○	◎	◎
香味令人满意的程度	-	○	◎	○
味道强度	-	○	◎	◎
味道令人满意的程度	-	○	◎	○
回味强度	-	○	◎	◎
回味令人满意的程度	-	○	◎	○

[0138] ＜脂肪酸组合物的效果＞

[0139] 制备了以下油脂组合物。

[0140] (1)0.0625% AATG/CO(AA 含量 250ppm)

[0141] (2)0.125% AATG/CO(AA 含量 500ppm)

[0142] (3)0.0625% AATG/LLC(AA 含量 250ppm)

[0143] (4)0.0625% AATG/HOS(AA 含量 250ppm)

[0144] 如下制备炸猪排：用盐 (0.6g) 和少量胡椒腌制猪腰肉 (75g)，用小麦粉、20%蛋液和面包屑涂敷，然后于 180℃在上述油脂制品中油炸 30 分钟。表 9 中的结果显示，即使 AA 含量相同，醇香味改善效果仍取决于所用油脂制品中的脂肪酸组成。更具体地说，其显示优选含有 5%或更少 α－亚麻酸 (C18:3(n-3)) 的油，例如 HOS 和 LLC。

[0145] [表 9]

[0146]

配制油	(1)	(2)	(3)	(4)
	CO	CO	LLC	HOS
AAG 含量（%）	250	500	250	250
香气强度	△	◎	◎	◎
香气令人满意的程度	△	○	○	○
香味强度	△	◎	◎	◎

Motif Exhibit 1002, Page 876 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

香味令人满意的程度	△	○	○	○
味道强度	△	◎	◎	◎
味道令人满意的程度	△	◎	◎	◎
回味强度	△	◎	◎	◎
回味令人满意的程度	△	○	○	◎

[0147] 发明优点

[0148] 可以通过将食品与醇香味改良剂或含有醇香味改良剂的植物油脂组合物一同烹调，而为食品提供醇香味。

Motif Exhibit 1002, Page 877 of 1290

Espacenet

Bibliographic data: CN102440302 (A) — 2012-05-09

Coated soybean cheese and making method thereof

Inventor(s): JINGLI XIE; QINGHUI LI; DONGZHI WEI ± (XIE JINGLI, ; LI QINGHUI, ; WEI DONGZHI)

Applicant(s): UNIV EAST CHINA SCIENCE & TECH ± (EAST CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY)

Classification: **- international:** ***A23C20/02***
- cooperative:

Application number: CN20111358380 20111111 Global Dossier

Priority number (s): CN20111358380 20111111

Also published as: CN102440302 (B)

Abstract of CN102440302 (A)

The invention discloses a technology for making coated soybean cheese. The technology for making the coated soybean cheese comprises the following three steps of: A) preparing soybean milk; B) making soybean cheese by mixed fermentation; and C) preparing the coated soybean cheese. The soybean milk is obtained by ultrasonic treatment after soybean is thermally pulped. The soybean cheese is obtained by squeezing soybean curd formed under the co-action of inoculated lactic acid bacteria leaven for fermenting and added D-gluconic acid-delta-lactone (GDL). The coated soybean cheese is prepared by using the soybean cheese subjected to limited enzymolysis as a raw material. The coated soybean cheese which is not added with any milk product components has good flavor property, coated property anddeliquescence in mouth.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 102440302 A
(43)申请公布日 2012.05.09

(21)申请号 201110358380.2

(22)申请日 2011.11.11

(71)申请人 华东理工大学
地址 200237 上海市徐汇区梅陇路 130 号

(72)发明人 谢静莉 李晴辉 魏东芝

(74)专利代理机构 上海智信专利代理有限公司 31002
代理人 薛琦

(51)Int.Cl.
A23C 20/02(2006.01)

权利要求书 1 页 说明书 8 页 附图 2 页

(54)发明名称

一种涂抹型大豆干酪及其制作方法

(57)摘要

本发明公开了一种涂抹型大豆干酪的制作工艺。本发明的涂抹型大豆干酪的制作过程分为三个工艺步骤：A) 豆乳的制备；B) 混合发酵制作大豆干酪；C) 再制涂抹型大豆干酪。所述豆乳是通过将大豆热磨浆后超声处理而获得。所述大豆干酪是由接种乳酸菌发酵剂发酵和加 D- 葡萄糖酸－δ－内酯(GDL)共同作用使豆乳形成凝乳压榨而获得。所述涂抹型大豆干酪是采用经限制性酶解的大豆干酪为原料而制成。本发明的不添加任何牛乳制品成分的涂抹型大豆干酪具备理想的风味特性、涂抹性及口中融化性。

CN 102440302 A

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

1. 一种涂抹型大豆干酪的制作方法，其特征在于包括步骤：

A) 豆乳的制备；

B) 混合发酵制作大豆干酪；

C) 再制涂抹型大豆干酪。

2. 如权利要求 1 所述的方法，其特征在于：所述步骤 A) 包括加入混合物总重量的 0.1 ～ 0.3% (w/v) 的小苏打以降低豆腥味。

3. 如权利要求 1 所述的方法，其特征在于：所述步骤 A) 还包括对豆乳进行超声处理。

4. 如权利要求 1 所述的方法，其特征在于：所述步骤 B) 包括接种乳酸菌发酵剂发酵和加 D- 葡萄糖酸 - δ - 内酯共同作用使豆乳形成凝乳。

5. 如权利要求 1 所述的方法，其特征在于：所述步骤 B) 还包括加入亲水胶体。

6. 如权利要求 5 所述的方法，其特征在于：所述亲水胶体选自：卡拉胶、果胶、黄原胶、瓜尔豆胶、CMC、魔芋粉、海藻酸钠和刺槐豆胶等。

7. 如权利要求 1 所述的方法，其特征在于：所述步骤 B) 还包括加入油脂。

8. 如权利要求 7 所述的方法，其特征在于：所述油脂为融化点接近或低于人体温的油脂，包括：半固态植物油如棕榈油、棕榈仁油、椰子油等；动物脂肪如猪油、黄油等；硬化植物油如大豆硬化油、氢化植物油等。

9. 如权利要求 4 所述的方法，其特征在于：所述步骤 B) 的乳酸菌发酵剂选自：嗜酸乳杆菌 Lactobacillus acidophilus、嗜热链球菌 Streptococcus thermophilus、干酪乳杆菌 Lactobacillus casei、保加利亚乳杆菌 Lactobacillus bulgaricus、双歧杆菌 Bifidobacterium、乳酸乳球菌乳亚种 Lactococcus lactis lactis 和乳酸链球菌 Streptococcus lactis 中的一种或多种。

10. 如权利要求 1 所述的方法，其特征在于：所述步骤 C) 包括蛋白酶解处理，所述蛋白酶选自：木瓜蛋白酶、菠萝蛋白酶、风味蛋白酶、中性蛋白酶、植物蛋白水解酶和碱性蛋白酶等。

11. 如权利要求 10 所述的方法，其特征在于：所述蛋白酶的加入量为 0.1 ～ 0.2% (w/w)。

12. 如权利要求 10 所述的方法，其特征在于：所述蛋白酶解处理安排在大豆干酪切碎融化后。

13. 权利要求 1 ～ 12 中任一项所述的方法制作的涂抹型大豆干酪。

Motif Exhibit 1002, Page 880 of 1290

一种涂抹型大豆干酪及其制作方法

技术领域

[0001] 本发明涉及食品工程领域，具体地说，是关于一种涂抹型大豆干酪及其制作方法。

背景技术

[0002] 随着奶酪涂抹面包逐渐成为一些国家，特别是亚洲国家的一种流行的新型食品食用方式，涂抹型食品（奶酪）的消费需求也随之增大。目前，大多数涂抹型奶酪都是以牛奶为主的动物奶源为原料生产的。然而，事实上，这种乳制品在很多国家的供应较少，并且由于适合饲养提供奶源的动物的土地资源越来越紧张，特别是受到环境的恶化和人口的快速增加的影响，在今后更会逐渐减少；再加上随着饮食健康观念及文化的多元化发展，现在世界上素食主义者也逐渐增多且构成了一个不容忽视的人群，对他们而言，传统的以动物奶源为原料的干酪将不再适用。另外，对于相当一部分人，在食用传统牛乳制品时由于缺乏β－半乳糖苷酶，不能水解和吸收乳糖从而导致了乳糖不耐症，引起腹部绞痛、腹胀、腹泻和恶心；以及一些人被发现对牛奶蛋白过敏。因而，寻求一种在满足普通人群消费的同时能适用于素质主义者、乳糖不耐症人群及对牛奶蛋白过敏人群的，具有类似于牛奶奶酪的质地及口感的植物蛋白来源干酪成了一个很大的创新研究点。

[0003] 众所周知，大豆的蛋白含量很高，可以作为很好的蛋白来源，相比动物奶源具有更大的生产潜力。在现有技术中，尝试添加豆乳代替部分牛乳生产混合奶酪，然而，在其特性上存在一些不理想的问题，例如不能消除豆制品的沙粒感，没有和牛奶涂抹奶酪相同的滑腻的质构，不能给人们感官上等同“奶酪”的感觉。

[0004] 在现有技术中，大豆干酪以豆腐或大豆蛋白、氢化植物油、水、盐类为原料混合融化制作而成，由于其未经发酵，不具备理想的涂抹性以及口中融化性，因而风味口感与牛奶奶酪相差甚远。为了使大豆干酪具有理想的涂抹性及口中融化性，需要添加一些牛乳制品成分，例如奶粉和奶酪。然而，要使不含牛乳制品成分的大豆干酪具备理想的涂抹性及口中融化性，需要克服一定的技术难点。由于大豆球蛋白的持水特性要高于酪蛋白，并在加热时易形成凝胶，因此得到的大豆蛋白凝胶涂抹性和口中融化性较差。与此同时，大豆制品容易形成豆腥味，且相比于牛乳制品的滑腻质构更倾向于形成颗粒质构，其品质和风味特性不够理想，因此有必要进一步加以改进。

发明内容

[0005] 本发明的首要目的就在于，提供一种具有很好风味特性、涂抹性与口中融化性的涂抹型大豆干酪产品。

[0006] 本发明的另一个目的在于，提供一种制作上述不添加任何牛乳制品成分的涂抹型大豆干酪的工艺方法。

[0007] 为实现上述目的，本发明的大豆干酪的制作方法包括以下步骤：

[0008] A）豆乳的制备；

[0009] B）混合发酵制作大豆干酪；

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0010] C) 再制涂抹型大豆干酪。

[0011] 根据本发明的优选实施例，所述步骤 A) 包括加入混合物总重量的 0.1 ～ 0.3% (w/v) 的小苏打以减少豆腥味。

[0012] 根据本发明，所述步骤 A) 还包括对豆乳进行了超声处理。

[0013] 根据本发明，所述步骤 B) 包括接种乳酸菌发酵剂发酵和加 D- 葡萄糖酸 - δ - 内酯共同作用使豆乳形成凝乳。

[0014] 根据本发明，所述步骤 B) 还包括加入亲水胶体；所述亲水胶体选自：卡拉胶、果胶、黄原胶、瓜尔豆胶、CMC、魔芋粉、海藻酸钠、刺槐豆胶等。

[0015] 根据本发明，所述步骤 B) 还包括加入油脂；所述油脂为融化点接近或低于人体温的植物油脂，包括：半固态植物油如棕榈油、棕榈仁油、椰子油等；硬化植物油如大豆硬化油、氢化植物油等。

[0016] 根据本发明，所述步骤 B) 的乳酸菌发酵剂选自：嗜酸乳杆菌 Lactobacillus acidophilus、嗜热链球菌 Streptococcus thermophilus、干酪乳杆菌 Lactobacillus casei、保加利亚乳杆菌 Lactobacillus bulgaricus、双歧杆菌 Bifidobacterium、乳酸乳球菌乳亚种 Lactococcus lactis lactis 和乳酸链球菌 Streptococcus lactis 中的一种或多种。

[0017] 根据本发明，所述步骤 C) 包括蛋白酶解处理，所述蛋白酶选自：木瓜蛋白酶、菠萝蛋白酶、风味蛋白酶、中性蛋白酶、植物蛋白水解酶、碱性蛋白酶等。优选的，所述蛋白酶的加入量为 0.1 ～ 0.2% (w/w)。

[0018] 根据一个优选实施例，所述蛋白酶解处理安排在大豆干酪切碎融化之后。

[0019] 本发明的涂抹型大豆干酪的制作方法具有以下特点：

[0020] 1、本发明的不添加任何牛乳制品成分制作的涂抹型大豆干酪，在风味特性、涂抹性以及口中融化性等方面具有如牛奶涂抹型奶酪一般的感官性质，克服了已有技术中以豆乳代替部分牛乳制作的混合干酪中存在的问题。

[0021] 2、本发明提供了一种在满足普通人群消费的同时能适用于素食主义者、乳糖不耐症人群及对牛奶蛋白过敏人群的涂抹型大豆干酪及其制作方法。

[0022] 3、由于大豆是一个营养价值丰富的高质量蛋白来源，在食品中已经广泛使用；因此本发明对于大豆干酪的开发具有非常重要的意义。

[0023] 4、大豆对人体健康有很多功效，如降低胆固醇，预防动脉硬化、癌症、糖尿病、骨质酥松症、肥胖症及肠道肾脏疾病，以及缓解女性更年期情况等。同时由于我国的大豆资源丰富，可以缓解对动物性奶源需求的压力。

[0024] 5、若不考虑到适用于素食主义者，根据本发明除应用植物油脂制作之外，添加动物脂肪如猪油、黄油等，都可以达到很好效果。

附图说明

[0025] 图 1 为本发明的大豆制作豆乳的流程图。

[0026] 图 2 为本发明的混合发酵制作大豆干酪的流程图。

[0027] 图 3 为本发明的涂抹型大豆干酪再制工艺的流程图。

[0028] 图 4 为本发明所述参加性能测试中的样品的 SDS-PAGE 分析结果图。

Motif Exhibit 1002, Page 882 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

具体实施方式

[0029] 以下结合具体实施例，对本发明作进一步说明。应理解，以下实施例仅用于说明本发明而非用于限定本发明的范围。

[0030] 实施例 1、大豆干酪的制备

[0031] 1.1、豆乳的制备

[0032] 本实施例中，豆乳的制备参考常规的方法，区别主要在于过程中加入了小苏打以去除豆腥味以及采用了超声处理，具体如下：

[0033] 如图 1 所示，取东北大豆，洗净后在水中常温浸泡 12 ～ 18h，为了降低豆腥味，同时加入小苏打，加入量为混合物总重量的 0.1 ～ 0.3％ (w/v)。

[0034] 浸泡后的大豆经加热煮沸 10min 后用清水清洗并去壳。使用热磨浆豆浆机，在 85℃、转速 11000rpm 的条件下将去壳的大豆间歇性磨浆 7 次，每次 30s，期间进行保温加热。为了增加豆乳溶解率和乳化作用，研磨后的豆乳经 4 层纱布过滤后降温至约 30℃，进行超声处理，频率为 40kHz，处理时间为 10 ～ 30min。

[0035] 制备过程中的具体条件如以下表 1 所示：

[0036] 表 1、豆乳的制备

[0037]

样品编号	1	2	3
浸泡时间（小时）	12	15	18
小苏打加入量 / 混合物总重量 (w/v)	0.3	0.2	0.1
超声处理时间（分钟）	10	20	30

[0038] 经测试，豆乳制备过程中加入混合物总重量的 0.1 ～ 0.3％ (w/v) 的小苏打能够有效降低豆腥味，从而为后续进一步制成大豆干酪打下良好的基础。

[0039] 1.2、混合发酵制备大豆干酪

[0040] 本实施例中，大豆干酪是由接种乳酸菌发酵剂发酵和加 D- 葡萄糖酸 - δ - 内酯 (GDL) 共同作用使豆乳形成凝乳压榨而得。乳酸菌在发酵豆乳中的作用有产酸、产香、除臭去腥以及改善其营养价值。豆乳中含有大量的能导致胃胀气的低聚糖如棉子糖和水苏糖，而水解利用这些低聚糖需要 α - 半乳糖苷酶去水解其中的 α - 半乳糖苷键。因而利用具有较高 α - 半乳糖苷酶活性的微生物菌种发酵大豆产品不仅可以减少大豆产品中的可导致胃胀气的低聚糖含量，而且一些益生菌种可以将大豆蛋白水解成一些功能性短肽和氨基酸，提高其营养价值。

[0041] 双歧杆菌和乳酸杆菌如发酵乳杆菌和嗜酸乳杆，都是较为理想的菌种。而 D- 葡萄糖酸 - δ - 内酯 (GDL) 具有很好的凝固豆乳的能力，因此本实施例中以乳酸菌发酵和添加 GDL 相结合，以致力于提高大豆干酪的营养价值以及凝乳块的性能。，同时使形成的凝乳块较为柔滑，风味较好。

[0042] 本实施例的混合发酵豆乳制备大豆干酪的过程如图 2 所示，具体如下：

Motif Exhibit 1002, Page 883 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0043] 将 1.1 中所得豆乳加热至 60 ～ 80℃ ；

[0044] 为改善豆乳质构，加入亲水胶体，加入量为 0.05 ～ 0.20％ (w/v)，优取卡拉胶，在 1000rpm 的条件下搅拌溶解 20min ；

[0045] 为提高豆乳的脂肪含量，使得最终产品有较好的涂抹性和口中融化性，需要加入一定的融化点接近或低于人体温的油脂，如 ：半固态植物油如棕榈油、棕榈仁油、椰子油等 ；硬化植物油如大豆硬化油、氢化植物油等，这里优选椰子油或棕榈油，本实施例的样品 1-9 分别为棕榈油、棕榈油、棕榈仁油、棕榈仁油、椰子油、椰子油、大豆硬化油、大豆硬化油、氢化植物油，添加量在 1 ～ 3％ (w/v)，在 1000rpm 的条件下搅拌溶解 20min ；

[0046] 然后对混合物进行均质，均质压力为 10 ～ 120MPa ；

[0047] 降温至 37℃后，接种入乳酸菌发酵剂，所述发酵剂菌种可以发酵豆乳产酸，加入量在 2 ～ 4％ (v/v)，发酵温度为 37 ～ 42℃ ；

[0048] 为了增加凝乳能力，一般在乳酸菌发酵 1.5 ～ 2.0h 时，当经乳酸菌发酵豆乳的滴定酸度达到约 0.20％ (乳酸) 时，加入 GDL，加入量为 0.2 ～ 0.3％ (w/v)。如乳酸菌预发酵过程过短达不到发酵利用低聚糖效果，过长导致凝乳形成，不利于 GDL 加入作用，然后将豆乳升温至 45 ～ 55℃ ；

[0049] 凝乳 1h 后进行切割，切割大小约为 5 ～ 10mm，边切割边排除乳清，切割时间为 5 ～ 10min ；

[0050] 将所得凝乳块用四层纱布包裹并置于压榨模具 (15×15×5cm) 中进行压榨定型，压力为 0.3 ～ 0.8MPa，由小到大逐渐增压，时间为 10 ～ 20min，获得大豆干酪块，冷藏待用。

[0051] 其中，所使用的乳酸菌发酵剂由 12％ (w/v) 脱脂奶粉水溶液，灭菌后接种 0.06％ (w/v) 的菌种，经 37℃发酵 18h 获得，所用菌种均购自丹尼斯克 (中国) 有限公司。

[0052] 制备过程中的具体条件如以下表 2 所示 ：

[0053] 表 2、混合发酵制备大豆干酪

[0054]

样品编号	1	2	3	4	5	6	7	8	9
亲水胶体 (w/v)	0.05	0.125	0.2	0.05	0.125	0.2	0.05	0.125	0.2
油脂 (w/v)	1	2	3	1	2	3	1	2	3
乳酸菌菌种	嗜酸乳杆菌 *Lactobacillus acidophilus*：双歧杆菌 *Bifidobacterium*=1：1			嗜热链球菌 *Streptococcus thermophilus*：干酪乳杆菌 *Lactobacillus casei*=1：1			保加利亚乳杆菌 *Lactobacillus bulgaricus*：乳酸乳球菌乳亚种 *Lactococcus lactis lactis*：乳酸链球菌 *Streptococcus lactis*=1：1：1		
乳酸菌发酵剂 (w/v)	2	3	4	2	3	4	2	3	4
发酵温度 (℃)	37	40	42	37	40	42	37	40	42
GDL (w/v)	0.2	0.25	0.3	0.2	0.25	0.3	0.2	0.25	0.3

[0055] 经过对获得的大豆干酪进行测试后发现，由于 GDL 具有很好的凝固豆乳的能力，过程中添加 GDL 后，形成的凝乳块较为柔滑，风味较好。同时，经过比较，单独使用乳酸菌发

Motif Exhibit 1002, Page 884 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

酵豆乳凝乳或 GDL 凝固豆乳得到的大豆干酪块，其产品质构效果不甚理想；而结合微生物菌种发酵与 GDL 共同使用进行凝乳，不仅可以减少胃胀气低聚糖的负面影响，而且所得的大豆干酪最终产品具有很好的质构及风味。

[0056] 1.3、再制工艺制备涂抹型大豆干酪

[0057] 豆乳一般在经过蛋白酶处理后可以达到脱腥作用，减少豆腥味；但是在发酵凝乳之前使用蛋白酶处理豆乳的话会影响凝乳的形成，因而不适用在大豆干酪发酵过程中采用酶解处理。经过实验研究发现，不经过酶处理工艺而得的大豆干酪其在质构性质上不够理想，而酶处理安排在不同的加工顺序位置，会有不同的效果。本实施例通过研究在不同的加工顺序位置的酶处理，最终确定了将酶处理在发酵凝乳之后进行，从而避免酶处理在发酵之前使用时对于凝乳形成的影响。

[0058] 如图 3 所示，将 1.2 制备的大豆干酪经过以下步骤制成涂抹型大豆干酪，具体如下：

[0059] 取一定量的上述所得的大豆干酪，切碎加热融化，加热温度为 70 ～ 100℃，加热时间为 10 ～ 20min，同时进行搅拌；

[0060] 降温至 35 ～ 50℃；

[0061] 加入蛋白酶制剂进行酶处理，优选木瓜蛋白酶或菠萝蛋白酶，添加量为 0.1 ～ 0.2％ (w/w)，处理时间为 4 ～ 5min，同时进行搅拌；

[0062] 然后进行升温处理，加热温度为 70℃～ 100℃，处理时间为 2 ～ 10min，同时进行搅拌；

[0063] 加入油脂和适量水，所述油脂种类与 1.2 中所取种类保持一致，添加量为 10 ～ 20％ (w/w)，在 1500rpm 的条件下搅拌 2min；

[0064] 加入乳化盐和盐，所述乳化盐优选多聚磷酸钠：磷酸三钠：柠檬酸钠＝ 2 ： 2 ： 1，总添加量为 2 ～ 2.5％ (w/w)，所述盐加入量为 0.03％ (w/w)，在 1500rpm 的条件下搅拌 20min；

