

(51) International Patent Classification:
A23J 1/14 (2006.01)(21) International Application Number:
PCT/US2017/018523(22) International Filing Date:
17 February 2017 (17.02.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/297,788 19 February 2016 (19.02.2016) US
62/440,723 30 December 2016 (30.12.2016) US(71) Applicant: HAMPTON CREEK, INC. [US/US]; 2000
Folsom Street, San Francisco, California 94110 (US).

(72) Inventors: BANSAL-MUTALIK, Ritu; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). BHIDE, Siddharth; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). GIBSON, Brenna; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). HALL, Camilla; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). JAKUBASCH, Malgorzata; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). KLEINER, Jake; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). LANQUAR, Viviane; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). MAHADEVAN, Swetha; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). NIEKOWAL, Trevor; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). PROULX, Jade; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). ROCHE, Ben; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US). XU, Meng; c/o Hampton Creek, Inc., 2000 Folsom Street, San Francisco, Colorado 94110 (US). FLATT, James; c/o

Hampton Creek, Inc., 2000 Folsom Street, San Francisco, California 94110 (US).

(74) Agents: LAI, Karen et al.; Squire Patton Boggs (US) LLP, 620 Hansen Way, Palo Alto, California 94304 (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, DJ, 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, KH, KN, KP, KR, KW, 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).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: FUNCTIONAL ADZUKI BEAN-DERIVED COMPOSITIONS

(57) Abstract: Provided herein are methods for producing an adzuki bean protein isolate having high functionality for a broad range of food applications. In some embodiments, the methods for producing the isolate comprise one or more steps selected from: (a) extracting one or more adzuki bean proteins from an adzuki bean protein source in an aqueous solution, for example, at a pH between about 6.5-10.0; (b) purifying protein from the extract using at least one of two methods: (i) precipitating protein from the extract at a pH near the isoelectric point of a globulin-rich fraction, for example a pH between about 5.0-6.0; and/or (ii) fractionating and concentrating protein from the extract using filtration such as microfiltration, ultrafiltration or ion-exchange chromatography; and (c) recovering purified protein isolate.



FUNCTIONAL ADZUKI BEAN-DERIVED COMPOSITIONS

1. FIELD OF THE INVENTION

[0001] The present disclosure relates to adzuki bean protein isolate compositions, methods for producing such compositions, and food products derivable from such compositions.

2. BACKGROUND

[0002] Conventional methods and processes used for extracting legume protein isolates and concentrates include alkaline extraction and acid precipitation or ultrafiltration (wet process) and air classification (dry process). The quality of the legume protein compositions produced by these methods is directly dependent on the operating conditions used to prepare them.

Application of an acidic, alkaline or neutral extraction process directly influences functional properties, e.g., the foaming or emulsifying properties of the protein compositions obtained, which makes the resulting protein compositions unsuitable for certain applications. It may therefore be necessary to modify the protein compositions so as to confer desired properties in the context of food applications.

[0003] Use of plant-based proteins such as soy and pea as animal protein substitutes have garnered increasing attention largely as consumers seek alternatives to conventional animal-based products, however, replicating functional properties while removing off-flavors are still challenges that need to be addressed.

[0004] What is needed, therefore, is a method and composition for producing purified plant protein isolates that exhibit one or more desired functional properties including replicating one or more desired organoleptic properties suitable for various applications. Disclosed herein are methods addressing the limitations of the current art.

3. SUMMARY OF THE INVENTION

[0005] Described herein are methods and compositions for producing a purified adzuki bean protein isolate. In some embodiments, the adzuki bean protein isolate comprises adzuki bean protein content of at least 60% by weight. In some embodiments, the adzuki bean protein isolate comprises a reduced oxidative enzyme activity relative to an otherwise unmodified source of the adzuki bean protein. In some embodiments, adzuki bean protein isolate comprises one or more modulated organoleptic properties that differ from the otherwise unmodified source of the adzuki bean protein.

[0006] Also provided herein are methods for producing an adzuki bean protein isolate having high functionality for a broad range of food applications. In some embodiments, the methods for producing the isolate comprise one or more steps selected from:

[0007] (a) Extracting one or more adzuki bean proteins from an adzuki bean protein source in an aqueous solution. In some embodiments, the extraction is performed at a pH between about 6.5-10.0.

[0008] (b) Purifying protein from the extract using at least one of two methods:

[0009] (i) precipitating protein from the extract at a pH near the isoelectric point of a globulin-rich fraction, for example a pH between about 5.0-6.0; and/or

[0010] (ii) fractionating and concentrating protein from the extract using filtration such as microfiltration, ultrafiltration or ion-exchange chromatography.