[0065] 然后进行搅拌熔融，温度为 70 ～ 100℃，搅拌速度为 1000 ～ 1500rpm，时间为 10 ～ 25min，充填包装，快速冷却后成品贮藏。

[0066] 制备过程中的具体条件如以下表 3 所示：

[0067] 表 3、再制工艺制备涂抹型大豆干酪

[0068]

样品编号	1	2	3	4	5	6	7	8	9	10	11	12
酶处理温度（℃）	35	40	45	50	35	40	45	50	35	40	45	50
酶	木瓜蛋白酶				菠萝蛋白酶				风味蛋白酶			
酶加入量(w/w)	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2
酶处理时间（分钟）	5	5	4	4	5	5	4	4	5	5	4	4
油脂(w/w)	10	10	20	20	10	10	20	20	10	10	20	20
乳化盐总加入量（w/w）	2	2	2.5	2.5	2	2	2.5	2.5	2	2	2.5	2.5

Motif Exhibit 1002, Page 885 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0069] （续表 3）

[0070]

样品编号	13	14	15	16	17	18	19	20	21	22	23	24
酶处理温度（℃）	35	40	45	50	35	40	45	50	35	40	45	50
酶	中性蛋白酶				植物蛋白水解酶				碱性蛋白酶			
酶加入量(w/w)	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2
酶处理时间（分钟）	5	5	4	4	5	5	4	4	5	5	4	4
油脂(w/w)	10	10	20	20	10	10	20	20	10	10	20	20
乳化盐总加入量（w/w）	2	2	2.5	2.5	2	2	2.5	2.5	2	2	2.5	2.5

[0071] 经测试，采用限制性酶解的大豆干酪为原料而制成的大豆干酪能够改善大豆制品的质构，不仅能消除以往大豆制品中存在的沙粒感的不足，形成和牛奶制品相似的滑腻的质构，而且由于大分子蛋白经酶解降解成小分子蛋白或肽段，更有利食用后的消化和吸收。

[0072] 实施例 2、性能测试

[0073] 为了研究其性能差异，对不同工艺制备的大豆干酪的性能进行测试。其中添加的胶体为卡拉胶 CH515（购自丹尼斯克（中国）有限公司），添加油脂为椰子油，乳酸菌发酵剂由脱脂牛奶接种 0.06％ (w/v) 的菌种（嗜酸乳杆菌 NCFM™：双歧杆菌 HOWARUTM Bifido ＝ 1 ： 1，购自丹尼斯克（中国）有限公司），经 37℃发酵 18h 而得；所用蛋白酶为木瓜蛋白酶。

[0074] 2.1、样品制备

[0075] 用于性能测试的样品制备过程如下：

[0076] 经乳酸菌 37℃发酵凝乳，然后切割排乳清压榨得大豆干酪样品 A，经 1.3 再制工艺得涂抹型大豆干酪样品 A#；

[0077] 经加入 GDL 50℃凝乳，然后切割排乳清压榨得大豆干酪样品 B，经 1.3 再制工艺得涂抹型大豆干酪样品 B#；

[0078] 经 1.2 乳酸菌和 GDL 混合工艺发酵凝乳，然后切割排乳清压榨得大豆干酪样品 C，经 1.3 再制工艺得涂抹型大豆干酪样品 C#；

[0079] 经 1.2 乳酸菌和 GDL 混合工艺发酵凝乳，然后切割排乳清压榨得大豆干酪样品 D，不经过酶处理过程，直接加油脂、乳化盐搅拌得涂抹型大豆干酪样品 D#。

[0080] 2.2、化学分析

[0081] 对 2.1 获得大豆干酪和涂抹型大豆干酪样品进行化学分析，结果如表 4 所示。

[0082] 表 4、化学分析

[0083]

Motif Exhibit 1002, Page 886 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

样品编号	脂肪含量 (g/100 g)	总蛋白含量 (g/100g)	水分含量 (g/100g)	盐含量 (g/100g)	灰分 (g/100g)	pH 值
A	20.37±0.02	22.39±0.02	56.29±0.56	-	0.53±0.01	4.68±0.02
B	18.51±0.02	19.72±0.01	57.58±0.30	-	0.26±0.01	5.81±0.03
C	20.24±0.02	20.11±0.02	55.26±0.16	-	0.96±0.01	4.80±0.02
D	20.24±0.02	20.11±0.02	55.26±0.16	-	0.96±0.01	4.80±0.02
A#	25.32±0.02	17.60±0.02	49.62±0.27	0.3±0	1.72±0.01	6.55±0.01
B#	23.94±0.02	17.10±0.02	51.09±0.36	0.3±0	1.30±0.01	7.20±0.02
C#	26.74±0.02	19.48±0.03	48.29±0.43	0.3±0	1.56±0.01	6.54±0.02
D#	26.80±0.02	17.20±0.02	59.15±0.20	0.3±0	1.53±0.01	6.63±0.02

[0084] 由表 4 的结果可见，不同的大豆干酪制备工艺对涂抹型大豆干酪的影响差异较大，其中，经乳酸菌和 GDL 混合发酵凝乳所制备大豆干酪的性能比其他单一的乳酸菌发酵或 GDL 工艺凝乳方式更优异，同时，相对比 C# 和 D# 样品之间的差异可知，酶解工艺对于涂抹型大豆干酪的性能也有重要的影响。

[0085] 2.3、SDS-PAGE

[0086] 对 2.1 获得的大豆干酪和涂抹型大豆干酪样品进行 SDS-PAGE 分析，结果如图 4 所示。由蛋白凝胶电泳的结果可知，酶处理过程将大分子量的大豆蛋白酶解成小分子量的蛋白或肽段，可以更好地溶解和被吸收，说明酶解工艺能有效解决了以往大豆产品的沙粒感缺陷。

[0087] 2.4、TPA 质构分析

[0088] 对 2.1 获得的大豆干酪和涂抹型大豆干酪样品进行 TPA 质构分析，结果如表 5 所示。

[0089] 表 5、TPA 质构分析

[0090]

样品编号	硬度	粘着性	弹性	内聚性	黏附性	咀嚼性	回复性
A#	287.456	-438.222	0.910	0.489	140.685	128.081	0.041
B#	217.407	-663.147	0.896	0.595	129.367	115.895	0.046
C#	378.365	-1384.114	0.915	0.567	245.656	186.704	0.039
D#	433.122	-1289.201	0.906	0.539	204.003	222.509	0.046

[0091] 如表 5 所示，经不同工艺制备的涂抹型大豆干酪的 TPA 质构特性差异明显。综合而言，涂抹型大豆干酪样品 C# 在粘着性、黏附性及回复性上都显著优于其他样品，且硬度较为适中，而与样品 C# 较为接近的为样品 D#，样品 A# 和 B# 都次之较远，说明前面混合发酵凝乳制备大豆干酪的工艺与单一的乳酸菌发酵或 GDL 工艺相比具有显著优势，而酶处理工艺对改善质构有着进一步的作用。

Motif Exhibit 1002, Page 887 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0092] 2.5、感官分析

[0093] 由 10 位专业人员组成的小组对各样品进行了感官品评分析，参数包括了涂抹型大豆干酪的外观、颜色、风味、融化性、硬度、涂抹性以及总体的印象评分，每项为 9 分制，结果如表 6 所示。

[0094] 表 6、感官评定

[0095]

感官参数	样品			
	A#	B#	C#	D#
外观	6.9±0.6	6.9±0.6	7.3±0.7	7.0±0.7
颜色	6.8±0.4	6.9±0.6	7.2±0.6	7.4±0.5
风味	6.9±0.6	6.7±0.7	7.2±0.8	6.5±0.9
融化性	7.3±0.5	7.1±0.7	7.5±0.5	7.2±0.8
硬度	5.9±0.6	6.0±0.7	6.0±0.5	6.8±0.6
涂抹性	7.1±0.6	6.9±0.6	7.5±0.5	6.3±0.5
总体印象评价	6.8±0.6	6.6±0.7	7.4±0.5	6.3±0.5

[0096] 由表 6 的结果可知，样品 C#，即由本发明所表述的工艺制备的涂抹型大豆干酪具有更优的感官接受性。

Motif Exhibit 1002, Page 888 of 1290

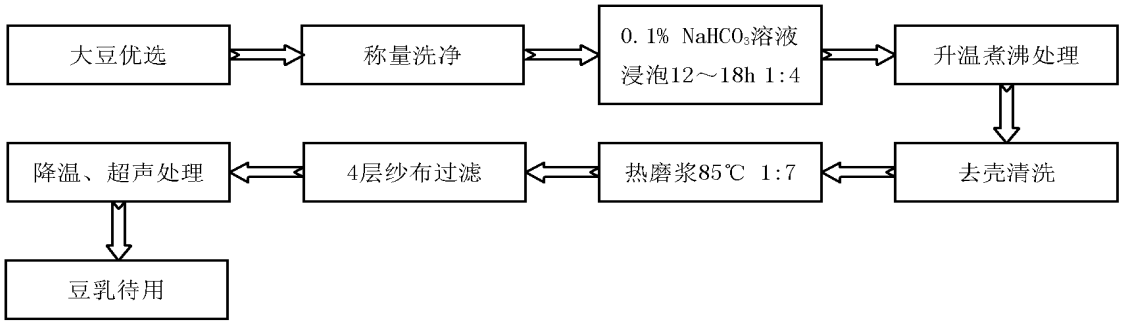

大豆优选
称量洗净
0.1% NaHCO3溶液
浸泡12～18h 1:4
升温煮沸处理
去壳清洗
热磨浆85℃ 1:7
4层纱布过滤
降温、超声处理
豆乳待用

图 1

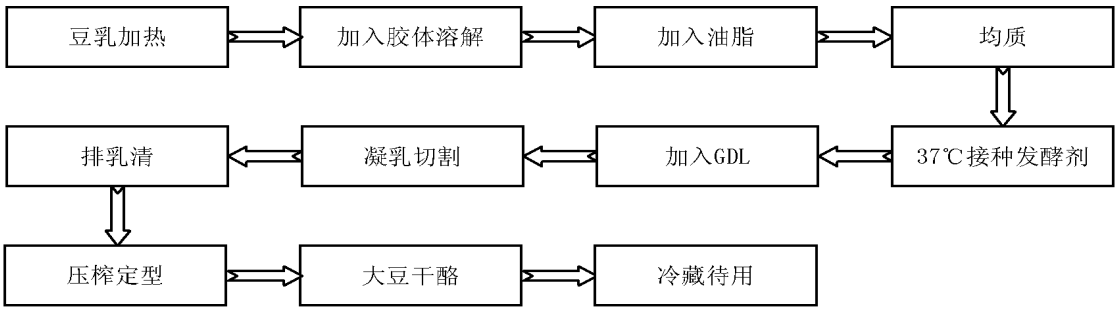

豆乳加热
加入胶体溶解
加入油脂
均质
37℃接种发酵剂
加入GDL
凝乳切割
排乳清
压榨定型
大豆干酪
冷藏待用

图 2

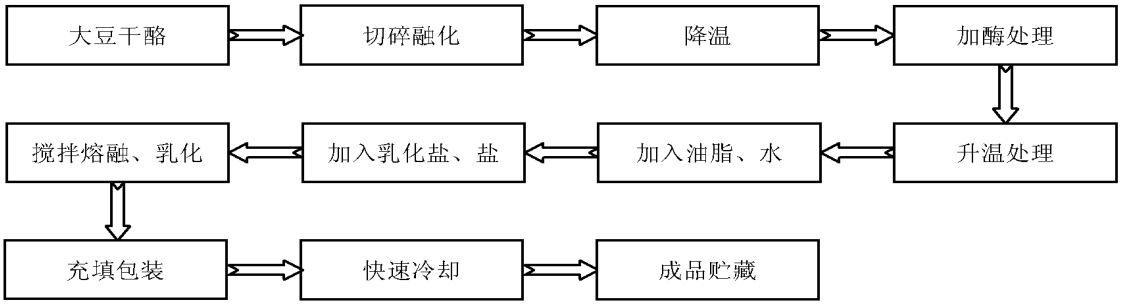

大豆干酪
切碎融化
降温
加酶处理
升温处理
加入油脂、水
加入乳化盐、盐
搅拌熔融、乳化
充填包装
快速冷却
成品贮藏

图 3

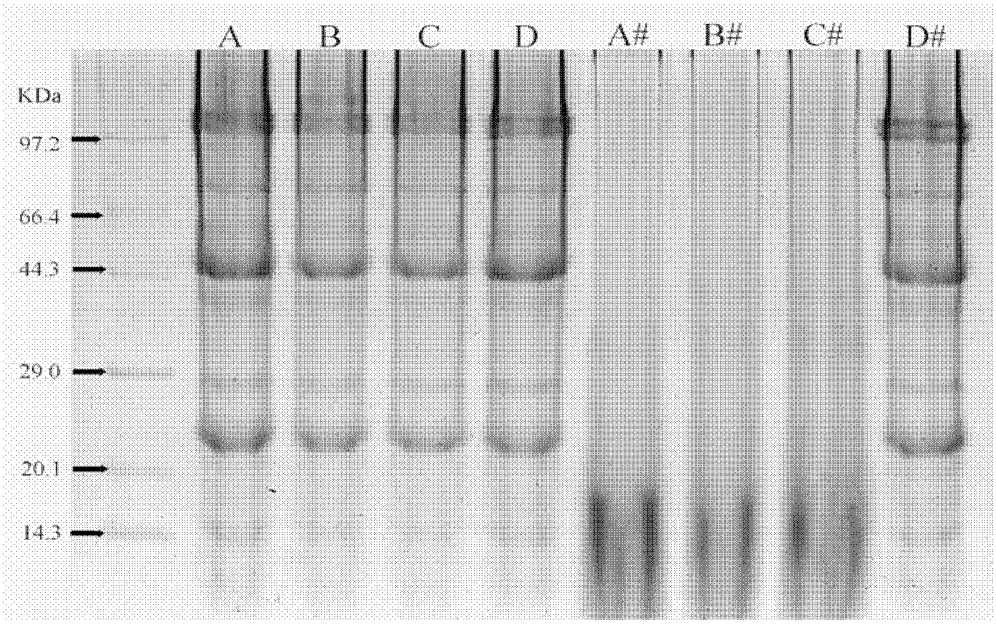


图 4

Espacenet

Bibliographic data: CN102578544 (A) — 2012-07-18

Seasoning as well as preparation method and use method thereof

Inventor(s): LIANCHENG LI ± (LI LIANCHENG)

Applicant(s): LIANCHENG LI ± (LI LIANCHENG)

Classification: **- international:** ***A23L27/24; A23L33/00***
- cooperative:

Application number: CN2012155709 20120306 Global Dossier

Priority number(s): CN2012155709 20120306

Abstract of CN102578544 (A)

The invention relates to a seasoning capable of improving the color, the flavor and the taste of fish, meat, vegetables and the like as well as a preparation method and a use method of the seasoning. The seasoning provided by the invention is characterized by comprising distiller's yeast, common salt and water, and the seasoning is healthy and has the advantages that the color, flavor and taste effects of food materials are obviously improved, and in addition, the seasoning is suitable for both old people and young people. The seasoning is suitable for being used by families, hotels, dining halls and the like during cooking.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 102578544 A
(43)申请公布日 2012.07.18

(21)申请号 201210055709.2

(22)申请日 2012.03.06

(71)申请人 李连成
地址 116023 辽宁省大连市高新园区爱贤街22号

(72)发明人 李连成

(74)专利代理机构 大连科技专利代理有限责任公司 21119
代理人 龙锋

(51)Int.Cl.
A23L 1/23(2006.01)
A23L 1/29(2006.01)

权利要求书 1 页 说明书 5 页

(54)发明名称

一种调味品及其制备方法和使用方法

(57)摘要

本发明涉及一种可提高鱼、肉、蔬菜等色香味的调味品及其制备方法和使用方法。本发明的调味品的特征在于含有酒曲、食盐和水，是一种提高食材色香味效果显著而且老少皆宜的健康型调味品，适合于家庭、饭店、食堂等在烹饪时使用。

CN 102578544 A

1. 一种调味品，其特征在于，所述调味品含有酒曲、食盐和水。

2. 根据权利要求 1 所述的调味品，其中，所述调味品的盐度为 8-10 质量 %，固形物含量为 38-42 质量 %，pH 为 4.3-4.7。

3. 根据权利要求 1 或 2 所述的调味品，其中，所述调味品中含有的水为山楂的水提取物。

4. 一种调味品的制备方法，其特征在于，所述制备方法包括如下工序：

（1）在蒸煮后的粮食中加入种曲菌进行发酵来制备种酒曲的工序；

（2）将由工序（1）得到的种酒曲与食盐均匀混合后，再加入水混合的工序；

（3）将由工序（2）得到的混合物进行发酵、熟化的工序。

5. 根据权利要求 4 所述的制备方法，其中，在工序（2）中，相对于 1 质量份的

种酒曲，食盐的加入量为 0.2-0.3 质量份，水的加入量为 1-1.5 质量份。

6. 根据权利要求 4 所述的制备方法，其中，在工序（1）中，发酵温度保持在 30-35℃，发酵时间为 48 小时以下；在工序（3）中，发酵温度保持在 20-30℃，发酵时间为 5-10 天。

7. 根据权利要求 4-6 中任意一项所述的制备方法，其中，在工序（2）中加入的水为山楂的水提取物。

8. 一种调味品的使用方法，其特征在于，将食材与权利要求 1-3 中任意一项所述的调味品混合、放置后，再对食材进行烹制或直接食用。

9. 根据权利要求 8 所述的使用方法，其中，将附着在食材上的调味品去除后再进行烹制或直接食用。

10. 根据权利要求 8 或 9 所述的使用方法，其中，所述食材选自鱼、肉或蔬菜。

一种调味品及其制备方法和使用方法

技术领域

[0001] 本发明涉及一种调味品及其制备方法和使用方法，具体而言是涉及一种可提高鱼、肉、蔬菜等色香味的调味品及其制备方法和使用方法，属于食品技术领域。

背景技术

[0002] 目前市场上有很多种类的调味品，如化学调味品、天然香辛调味品、复合调味品等。

[0003] 味精是人们常用的增鲜增味调味品。但使用味精有很多限制，使用不当甚至会对人体产生危害。味精为碱性物质，在酸性环境中不易溶解，因此在烹制糖醋、醋熘等酸性强的菜肴时，不宜使用。在含碱性原料的菜肴中也不宜使用味精，因为味精遇碱会化合成谷氨酸二钠，产生氨水臭味。而且，由于味精在高温下会分解为焦谷氨酸钠，危害人体健康，因此也不宜长时间高温加热。另外，还有研究表明，味精可能会引起胎儿缺陷，因此婴幼儿和正在哺乳期的母亲不宜吃味精。

[0004] 嫩肉粉也是人们所熟悉的调味品，它主要用于提高动物性食材的嫩度，并增鲜增味。但在嫩肉粉中通常都含有作为防腐剂和肉类发色剂的亚硝酸盐，过量食用将会导致亚硝酸盐中毒。亚硝酸盐是剧毒物质，它能使血液中正常携氧的低铁血红蛋白氧化成高铁血红蛋白，因而失去携氧能力而引起组织缺氧，成人摄入 0.2-0.5 克即可引起中毒，3 克即可致死。亚硝酸盐同时还是一种致癌物质，据研究，食道癌与患者摄入的亚硝酸盐量呈正相关性。

[0005] 随着人们生活水平的不断提高和对食品安全的不断重视，人们对健康型调味品的渴求越来越强烈。

发明内容

[0006] 本发明的目的在于提供一种用于提高鱼、肉、蔬菜等色香味的健康型调味品及其制备方法和使用方法。

[0007] 本发明的调味品含有酒曲、食盐和水。其中，调味品的盐度优选为 8-10 质量 %，固形物含量优选为 38-42 质量 %，pH 优选为 4.3-4.7。另外，本发明的调味品中含有的水优选为山楂的水提取物。

[0008] 酒曲中含有各种酶，不但可适当分解动物性食材的结缔组织、肌纤维中的胶原蛋白及弹性蛋白等，也可适当分解植物性食材的植物蛋白和纤维等，并将之部分水解为氨基酸等增鲜物质，因而使用本发明的调味品可获得嫩而不韧、味道鲜美的效果，提高食材的“香”和“味”。而且本申请的发明人发现，使用本发明的调味品还可改善食材外观，提高食材的“色”。另外，本发明的调味品中不含亚硝酸盐等有害物质，含有的食盐可起到防腐作用。因此，本发明的调味品是一种提高食材色香味效果显著而且老少皆宜的健康型调味品。

[0009] 本发明的调味品的制备方法包括如下工序：

（1）在蒸煮后的粮食中加入种曲菌进行发酵来制备种酒曲的工序；

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

（2）将由工序（1）得到的种酒曲与食盐均匀混合后，再加入水混合的工序；

（3）将由工序（2）得到的混合物进行发酵、熟化的工序。

[0010] 在工序（1）中，发酵温度优选保持在 30-35℃，发酵时间优选为 48 小时以下。在工序（2）中，相对于 1 质量份的种酒曲，食盐的加入量优选为 0.2-0.3 质量份，水的加入量优选为 1-1.5 质量份；另外，加入的水优选为山楂的水提取物。在工序（3）中，发酵温度优选保持在 20-30℃，发酵时间优选为 5-10 天。

[0011] 本发明的制备方法工艺简单，不但适合小型生产，也适合大规模化生产。

[0012] 本发明的调味品的使用方法是将食材与本发明的调味品混合、放置后，再对食材进行烹制或直接食用的方法。其中，优选将附着在食材上的调味品去除后再进行烹制或直接食用，所述食材优选为鱼、肉或蔬菜。

[0013] 本发明的使用方法非常简单易操作，只需将本发明的调味品与食材混合即可做出味美且健康的菜肴来，适合于家庭、饭店、食堂等在烹饪时使用。

具体实施方式

[0014] 调味品]

本发明的调味品含有酒曲、食盐和水。

[0015] 由于酒曲中含有大量微生物分泌产生的蛋白酶、淀粉酶等，当使用本发明的调味品调制鱼、肉、蔬菜等食材时，不但可适当分解鱼、肉的结缔组织、肌纤维中的胶原蛋白及弹性蛋白等，也可适当分解蔬菜的纤维和植物蛋白等，因此可以软化肉质，大大提高食材的嫩度，特别是对肉质较老的食材效果更为显著。另外，由于食材中一部分淀粉、纤维和蛋白质被水解为糖和氨基酸（增鲜物质），因此可以达到味道鲜美的效果，提高食材的“香”和“味”。

[0016] 此外，虽然原理并不清楚，但本申请的发明人发现，使用本发明的调味品还可改善食材外观，使鱼类食材看起来更为鲜亮，肉类食材看起来更为红润，蔬菜类食材看起来更为鲜嫩，提高食材的“色”，促进食欲。

[0017] 对本发明的调味品中的盐度没有特别限制，但优选为 8-10 质量 %。当盐度保持在上述范围时，可以得到保存性良好且咸度适中的调味品。本发明的调味品中不必另外添加作为防腐剂的亚硝酸盐等有害物质。

[0018] 对本发明的调味品中的固形物含量也没有特别限制，但优选为 38-42 质量 %。当固形物含量保持在上述范围时，可以得到酒曲浓度和盐度合适的调味品。

[0019] 由于本发明的调味品是经发酵得到的，而发酵一般是在酸性条件下进行的，因此本发明的调味品的 pH 一般在 4.3-4.7 的范围内。

[0020] 另外，本发明的调味品中含有的水优选为山楂的水提取物。山楂味酸、甘、性温，不仅营养价值丰富，还有助消化、增食欲、消积食等药用价值。而且，山楂还具有软化肉质、去除肉类异味的作用，与肉同食有很好的效果。

[0021] 综上所述，本发明的调味品是一种提高食材色香味效果显著而且老少皆宜的健康型调味品，具有良好的社会和经济效益，市场前景广阔。

[0022] [制备方法]

本发明的调味品的制备方法包括如下工序：

（1）在蒸煮后的粮食中加入种曲菌进行发酵来制备种酒曲的工序；

Motif Exhibit 1002, Page 895 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

（2）将由工序（1）得到的种酒曲与食盐均匀混合后，再加入水混合的工序；

（3）将由工序（2）得到的混合物进行发酵、熟化的工序。

[0023] 工序（1）是制备种酒曲的传统方法，具体是在蒸煮后的粮食中加入种曲菌，通过发酵来制备种酒曲。

[0024] 对用于制备种酒曲的粮食没有特别限制，可列举稻类（水稻等）、麦类（小麦、大麦、燕麦等）、谷类（粟、黍等）、豆类（大豆、蚕豆、豌豆、绿豆等）、薯类（红薯、马铃薯、山药等）、玉米、高粱等。其中的稻类、麦类等带壳的粮食需经脱壳处理后再进行蒸煮。

[0025] 对于使用的种曲菌没有特别限制，只要是制酒用的种曲菌即可，可自制，也可买市售的商品。其用量可根据使用的粮食种类、发酵温度和时间等适当调节，一般为 2g 种曲菌 /kg 蒸煮后的粮食。

[0026] 在工序（1）中，发酵温度优选保持在 30-35℃，发酵时间优选为 48 小时以下。通过将发酵温度和时间保持在上述范围，可得到微生物含量高且代谢能力强的种酒曲。发酵过程中还要适当翻动发酵物，以保证发酵均匀、完全。

[0027] 工序（2）将由工序（1）得到的种酒曲与食盐均匀混合后，再加入水混合的工序。要注意在盛装该混合物及发酵该混合物的过程中避免使用不锈钢或其他金属制容器，例如可以使用塑料容器。

[0028] 首先将种酒曲搅拌开，加入食盐后，使二者均匀混合。食盐在酒曲发酵过程中可以抑制杂菌生长，并在调味品中起到调味的作用。对于食盐用量没有特别限制，但如果食盐用量过少，在工序（3）的发酵过程中杂菌生长难以得到抑制而导致得不到所需的酒曲，从而造成发酵失败。如果过高，对酒曲的发酵也会产生不良影响，而且得到的调味品会过咸。因此，从保存性和调味性二者来看，相对于 1 质量份的种酒曲，食盐的加入量优选为 0.2-0.5 质量份，更优选为 0.2-0.3 质量份。

[0029] 接下来，在种酒曲和盐的混合物中加入水，并充分混合。虽然对水的用量没有特别限制，但以刚刚没过种酒曲和盐的混合物为宜，一般来讲，相对于 1 质量份的种酒曲，水的加入量优选为 1-2 质量份，更优选为 1-1.5 质量份。如果水量过少，处于表面的种酒曲有可能暴露在空气中，造成发酵不完全。如果水量过多，则会造成盐度降低，使保存性下降。

[0030] 另外，在工序（2）中加入的水优选为山楂的水提取物。山楂的水提取物可按常规方法制备，例如将鲜山楂洗净去核后加入适量水，文火熬煮至山楂烂熟后，再用过滤设备将山楂肉和皮等过滤掉，得到的汁液即可作为山楂的水提取物。对山楂的水提取物的浓度没有特别限制，可依口味适当调节。

[0031] 工序（3）是将由工序（2）得到的混合物进行发酵、熟化的工序。

[0032] 在工序（3）中，发酵温度优选保持在 20-30℃，更优选保持在 25-30℃。发酵时间优选为 5-10 天，更优选为 7-10 天。通过将发酵温度和时间保持在上述范围，可得到充分发酵和熟化的酒曲。

[0033] 在发酵、熟化过程中，最好大概以每日 1-2 次的频度翻动发酵物，使之与空气接触，以保证发酵、熟化充分。另外，在发酵、熟化的初期阶段，由于种酒曲吸水而造成水量减少，可以适当添加水，使水总保持为刚刚没过发酵物的程度，并充分混合。

[0034] 本发明的制备方法具有工艺简单、配方简单的特点，不但适合小型生产，也适合大规模化生产。

Motif Exhibit 1002, Page 896 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0035] [使用方法]

本发明的调味品的使用方法是将食材与调味品混合、放置后，再对食材进行烹制或直接食用的方法。所述食材可使用鱼、肉或蔬菜。

[0036] 对混合方法没有特别限制，可依食材大小而定，当为体积较大的食材时，一般采用将调味品涂抹在食材上的方法。当为体积较小的食材时，一般采用将食材包埋在调味品中的方法、或者取适量的调味品加到食材中后搅拌均匀的方法。

[0037] 对放置时间也没有特别限制，一般为 2 小时以上，优选为 3 小时以上。

[0038] 调味品用量可依口味适当调节，但一般以食材重量的 7-10% 为宜。

[0039] 经调制后的食材可直接进行烹制或食用，也可先将附着在食材上的调味品去除后再进行烹制或食用。例如，使用本发明的调味品拌制蔬菜沙拉时，先将蔬菜切成小块，加入本发明的调味品搅拌均匀，放置 2 小时后，可直接食用，也可从菜肴美观性的角度出发，将附着在食材上的调味品去除后再直接食用。另外，由于酒曲在高温下容易变焦，因此在煎炸食材时，优选将食材上的调味品去除后再进行烹制。

[0040] 本发明的使用方法非常简单易操作，只需将食材与本发明的调味品混合、放置即可做出味美且健康的菜肴来，适合于家庭、饭店、食堂等在烹饪时使用。

实施例

[0041] 下面通过实施例和比较例对本发明进行详细说明，但本发明不受这些实施例的限制，在符合前后宗旨的范围内，可作适当变化。

[0042] 实施例 1

在煮好的大米饭中加入自制的种曲菌（2g 种曲菌 /kg 大米饭），在 35℃的条件下发酵 48 小时，并在发酵过程中每日翻动发酵物，得到种酒曲（工序（1））。取塑料盒，向其中加入 300g 制得的种酒曲，将酒曲搅拌开后，向其中加入 60g 食盐均匀混合，再加入 300g 温水（30℃）充分混合（工序（2））。将得到的混合物在 25℃的条件下发酵 1 星期，每天搅拌 1-2 次，并在初期阶段适当添加水，使水总保持为刚刚没过发酵物的程度，得到调味品 1（工序（3））。调味品 1 的盐度为 9 质量 %，固形物含量为 40 质量 %，pH 为 4.5。

[0043] 取鳕鱼、牛肉切成小块，芹菜切成小段，以食材重量 10% 的比例，将调味品 1 分别加到各个食材中搅拌均匀，放置 2 小时后，经厨师烹制，烹制的菜肴请 10 个志愿者进行品尝。按照菜肴鲜美、软嫩程度，分为 +、++、+++、++++ 四个级别（6 人以上均一致评价的级别作为最后结果），结果如表 1 所示。

[0044] 实施例 2

将 100g 鲜山楂洗净去核后加入 100g 水，文火熬煮至山楂烂熟后，再用硅藻土过滤器（青岛光大装备科技有限公司制造）将山楂肉和皮等过滤掉，得到的汁液即为山楂的水提取物。除了将制备调味品 1 的工序（2）中使用的水替换为该山楂的水提取物以外，其余的制备工序与实施例 1 同样，得到调味品 2。调味品 2 的盐度为 9 质量 %，固形物含量为 39 质量 %，pH 为 4.3。

[0045] 除了将调味品 1 换成调味品 2 之外，其他与实施例 1 同样，菜肴的其他辅料相同，并经同一厨师对各个食材进行烹制，请同样的志愿者进行品尝，评价结果如表 1 所示。

[0046] 比较例 1

Motif Exhibit 1002, Page 897 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

除了不使用调味品 1 之外，其他与实施例 1 同样，菜肴的其他辅料相同，并经同一厨师对各个食材进行烹制，请同样的志愿者进行品尝，评价结果如表 1 所示。

[0047] 比较例 2

除了将调味品 1 换成味精并将其用量调整为食材重量的 0.5% 之外，其他与实施例 1 同样，菜肴的其他辅料相同，并经同一厨师对各个食材进行烹制，请同样的志愿者进行品尝，评价结果如表 1 所示。

[0048] [表 1]

实施例或比较例	实施例 1			实施例 2			比较例 1			比较例 2		
食材	鳝鱼	牛肉	斧菜	鳝鱼	牛肉	斧菜	鳝鱼	牛肉	斧菜	鳝鱼	牛肉	斧菜
鲜美程度	+++	+++	+++	+++	+++	+++	+	+	+	++	++	++
软嫩程度	+++	+++	+++	+++	++++	+++	+	+	+	+	+	+

从表 1 的结果可以看出，与未使用本发明的调味品的菜肴（比较例 1）和仅使用了味精的菜肴（比较例 2）相比，使用了本发明的调味品的菜肴（实施例 1 和 2）不但味道鲜美，而且肉质软嫩，口感非常好。此外，使用了本发明的调味品的菜肴（实施例 1 和 2）色彩更为鲜亮，很有食欲。

Motif Exhibit 1002, Page 898 of 1290

Espacenet

Bibliographic data: CN102835460 (A) — 2012-12-26

Smearing cheese analogue and preparation method thereof

Inventor(s): GAO HONGYAN; LIU ZHENMIN; MO BEIHONG; XIAO YANG; CHEN SHUAI; SHI CHUNQUAN; ZHENG YUANRONG; SUN KEJIE; GUO BENHENG ± (GAO HONGYAN, ; LIU ZHENMIN, ; MO BEIHONG, ; XIAO YANG, ; CHEN SHUAI, ; SHI CHUNQUAN, ; ZHENG YUANRONG, ; SUN KEJIE, ; GUO BENHENG)

Applicant(s): BRIGHT DAIRY & FOOD CO LTD ± (BRIGHT DAIRY & FOOD CO., LTD)

Classification: **- international:** ***A23C20/02***
- cooperative:

Application number: CN20111168270 20110621 Global Dossier

Priority number(s): CN20111168270 20110621

Also published as: CN102835460 (B)

Abstract of CN102835460 (A)

The invention discloses a smearing cheese analogue and a preparation method thereof. The smearing cheese analogue comprises the following raw materials, by weight: 15%-28% of vegetable fat, 0.2%-1.3% of a stabilizer, 0.2%-1% of an emulsifier, 5% -18% of a plant protein powder, 3%-10% of a modified starch and 45%-65% of water. The smearing cheese analogue completely uses non milk-derived ingredients as raw materials, most of which are composed of plant ingredients, does not contain cholesterol, lactose or milk fat, can satisfy consumers requiring no cholesterol or saturated fat and consumers having lactose intolerance, and is a quite healthy food. The smearing cheese analogue with light flavor is very suitable for the taste of Chinese consumers, uses the raw materials with wide sources, and has a strong price advantage. The preparation method is simple, uses easily available factory equipment, and can be applied to scale and industrial production.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 102835460 A
(43)申请公布日 2012.12.26

(21)申请号 201110168270.X

(22)申请日 2011.06.21

(71)申请人 光明乳业股份有限公司
地址 201103 上海市吴中路 578 号

(72)发明人 高红艳 刘振民 莫蓓红 肖杨 陈帅 石春权 郑远荣 孙克杰 郭本恒

(74)专利代理机构 上海智信专利代理有限公司 31002
代理人 薛琦 朱水平

(51)Int.Cl.
A23C 20/02(2006.01)

权利要求书 2 页 说明书 12 页

(54)发明名称

一种涂抹干酪模拟物及其制备方法

(57)摘要

本发明公开了一种涂抹干酪模拟物及其制备方法。包括以下重量百分比的原料：植物脂肪 15%～28%，稳定剂 0.2%～1.3%，乳化剂 0.2%～1%，植物蛋白粉 5%～18%，变性淀粉 3%～10%和水 45%～65%。本涂抹干酪模拟物完全采用非乳来源的配料为原料，大部分原料由植物性配料组成，不含胆固醇、乳糖以及乳脂肪，可以满足对胆固醇、饱和脂肪有要求的消费者，以及对乳糖不耐的消费者，是一款非常健康的食品。风味较清淡，非常适合中国消费者的口味。原料来源十分丰富，有很强的价格优势。制备方法工艺简单、工厂设备容易满足，能够实现规模化、产业化生产。

CN 102835460 A

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

1. 一种涂抹干酪模拟物，其特征在于，包括以下重量百分比的原料：植物脂肪 15%～28%，稳定剂 0.2%～ 1.3%，乳化剂 0.2%～ 1%，植物蛋白粉 5%～ 18%，变性淀粉 3%～10%和水 45%～ 65%；

所述的植物脂肪为氢化植物油、植物油和植物油分提物中的一种或多种；

所述的稳定剂为卡拉胶、明胶、刺槐豆胶、瓜尔豆胶、黄原胶、海藻酸钠、阿拉伯胶和结冷胶中的一种或多种；

所述的乳化剂为单、双、三甘油脂肪酸酯、山梨醇酯、聚氧乙烯山梨醇酐酯、磷脂、聚山梨醇酯和蔗糖酯中的一种或多种；

所述的植物蛋白粉是大豆蛋白粉、花生蛋白粉、芝麻蛋白粉、核桃蛋白粉和酶解植物蛋白粉中的一种或多种；

所述的变性淀粉为麦芽糊精、预糊化玉米变性淀粉和小麦变性淀粉中的一种或多种。

2. 如权利要求 1 所述的涂抹干酪模拟物，其特征在于，

所述的植物脂肪为氢化大豆油、玉米油二者的混合物，二者的质量比例为 2.5 ： 1；

所述的稳定剂为卡拉胶、明胶和黄原胶的混合物，所述的卡拉胶、明胶和黄原胶的复配比例为质量比 2 ： 1 ： 1；

所述的乳化剂为单、双、三甘油脂肪酸酯、磷脂和聚山梨醇酯的混合物，所述的单、双、三甘油脂肪酸酯、磷脂和聚山梨醇酯的复配比例为质量比 1 ： 1 ： 1；

所述的酶解植物蛋白粉为酶解大豆蛋白粉和酶解花生蛋白粉；

所述的变性淀粉为麦芽糊精和预糊化玉米变性淀粉。

3. 如权利要求 1 或 2 所述的涂抹干酪模拟物，其特征在于，所述的涂抹干酪模拟物还包括以下重量百分比的原料：5%～ 20%豆浆。

4. 如权利要求 1～3 任一项所述的涂抹干酪模拟物，其特征在于，所述的涂抹干酪模拟物还进一步包括以下原料：食盐 0.4%～ 1.0%和 / 或甜味剂 0%～ 8%。

5. 如权利要求 4 任一项所述的涂抹干酪模拟物，其特征在于，所述的甜味剂为白砂糖、阿斯巴甜、安赛蜜和三氯蔗糖中的一种或几种。

6. 如权利要求 1～5 任一项所述的涂抹干酪模拟物，其特征在于，所述的涂抹干酪模拟物，还进一步包括以下本领域常规干酪的添加剂，为风味呈现物质、营养强化剂、色素、果蔬汁、果蔬粉、果粒果酱、防腐剂、抗氧化剂、酸度调节剂和膳食纤维中的一种或多种。

7. 如权利要求 1～6 任一项所述的涂抹干酪模拟物，其特征在于，所述的涂抹干酪模拟物的指标为：32.9%～ 52.3%的固形物、15.7%～ 28.4%的脂肪、3.8%～ 14.7%的蛋白质，百分比各成分占再制干酪总量的质量百分比；所述的涂抹干酪模拟物的 pH 为 4.5～6.0。

8. 一种如权利要求 1～7 任一项所述的涂抹干酪模拟物的制备方法，其特征在于，包括以下步骤：

①将植物脂肪加热熔化，无脂肪结晶存在。

②将稳定剂和乳化剂、溶解甜味剂的水溶液依次加入到步骤①所得的植物脂肪中，形成乳化体系。

③乳化体系中缓慢加入植物蛋白粉以及变性淀粉，形成所需的质构。

④加入豆浆、食盐和各种常规添加剂，调节风味和 pH 值，后进行杀菌。

Motif Exhibit 1002, Page 901 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

⑤均质、灌装、冷却。

9. 如权利要求 8 所述的制备方法，其特征在于，步骤②为在加热剪切搅拌条件下，充分混合乳化，所述的剪切搅拌条件是 900rpm ～ 1200rpm，70℃～ 90℃，乳化时间为 5min ～ 30min ；步骤③为 1200rpm ～ 1800rpm，70℃～ 90℃，15min ～ 40min ；所述的植物蛋白粉的加入速度为 2g ～ 10g/min。

10. 如权利要求 8 所述的制备方法，其特征在于，步骤⑤所述的均质的压力为 50bar ～ 200bar ；所述的灌装为不低于 70℃的条件下灌装，之后冷却至 2℃～ 8℃入库。

Motif Exhibit 1002, Page 902 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

一种涂抹干酪模拟物及其制备方法

技术领域

[0001] 本发明属于乳制品领域，特别涉及一种涂抹干酪模拟物及其制备方法。

背景技术

[0002] 干酪又称奶酪，是一种在牛乳中加入适量乳酸菌发酵剂和凝乳酶制剂使乳中的蛋白质（主要是酪蛋白）凝固，变成不溶性酪蛋白钙，排出乳清、并经一定时间的成熟或不经成熟而制成的食品。制备干酪过程中酪蛋白通常是主要的蛋白源，这是由其特殊的流变性质和融化特性决定的。

[0003] 涂抹干酪是一种由牛奶、稀奶油发酵、成熟或不经成熟的干酪，其质地均匀细腻、易于涂抹、可直接食用，或用于制作甜点和蛋糕，一般具有独特的口感和风味，非常受消费者喜爱。

[0004] 干酪模拟物通常被定义为一些非乳来源的脂肪、蛋白质、碳水化合物等配料相混合，经加工制得的类似于干酪的产品。目前，随着消费者对干酪能量、胆固醇、脂肪的担心，干酪模拟物开始受到越来越多的关注。此外，该种产品采用植物性来源的配料，满足了食品快餐业的成本的需要，具有很广泛的应用前景。

发明内容

[0005] 因此，本发明要解决的技术问题就是针对受消费者喜爱的涂抹干酪存在能量、胆固醇、饱和脂肪过高的缺陷，提供一种涂抹干酪模拟物及其制备方法。该涂抹干酪模拟物完全采用非乳来源的配料为原料，以植物原料为主，健康，清淡。

[0006] 本发明为解决上述技术问题采用的技术方案如下：

[0007] 一种涂抹干酪模拟物，包括以下重量百分比的原料：植物脂肪 15%～ 28%，稳定剂 0.2%～ 1.3%，乳化剂 0.2%～ 1%，植物蛋白粉 5%～ 18%，变性淀粉 3%～ 10%和水 45%～ 65%。

[0008] 较佳的，本发明所述的涂抹干酪模拟物，包括以下重量百分比的原料：植物脂肪 19%～ 24%，稳定剂 0.2%～ 1.3%，乳化剂 0.2%～ 1%，植物蛋白粉 8%～ 15%，变性淀粉 4%～ 8%和水 55%～ 65%。

[0009] 本发明中，所述的植物脂肪为氢化植物油、植物油和植物油分提物中的一种或多种的混合物。较佳的为氢化大豆油、氢化菜籽油、氢化葵花籽油、棕榈油及其分提物、棕榈仁油、大豆油、玉米油和棉籽油中的一种或多种的混合物。考虑到最终产品的涂抹特性，更佳的为氢化大豆油、玉米油二者的混合物，较佳的质量比例为 2.5 ： 1。

[0010] 本发明中，特别优选的稳定剂对涂抹干酪模拟物的质构、以及产品的稳定体系发挥重要作用。所述的稳定剂为卡拉胶、明胶、刺槐豆胶、瓜尔豆胶、黄原胶、海藻酸钠、阿拉伯胶和结冷胶中的一种或多种的混合物。较佳的为卡拉胶、明胶和黄原胶的混合物，所述的卡拉胶、明胶和黄原胶的复配比例较佳的为质量比 2 ： 1 ： 1。

[0011] 本发明中，特别优选的乳化剂对涂抹干酪模拟物水包油体系的均一乳化发挥重要

Motif Exhibit 1002, Page 903 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

作用。所述的乳化剂为单、双、三甘油脂肪酸酯、山梨醇酯、聚氧乙烯山梨醇酐酯、磷脂、聚山梨醇酯和蔗糖酯中的一种或多种的混合物。较佳的为单、双、三甘油脂肪酸酯、磷脂和聚山梨醇酯的混合物，所述的单、双、三甘油脂肪酸酯、磷脂和聚山梨醇酯的复配比例较佳的为质量比 1 ： 1 ： 1。