[0011] (c) Recovering purified protein isolate.

[0012] In particular embodiments, the extraction is performed at a pH of about 7.0. In other particular embodiments, the isoelectric precipitation of adzuki bean protein is performed at pH 5.6 +/- 0.2.

[0013] Also disclosed is a process to produce an edible adzuki bean protein isolate from a source of an adzuki bean protein, the process comprising: subjecting the source of the adzuki bean protein to a fractionation process to obtain a protein-rich fraction, wherein at least 50% by weight of the protein-rich fraction comprises or consists of one or more globulin-type proteins; reducing at least one impurity, the at least one impurity associated with an off-odor or an off-flavor in the edible adzuki bean protein isolate; and purifying the protein-rich fraction to obtain the edible adzuki bean protein isolate, wherein: at least 60% by weight of the edible protein isolate is adzuki bean protein, an oxidative enzymatic activity of the edible protein isolate is lower than a corresponding oxidative enzymatic activity of the source of the adzuki bean protein, and an organoleptic property of the edible protein isolate differs from a corresponding organoleptic property of the source of the adzuki bean protein.

[0014] In accordance with preferred aspects of the present invention, methods and compositions for egg replacement are provided, said composition comprising a plant-based protein isolate modified by transglutaminase; wherein said composition is essentially egg-free and, wherein said composition comprises one or more functional properties of a natural egg. Preferably, composition comprises emulsifying properties of a natural egg. More preferably, the composition provides plant-based protein isolate modified by 0.0001% to 0.0125% transglutaminase and exhibits significantly reduced activity lipoygenase or other enzymes that can oxidize lipids.

[0015] In certain aspects, the methods and compositions described herein provide purified protein isolates having modulated organoleptic properties of one or more of the following characteristics: astringent, beany, bitter, burnt, buttery, nutty, sweet, sour, fruity, floral, woody, earthy, beany, spicy, metallic, sweet, musty, grassy, green, oily, vinegary, neutral

and bland flavor or aromas. Preferably, the purified protein isolates exhibit modulated organoleptic properties such as a reduction or absence in one or more of the following: astringent, beany, bitter, burnt, buttery, nutty, sweet, sour, fruity, floral, woody, earthy, beany, spicy, metallic, sweet, musty, grassy, green, oily, vinegary neutral and bland flavor or aromas.

[0016] The purified protein isolates are suitable for various food applications and have been incorporated into, e.g., edible egg-free emulsion, egg analog, egg-free scrambled eggs, egg-free patty, egg-free pound cake, egg-free angel food cake, egg-free yellow cake, egg- and dairy-free cream cheese, egg-free pasta dough, egg-free custard, egg-free ice cream, and dairy-free milk. The purified protein isolates are also suitable for use as plant-based analogs for cream cheese, pasta dough, pasta, milk or milk-like beverage, a food product comprising said milk or milk-like beverage, custard, ice cream, frozen desert, meat replicas (e.g., deli meat replicas; emulsified extruded meats (e.g., sausages, fish cake replicas); dips, fillings and spreads, chips, and crackers. Other applications are also suitable for the functional adzuki bean protein isolates described herein, and the foregoing list is non-limiting.

4. BRIEF DESCRIPTION OF THE FIGURES

[0017] **FIG. 1A** depicts a general process diagram for adzuki bean protein isolation in accordance with the methods described herein.

[0018] **FIG. 1B** depicts a general process diagram for protein isolation using transglutaminase in the aqueous extraction step.

[0019] **FIG. 1C** depicts a general process diagram for protein isolation using transglutaminase in the purification step.

[0020] **FIG. 1D** depicts a general process diagram for dry-fractionation protein isolation using transglutaminase in the purification step.

[0021] **FIG. 2** provides one embodiment of a process for preparation of protein isolate in accordance with the methods provided herein.

[0022] **FIG. 3** depicts a general process block flow diagram for pilot scale protein isolation.

[0023] **FIG. 4** graphically depicts denaturation temperature profiles of different purified protein isolates.

[0024] **FIG. 5** depicts a protein profile chromatogram resulting from a capillary gel electrophoresis analysis of an adzuki protein isolate provided herein.

[0025] **FIG. 6** depicts a thermogram indicating a thermal denaturation temperature of 81.2° C for an adzuki bean protein isolate provided herein.

[0026] **FIG. 7** depicts solubility of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) and not washing the pellet of precipitation buffer (FXL0001), as well as several reference materials.