[0012] 本发明中，所述的植物蛋白粉是植物来源的蛋白粉，是按照现有技术，以植物为原料，经提取、纯化、浓缩等工艺，得到的其中的蛋白部分的产品。其成分指标为：蛋白质 60％～ 100％，水不超过 12％，百分比为质量百分比。所述的植物蛋白粉较佳的为大豆蛋白粉、花生蛋白粉、芝麻蛋白粉和核桃蛋白粉中的一种或多种的混合物。所述的植物蛋白粉之所以选择上述品种是在于具有较高的蛋白营养价值，如大豆蛋白其蛋白质的氨基酸组成与牛奶蛋白相近，除蛋氨酸略低外，其余必需氨基酸含量均较丰富，是植物性的完全蛋白质，在营养价值上，可与动物蛋白等同。此外，植物蛋白的来源十分的丰富，价格亦较低。但是，植物蛋白的分子较大，且不具备牛乳蛋白的磷酸结构，对涂抹干酪模拟物的风味和质构有一定的影响。所以为得到更佳的产品，所述的植物蛋白粉更佳的是酶解植物蛋白粉。酶水解技术按照现有技术，处理后再作为配料应用到涂抹干酪模拟物中。经发明人进一步优化实验，酶解大豆蛋白粉和酶解花生蛋白粉能够提供良好的涂抹质构。

[0013] 本发明中，所述的变性淀粉的作用是酪蛋白替代物。所述的变性淀粉可以为本领域常规，较佳的为麦芽糊精、预糊化玉米变性淀粉和小麦变性淀粉中的一种或多种，经发明人优化实验，更佳的为麦芽糊精和预糊化玉米变性淀粉。

[0014] 本发明所述的涂抹干酪模拟物，较佳的包括以下原料：豆浆。豆浆的用量较佳的是占原料总质量的 5％～ 20％ (wt)。

[0015] 本发明中，所述的豆浆为大豆用水浸泡后磨碎、过滤、煮沸而成的浆状液体，营养非常丰富，且易于消化吸收。所述的豆浆优选成分指标：脂肪 0.3％～ 2.2％，蛋白质 0.6％～ 4.5％，百分比为质量百分比。

[0016] 本发明所述的涂抹干酪模拟物，更佳的还进一步包括以下重量百分比的原料：食盐 0.4％～ 1.0％和 / 或甜味剂 0％～ 8％。食盐、甜味剂起调味作用。本发明中，所述的甜味剂为本领域常规甜味剂，较佳的为白砂糖、阿斯巴甜、安赛蜜、三氯蔗糖中的一种或几种，更佳的为白砂糖。

[0017] 本发明所述的涂抹干酪模拟物，更佳的还可以根据产品风味和口感等需要，进一步包括以下本领域常规干酪的添加剂，较佳的为风味呈现物质、营养强化剂、色素、果蔬汁、果蔬粉、果粒果酱、防腐剂、抗氧化剂、酸度调节剂和膳食纤维中的一种或多种。上述各原料用量均可为本领域常规用量，较佳的为：风味呈现物质用量≤ 0.1％；营养强化剂用量≤ 1％；色素用量≤ 0.1％；果蔬汁用量≤ 20％；果蔬粉用量≤ 10％；果粒果酱用量≤ 25％；防腐剂用量≤ 0.1％；抗氧化剂用量≤ 0.1％；酸度调节剂用量≤ 1.0％；膳食纤维≤ 3％；上述用量范围不包括 0％；百分比为各成分占各原料总量的质量百分比。

[0018] 本发明第二方面提供一种上述涂抹干酪模拟物的制备方法，包括以下步骤：

[0019] ①将植物脂肪加热熔化，无脂肪结晶存在。

[0020] ②将稳定剂和乳化剂、溶解甜味剂的水溶液依次加入到步骤①所得的植物脂肪中，形成乳化体系。

[0021] ③乳化体系中缓慢加入植物蛋白粉以及变性淀粉，形成所需的质构。

Motif Exhibit 1002, Page 904 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0022] ④加入其他辅料（即豆浆、食盐和各种常规添加剂），调节风味和 pH 值，后进行杀菌。

[0023] ⑤均质、灌装、冷却。

[0024] 本发明中，步骤①为植物脂肪加热熔化过程。其中，所述较佳的熔化温度为 70℃～ 90℃，熔化时间为 20 ～ 30min，熔化过程中并伴随搅拌，搅拌速度为 100rpm ～ 300rpm。

[0025] 本发明中，步骤②为在加热剪切搅拌条件下，充分混合乳化，从而形成乳化体系。其中，所述的剪切搅拌条件较佳的是在 900rpm ～ 1200rpm 条件下加热至 70℃～ 90℃。为保证更好的乳化，在稳定剂和乳化剂、溶解甜味剂的水依次加入到脂肪中前，需将稳定剂和乳化剂提前混合均匀，并对水预先加热，加热温度至 70℃～ 90℃。所述的乳化时间较佳的为 5min ～ 30min，更佳的为 10min ～ 20min。

[0026] 本发明中，步骤③为质构形成过程。其中，要求快速剪切搅拌，较佳的剪切速度为 1200rpm ～ 1800rpm，较佳的温度控制为 70℃～ 90℃，剪切时间 15min ～ 40min。所述的植物蛋白粉和变性淀粉缓慢加入至乳化体系中，以防止粉料结块对产品质地产生影响，较佳的加入速度为 2g ～ 10g/min，更佳的为 3g ～ 5g/min。

[0027] 本发明中，步骤④中所述的加入其他辅料的搅拌速度为 1200rpm ～ 1800rpm，搅拌时间为 3min ～ 5min。所述的杀菌方式较佳的为巴氏杀菌或超高温灭菌。其中，所述的巴氏杀菌的温度较佳的为 65℃～ 90℃，时间较佳的为 10min ～ 20min ；所述的超高温灭菌的温度较佳的为 120℃～ 145℃，更佳的为 140℃ ；时间较佳的为 2s ～ 7s，更佳的为 5s。

[0028] 本发明中，步骤⑤中在灌装之前按照本领域常规方式进行均质，较佳的均质压力为 50bar ～ 200bar。所述的灌装冷却较佳的为不低于 70℃的条件下灌装，之后冷却至 2℃～ 8℃入库即可。

[0029] 较佳地，本发明的涂抹干酪模拟物的指标为 ：32.9％～ 52.3％的固形物、15.7％～ 28.4％的脂肪、3.8％～ 14.7％的蛋白质，百分比各成分占再制干酪总量的质量百分比。

[0030] 较佳的，本发明的涂抹干酪模拟物的 pH 为 4.5 ～ 6.0。

[0031] 本发明所用原辅料均市售可得。

[0032] 本发明的积极进步效果在于 ：本发明提供了一种涂抹于酪模拟物及其制备方法。最终产品在完全非乳体系的情况下，既具有细腻、均匀的涂抹质构，又拥有良好的风味和口感。本涂抹干酪模拟物完全采用非乳来源的配料为原料，打消了消费者对动物来源配料脂肪、胆固醇以及能量的顾虑。大部分原料由植物性配料组成，不含胆固醇、乳糖以及乳脂肪，可以满足对胆固醇、饱和脂肪有要求的消费者，以及对乳糖不耐的消费者，具有健康的功能特性，是一款非常健康的食品。本涂抹干酪模拟物风味较清淡，非常适合中国消费者的口味。该模拟物以植物为原料，来源十分丰富，有很强的价格优势。同时，涂抹干酪模拟物的制备方法工艺简单、工厂设备容易满足，能够实现规模化、产业化生产。

具体实施方式

[0033] 下面用实施例来进一步说明本发明，但本发明并不受其限制。下列实施例中未注明具体条件的实验方法，通常按照常规条件，或按照制造厂商所建议的条件。

[0034] 下述实施例中，所用原料来源为 ：

Motif Exhibit 1002, Page 905 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0035] 大豆油、玉米油、棉籽油、甜味剂、植物蛋白粉、豆浆：市售。氢化大豆油、氢化菜籽油、氢化葵花籽油、棕榈仁油、棕榈油分提物：上海嘉里粮油工业有限公司。

[0036] 酶解大豆蛋白粉：哈高科大豆食品公司。

[0037] 稳定剂、乳化剂：丹尼斯克中国有限公司、斯比凯克中国有限公司。

[0038] 风味物质：奇华顿香精香料有限公司。

[0039] 变性淀粉：国民淀粉。

[0040] 防腐剂、酸度调节剂、矿物盐：上海励成食品工业有限公司。

[0041] 维生素：帝斯曼中国有限公司。

[0042] 果蔬粉：上海诚一食品原料有限公司。

[0043] 果蔬汁、果粒：上海统园食品技术有限公司。

[0044] 膳食纤维：捷成中国贸易有限公司。

[0045] 实施例 1

[0046] 原料配方：

[0047]

原料种类	含量 wt%
氢化大豆油	20
玉米油	8
大豆蛋白粉	3.5
芝麻蛋白粉	2
麦芽糊精	3
葡萄糖内酯	1
白砂糖	8
食盐	0.4
卡拉胶	0.3
刺槐豆胶	0.2
单、双、三甘油脂肪酸酯	0.2
山梨酸钾	0.09
水	53.31

[0048]

Motif Exhibit 1002, Page 906 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0049] 首先将氢化大豆油、玉米油升温至 70℃，此温度下保持 20min，并同时伴随搅拌，搅拌速度为 100rpm。后将卡拉胶、刺槐豆胶、单、双、三甘油脂肪酸酯均匀混合，水预热至 70℃加入白砂糖溶解，再与上述脂肪混合，剪切速度为 1200rpm，持续时间为 10min。后将大豆蛋白粉、芝麻蛋白粉、麦芽糊精以 5g/min 的速度加入到乳化体系中，控制温度 70℃，剪切速度为 1800rpm，剪切 15min，然后葡萄糖内酯、食盐、山梨酸钾全部加入，搅拌速度为 1400rpm，5min 后，进行杀菌和均质。杀菌条件为 90℃保温 15min，均质压力为 100bar，最后进行灌装、冷却。

[0050] 本实施例中制得的涂抹干酪模拟物：固形物 45.2%；脂肪 28.4%；蛋白质 3.8%；pH 为 4.8，百分比为各成分占再制干酪质量总量的百分比。

[0051] 实施例 2

[0052] 原料配方：

[0053]

原料种类	含量 wt%
氢化葵花籽油	20
豆浆	7
酶解大豆分离蛋白	18
预糊化玉米变性淀粉	5
海藻酸钠	0.1
黄原胶	0.1
单、双、三甘油脂肪酸酯	0.2
磷脂	0.2
聚山梨醇酯	0.2
食盐	1.8
柠檬酸	0.12
水	47.28

[0054]

[0055] 首先将氢化葵花籽油升温至 90℃，此温度下保持 25min，并同时伴随搅拌，搅拌速度为 200rpm。后将海藻酸钠、黄原胶、单、双、三甘油脂肪酸酯、磷脂、聚山梨醇酯均匀混合，水预热至 90℃，与上述脂肪混合，剪切速度为 1000rpm，持续时间为 20min。再将大豆分离蛋白粉、预糊化玉米变性淀粉以 10g/min 的速度加入到乳化体系中，控制温度 90℃，剪切速度为 1600rpm，剪切 40min，然后豆浆、食盐、柠檬酸全部加入，搅拌速度为 1800rpm，3min 后，进

Motif Exhibit 1002, Page 907 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

行杀菌和均质。杀菌条件为 90℃保温 10min，均质压力为 120bar，后进行灌装、冷却。

[0056] 本实施例中制得的涂抹干酪模拟物：固形物 46.9%；脂肪 20.2%；蛋白质 14.7%；pH 为 5.6，百分比为各成分占再制干酪质量总量的百分比。

[0057] 实施例 3

[0058] 原料配方：

[0059]

原料种类	含量 wt%
棕榈油硬脂分提物	20
大豆蛋白粉	11
麦芽糊精	5
豆浆	5
白砂糖	5
食盐	1.8
磷酸	1.0
单、双、三甘油脂肪酸酯	0.5
山梨醇酯	0.5
卡拉胶	0.2
明胶	0.2
结冷胶	0.1
水	49.7

[0060]

[0061] 首先将棕榈油硬脂分提物升温至 80℃，此温度下保持 30min，并同时伴随搅拌，搅拌速度为 150rpm。后将单、双、三甘油脂肪酸酯、山梨醇酯、卡拉胶、明胶、结冷胶均匀混合，水预热至 80℃加入白砂糖溶解，与上述脂肪混合，剪切速度为 900rpm，持续时间为 30min。再将大豆蛋白粉、麦芽糊精以 4g/min 的速度加入到乳化体系中，控制温度 80℃，剪切速度为 1200rpm，剪切 30min，然后豆浆、食盐、磷酸全部加入，搅拌速度为 1600rpm，4min 后，进行杀菌和均质，杀菌条件为 65℃保温 20min，均质压力为 150bar，后进行灌装、冷却。

[0062] 本实施例中制得的涂抹干酪模拟物：固形物 43.9%；脂肪 20.8%；蛋白质 8.7%；pH 为 4.5，百分比为各成分占再制干酪质量总量的百分比。

[0063] 实施例 4

Motif Exhibit 1002, Page 908 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

[0064] 原料配方：

[0065]

原料种类	含量 wt%
氢化菜籽油	20
酶解大豆蛋白粉	5
酶解花生蛋白粉	5
阿斯巴甜	480ppm
小麦变性淀粉	3
食盐	0.7
磷脂	0.3
蒸馏单甘酯	0.2
卡拉胶	0.2
瓜尔豆胶	0.2
阿拉伯胶	0.1
水	65.3

[0066]

[0067] 首先将氢化菜籽油升温至 80℃，此温度下保持 25min，并同时伴随搅拌，搅拌速度为 300rpm。后将蒸馏单甘酯、磷脂、卡拉胶、瓜尔豆胶、阿拉伯胶均匀混合，水预热至 80℃加入阿斯巴甜溶解，然后与上述脂肪混合，剪切速度为 1200rpm，持续时间为 5min。再将酶解大豆蛋白粉、酶解花生蛋白粉、小麦变性淀粉以 4g/min 的速度加入到乳化体系中，控制温度 80℃，剪切速度为 1800rpm，剪切 25min，然后豆浆、食盐全部加入，搅拌速度为 1400rpm，4min 后，进行杀菌和均质。杀菌条件为 65℃保温 20min，均质压力为 200bar，杀后进行灌装、冷却。

[0068] 本实施例中制得的涂抹干酪模拟物：固形物 34.5%；脂肪 20.7%；蛋白质 7.2%；pH 为 6.0，百分比为各成分占再制干酪质量总量的百分比。

[0069] 实施例 5

[0070] 原料配方：

[0071]

Motif Exhibit 1002, Page 909 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

原料种类	含量wt%
棕榈仁油	10
棉籽油	8
大豆蛋白粉	4
核桃蛋白粉	2
白砂糖	4
小麦变性淀粉	3
柠檬酸	0.8
食盐	0.7
磷脂	0.3
聚氧乙烯山梨醇酐酯	0.4
卡拉胶	0.6
刺槐豆胶	0.3
黄原胶	0.4
水	65.5

[0072]

[0073] 首先将棕榈仁油、棉籽油升温至80℃，此温度下保持18min，并同时伴随搅拌，搅拌速度为150rpm。后将磷脂、聚氧乙烯山梨醇酐酯、卡拉胶、刺槐豆胶、黄原胶均匀混合，水预热至80℃加入阿斯巴甜溶解，与上述脂肪混合，剪切速度为1200rpm，持续时间为15min。再将大豆蛋白粉、核桃蛋白粉、小麦变性淀粉以2g/min的速度加入到乳化体系中，控制温度80℃，剪切速度为1200rpm，剪切20min。然后柠檬酸、食盐全部加入，搅拌速度为1200rpm，4min后，进行杀菌和均质。杀菌条件为140℃保温2s，均质压力为50bar，后进行灌装、冷却。

[0074] 本实施例中制得的涂抹干酪模拟物：固形物33.1%；脂肪18.6%；蛋白质4.5%；pH为5.2，百分比为各成分占再制干酪质量总量的百分比。

[0075] 实施例6

[0076] 原料配方：

[0077]

Motif Exhibit 1002, Page 910 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

原料种类	含量 wt%
氢化大豆油	15
豆浆	20
酶解大豆蛋白粉	9
安赛蜜	260ppm
预糊化玉米变性淀粉	6
食盐	0.7
磷脂	0.3
蔗糖酯	0.3
卡拉胶	0.2
明胶	0.1
黄原胶	0.1
水	48.3

[0078]

[0079] 首先将氢化大豆油升温至 80℃，此温度下保持 18min，并同时伴随搅拌，搅拌速度为 150rpm。后将蔗糖酯、磷脂、卡拉胶、明胶、黄原胶均匀混合，水预热至 80℃加入安赛蜜溶解，然后与上述脂肪混合，剪切速度为 1200rpm，持续时间为 15min。再将酶解大豆蛋白粉、预糊化玉米变性淀粉以 3g/min 的速度加入到乳化体系中，控制温度 80℃，剪切速度为 1800rpm，剪切 35min。最后将食盐全部加入，搅拌速度为 1600rpm，4min 后，进行杀菌和均质。杀菌条件为 140℃保温 7s，均质压力为 150bar，后进行灌装、冷却。

[0080] 本实施例中制得的涂抹干酪模拟物：固形物 32.9％；脂肪 15.7％；蛋白质 8.0％；pH 为 5.7，百分比为各成分占再制干酪质量总量的百分比。

[0081] 实施例 7

[0082] 原料配方：

[0083]

原料种类	含量 wt%
氢化大豆油	12
大豆油	4

Motif Exhibit 1002, Page 911 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

大豆蛋白粉	5
烟熏火腿	4.5
麦芽糊精	10
三氯蔗糖	80ppm
大蒜风味料	0.5
食盐	1.0
磷脂	0.3
单、双、三甘油脂肪酸酯	0.4
刺槐豆胶	0.2
瓜尔豆胶	0.1
卡拉胶	0.1
山梨酸	0.09
水	61.81

[0084]

[0085] 首先将氢化大豆油、大豆油升温至 80℃，此温度下保持 18min，并同时伴随搅拌，搅拌速度为 150rpm。后将磷脂、单、双、三甘油脂肪酸酯、刺槐豆胶、瓜尔豆胶、卡拉胶均匀混合，水预热至 80℃加入三氯蔗糖溶解，然后与上述脂肪混合，剪切速度为 1200rpm，持续时间为 15min。再将大豆蛋白粉、麦芽糊精以 4g/min 的速度加入到乳化体系中，控制温度 80℃，剪切速度为 1800rpm，剪切 25min。再将食盐、山梨酸加入，搅拌速度为 1500rpm，4min 后，进行杀菌和均质。杀菌条件为 140℃保温 5s，均质压力为 150bar，最后拌入烟熏火腿和大蒜风味料、灌装、冷却。

[0086] 本实施例中制得的涂抹干酪模拟物：固形物 37.9％；脂肪 16.8％；蛋白质 4.5％；pH 为 5.5，百分比为各成分占再制干酪质量总量的百分比。

[0087] 实施例 8

[0088] 原料配方：

[0089]

原料种类	含量 wt％
氢化菜籽油	10

Motif Exhibit 1002, Page 912 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

玉米油	5
酶解大豆蛋白粉	18
预糊化玉米变性淀粉	5.5
麦芽糊精	4.5
白砂糖	8
食盐	0.7
单、双、三甘油脂肪酸酯	0.4
磷脂	0.3
蔗糖酯	0.3
黄原胶	0.5
明胶	0.2
卡拉胶	0.2
山梨酸	0.09
水	46.31

[0090]

[0091] 首先将氢化菜籽油、玉米油升温至 70℃，此温度下保持 20min，并同时伴随搅拌，搅拌速度为 200rpm。后将单、双、三甘油脂肪酸酯、磷脂、蔗糖酯、黄原胶、明胶、卡拉胶均匀混合，水预热至 75℃加入白砂糖溶解，再与上述脂肪混合，剪切速度为 1000rpm，持续时间为 10min。再将酶解大豆蛋白粉、预糊化玉米变性淀粉、麦芽糊精以 3g/min 的速度加入到乳化体系中，控制温度 70℃，剪切速度为 1800rpm，剪切 40min。再将食盐、山梨酸加入，搅拌速度为 1600rpm，3min 后，进行杀菌和均质。杀菌条件为 80℃保温 15min，均质压力为 100bar，后进行灌装、冷却。

[0092] 本实施例中制得的涂抹干酪模拟物：固形物 52.3%；脂肪 16.1%；蛋白质 11.7%；pH 为 6.0，百分比为各成分占再制干酪质量总量的百分比。

[0093] 效果实施例 1

[0094] 对本发明的涂抹干酪模拟物（实施例为例）进行质构效果检验，以卡夫稀奶油奶酪、总统牌金文奶油奶酪、casarelli mascarpone 和光明健康成长奶酪作对照。

[0095] 质构测试方法：

[0096] 质构测试通过模拟人口腔的咀嚼运动，对样品进行压缩，通过界面输出质地测试曲线，分析硬度、涂抹性、粘着性等质构特性。所有样品取样后至测试前均统一放置于 0℃～

Motif Exhibit 1002, Page 913 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

4℃冰箱中以防止温度对产品质地产生影响。物性测试仪（TA-LH 型）参数设置：测量前探头下降速度 1.0mm/s；测试速度 1.0mm/s；测量后探头回程速度 2.0mm/s；测试距离 10mm；感应力 10g；探头类型 p/25。

[0097] TPA 曲线参数：

[0098] ①硬度：TPA 曲线第一压缩周期中的最大力值 N 表示，表示干酪的软硬程度。

[0099] ②涂抹性：探头下行过程中受力曲线与时间轴所形成的峰面积表示，面积值越小越易涂抹。

[0100] ③粘着性：探头上行过程中受力曲线与时间轴所形成的峰面积表示，面积值越大，粘度越大，咀嚼时口腔的舒适感越差。

[0101] 测试结果记录于表 1。

[0102] 表 1. 不同产品的质构测试结果

[0103]

测试样品	硬度（g）	涂抹性（g·s）	粘着性（g·s）
卡夫稀奶油奶酪	38	445	309
总统牌金文奶油奶酪	52	589	412
casarelli mascarpone	27	356	288
光明健康成长奶酪	41	496	372
实施例 1	36	428	291
实施例 4	40	461	363
实施例 5	28	342	265

[0104] 由上表 1 的测试数据可知：本发明实施例制得的涂抹干酪模拟物样品，质构特性与市售的原制涂抹干酪、再制涂抹干酪数据相当，尤其是实施例 4，展示优良的涂抹特性，故本发明的涂抹干酪模拟物完全符合预期要求。

Motif Exhibit 1002, Page 914 of 1290

J-PlatPat
Japan Platform for Patent Information
Help desk (9:00-21:00)
(+81)3-6666-8801
Japanese Top page Help list Site map JPO IN
National Center for Industrial Property Information and Tra
Patent & Utility Model Design Trademark Trial & Appeal
Top page Patent& Utility Model Patent& Utility Model Number Search
Patent & Utility Model Number Search Back Help
Search List De
You can retrieve a variety of patent and utility model gazettes by their numbers.
Selected Gazette
Previous Document 1/1 Next Documer
JP,53-115846,A
PAJ Detail Image
CLAIMS DETAILED DESCRIPTION
DESCRIPTION OF DRAWINGS

*** NOTICES ***

JPO and INPIT are not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

the Description 1. title-of-the-invention protein prize artificial color agent 2, the range aromatic-amino-acid derivative of % *******, or a different ****-like amino acid screen -- the protein diet article artificial color agent which contains 2 or more type of kind **** of a conductor.

3. Detailed opinion Akemoto invention of invention relates to the new artificial color agent of a protein diet article.

the present processed meat, milling products, fish meat, and .. MU, a sausage, and the development of the artificial color agent of a protein diet article that has five pigments to salted salmon roe, cod roe, roe, etc. how much are demanded strongly.

The inventor is an aromatic-amino-acid derivative which finds out that different ****-like amino # ******* can use aromatic amino 1M ********* as a protein prize artificial color agent, and completes this @ **, and it uses for the 9 present invention. a different ****-like amino acid derivative is constituted -- if amino acid+7M TO carries out, phenylalanine, tyrosine, chill KISHIN, Tori Bouto Hwang, and histidine are raised.

As for the aromatic-amino-acid derivative and different ****-like amino acid derivative which are used for the present invention, at least one hydrogen atom of an amino group is not replaced at all.

The ** film of the carboxyl group is carried out in a certain form.

As the protected type **, a peptide bond with alkyl ester, such as methyl and ethyl, and other amino acid is **********.

t* There is no restriction in the structure of other above-mentioned amino acid in the case of adopting peptide, for example, the carboxyl group may be a free state, and it is not necessary to have an amino group (- MH2), and when it has an amino group, hydrogen atoms, such as acylation, may be carried out.

Protein diet article 100 made into the L object using the amino acid derivative used for the present invention as an artificial color agent? In addition, it is at the five to Si 50 millimol i1, the directions for use face a metaphor preserving in salt -- a protein prize -- * -- ** Si or **** 1 is also good in a Minot ** conductor -- it may carry out, it may be made a drop liquid condition, and may inject into the inside of foodstuffs -- ** -- ** -- the protein diet article may be dipped into the amine acid derivative solution.

The color tone of the target protein diet article was further prepared, and it found out that it was good for eye nine prevent tenebrescence further sufficiently to use together anti-oxidants, such as L-ascorbic acid, erythorbic acid, those salts, dibutylhydroxytoluene, and burylhydroxyanisole.

When using an anti-oxidant together, the amount used is 0.1 to 1% machine anti-****** to the object protein diet article.

In an idea that it can be made to be able to color for foodstuffs by FUERIHEMOKU cx-MU (fsrrihemocnrome) and the tenebrescence by air oxidation can be avoided during light and storage, FERIHEMO chromium generation ability to MACHIN (hematin), a metmyoglobin (metMb), and urea denaturation myoglobin was ****(ed) in the test tube to that of each ** amino 1111 conductor.

A FERIHEMO chromium generation ability test result with completion-of-the-work denaturation myoglobin of an amino acid derivative is shown in Table 1 as the example of -, and it is 9.

In the PH vote 7.0, it calculated to what time the absorption value increased the wavelength of the alpha band of a visible absorption spectrum as compared with contrast (when not using a sample), and the R value showed it.

It turned out that no amino acid derivatives other than an one or more-table examination end, aromatic-amino-acid ******, or a different ****-like amino it[derivative generate red 7 ERIHEMO chromium.

Generating red 7 ERIHEMO chromium is confirmed and an aromatic-amino-acid derivation book and different **-like amino fR ****** are 9.

However, it was also confirmed that the amino acid from which the carbo CHIFURU group is not protected does not generate red FERIHEMO chromium.

the pH condition made to react -- 6.5 or more -- ** Si -- it was preferable.
If processed meat, such as a ham and a sausage, and ** t are used together and used [wheat bleaching agents, such as ****** potash,] with the above-mentioned anti-oxidant before heat-treatment, when using an amino acid derivative for what the manufacturing stage of marine paste products, such as **, takes heat-treatment, and heightening the coloring effect, they are preferable.
The amount of the improving agent used is the degree of 0.01 to 0.2%@.
This **** is described in detail according to an working example below.
The color test was done about working-example L-histidine methyl ester, L-phenylalanine ethyl ester, tyrosine ethyl ester, and HISUCHI rel histidine.
**** storage was carried out -- it preserves in salt, while an egg will be thawed at a room temperature on the occasion of pure **, the egg grain IoOff will be immersed in underwater [containing salt 12? and a sample / 15-m] and hand return will be carried out at a room temperature for about 3 to 4 hours, if it obtains "become empty", and there are not rinsing after a friend and a water break coconut -- it came out, put into small Chalais, and stored in the refrigerator before and behind 5C.
The inside which does not contact the surface portion which touches the air of an egg grain directly was independently filled in the standard cell of path 30**, using Nippon Denshoku colorimetry ****** ID-101DW, the R value was measured and the color tone of a red system of coloring of cod roe was evaluated.
A result is shown in Table 2 and it is a round head.

Previous Document 1/1 Next Documer

To return to the top of this i

Notice on Usage Privacy Policy Questionnaire

[Translation done.]

⑲日本国特許庁　　⑪特許出願公開

公開特許公報　　昭53—115846

⑤Int. Cl.²	識別記号	⑫日本分類	庁内整理番号	⑬公開　昭和53年(1978)10月9日
A 23 L 1/27		34 F 04	7110—49	
A 23 L 1/31				

発明の数　1
審査請求　未請求

（全 3 頁）

⑭蛋白食品発色剤

⑳特　　願　昭52—29304
㉒出　　願　昭52(1977) 3月18日

㉑発 明 者　野中順三九
横浜市港北区仲手原2—43—14
同　小泉千秋
藤沢市藤が岡3丁目24番地
㉑出 願 人　野中順三九
横浜市港北区仲手原2—43—14
同　小泉千秋
藤沢市藤が岡3丁目24番地
㉔代 理 人　弁理士　後藤道生

明　細　書

1. 発明の名称

蛋白食品発色剤

2. 特許請求の範囲

芳香族アミノ酸誘導体又は異節環状アミノ酸誘導体の一種又は二種以上を含有する蛋白食品発色剤。

3. 発明の詳細な説明

本発明は蛋白食品の新規発色剤に関する。

現在肉製品、鯨肉製品、魚肉、ハム、ソーセージ、いくら、すじこ、たらこ、魚卵等ヘム色素を有する蛋白食品の発色剤の研究が強く要望されている。

本発明者等は芳香族アミノ酸誘導体又は異節環状アミノ酸誘導体が蛋白食品発色剤として使用できることを見出し本発明を完成した。

本発明に使用する芳香族アミノ酸誘導体、異節環状アミノ酸誘導体を構成するアミノ酸の例としてはフェニルアラニン、チロシン、チロキシン、トリプトファン、ヒスチジンがあげられる。

本発明に使用する芳香族アミノ酸誘導体、異節環状アミノ酸誘導体はアミノ基の少くとも一個の水素原子は何ら置換されていないものである。またそのカルボキシル基は何らかの形で保護されているものである。その保護形体としては例えばメチル、エチル等のアルキルエステル、他のアミノ酸とのペプチド結合があげられる。ペプチドを採用する場合の上記の他のアミノ酸の構造には制限がなく、例えばそのカルボキシル基は遊離状態であつてもよく、またアミノ基（$-NH_2$）を有していなくともよく、アミノ基を有している場合アシル化等水素原子が置換されていてもよい。

本発明に使用するアミノ酸誘導体を発色剤として使用するには目的とする蛋白食品100g当り5～50ミリモル程度で、その使用方法は例えば塩漬に際し蛋白食品にアミノ酸誘導体をふりかけてもよいし、溶液状態にして食品内部に注入してもよく、またそのアミノ

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

酸誘導体溶液中に被蛋白食品を浸してもよい。

目的とする蛋白食品の色調をさらに整え、褐色をさらに十分に防止するために、L－アスコルビン酸、エリソルビン酸及びそれらの塩、ジブチルヒドロキシトルエン、ブチルヒドロキシアニソール等の抗酸化性物質を併用するとよいことを見出した。抗酸化性物質を併用する場合、その使用量は目的蛋白食品に対し0.1～1％程度である。

食品をフエリヘモクローム（ferrihemocarome）によつて発色させることができれば、貯蔵中に空気酸化による褐色を避けることができるとの考えで、各種アミノ酸誘導体のヘマチン（hematin）、メトミオグロビン（metMb）及び変性ミオグロビンに対するフエリヘモクローム生成能を試験管内で比較した。その一例としてアミノ酸誘導体の変性ミオグロビンとのフエリヘモクローム生成能試験結果を表1に示した。pH=7.0において、可視吸収スペクトルのアルファーバンドの波長を対照（試料を用いない場合）と比較して吸収値が何倍に増大したかを計算しR値で示した。

表　1

試　料	アルファーバンド	
	nm	R
ヒスチジル ヒスチジン	560	1.74
カルノシン	570	1.35
ヒスチジン メチルエステル	562	1.69
フエニルアラニン エチルエステル	560	1.65
チロシン エチルエステル	560	1.72
ヒスチジン	563	1.10

以上の試験結果、芳香族アミノ酸誘導体又は異節環状アミノ酸誘導体以外のアミノ酸誘導体は赤色のフエリヘモクロームを生成しないことがわかつた。また芳香族アミノ酸誘導体、異節環状アミノ酸誘導体は赤色のフエリヘモクロームを生成することが確められた。しかしカルボキシル基が保護されていないアミノ酸は赤色のフエリヘモクロームを生成しないことも確められた。反応させたpH条件は6.5以上がより好ましかつた。

ハム、ソーセージ等の肉製品、かまぼこ等の水産練製品の製造段階で加熱処理を要するものにアミノ酸誘導体を使用する場合、加熱処理前に炭素酸カリ等の小麦粉等改良剤を前述の抗酸化性物質とともに併用して使用すると発色効果を高める上で好ましい。その改良剤の使用量は0.01～0.2％程度である。

以下実施例により本発明を詳細に説明する。

実施例

L－ヒスチジンメチルエステル、L－フエニルアラニンエチルエステル、チロシンエチルエステル及びヒスチジルヒスチジンについて発色試験を行つた。

凍結貯蔵したすけとうたら卵を試験に際して室温で解凍し、その卵粒100gを食塩12gと試料を含む15mlの水中に浸漬し、室温で約3～4時間手返ししながら塩漬した後、水洗、水切りしないで小型シヤレーに入れ5℃前後の冷蔵庫に貯蔵した。

卵粒の空気に直接触れる表面部分と接触しない内部を別々に径30mmの標準セルに詰め日本電色製測色色差計ND-101D型を用い、a値を測定し、たらこの発色の赤色系の色調を評価した。結果を表2に示した。

Motif Exhibit 1002, Page 918 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

表　2

試料	貯蔵日数 2	6	10	14
1.8%ヒスチジンメチルエステル	6.7	5.5 (7.0)	5.9 (7.4)	5.2 (6.9)
1.8%ヒスチジンメチルエステル +0.3%アスコルビン酸	7.7	6.6 (7.5)	7.5 (8.4)	7.3 (8.4)
2.0%ヒスチジルヒスチジン	7.7	6.0 (8.0)	5.4 (7.6)	4.4 (6.9)
2.0%ヒスチジルヒスチジン +0.3%アスコルビン酸	8.5	7.0 (7.4)	8.3 (8.6)	7.0 (7.6)
1.7%フエニルアラニンエチルエステル	7.9	6.2 (8.2)	6.5 (8.0)	6.2 (7.3)
1.7%フエニルアラニンエチルエステル +0.3%アスコルビン酸	6.9	6.0 (7.8)	6.3 (8.4)	5.9 (8.1)
1.8%チロシンエチルエステル	6.5	5.6 (7.3)	6.0 (7.5)	5.6 (7.2)
1.8%チロシンエチルエステル +0.3%アスコルビン酸	6.4	5.5 (7.9)	5.8 (8.4)	5.1 (7.9)
0.9%ニコチン酸アミド +0.3%アスコルビン酸	7.0	5.2 (8.3)	5.2 (9.9)	4.3 (8.5)

( )内は顆粒の内部部分について測定した値である。

この結果から、アスコルビン酸との併用が好ましいこと。対照試験のニコチン酸アミドと比較して表面部分の発色が安定していることがわかる。

特許出願人　野　中　順三九
　　小　桑　千　秋

代　理　人　後　藤　達　生

Motif Exhibit 1002, Page 919 of 1290

Espacenet

Bibliographic data: JPH08140627 (A) — 1996-06-04

HEME IRON-CONTAINING FOOD AND ITS PREPARATION

Inventor(s):	INOUE KOICHI; YAMAGUCHI SHINYA ± (INOUE KOICHI, ; YAMAGUCHI SHINYA, ; INOE KOICHI)
Applicant(s):	HOUSE FOODS CORP ± (HOUSE FOODS CORP, ; HAUSU SHOKUHIN KK)
Classification:	**- international:** ***A23L1/304; A23L1/39;*** (IPC1-7): A23L1/304 **- cooperative:**
Application number:	JP19940292736 19941128 Global Dossier
Priority number (s):	JP19940292736 19941128
Also published as:	JP2883823 (B2)

Abstract of JPH08140627 (A)

PURPOSE: To obtain a heme iron containing food useful for preparation of curry, etc., without losing color tone and flavor essential in a food by mixing a heme iron, an emulsifier, paprika pigment and ginger, etc., and blending with fats and oils under heating. CONSTITUTION: This heme iron-containing food is obtained by mixing a heme iron such as a material derived from an enzymolysis of hemoglobin preferably in an amount of 1.0-5.0ppm with an emulsifier such as fatty acid ester of sugar, paprika pigment and ginger and/or garlic and blending fats and oils such as corn oil or starch such as wheat flour therewith under heating.

【発行国】

日本国特許庁（ＪＰ）

【公報種別】 (19)日本国特許庁（ＪＰ） (12) 公開特許公報（Ａ） (11)特許出願公開番号

特開平8－140627

(43)公開日 平成８年(1996)６月４日

公開特許公報（Ａ）

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ａ２３Ｌ 1/304				

【公開番号】

特開平８－１４０６２７

審査請求 未請求 請求項の数３ ＯＬ （全 ４ 頁）

(21)出願番号 特願平6－292736

【公開日】 (22)出願日 平成６年(1994)11月28日

平成８年（１９９６）６月４日

(71)出願人 000111487
ハウス食品株式会社
大阪府東大阪市御厨栄町１丁目５番７号
(72)発明者 井上 浩一
大阪府東大阪市御厨栄町１丁目５番７号
ハウス食品株式会社内
(72)発明者 山口 慎也
大阪府東大阪市御厨栄町１丁目５番７号
ハウス食品株式会社内
(74)代理人 弁理士 中村 稔 （外７名）

【発明の名称】

ヘム鉄含有食品及びその製造方法

(54)【発明の名称】 ヘム鉄含有食品及びその製造方法

【国際特許分類第６版】

A23L 1/30

【審査請求】

【請求項の数】

【出願形態】ＯＬ

【全頁数】４

(57)【要約】

【目的】 食品本来の色調と風味を損ねることのない、ヘム鉄を含有する食品を提供すること。

【構成】 ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを含有するヘム鉄含有食品、及びヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合するヘム鉄含有食品の製造方法。

【出願番号】

特願平６－２９２７３６

【出願日】

平成６年（１９９４）１１月２８日

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

【特許請求の範囲】
【請求項1】　ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを含有することを特徴とするヘム鉄含有食品。
【請求項2】　ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合することを特徴とするヘム鉄含有食品の製造方法。
【請求項3】　ヘム鉄、乳化剤、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合し、次いでパプリカ色素を混合することを特徴とするヘム鉄含有食品の製造方法。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、ヘム鉄を含有する食品、例えば、カレー、ミートソース、ビーフシチュー、マーボ豆腐などの食品、及び該食品の製造方法に関する。
【０００２】
【従来の技術】従来、食品の鉄分を強化のためにヘム鉄を利用することは知られており、また、ヘム鉄に起因する生臭み・金属味等を防止する技術として種々の提案がなされている（特開昭６３－２９４７６１号、特開平１－１５７３５９号、特開平４－３０７７９号等）。しかしながら、ヘム鉄をカレーやミートソースなどに添加すると、これらが黒緑色の帯びた状態となり、食品本来の色調が損なわれるとの問題が生じるが、これまで、このようなヘム鉄に起因する食品の色調の改善はなされていない。
【０００３】
【発明が解決しようとする課題】本発明は、食品本来の色調と風味を損ねることなく、ヘム鉄を含有する食品を提供することを目的とする。本発明は、又、このような食品の効率的な製造方法を提供することを目的とする。
【０００４】
【課題を解決するための手段】本発明は、ヘム鉄に、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを併用すると、食品本来の色調と風味を良好に保持することができ、特に、ヘム鉄とパプリカ色素とを特定の条件下で混合すると一層食品本来の色調を良好に保持することができるとの知見に基づいてなされたのである。すなわち、本発明は、ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを含有することを特徴とするヘム鉄含有食品を提供する。本発明は、又、ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合することを特徴とするヘム鉄含有食品の製造方法を提供する。本発明は、又、ヘム鉄、乳化剤、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合し、次いでパプリカ色素を混合することを特徴とするヘム鉄含有食品の製造方法を提供する。
【０００５】本発明で用いるヘム鉄はヘモグロビンの酵素分解物であり、鉄分を例えば０．０５～５重量％（以下、％と略称する）を含むものである。その製造方法としては、例えば動物血液の赤血球を溶血、変性し、例えばアルカラーゼのような蛋白質分解酵素によりヘモグロビンを酵素分解し、ヘム鉄を含む画分を取り出して乾燥することにより得られる。ヘム鉄を含む画分を取り出すには例えば１００００ダルトンの限外沪過により、ヘム鉄を含まない画分を沪過する方法と酵素分解終了後ｐＨを蛋白質の等電点近くまで下げ、沈降したヘムを含む画分を遠心分離機により補集する方法等があげられる。
【０００６】また、本発明ではヘム鉄としては、キトサンで処理したものを使用することが好ましい。その製造方法としては、ヘモグロビン分解物として得られるヘム鉄の水懸濁液に、キトサン水溶液を添加し、撹き混ぜながらｐＨを調整して、懸濁状態で得られるヘム鉄を、フィルタープレス等で分離する方法等があげられる。本発明ではヘム鉄として任意の形態のものを使用することができる。例えば、粉末、顆粒、液体等である。本発明において、食品に添加するヘム鉄の量は任意とすることができるが、鉄分として食品全体の０．１～１０．０ｐｐｍとするのが好ましく、より好ましくは１．０～５．０ｐｐｍである。
【０００７】本発明では乳化剤を使用する。乳化剤はヘム鉄を食品中に均一に分散させる作用を有するものであり、例えばショ糖脂肪酸エステル、モノグリセリン脂肪酸エステル、ポリグリセリン脂肪酸エステル、ソルビタン脂肪酸エステル、プロピレングリコール脂肪酸エステル、レシチン等があげられる。中でも親水性のものが好ましく、ＨＬＢ９以上ものが良い。乳化剤は粉体のまま使用することもできるが、乳化剤を水又は油脂に溶解した液状物として使用するのが好ましい。乳化剤溶液の濃度は、特に制限されないが０．０１～５％が好ましく、より好ましくは０．１～２％である。また、ヘム鉄と乳化剤との割合（重量比）は、１００：１～１：１００が望ましく、好ましくは２０：１～１：１０である。ここで、油脂を使用する場合には、例えばコーン油、大豆油、ナタネ油、ゴマ油、コメ油、サフラワー油、ヤシ油、パーム核油、カカオ油、ヒマワリ油、綿実油等の植物性油脂、バター、ヘット、ラード等の動物性油脂の１種又は２種以上の混合物などを使用するのがよい。
【０００８】本発明で用いるパプリカ色素としては、液状、粉末状などがあげられるが、決してこれらに限定されるものではない。しかしパプリカ色素の好ましい形態は液状であり、パプリカ色素の使用量は、ヘム鉄に対して、パプリカ色素／ヘム鉄の重量比が１／１００～１／１、好ましくは１／１０～１／２となるようにするのがよい。
【０００９】本発明で用いるジンジャー及び／又はガー

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

リックとして、生のもの、これを冷凍したもの、乾燥したものなどが使用できる。好ましくは、生のもの（又はこれを冷凍したもの）である。ジンジャー及び／又はガーリックは、ヘム鉄由来の苦味、生臭味、金属味を抑制する作用を有する。ジンジャー及び／又はガーリックは、ヘム鉄に対して、（ジンジャー及び／又はガーリック）／ヘム鉄の重量比が１／１～１０／１、好ましくは２／１～４／１となるようにするのがよい。

【００１０】本発明では上記成分を必須とするが、必要に応じて種々の成分を用いることができる。具体的には、ヘム鉄を含有させる食品で用いられる各種成分があげられる。例えば、ヘム鉄は、カレー、ハヤシ、シチュー、スープ、マーボー豆腐、酢豚等に添加でき、その他にもミートソース、デミグラソース、カレーソース等の各種ソース類、田楽みそ、加工みそ、トウバンジャン、テンメンジャン、みそ汁の素、丼物用ソース、鍋物用ソース等にも添加できるので、これらの食品で用いられている成分である。このうち、塩などの調味料、澱粉や油脂（ルウ）などがあげられる。

【００１１】塩の量は特に制限されないが、対象となる液状食品の具材を除いた液状部分の0.１％以上更には0.５重量％以上である場合、ヘム鉄の黒い凝集物を防止できるので好ましい。具体的には、0.５～1.０％程度添加するのがよい。この際、塩（食塩）自体を使用する代わりに、例えば醤油、みそ、ウスターソース等を使用することもできる。本発明で用いる澱粉としては、例えば小麦粉、米粉、小麦澱粉、トウモロコシ澱粉、馬鈴薯澱粉、米澱粉、タピオカ澱粉等があげられる。澱粉の使用量も、任意であるが、対象となる食品の具材を除いた部分の１％以上とするのが好ましく、より好ましくは３％以上である。具体的には、３～１０％である。この量で澱粉を使用するとヘム鉄の黒い凝集物を防止できるので好ましい。

【００１２】本発明で用いる油脂としては、例えばコーン油、大豆油、ナタネ油、ゴマ油、コメ油、サフラワー油、ヤシ油、パーム核油、カカオ油、ヒマワリ油、綿実油等の植物性油脂、バター、ヘット、ラード等の動物性油脂の１種又は２種以上の混合物があげられる。油脂の量も任意であるが、食品全体中、１～２０％とするのが好ましく、より好ましくは６～１０％である。本発明ではさらにシナモン、セージ、タイム、クローブ、ローレルカルダモン、オレガノ、ナツメグ、メース、レッドペッパー、ホワイトペッパー、ブラックペッパー等の香辛料、ショ糖、果糖、ブドウ糖、乳糖等の甘味料の他、果実ペースト、野菜ペースト、水、着香料、着色料、調味料等を適宜使用することができる。

【００１３】本発明で上述したカレーなどのヘム鉄含有食品をつくるための具材としては、キャベツ、レタス、シュンギク、ゴボウ、ニンジン、セロリ、玉葱、長ネギ、フキ、カリフラワー、ブロッコリー等の野菜類、ジャガイモ、サツマイモ等のイモ類、大豆、小豆、エンドウ豆等の豆類、豆腐等の大豆加工品、鮭、アサリ、カニ、イカ、タコ等の魚介類、牛肉、豚肉、鶏肉等の畜肉類等を例示し得、それらの１種又は２種以上を適宜選択して使用することができる。

【００１４】本発明では上記必須成分を使用する限り任意の方法でヘム鉄含有食品を製造することができるが、ヘム鉄、乳化剤、パプリカ色素、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉などの他の成分と加熱混合するのが好ましい。又は、ヘム鉄、乳化剤、ジンジャー及び／又はガーリックを混合した後、油脂又は澱粉と加熱混合し、次いでパプリカ色素混合するのが好ましい。ここで、混合は各成分が均一となるように、高速撹拌機やサイレントカッターなどを用いて行うのが好ましく、ついで加熱混合は例えば、横型レオニーダー（槇原工業株式会社製）などを用いて、９０～９５℃に達した後、９０～９５℃で３～１０分間加熱を行うのが好ましい。次に、得られたヘム鉄含有食品に、必要によりレトルトパウチなどの密封包装容器に充填し加圧加熱殺菌等の加熱殺菌処理を施すことができ、又、加熱殺菌処理の他に、殺菌することなく無菌充填により包装されるか、あるいは、冷凍処理や乾燥処理等を施しても良い。

【００１５】

【発明の効果】本発明によれば、色調と風味の良好なヘム鉄含有食品を得ることができる。次に実施例により本発明を説明する。

【００１６】

【実施例】

実施例１

ヘム鉄４重量部とショ糖脂肪酸エステル（ＨＬＢ１５）水溶液（濃度０．４％）１００重量部、パプリカ色素１重量部、ジンジャー４重量部及びガーリック４重量部を、高速撹拌機を用いて均一に混合した（ヘム鉄混合物という）。一方、玉葱３００重量部をみじん切りにした後、バター１２５重量部で３０分間炒めた。これに、カレー粉５０重量部、小麦粉１３０重量部をバター１００重量部で２０分間炒めて得た小麦粉ルー、ブイヨン２０００重量部、リンゴ（すりおろしたもの）２００重量部、トマト（みじん切りにしたもの）２１０重量部、チャツネ２００重量部、食塩２０重量部、具材として牛肉（予めバターで炒めたもの）８５０重量部、ニンジン（１０分間ボイルしたもの）３００重量部、ジャガイモ（１０分間ボイルしたもの）８５０重量部を添加混合し、さらに上記ヘム鉄混合物を加えて弱火で沸騰するまで煮込んだ。得られたカレーを２００ｇずつレトルトパウチ（ポリエチレンテレフタレート／ナイロン／アルミニウム／無延伸ポリプロピレン）に充填密封し、１２１℃、２０分間の条件で加圧加熱殺菌処理を施した。得られたレトルトカレーを５分間熱湯で加温した後、パウ

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

チを開封し、カレーを皿に注ぎ出した。得られたカレーは、通常のカレーと同様の良好な外観を呈するものであった。尚、使用したヘム鉄は、ヘモグロビン分解物として得られたヘム鉄の水懸濁液に、キトサン水溶液を添加し、撹き混ぜながらｐＨを調整して、懸濁状態で得られるヘム鉄を、フィルタープレス等で分離することにより製造されたものである。

【００１７】実施例２

パプリカ色素をヘム鉄混合物中に添加せず、具材を除いた他の全ての材料を混合した後に添加して混合し、その後具材を加えた以外は実施例１と同様の方法によりレトルトカレーを得た。得られたカレーは、通常のカレーと同様の良好な外観と風味を呈するものであった。

比較例１

パプリカ色素、ジンジャー及びガーリックを使用しないことを除き、実施例１と同様にしてレトルトカレーを得た。得られたカレーは、黒緑っぽい外観を有し、又、ヘム鉄特有の生臭味と金属臭が感じられるものであった。

Espacenet

Bibliographic data: JP2012016336 (A) — 2012-01-26

FOOD MATERIAL CONTAINING HEME IRON AND METHOD FOR PRODUCING THE SAME

Inventor(s): TANAKA KENICHI; IGARASHI OSAMU ± (TANAKA KENICHI, ; IGARASHI OSAMU)

Applicant(s): EKOOPASU LAB KK; IDEMITSU TECHNOFINE CO LTD ± (EKOOPASU LABORATORIES:KK, ; IDEMITSU TECHNOFINE CO LTD)

Classification: **- international:** ***A23L1/30***
- cooperative:

Application number: JP20100157321 20100709 Global Dossier

Priority number (s): JP20100157321 20100709

Abstract of JP2012016336 (A)

PROBLEM TO BE SOLVED: To provide a food material containing a high density heme iron of which color or aroma is easily modulated, and to provide a method for easily and effectively producing the food material containing the high density heme iron. ;SOLUTION: The food material containing the heme iron is made by crushing a dried fish meat. The food material containing the heme iron is made by crushing a remaining extraction after that the dried meat is extracted with hot water. An average particle diameter of the food material containing the heme iron ranges from 1 [mu]m to 1,000 [mu]m. ;COPYRIGHT: (C)2012,JPO&INPIT;PROBLEM TO BE SOLVED: To provide a food material containing a high density heme iron of which color or aroma is easily modulated, and to provide a method for easily and effectively producing the food material containing the high density heme iron.SOLUTION: The food material containing the heme iron is made by crushing a dried fish meat. The food material containing the heme iron is made by crushing a remaining extraction after that the dried meat is extracted with hot water. An average particle diameter of the food material containing the heme iron ranges from 1 [mu]m to 1,000 [mu]m.

(19)日本国特許庁(JP)　(12)公開特許公報(A)　(11)特許出願公開番号
特開2012-16336
(P2012-16336A)
(43)公開日　平成24年1月26日(2012.1.26)

(51)Int.Cl.		F I		テーマコード（参考）
A23L 1/30 (2006.01)		A23L 1/30	A	4B018

審査請求 未請求　請求項の数 12　OL　（全 10 頁）

(21)出願番号　特願2010-157321 (P2010-157321)
(22)出願日　平成22年7月9日 (2010.7.9)

(71)出願人　510191285
有限会社エコーバス・ラボラトリーズ
東京都西東京市南町一丁目５番２４号
(71)出願人　500242384
出光テクノファイン株式会社
東京都港区北青山一丁目３番６号
(74)代理人　110000637
特許業務法人樹之下知的財産事務所
(72)発明者　田仲　健一
東京都西東京市南町一丁目５番２４号
(72)発明者　五十嵐　脩
東京都墨田区東向島四丁目３９番２号

Ｆターム(参考)　4B018 MD07 MD08 MD20 MD74 ME14 MF01 MF06 MF07

(54)【発明の名称】ヘム鉄含有食品素材およびその製造方法

(57)【要約】
【課題】色や臭いの調整も容易な高濃度のヘム鉄含有食品素材を提供すると共に、簡易かつ効率的に高濃度のヘム鉄を含有する食品素材を製造する方法を提供すること。
【解決手段】本発明のヘム鉄含有食品素材は、魚類の乾燥肉を粉砕してなるヘム鉄含有食品素材であって、該ヘム鉄含有食品素材は、前記乾燥肉を熱水抽出した後の抽出残を粉砕してなるものであり、該ヘム鉄含有食品素材の平均粒子径が１μｍから１０００μｍまでの範囲であることを特徴とするものである。
【選択図】なし

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

【特許請求の範囲】
【請求項１】
魚類の乾燥肉を粉砕してなるヘム鉄含有食品素材であって、
該ヘム鉄含有食品素材は、前記乾燥肉を熱水抽出した後の抽出残を粉砕してなるものであり、
該ヘム鉄含有食品素材の平均粒子径が１μｍ以上１０００μｍ以下の範囲である
ことを特徴とするヘム鉄含有食品素材。
【請求項２】
請求項１に記載のヘム鉄含有食品素材において、
前記抽出残を、粉砕の前後のうちの少なくともいずれかで溶剤にて洗浄する
ことを特徴とするヘム鉄含有食品素材。
【請求項３】
請求項１または請求項２に記載のヘム鉄含有食品素材において、
前記抽出残を、粉砕する前に乾燥する
ことを特徴とするヘム鉄含有食品素材。
【請求項４】
請求項１から請求項３までのいずれか１項に記載のヘム鉄含有食品素材において、
前記ヘム鉄含有食品素材１００ｇ中のヘム鉄含有量が、金属鉄換算で、７ｍｇ／１００ｇ以上である
ことを特徴とするヘム鉄含有食品素材。
【請求項５】
請求項１から請求項４までのいずれか１項に記載のヘム鉄含有食品素材において、
前記乾燥肉が魚節である
ことを特徴とするヘム鉄含有食品素材。
【請求項６】
請求項２から請求項５までのいずれか１項に記載のヘム鉄含有食品素材において、
前記溶剤がエチルアルコールである
ことを特徴とするヘム鉄含有食品素材。
【請求項７】
魚類の乾燥肉を熱水抽出して抽出残を得る抽出残製造工程と、
前記抽出残の平均粒子径が１μｍ以上１０００μｍ以下の範囲となるように粉砕する粉砕工程と、
を備えることを特徴とするヘム鉄含有食品素材の製造方法。
【請求項８】
請求項７に記載のヘム鉄含有食品素材の製造方法において、
前記粉砕工程前に、さらに、前記抽出残を溶剤にて洗浄する前洗浄工程を備える
ことを特徴とするヘム鉄含有食品素材の製造方法。
【請求項９】
請求項７または請求項８に記載のヘム鉄含有食品素材の製造方法において、
前記粉砕工程後に、さらに、粉砕後の抽出残を溶剤にて洗浄する後洗浄工程を備える
ことを特徴とするヘム鉄含有食品素材の製造方法。
【請求項１０】
請求項７から請求項９までのいずれか１項に記載のヘム鉄含有食品素材の製造方法において、
前記粉砕工程前に、さらに、前記抽出残を乾燥させる乾燥工程を備える
ことを特徴とするヘム鉄含有食品素材の製造方法。
【請求項１１】
請求項７から請求項１０までのいずれか１項に記載のヘム鉄含有食品素材の製造方法において、
前記乾燥肉が魚節である

Motif Exhibit 1002, Page 927 of 1290

ことを特徴とするヘム鉄含有食品素材の製造方法。
【請求項１２】
請求項８から請求項１１までのいずれか１項に記載のヘム鉄含有食品素材の製造方法において、
前記溶剤がエチルアルコールである
ことを特徴とするヘム鉄含有食品素材の製造方法。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は，微粒子状のヘム鉄含有食品素材およびその製造方法に関する。
【背景技術】
【０００２】
動物の血液中に含まれるヘモグロビンのヘム部を濃縮して得られたいわゆる「ヘム鉄」は、体内での消化吸収に優れるので鉄補給用の補助食品として有用である。一方、ヘム鉄は、水に溶解しないため必ずしも濃縮が容易ではなく、これまで種々の製法が提案されている。例えば、溶血したヘモグロビンをｐＨ１０以上、または３以下に調整しながら，７０℃以上の温度にて少なくとも１０分間以上保持し、その後使用するプロテアーゼの最適なｐＨおよび温度で酵素分解し、酵素を失活させ、分解液のｐＨを５～６に調整することにより得られる等電点沈殿物をヘム鉄として回収する方法が提案されている（特許文献１参照）。また、魚節を粉末状とし、ｐＨを１程度の強酸性にした水溶液でヘム鉄を可溶化して抽出し、抽出液をアルカリ性にしてヘム鉄を沈殿させ回収する方法が提案されている（特許文献２参照）。
【先行技術文献】
【特許文献】
【０００３】
【特許文献１】特開平５－２４４８９９号公報
【特許文献２】特開２００４－１８０６０２号公報
【発明の概要】
【発明が解決しようとする課題】
【０００４】
しかしながら、特許文献１に記載の方法は、ｐＨの調整や，酵素を使うなど作業が煩雑であり、得られるヘム鉄の収率も高いものではなく、さらに，黒褐色の色や血生臭さを除去（調整）するのが困難であるため、食品への応用に制限があった。また、特許文献２に記載の方法も、ｐＨの調整を行ってヘム鉄の可溶化および再沈殿を行うものであり、効率的な方法とは言えない。
【０００５】
本発明の目的は、色や臭いの調整も容易な高濃度のヘム鉄含有食品素材を提供すると共に、簡易かつ効率的に高濃度のヘム鉄を含有する食品素材を製造する方法を提供することにある。
【課題を解決するための手段】
【０００６】
従来、魚節から熱水抽出などにより出汁をとった後の抽出残は腐敗しやすいために食品素材として利用することは困難と考えられており、肥料として利用したり、産業廃棄物として廃棄したりしていた。これに対し、本発明者らは、従来の技術常識を覆し、前記抽出残を特定の範囲の平均粒子径となるように粉砕すれば、粉砕後の抽出残中の水分量を少なくすることができるために保存しやすくなり、食品素材としても利用できることを見出した。そして、このような食品素材は、驚くべきことに、高濃度のヘム鉄を含有しており、食品としての色調および加工性の点でも好ましいものであり、しかも動物などの血液を原料としていないために血生臭さもなく、臭気の点でも好ましいものであることを見出した。本発明は、このような知見に基づいて完成されたものである。

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

すなわち、本発明のヘム鉄含有食品素材は、魚類の乾燥肉を粉砕してなるヘム鉄含有食品素材であって、該ヘム鉄含有食品素材は、前記乾燥肉を熱水抽出した後の抽出残を粉砕してなるものであり、該ヘム鉄含有食品素材の平均粒子径が１μｍ以上１０００μｍ以下の範囲であることを特徴とするものである。

【０００７】

本発明のヘム鉄含有食品素材においては、前記抽出残を、粉砕の前後のうちの少なくともいずれかで溶剤にて洗浄することが好ましい。

本発明のヘム鉄含有食品素材においては、前記抽出残を、粉砕する前に乾燥することが好ましい。

本発明のヘム鉄含有食品素材においては、ヘム鉄含有食品素材１００ｇ中のヘム鉄含有量が、金属鉄換算で、７ｍｇ／１００ｇ以上であることが好ましい。

本発明のヘム鉄含有食品素材においては、前記乾燥肉が魚節であることが好ましい。

本発明のヘム鉄含有食品素材においては、前記溶剤が水または有機溶媒であることが好ましく、前記有機溶媒はエチルアルコールであることが好ましい。

【０００８】

本発明のヘム鉄含有食品素材の製造方法は、魚類の乾燥肉を熱水抽出して抽出残を得る抽出残製造工程と、前記抽出残の平均粒子径が１μｍ以上１０００μｍ以下の範囲となるように粉砕する粉砕工程と、を備えることを特徴とする方法である。

【０００９】

本発明のヘム鉄含有食品素材の製造方法においては、前記粉砕工程前に、さらに、前記抽出残を溶剤にて洗浄する前洗浄工程を備えることが好ましい。

本発明のヘム鉄含有食品素材の製造方法においては、前記粉砕工程後に、さらに、粉砕後の抽出残を溶剤にて洗浄する後洗浄工程を備えることが好ましい。

本発明のヘム鉄含有食品素材の製造方法においては、前記粉砕工程前に、さらに、前記抽出残を乾燥させる乾燥工程を備えることが好ましい。

本発明のヘム鉄含有食品素材の製造方法においては、前記乾燥肉が魚節であることが好ましい。

本発明のヘム鉄含有食品素材の製造方法においては、前記溶剤がエチルアルコールであることが好ましい。

【発明の効果】

【００１０】

本発明のヘム鉄含有食品素材によれば、そのまま高濃度のヘム鉄を摂取できる食品にできるだけでなく、微粒子状であるため各種の食品などの素材としても好適に使用できる。また、本発明のヘム鉄含有食品素材の製造方法によれば、簡易かつ効率的に、色や臭いの調整も容易な高濃度のヘム鉄を含有する食品素材を製造することができる。

【発明を実施するための形態】

【００１１】

以下、本発明をその好適な実施形態に即して詳細に説明する。

先ず、本発明のヘム鉄含有食品素材の製造方法について説明する。すなわち、本発明のヘム鉄含有食品素材の製造方法は、魚類の乾燥肉を熱水抽出して抽出残を得る抽出残製造工程と、前記抽出残の平均粒子径が１μｍ以上１０００μｍ以下の範囲となるように粉砕する粉砕工程と、を備えることを特徴とする方法である。

【００１２】

抽出残製造工程においては、魚類の乾燥肉を熱水抽出して抽出残を得る。

本発明で用いる魚類の乾燥肉としては、魚類を乾燥させたものであれば特に限定されないが、得られる食品素材のヘム鉄含有量が高いという観点から、魚節を用いることが好ましい。また、前記魚類としては、例えば、鰹、鯖、秋刀魚、鰯、鰤などの青魚が挙げられる。これらの中でも、入手しやすく、また得られる食品素材のヘム鉄含有量を高くできるという観点から、鰹、鯖が好ましく、鰹が特に好ましい。これらの魚類の乾燥肉は１種を単独で用いてもよく、２種以上を組み合わせて用いてもよい。

Motif Exhibit 1002, Page 929 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

【００１３】
前記魚類の乾燥肉を熱水抽出する場合には、熱水抽出における処理温度は８０℃以上１００℃以下の範囲とすることが好ましく、９０℃以上１００℃以下の範囲とすることがより好ましい。処理温度が前記下限未満では、抽出が不十分となるために得られる食品素材のヘム鉄含有量を高くしにくくなる傾向にあり、他方、前記上限を超えるようにする場合には、特別な設備が必要となるため、製造コストが高くなる傾向にある。また、熱水抽出における処理時間は１０分間以上２時間以下の範囲とすることが好ましく、２０分間以上５０分間以下の範囲とすることがより好ましい。処理時間が前記下限未満では、抽出が不十分となるために得られる食品素材のヘム鉄含有量を高くしにくくなる傾向にあり、他方、前記上限を超えると、製造コストが高くなる傾向にある。

【００１４】
粉砕工程においては、前記抽出残の平均粒子径が１μｍ以上１０００μｍ以下の範囲となるように粉砕する。
本発明において平均粒子径は、５０％質量粒子径のことをいう。そして、５０％質量粒子径とは、測定対象の粉体の集団の全質量を１００％として累積カーブを求めたとき、その累積カーブの質量の値が５０％となる点の粒子径のことをいう。このような平均粒子径は、光散乱法により測定することができる。

【００１５】
前記抽出残を粉砕する方法としては、例えば、カッターミル、ハンマーミル、ビーズミル、ジェットミル、ボールミルなどを用いる方法を採用することができる。
また、本発明においては、前記抽出残を粉砕する前に、前記抽出残を乾燥しておくことが好ましい。これによれば、抽出残の粉砕工程をさらに効率よく行うことが可能となる。また、粉砕前の抽出残から水分を除去することが可能となるため、抽出残の腐敗を抑制することが可能となる。このように抽出残を乾燥する場合には、乾燥および粉砕の効率の観点から、乾燥温度は５０℃以上１００℃以下の範囲であることが好ましく、６０℃以上８０℃以下の範囲であることがより好ましい。また、乾燥時間は、３０分間以上２４時間以下の範囲であることが好ましく、３時間以上１２時間以下の範囲であることがより好ましい。

【００１６】
本発明においては、前記粉砕工程前に、さらに、前洗浄工程を備えることが好ましい。このような工程により、色や臭いを除去することできる。
前洗浄工程においては、前記抽出残を溶剤にて洗浄する。
前洗浄工程で用いる溶剤としては、例えば、水、有機溶媒が挙げられる。有機溶媒としては、例えば、メチルアルコール、エチルアルコール、イソプロピルアルコールなどのアルコール類；アセトン、エチルメチルケトンなどのケトン類；ジメチルエーテル、ジエチルエーテルなどのエーテル類；酢酸メチル、酢酸エチルなどのエステル類；ヘキサンなどの炭化水素類が挙げられる。これらの溶剤の中でも、得られる食品素材の安全性の観点から、水、エタノールなどが好ましい。また、得られる食品素材におけるヘム鉄含有量を高めるという観点から、エタノールが特に好ましい。また、これらの溶剤は１種を単独で用いてもよく、２種以上を組み合わせて用いてもよい。

【００１７】
前記抽出残を溶剤にて洗浄する場合には、前洗浄における処理温度は２０℃以上前記溶剤の沸点以下の範囲とすることが好ましく、４０℃以上前記溶剤の沸点以下の範囲とすることがより好ましく、６０℃以上前記溶剤の沸点以下の範囲とすることが特に好ましい。処理温度が前記下限未満では、洗浄が不十分となるために得られる食品素材のヘム鉄含有量を高くしにくくなる傾向にあり、他方、前記上限を超えるようにする場合には、特別な設備が必要となるため、製造コストが高くなる傾向にある。また、前洗浄における処理時間は２０分間以上２４時間以下の範囲とすることが好ましく、３時間以上２０時間以下の範囲とすることがより好ましく、９時間以上１５時間以下の範囲とすることが特に好ましい。処理時間が前記下限未満では、洗浄が不十分となるために得られる食品素材のヘム鉄

Motif Exhibit 1002, Page 930 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

含有量を高くしにくくなる傾向にあり、他方、前記上限を超えると、製造コストが高くなる傾向にある。

【００１８】

本発明においては、前記粉砕工程後に、さらに、後洗浄工程を備えることが好ましい。このような工程により、色や臭いをさらに除去することができる。

後洗浄工程においては、粉砕後の抽出残を溶剤にて洗浄する。

後洗浄工程で用いる溶剤としては、前記前洗浄工程で用いる溶剤で用いる溶剤と同様のものを、同様の観点から用いることができる。

【００１９】

前記粉砕後の抽出残を溶剤にて洗浄する場合には、後洗浄における処理温度は２０℃以上前記溶剤の沸点以下の範囲とすることが好ましく、４０℃以上前記溶剤の沸点以下の範囲とすることがより好ましく、６０℃以上前記溶剤の沸点以下の範囲とすることが特に好ましい。処理温度が前記下限未満では、洗浄が不十分となるために得られる食品素材のヘム鉄含有量を高くしにくくなる傾向にあり、他方、前記上限を超えるようにする場合には、特別な設備が必要となるため、製造コストが高くなる傾向にある。また、後洗浄における処理時間は２０分間以上２４時間以下の範囲とすることが好ましく、３時間以上２０時間以下の範囲とすることがより好ましく、９時間以上１５時間以下の範囲とすることが特に好ましい。処理時間が前記下限未満では、洗浄が不十分となるために得られる食品素材のヘム鉄含有量を高くしにくくなる傾向にあり、他方、前記上限を超えると、製造コストが高くなる傾向にある。

【００２０】

以上説明した本発明のヘム鉄含有食品素材の製造方法によれば、前記抽出残製造工程および前記粉砕工程を行うことにより、簡易かつ効率的に高濃度のヘム鉄を含有する食品素材を製造することができる。また、前記粉砕工程の前後に、必要に応じて、前記前洗浄工程および前記後洗浄工程を行うことができ、これらの工程により、得られる食品素材のヘム鉄含有量を高めることができると共に、色や臭いを除去することもできる。

【００２１】

次に、本発明のヘム鉄含有食品素材について説明する。本発明のヘム鉄含有食品素材は、前述した本発明のヘム鉄含有食品素材の製造方法により製造することができる。

すなわち、本発明の第１のヘム鉄含有食品素材は、前記抽出残製造工程および前記粉砕工程を行うことにより得ることができる。また、本発明の第２のヘム鉄含有食品素材は、前記抽出残製造工程、前記前洗浄工程および前記粉砕工程を行うことにより得ることができる。また、前記第１および第２のヘム鉄含有食品素材には、さらに、前記後洗浄工程を行うことが好ましい。

【００２２】

本発明のヘム鉄含有食品素材に用いる魚類の乾燥肉および溶剤としては、前述した本発明のヘム鉄含有食品素材の製造方法に用いるものと同様のものを用いることができる。また、本発明のヘム鉄含有食品素材においては、前記抽出残製造工程、前記前洗浄工程、前記粉砕工程および前記後洗浄工程における処理条件を、前述した本発明のヘム鉄含有食品素材の製造方法における場合と同様の処理条件とすることが好ましい。

【００２３】

そして、本発明のヘム鉄含有食品素材の平均粒子径は１μｍ以上１０００μｍ以下の範囲であることが必要である。平均粒子径が１０００μｍを超えると、得られる食品素材の色調および加工性や食感が悪くなる。他方、平均粒子径を１μｍ未満とすることは困難であり、製造コストが高くなる。また、本発明のヘム鉄含有食品素材の平均粒子径は、食品素材としてのハンドリング性の観点から、１０μｍ以上５００μｍ以下の範囲であることが好ましく、２０μｍ以上３００μｍ以下の範囲であることが特に好ましい。

また、本発明のヘム鉄含有食品素材中の水分量（質量基準）は、ヘム鉄含有食品素材を腐敗しにくくするという観点から、１０質量％以下であることが好ましく、７質量％以下であることがより好ましい。

Motif Exhibit 1002, Page 931 of 1290

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

さらに、本発明のヘム鉄含有食品素材はヘム鉄含有量が高いものであることが好ましい。具体的には、ヘム鉄含有食品素材１００ｇ中のヘム鉄含有量が、金属鉄換算で、７ｍｇ／１００ｇ以上であることが好ましい、１５ｍｇ／１００ｇ以上であることがより好ましい。なお、このようなヘム鉄含有量は、前記ヘム鉄含有食品素材中の水分量を０質量％に換算して求めたものである。

本発明のヘム鉄含有食品素材は、例えば、パン、クッキー、ふりかけ、ヨーグルト、ドリンク、マーガリン、栄養素を補うためのサプリメントなどの食品の素材として特に好適に使用することができる。

【実施例】

【００２４】

次に、本発明を実施例および比較例によりさらに詳細に説明するが、本発明はこれらの例によってなんら限定されるものではない。

【００２５】

［実施例１］

フレーク状の鰹節を温度９５℃の熱水中に４０分間浸漬して熱水抽出した。その後、抽出物を分離して、抽出残を回収した（抽出残製造工程）。得られた抽出残を、温度７０℃にて７時間乾燥した。乾燥後の抽出残を、カッターミルを用いて粉砕して食品素材を得た（粉砕工程）。なお、得られた食品素材における水分量は６．９質量％であった。

【００２６】

［実施例２］

フレーク状の鰹節を温度９５℃の熱水中に４０分間浸漬して熱水抽出した。その後、抽出物を分離して、抽出残を回収した（抽出残製造工程）。得られた抽出残を、温度７０℃にて７時間乾燥した。乾燥後の抽出残を、カッターミルを用いて粉砕した（粉砕工程）。粉砕後の抽出残を、室温（２５℃）のエチルアルコール中に７時間浸漬して洗浄した（後洗浄工程）。その後、洗浄後の抽出残を温度７０℃にて７時間乾燥して食品素材を得た。なお，得られた食品素材における水分量は６．０質量％であった。

【００２７】

［実施例３］

フレーク状の鰹節を温度９５℃の熱水中に４０分間浸漬して熱水抽出した。その後、抽出物を分離して、抽出残を回収した（抽出残製造工程）。得られた抽出残を、室温（２５℃）のエチルアルコール中に７時間浸漬して洗浄した（前洗浄工程）。洗浄後の抽出残を、温度７０℃にて７時間乾燥した。乾燥後の抽出残を、カッターミルを用いて粉砕して食品素材を得た（粉砕工程）。なお、得られた食品素材における水分量は５．９質量％であった。

【００２８】

［実施例４］

フレーク状の鰹節を温度９５℃の熱水中に４０分間浸漬して熱水抽出した。その後、抽出物を分離して、抽出残を回収した（抽出残製造工程）。得られた抽出残を、室温（２５℃）のエチルアルコール中に７時間浸漬して洗浄した（前洗浄工程）。洗浄後の抽出残を、温度７０℃にて７時間乾燥した。乾燥後の抽出残を、カッターミルを用いて粉砕した（粉砕工程）。粉砕後の抽出残を、室温（２５℃）のエチルアルコール中に７時間浸漬して洗浄した（後洗浄工程）。その後、洗浄後の抽出残を温度７０℃にて７時間乾燥して食品素材を得た。なお、得られた食品素材における水分量は４．９質量％であった。

【００２９】

［比較例１］

フレーク状の鰹節を温度９５℃の熱水中に４０分間浸漬して熱水抽出した。その後、抽出物を分離して、抽出残を回収し、乾燥して食品素材を得た（抽出残製造工程）。得られた食品素材における水分量は８．０質量％であった。

【００３０】

＜性能評価＞

Motif Exhibit 1002, Page 932 of 1290

実施例１から実施例４まで、および比較例１で得られた食品素材の性状（平均粒子径、臭気、黄色度、ヘム鉄含有量、安息角、嵩密度）を以下に示す方法により評価した。得られた結果および水分量に関する結果を表１に示す。

（ｉ）平均粒子径

得られた食品素材の平均粒子径を、粒子径分布測定装置（日機装株式会社製、「マイクロトラック　ＭＴ３０００」）を用いて測定した。

（ii）臭気の評価

得られた食品素材の臭気を以下に示す基準に基づいて評価した。

◎：臭いは殆どない。

○：臭いはあるが、気にはならない程度である。

△：やや強い臭いはあるが、血生臭くはない。

×：強い臭いがあり、しかも血生臭い。

（iii）黄色度の測定

得られた食品素材の黄色度（ＹＩ値）を、測色色差計（コニタミノルタ株式会社製、「色彩色差計　ＣＲ－２００，ＣＲ－２３１」）を用いて測定した。なお、黄色度が低いほど、食品としての色調に優れている。

（iv）ヘム鉄含有量

得られた食品素材のヘム鉄含有量を、誘導結合プラズマ発光分光分析法（ＩＣＰ発光分光分析法）により、測定した。具体的には、ＩＣＰ発光分光分析装置（ＶＡＲＩＡＮ社製、「ＶｉｓｔａＰｒｏ－ＲＬ型」）を用いた。なお、食品素材１００ｇ中のヘム鉄含有量は、ヘム鉄を金属鉄換算し、食品素材中の水分量を０質量％に換算して求めたものである。単位は（ｍｇ／１００ｇ）である。

（v）安息角の測定

得られた食品素材の安息角を次のようにして測定した。すなわち、１００ｇの食品素材を試料として安息角測定装置（株式会社セイシン企業製、「ＭＴ－１０００」）に投入し、該試料を水平面へ落下させた。そして、落下した試料により形成された斜面と、水平面とがなす角度を、試料の安息角として測定した。なお、試料の水分量は５．０質量％から１０．０質量％までの範囲に入るように調整したものを用いた。なお、安息角が小さいほど、流動性に優れ、加工性に優れている。単位は（°）である。

（vi）嵩密度

得られた食品素材を試料として、該試料の嵩密度を、嵩密度測定器（株式会社セイシン企業製、「川北式カサ密度計　ＩＨ２０００型」）を用いて測定した。なお、試料の水分量は５．０質量％から１０．０質量％までの範囲に入るように調整したものを用いた。なお、嵩密度が高いほど、流動性および分散性に優れ、加工性に優れている。単位は（ｇ／ｃｍ^3）である。

【００３１】

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

【表１】

	平均粒子径 (μm)	臭気	黄色度	ヘム鉄含有量 (mg／100g)	安息角 (°)	嵩密度 (g／cm^3)	水分量 (質量%)
実施例1	42.4	△	67.4	8.53	60	0.457	6.9
実施例2	37.8	○	48.9	20.40	45	0.527	6.0
実施例3	38.2	○	42.2	20.50	45	0.512	5.9
実施例4	35.2	◎	41.8	22.00	39	0.541	4.9
比較例1	—	△	77.9	7.53	—	—	8.0

【００３２】

Motif Exhibit 1002, Page 934 of 1290

表１に示す結果からも明らかなように、本発明のヘム鉄含有食品素材（実施例１から実施例４まで）は、高濃度のヘム鉄を含有しており、食品としての色調および加工性の点でも好ましいものであり、しかも臭気の点でも好ましいものであることが確認された。また、前洗浄処理や後洗浄処理を行った場合（実施例２から実施例４まで）には、臭気、ヘム鉄含有量、並びに食品としての色調および加工性のうちの全ての点において向上が見込めることが確認された。

【産業上の利用可能性】

【００３３】

本発明のヘム鉄含有食品素材は、例えば、パン、クッキー、ふりかけ、ヨーグルト、ドリンク、マーガリン、栄養素を補うためのサプリメントなどの食品の素材として特に好適に使用することができる。

Electronic Patent Application Fee Transmittal

Application Number:	15398479
Filing Date:	04-Jan-2017
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Filer:	M. Angela Parsons/Shannon Glaeser
Attorney Docket Number:	38767-0015002

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	30335338
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Shannon Glaeser
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	12-SEP-2017
Filing Date:	04-JAN-2017
Time Stamp:	12:53:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$180
RAM confirmation Number	091217INTEFSW00010971061050
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	Transmittal.pdf	70123 42aff1cd96348bba747ef3a94335e9545d1a38b3	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
2	Information Disclosure Statement (IDS) Form (SB08)	1449.pdf	124225 8477254d637c30769b2fc7e4a86a62f351f18ca2	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	CN101156632.pdf	582162 d1b6e4a46018e3f855a54d78784516945d11905d	no	8
Warnings:					
Information:					
4	Foreign Reference	CN101489422.pdf	3234714 f59337c83d5c051b088307eceda2d0685a87095c	no	61
Warnings:					
Information:					
5	Foreign Reference	CN101861895.pdf	838339 2dc56b8384533d7d9128b02b5b8403ceaa1d02d6	no	13
Warnings:					
Information:					
6	Foreign Reference	CN101897418.pdf	1157661 8dc103475b9c337e48d2cfb2697001e2b1a4a1df	no	17

Warnings:					
Information:					
7	Foreign Reference	CN102440302.pdf	1244855 b13216bcc187bc35a36e14f6e41555f5191aa546	no	13
Warnings:					
Information:					
8	Foreign Reference	CN102578544.pdf	616731 552dbb3044e90cc149a94a7f136ffdf1d8c87d4a	no	8
Warnings:					
Information:					
9	Foreign Reference	CN102835460.pdf	833806 e910188f4c48d12b948fee2f60fe4bf36fac4f20	no	16
Warnings:					
Information:					
10	Foreign Reference	JPS53115846A.pdf	551391 358cf0c2707d10dae0c21703e4fcd271b9c7ca74	no	5
Warnings:					
Information:					
11	Foreign Reference	JPH08140627A.pdf	516123 5544967d1e1dc39f98f553ad5d4e9140ef5db342	no	5
Warnings:					
Information:					
12	Foreign Reference	JP2012016336.pdf	4872119 9087ac617395af39334d6492d73d0dcc746a2dc0	no	11
Warnings:					
Information:					
13	Non Patent Literature	Naike_1996.pdf	3415330 415ed32dd6b259ad6086db6457f79c03c79a1c8d	no	17
Warnings:					
Information:					

14	Non Patent Literature	CN_OA_2014800143499_2017_07_24.pdf	2825284 a5c4197df8a8003ac8aa00256823a3d75d691236	no	52
Warnings:					
Information:					
15	Non Patent Literature	CN_OA_2012800417131_2017_07_13.pdf	6746604 f1fcc718b7503f56e6b768db5173a41855a080b3	no	28
Warnings:					
Information:					
16	Non Patent Literature	CN_OA_2014800137784_2017_08_09.pdf	1251009 0d9c22624f502a2c083faa815b03a9f37f0c0897	no	24
Warnings:					
Information:					
17	Non Patent Literature	Xiaoling.pdf	266416 f3f7aabbb1267df67a8cfbcae6bc5f8e6c00e227	no	10
Warnings:					
Information:					
18	Fee Worksheet (SB06)	fee-info.pdf	30775 0dc9a7f5de83bd95756c00a2e859dd91d422e0ea	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	29177667	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL REPLY TO ACTION OF APRIL 14, 2017

Please consider the following reply.

Amendments to the Specification:

Please amend the paragraph on page 14, lines 5-29, as follows:

Marked Up

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* sps. (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* sps. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium* sp. (quinoa); *Sesamum* sp. (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, *Aspergillus oryzae*, *Trichoderma reesei*, *Myceliopthera thermophile*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Synechocistis* sp., *Aquifex aeolicus*, *Methylacidiphilum infernorum*, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, et al., *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at [[http://]] hemeprotein.info/heme.php.

Clean

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox,

or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* sps. (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* sps. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium* sp. (quinoa); *Sesamum* sp. (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, *Aspergillus oryzae*, *Trichoderma reesei*, *Myceliopthera thermophile*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Synechocistis* sp., *Aquifex aeolicus*, *Methylacidiphilum infernorum*, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, et al., *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at hemeprotein.info/heme.php.

Please insert the paragraphs below immediately following page 18, line 23.

In some embodiments, the food product contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the food product contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the food products can be produced to approximate the natural variation in meat.

In some embodiments, the food product comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 5 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein (e.g., a heme-containing protein) by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source.

Please amend the paragraph on page 52, lines 16-22, as follows:

Marked Up

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (~~FIG. 1~~FIG. 2).

Clean

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (FIG. 2).

Amendments to the Drawings:

Please replace the original FIG. 2 with the Replacement Sheet filed herewith.

List of claims (replacing prior versions).

1. – 30. (Canceled)

31. (Currently Amended) A food flavor additive composition comprising:

a) an isolated heme-containing protein;

b) a compound ~~sugar, sugar alcohol, sugar acid, or sugar derivative~~ selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, maltodextrin, and galactose, and mixtures of two or more thereof; and

c) a compound selected from cysteine, cystine, ~~selenocysteine,~~ thiamine, methionine, and mixtures of two or more thereof;

wherein the flavor additive composition contains no animal products ~~tissue derived components~~; and

~~wherein the flavor additive composition is packaged and is in the form of a liquid, a gel, an emulsion, a powder, or a bouillon cube~~

wherein cooking the food flavor additive composition results in the production of at least two volatile compounds which have a meat-associated aroma.

32. (Canceled) ~~The food flavor additive composition of claim 31, wherein the heme-containing protein is present in an amount between about 0.01% and about 5% by weight.~~

33. (Previously Presented) The food flavor additive composition of claim 31, wherein the heme-containing protein is selected from the group consisting of a androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, a leghemoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin, a truncated 2/2 globin, and a hemoglobin 3.

34. (Previously Presented) The food flavor additive composition of claim 31, wherein the heme-containing protein comprises amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 1-26.

35. (Currently Amended) The food flavor additive composition of claim 31, wherein the food flavor additive composition is a powder[[,]] or a liquid~~, an emulsion or a gel~~ composition.

36. (Currently Amended) The food flavor additive composition of claim 31, wherein the heme-containing protein is a plant, ~~a mammalian,~~ a yeast or filamentous fungi, or bacterial heme-containing protein.

37. (Previously Presented) The food flavor additive composition of claim 31, further comprising one or more of an extract or hydrolysate selected from a vegetable protein hydrolysate, a soy protein hydrolysate, a yeast extract, a yeast protein hydrolysate, and an algal protein hydrolysate.

38. (Previously Presented) The food flavor additive composition of claim 31, further comprising one or more of a compound selected from alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

39. (Previously Presented) The food flavor additive composition of claim 31, further comprising one or more of an oil selected from coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, and oil from bacteria or fungi.

40. (Previously Presented) The food flavor additive composition of claim 31, further comprising one or more of albumin or a globulin.

41. (Currently Amended) A food flavor additive composition prepared by a process of:

a) combining an isolated heme-containing protein; a sugar, sugar alcohol, sugar acid, or sugar derivative selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof; and a compound selected from cysteine, cystine, selenocysteine, thiamine, methionine, and mixtures of two or more thereof, to form a heme flavor reaction mixture, and

b) heating the heme flavor reaction mixture to result in the food flavor additive composition;

wherein the food flavoring additive composition contains products no animal tissue derived components; and

wherein the heme flavor reaction mixture has a meaty flavor and/or meaty aroma during the heating of step b) and/or the food flavoring additive composition has a meaty flavor and/or meaty aroma after the heating of step b).

42. (Previously Presented) The food flavor additive composition of claim 41, wherein the process further comprises heating the heme flavor reaction mixture to at least 150°C for at least 1 minute.

43. (Canceled) ~~The food flavor additive composition of claim 41, wherein the heme flavor reaction mixture has a meaty flavor and/or meaty aroma during the heating of step b) and/or the food flavoring additive composition has a meaty flavor and/or meaty aroma after the heating of step b).~~

44. (Previously Presented) The food flavor additive composition of claim 42, wherein the food flavoring additive composition further comprises a compound selected from the group consisting of 2-methyl-furan, bis(2-methyl-3-furyl)disulfide, 2-pentyl-furan, 3,3'-dithiobis-2-

methyl-furan, 2,5-dimethyl-pyrazine, 2-methyl-3-furanthiol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, dihydro-3-(2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide, decanal, 5-ethyldihydro-2(3H)-furanone, dihydro-5-pentyl-2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, p-Cresol, and hexanoic acid.

45. (Previously Presented) The food flavor additive composition of claim 44, wherein the compound is detectable in the headspace above the heme flavor reaction mixture during the heating of step b) or in the headspace above the food flavoring additive composition after the heating of step b).

46. (Previously Presented) The food flavor additive composition of claim 41, where the process further comprises removing the heme-containing protein from the food flavoring additive composition after the heating in step b) of the heme flavor reaction mixture.

47. (Previously Presented) The food flavor additive composition of claim 41, wherein the process further comprises altering the pH of the heme flavor reaction mixture prior to the heating of step b).

48. (Previously Presented) The food flavor additive composition of claim 41, wherein the process further comprises dehydrating the heme flavor reaction mixture to a powder form after the heating of step b).

49. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme-containing protein is present in an amount between about 0.01% and about 5% by weight in the heme flavor reaction mixture.

50. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme-containing protein is selected from the group consisting of an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, a leghemoglobin, an

erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin, a truncated 2/2 globin, and a hemoglobin 3.

51. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme-containing protein comprises an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 1-26.

52. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme flavor reaction mixture further comprises one or more oils selected from coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, and oil from bacteria or fungi.

53. (Previously Presented) The food flavor additive composition of claim 41, further comprising an ingredient selected from an emulsifier and a gelling agent.

54. (Previously Presented) The food flavor additive composition of claim 41, the process further comprising adding an emulsifier and a gelling agent to the heme flavor reaction mixture prior to the heating of step b).

55. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme flavor reaction mixture is an aqueous solution.

56. (Previously Presented) The food flavor additive composition of claim 41, wherein the heme flavor reaction mixture is an oil-based mixture.

57. (Previously Presented) The food flavor additive composition of claim 41, further comprising removing the heme-containing protein from the food flavor additive composition.

58. (Previously Presented) The food flavor additive composition of claim 41, further comprising one or more of an extract or hydrolysate selected from a vegetable protein hydrolysate, a soy protein hydrolysate, a yeast extract, a yeast protein hydrolysate, and an algal protein hydrolysate, and or an albumin or a globulin.

59. (Previously Presented) A process for imparting a meaty flavor and/or meaty aroma to a consumable food product, comprising:

adding to the consumable food product a food flavor additive composition of claim 31.

60. (Previously Presented) The process of claim 59, further comprising heating the consumable food product.

61. (New) The food flavor additive composition of claim 31, in the form of a soup, stew base, a bouillon powder, bouillon cube, flavor packets or seasoning packet.

REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein.

The present amendment assumes that the claim amendments made in Applicants Reply to Office Action of April 14, 2017 filed on July 14, 2017 have **not** been entered. Therefore, the amendments made to the claims herein are based on the claims pending on the mailing date of the present Office Action, i.e., April 14, 2017.

Upon entry of the above amendment, claims 31-60 will be pending. Claims 59 and 60 were previously withdrawn, so are not currently under examination. Claims 31, 35 and 41 have been amended. Most of the amendments are simply to clarify scope and are supported throughout the specification. For example, the amendment to claim 1 finds support at least at page 4, line 27 to page 5, line 2; page 5, lines 3-9; page 12, lines 2-5, 15-18 (Table 1, rows 1, 4 and 6); Example 1; Example 4; Example 5; Example 6; Example 7; Example 8; Example 9 Example 10; Example 11 and Table 13; page 7, lines 5-8; Examples 4-12; page 5, lines 10-12; page 7, lines 5-7; and page 25, lines 24-28. Claim 35 finds support at least at page 5, lines 10-12; page 7, lines 5-7; and page 25, lines 24-28. The amendments to claim 41 find support at least at page 3, lines18 – page 4, line 3; page 7, line 9 – page 9, line 28; page 4, lines 16-21; and page 7, lines 5-8; Examples 4-12.

No new matter has been introduced by these amendments. Reconsideration and allowance of the claims are respectfully requested in view of the above amendments and the following remarks.

Amendments to the Specification

Applicant have amended the Specification for consistency with the amendments made in parent U.S. Application Serial No. 14/797,006. Support for the amendments to the specification can be found at least at page 18, lines 16-27 of U.S. Application Serial No. 14/797,006; at [00354] of U.S. Application Serial No. 13/941,211 (published as US 2014/0220217); and at least at [00258] of U.S. Application Serial No. 61/751,816. The present application claims priority to U.S. Application Serial No. 14/797,006, U.S. Application Serial No. 13/941,211 and to U.S.

Application Serial No. 61/751,816, each of which were incorporated by reference. Accordingly, no new matter has been introduced by these amendments.

Interview Summary

Applicants acknowledge the courtesy extended by Examiner Gwartney for the in-person interview with Applicants' representatives, Ian Lodovice and Teresa Lavoie on August 14, 2017. Applicants also acknowledge the courtesy extended by Examiner Gwartney for the telephonic interview with Applicants' representatives, Ian Lodovice and Teresa Lavoie on September 11, 2017. During the interviews, Applicants discussed Applicants' Reply to Office Action of April 14, 2017 filed on July 14, 2017 and further amendments for placing the application in condition for allowance. The amendments presented herein are consistent with those discussions.

Election/Restriction

Applicant acknowledges and thanks Examiner Gwartney for the telephone conversation on April 6, 2017 with Applicant's representative Ian Lodovice where the participants discussed Restriction to one of the following inventions is required under 35 U.S.C. 121:

The Examiner restricted the claims into two groups:

I. Claims 31-58, drawn to a food flavor additive comprising an isolated heme-containing protein, a sugar, sugar alcohol, sugar acid, or sugar derivative classified in A23L27/00.

II. Claims 59 and 60, drawn to a process of imparting a meaty flavor and/or meaty aroma to a consumable food product, classified in A23L13/426.

In a telephone call on April 6, 2017, the undersigned elected Group I, claims 31-58. Applicants affirm this election, without traverse.

Specification

The disclosure is objected to because the Examiner indicated that the specification contains an embedded hyperlink and/or other form of browser-executable code.

Applicants have amended the specification herein to remove the embedded hyperlink. In view of these amendments, Applicants respectfully request that the objections to the specification be withdrawn.

Drawings

The drawings are objected to because the Examiner indicated that Figure 2 does not contain a label for the Y-axis. Filed herewith is a Replacement Sheet for FIG. 2 addressing the present rejection. Reconsideration and withdrawal of the objection is requested.

Claim Rejections - 35 U.S.C. § 112

Claims 31-58 stand rejected under 35 U.S.C. § 112 (pre-AIA), first paragraph, as allegedly failing to comply with the written description requirement. Applicant respectfully disagrees.

The Federal Circuit specifically states that the test for written description under 35 U.S.C. § 112, first paragraph, "is whether the disclosure of the application relied upon *reasonably* conveys to those *skilled in the art* that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc., 10 v. Eli Lilly & Co.*, 598 F3d 1336, 1351 (Fed. Cir. 2010) (emphasis added). See, also, *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 1575 (Fed. Cir. 1985); and *In re Kaslow*, 707 F.2d 1366, 1375 (Fed. Cir. 1983). Whether the description requirement is met is a question of fact. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555 (Fed.Cir.1991). "We have made clear that the written description requirement does not demand either examples or an actual reduction to practice; a constructive reduction to practice that in a definite way identifies the claimed invention can satisfy the written description requirement." *Ariad Pharm* at 1352 (*citing Falko-Gunter Falkner v. Inglis*, 448 F.3d 1357, 1366-67 (Fed.Cir.2006)). "The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not have recognized in the applicant's disclosure a description of the invention defined by the claims." MPEP § 2163.04 (*citing In re Werheim*, 541 F.2d 257, 263 (CCPA 1976)).

The Examiner objected to the phrase "*wherein the flavor additive composition contains no animal tissue derived components*" recited in claims 31 and 41, alleging "there is no support

to claim that the flavor additive composition contains no animal tissue derived components." Without conceding the appropriateness of the rejection, and solely in the interested of advancing prosecution, the Applicant has amended claims 31 and 41 to recite "wherein the flavor additive composition contains no animal products." The amended claim has written description support in the specification as filed at, for example, page 7, lines 2-8; and Examples 4-12. Throughout Examples 4-12, a number of different flavor additive compositions are described, each of which does not contain animal products. Further, as the Specification expressly provides that the food products (e.g., food additive compositions) described therein can be prepared in various ways and can be formulated to be free of animal products.

The Examiner also objected to the phrase "wherein the flavor additive composition is packaged and is in the form of a liquid, a gel, an emulsion, a powder or a bouillon cube." Without out conceding the appropriateness of the rejection, and solely in the interested of advancing prosecution, the Applicant has amended claim 31 to obviate this rejection.

The Examiner also objected to the phrase "wherein the heme-containing protein is present in an amount between about 0.01% to about 5%" recited in claim 32. Without conceding the appropriateness of the rejection, and solely in the interest of advancing prosecution, the Applicant has amended claims 32 to obviate this rejection.

The Examiner also objected to the phrase "further comprising an ingredient selected from an emulsifier and a gelling agent" recited in claim 53, as well as the phrase "adding an emulsifier and a gelling agent to the heme flavor reaction mixture prior to the heating of step b)" recited in claim 54. Support for claims 53 and 54 can be found, at least at page 7, lines 1-3; page 26, line 23, and throughout the specification. As above, Applicant respectfully that the Specification reasonably conveys that the term "food product" recited in the specification properly includes a "food flavor additive composition" as claimed. For at least this reason, Applicant maintains that claims 53-54 meet the test for written description under 35 U.S.C. § 112, first paragraph, and reasonably conveys to those skilled in the art that the inventors had possession of the claimed subject matter as of the filing date.

In summary, based at least on the disclosure cited above, the present application meets the test for written description under 35 U.S.C. § 112, first paragraph, and reasonably conveys to

those skilled in the art that the inventors had possession of the claimed subject matter as of the filing date

Claims 31-58 stand rejected under 35 U.S.C. 112 (pre-AIA), second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor regards as the invention. Without conceding the appropriateness of the rejection, and solely in the interested of advancing prosecution, Applicant has amended the claims to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim Rejections - 35 U.S.C. § 102

Claims 31, 33-36, 38 and 40 are rejected under pre-AIA 35 U.S.C. § 102(b) as being anticipated by Tong et al. ("Blood Composition of Different Beef Breed Types," Can. J. Anim. Sci, 66, Dec. 1986, pp. 915-924)("Tong") as evidenced by Shimbayashi et al. ("Free Amino Acids and Phsphorylethanolamine in Milk Whey of Cow," Agr. Biol. Chem, Vol. 29, No. 1, 1965, pp. 13-19)("Shimbayashi"). To the extent that the rejection may be applied to the amended claims, Applicants traverse.

Under Federal Circuit law, "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Also, "unless a reference discloses within the four corners of the document not only all of the limitations claimed but also all of the limitations arranged or combined in the same way as recited in the claim, it cannot be said to prove prior invention of the thing claimed and, thus, cannot anticipate under 35 U.S.C. § 102." *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1371 (Fed. Cir. 2008).

Tong was cited for disclosing that blood from cattle comprise hemoglobin, glucose, protein, and albumin. Shimbayashi was cited as evidence that blood from cattle was known to comprise free amino acids including methionine, taurine, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine. Neither Tong, nor Shimbayashi, disclose a food flavor additive composition that

contains no animal products as presently claimed. Thus, Tong, when considered alone or in combination with Shimbayashi, fails to teach each and every element as set forth in the claims.

For at least the reasons above, Tong does not anticipate the pending claims. Reconsideration and withdrawal of the rejection under 35 U.S.C. 102(b) of claims 31, 33-36, 38 and 40 is requested.

Claims 31-36 and 38 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Koutsidis et al. ("Water-soluble precursors of beef flavor: I. Effect of diet and breed," Meat Science, 79, 2008, pp. 124-130)("Koutisids") as evidenced by Field et al. ("Heme Pigment Content of Bovine Hemopoietic Marrow and Muscle," J. Food Sci., Volume 45, 1980, pp. 1109-1112)("Field"). To the extent that the rejection may be applied to the amended claims, Applicants traverse.

Koutsidis was cited for disclosing that beef comprises sugars and free amino acids. Field was cited as evidence that "beef muscle is known to comprise about 0.06 % by weight hemoglobin." Neither Koutsidis, nor Field, disclose a food flavor additive composition that that contains no animal products as presently claimed. Thus, Koutsidis, fails to teach each and every element as set forth in the claims.

For at least the reasons above, Koutsidis, as evidenced by Field or otherwise, does not anticipate the pending claims. Reconsideration and withdrawal of the rejection under 35 U.S.C. 102(b) of claims 31-36 and 38 is requested.

Claims 31, 33-36, 38, 39-45, 50, 51 and 58 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Jamieson ("Iroquois Stew With Beef, Chicken and Pork," Bon Appetit, November 1995, http://www.epicurious.conilrecipesifoodiviewsiiroquois-stewwith-beef-chicken-and-pork-865) as evidenced by Koutsidis, Field and Griffiths ("XCIII. The Action of Gastric Juice on Beef Muscle-Globulin, With Reference to Anaemia," Biochemistry Journal, Vol. 28, 1934, pp. 671-675)("Griffiths").

According to the Examiner, Jamieson discloses Iroquois stew with beef and method for making the stew comprising adding beef to a composition of pork, chicken, flour, vegetable oil, onion, chicken broth, carrots, bay leaves, all spice and potatoes." Koutsidis and Field were cited

for the reasons outlined above. Griffiths was cited for teaching that "beef is known to comprise globulin."

Jamieson does not disclose a food flavor additive composition that "contains no animal products" as presently claimed. Thus, Jamieson fails to teach all elements of the claims. Koutsidis, Field and Griffiths were not cited for, nor do they teach, the deficiencies of Jamieson discussed above.

For at least the reasons above, Jamieson, as evidenced by Koutsidis, Field and Griffiths, or otherwise, does not anticipate the pending claims. Reconsideration and withdrawal of the rejection under 35 U.S.C. 102(b) of claims 31-36 and 38 is requested.

CONCLUSION

Applicants respectfully request that claims 30-58 be allowed. If any issues remain, the Examiner is asked to telephone the undersigned to arrange a time for an interview.

Applicants do not concede any positions of the Office that are not expressly addressed above, nor do Applicants concede that there are no other good reasons for patentability of the presented claims or other claims.

The fees for a three month extension of time is being paid with this reply on the Electronic Filing System. Apply those fees and any other necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: September 24, 2017

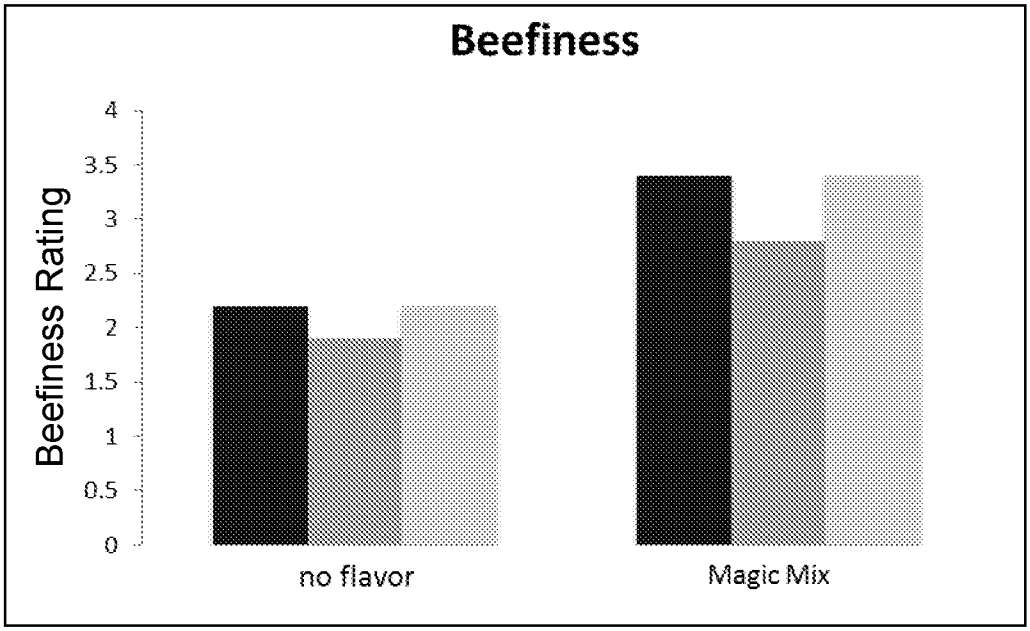
/Ian J.S. Lodovice/

Ian J. Lodovice
Reg. No. 59,749

Customer Number 26191
Fish & Richardson P.C.
Telephone: (617) 956-5972
Facsimile: (877) 769-7945

61554863.doc

FIG. 2


Beefiness
Beefiness Rating
4
3.5
3
2.5
2
1.5
1
0.5
0
no flavor
Magic Mix

Electronic Acknowledgement Receipt

EFS ID:	30464249
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	Ian J.S. Lodovice/Judith Witzel
Filer Authorized By:	Ian J.S. Lodovice
Attorney Docket Number:	38767-0015002
Receipt Date:	25-SEP-2017
Filing Date:	04-JAN-2017
Time Stamp:	15:56:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		SuppReply.pdf	172963 04f7cbed308d3ec9b6c1c95024b221359b585dc3	yes	20

	Multipart Description/PDF files in .zip description				
	Document Description	**Start**	**End**		
	Supplemental Response or Supplemental Amendment	1	1		
	Specification	2	6		
	Claims	7	12		
	Applicant Arguments/Remarks Made in an Amendment	13	20		
Warnings:					
Information:					
2	Drawings-only black and white line drawings	Fig2.pdf	150455 ce3e54c5c752f5e15598bd41ad806d61f36fa60c	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	323418		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/398,479	Filing Date 01/04/2017	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

		(Column 1)		(Column 2)	(Column 3)		
AMENDMENT	09/25/2017	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 29	Minus	** 30	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)		
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
TRINA STEPTOE

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 15398479 Document Date: 09/25/2017

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

Attorney Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed; U.S. patents will be provided upon request. Copies of a Communication from a foreign Patent Offices in a counterpart application also is enclosed.

This statement is being filed after a first Office Action on the merits, but before receipt of a Final Office Action or a Notice of Allowance. A submission fee in the amount of $180 as required under 37 CFR § 1.17(p) is being paid herewith. Please apply any other charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: /November 17, 2017/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61594507.doc

Sheet 1 of 1

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0015002	Application No. 15/398,479
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Impossible Foods Inc.	
(37 CFR §1.98(b))		Filing Date January 4, 2017	Group Art Unit 1791

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	1.	2,934,437	04/26/1960	Morton et al.			
	2.	3,394,017	07/23/1968	Giacino et al.			
	3.	5,039,543	08/13/1991	Lee et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	4.	EP 0136428	04/10/1985	EPO				
	5.	EP 2943072	11/18/2015	EPO				
	6.	JP S42-22194	10/31/1942	JAPAN				
	7.	JP S49-39824	10/29/1974	JAPAN				
	8.	JP S51-63971	10/24/1975	JAPAN			Engl. Tranls.	
	9.	WO 2014/110532	07/17/2014	WIPO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document
	10.	Supplementary European Search Report in European Application No. 15774164, dated October 27, 2017, 11 pages

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 136 428**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **84108340.5**

(22) Date of filing: **16.07.84**

(51) Int. Cl.⁴: **A 23 L 1/226**
A 23 L 1/231

(30) Priority: **01.09.83 US 528561**

(43) Date of publication of application:
10.04.85 Bulletin 85/15

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(71) Applicant: **SOCIETE DES PRODUITS NESTLE S.A.**
Case postale 353
CH-1800 Vevey(CH)

(72) Inventor: **Lee, Eldon Chen-Hsiung**
10 High Trail Road
New Milford Connecticut 06776(US)

(72) Inventor: **Van Pottelsberghe de la Potterie Pierre Joseph**
365 Compton Road
Cincinnati Ohio 45215(US)

(72) Inventor: **Tandy, John Stewart**
Tamshell Drive
Kent Connecticut 06757(US)

(54) **Flavoring agents.**

(57) A process for the production of meat or fish flavoring agents comprises reacting a sulfur-containing compound with a lipid which is oxidized prior to or during the reaction, desirably to a peroxide value equal or close to zero. The reaction mixture may include an amino acid source.

EP 0 136 428 A2

Croydon Printing Company Ltd

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention pertains to the field of flavorants and particularly pertains to flavorants which impart cooked meat flavor to foodstuffs. More specifically, the present invention is concerned with the preparation of meat flavorants having increased flavor and aromatic intensity and which more closely resemble the natural flavor and aroma of cooked meat.

2. Description of Related Art

The desirability of providing flavoring agents which closely simulate the flavor and aromatic characteristics of cooked meat has long been recognized. Such flavoring agents may, for example, be employed with non-meat sources of protein so as to make them more palatable and as meat-like as possible. So, too, they may also be employed with meat-containing or meat-based foods or vegetable-type foodstuffs such as condensed soups, dried meats, packaged gravies, casseroles, etc., in order to supplement or enhance these foods whose organoleptic properties may have been affected by their processing.

Various expedients have been suggested in the prior art in attempts to provide flavoring agents having the organoleptic profile of cooked meat. Thus, for example, U.S. Patent No. 2,934,437 describes the preparation of a meat-like flavor by the

reaction of a mixture of monosaccharide and a source of amino acid. U.S. Patent No. 3,394,015 describes the preparation of a meat-like flavorant from the reaction of a proteinaceous substance with a sulfur-containing compound in the absence of a monosaccharide. U.S. Patent No. 3,532,514 describes the preparation of a meat-like flavorant from a mixture of an amino acid source, a mono-, di-, tri-, or polysaccharide and an animal or vegetable fat. U.S. Patent No. 3,394,017 describes the preparation of a meat-like flavorant by reacting thiamine with a sulfur-containing polypeptide or an amino acid mixture derived therefrom and thereafter adding aldehydes and ketones to the product.

These and similar techniques may suffer from a number of disadvantages. Firstly, these meat flavorants may possess only a small fraction of the total flavor and aromatic notes possessed by natural cooked meat. This is attributable, in part, to the particular reactive ingredients employed to produce the flavorants which can provide only a limited number of the required chemical precursors -- such as various aldehydes and ketones -- which are necessary to obtain the full organoleptic profile of cooked meat.

Moreover, the addition of separately prepared aldehydes and/or ketones to the flavorants in an effort to enhance the organoleptic profile of the flavorant produced is generally uneconomical.

Still further, the intensity or strength of these flavorants may be relatively weak so that a considerable amount of such flavorants would be required to obtain a perceptible flavoring effect upon a foodstuff. This, of course, may be economically undesirable. It would be far more advantageous to employ as little flavorant as possible and still obtain the desired flavoring effect.

SUMMARY OF THE INVENTION

Applicants have discovered a process for preparing flavorants for imparting a cooked meat flavor to foodstuffs which avoids substantially all of the disadvantages that may be associated with the prior art flavorants discussed above.

The process of the present invention economically and desirably utilizes a lipid material, and more specifically an oxidized lipid material, to provide a wide range of chemical precursors which facilitate the preparation of flavorants having the characteristic species which more closely resemble that of natural cooked meat. Still further, the flavorants produced by this process are enhanced and intensified so that a relatively small amount of these flavorants is needed to obtain a desired flavoring effect upon a foodstuff.

More particularly, applicants have found that superior meat flavorants may be obtained by reacting at elevated temperatures a sulfur-containing compound and an oxidized lipid material, such as oxidized triglycerides and/or oxidized unsaturated fatty acid components thereof. The lipid material may be separately oxidized prior to being combined with the sulfur-containing compound or, alternatively, may be oxidized during the reaction step itself after having been combined with the sulfur-containing compound.

The oxidation of the lipid material leads to the formation of a wide range of various hydroperoxides which in turn form a broad range of fatty unsaturated aldehydes and ketones. These carbonyl compounds then react with the sulfur-containing compound(s) to form heterocyclic ring structures which greatly enhance and intensify the characteristic meat flavor and aroma in the flavorants. Moreover, by virtue of employing an oxidized lipid material in the process of the present invention, the unsaturated aldehydes and ketones which are formed are endogenous to the starting lipid material and therefore need not be separately added from some other source.

In a preferred embodiment of the present invention, a source of amino acid is additionally reacted with the sulfur-containing compound(s) and the oxidized lipid material to even further enhance the flavorants produced. In this embodiment, the fatty aldehydes and ketones derived from the oxidized lipid

material undergo non-enzymatic browning reactions with the amino acid source to provide an even broader organoleptic profile to the flavorant. As a source of amino acid, enzymatically treated meat hydrolysates are especially preferred. It is noted that in the context of the present invention, the term "meat" also includes fish.

The ability to produce an acceptable flavorant at all, much less, a flavorant which has an even broader organoleptic profile and increased intensity when employing an oxidized lipid material is quite unexpected and contrary to what would be predicted by those skilled in the art. Thus, it has long been recognized that lipid oxidation is a major cause of deterioration in meat, particularly of its organoleptic qualities, resulting in off-flavors and rancidity. Generally, steps are taken to inhibit such undesirable lipid oxidation through the use of metal chelators (e.g., polyphosphates, citric acid, ascorbic acid and EDTA), phenolic antioxidants (propyl gallate, BHA and BHT), tocopherols, vacuum packaging, etc. Still, some oxidation nevertheless takes place during the cooking of the meat.

Yet, in the process of the present invention, rather than avoiding lipid oxidation, steps are actually taken to ensure that oxidized lipids are indeed present in order to form the novel flavorants discovered by applicants. These oxidized lipid materials, typically thought of as being the cause of rancidity, are actually utilized in the process of the present invention to