[0027] **FIG. 8** depicts solubility of adzuki bean isolates that were prepared without washing the isolate pellet of precipitation buffer (FXL0001), under various solvent conditions.

[0028] **FIG. 9** depicts solubility of adzuki bean isolates that were prepared by washing the isolate pellet of precipitation buffer (DMT0001), under various solvent conditions.

[0029] **FIG. 10** depicts foam stability of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) and not washing the pellet of precipitation buffer (FXL0001) in various buffer and protein conditions, as well as several reference materials.

[0030] **FIG. 11** depicts emulsion stability of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) and not washing the pellet of precipitation buffer (FXL0001) in various buffer and protein conditions, as well as several reference materials. Lower stability index values indicate emulsions that are more stable over time.

[0031] **FIG. 12** depicts gelation onset temperature of gels formed by heating of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) in various buffer and protein conditions, as well as several reference materials.

[0032] **FIG. 13** depicts gel elasticity versus gel strength of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) in various buffer and protein conditions, as well as several reference materials.

[0033] **FIG. 14** depicts viscosity at zero shear rate of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) in various buffer and protein conditions, as well as several reference materials.

[0034] **FIG. 15** depicts yield stress of adzuki bean isolates that were prepared by washing the isolate pellet (DMT0001) in various buffer and protein conditions, as well as several reference materials.

5. DETAILED DESCRIPTION OF THE EMBODIMENTS

5.1 Terminology

[0035] As used herein, the singular forms “a,” “an,” and “the” include the plural referents unless the context clearly indicates otherwise.

[0036] The term “about” indicates and encompasses an indicated value and a range above and below that value. In certain embodiments, the term “about” indicates the designated value $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$. In certain embodiments, the term “about” indicates the designated value $\pm$ one standard deviation of that value.

[0037] The term “reduce” indicates a lessening or decrease of an indicated value relative to a reference value. In some embodiments, the term “reduce” (including “reduction”) refers to a lessening or a decrease of an indicated value by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% relative to a reference value. In some embodiments, the term “reduce” (including “reduction”) refers to a lessening or a decrease of an indicated value by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% relative to a reference value.

[0038] As used herein, the term “eggs” includes but is not limited to chicken eggs, other bird eggs (such as quail eggs, duck eggs, ostrich eggs, turkey eggs, bantam eggs, goose eggs), and fish eggs such as fish roe. Typical food application comparison is made with respect to chicken eggs.

[0039] As used herein, the term “enriched” refers to an increase in a percent amount of a molecule, for example, a protein, in one sample relative to the percent amount of the molecule in a reference sample. For example, an isolate enriched in a certain type of globulin protein relative to whole adzuki bean means that, the amount of the globulin protein in the isolate expressed as a percentage of the amount of total protein in the isolate, is higher than the amount of the globulin protein in a whole adzuki bean expressed as a percentage of the amount of total protein in the whole adzuki bean. In some embodiments, the enrichment is on a weight to weight basis. In some embodiments, the enrichment refers to an increase of about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% relative to the reference value or amount. In some embodiments, the enrichment refers to an increase of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% relative to the reference value or amount.

[0040] As used herein, “plant source of the isolate” refers to a whole plant material such as whole adzuki bean, or from an intermediate material made from the plant, for example, a dehulled bean, a flour, a powder, a meal, ground grains, a cake (such as, for example, a defatted or de-oiled cake), or any other intermediate material suitable to the processing techniques disclosed herein to produce a purified protein isolate.

[0041] The term “transglutaminase” refers to an enzyme (R-glutamyl-peptide:amine glutamyl transferase) that catalyzes the acyl-transfer between γ -carboxyamides and various primary amines, classified as EC 2.3.2.13. It is used in the food industry to improve texture of some food products such as dairy, meat and cereal products. It can be isolated from a bacterial source, a fungus, a mold, a fish, a mammal and a plant.

[0042] The terms “majority” or “predominantly” with respect to a specified component, e.g., protein content refer to the component having at least 50% by weight of the referenced batch, process stream, food formulation or composition.

[0043] Unless indicated otherwise, percentage (%) of ingredients refer to total % by weight typically on a dry weight basis unless otherwise indicated.

[0044] The term “purified protein isolate”, “protein isolate”, “isolate”, “precipitate”, “protein extract”, “isolated protein” or “isolated polypeptide” refers to a protein fraction, a protein or polypeptide that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) exists in a purity not found in nature, where purity can be adjudged with respect to the presence of other cellular material (e.g., is free of other proteins from the same species) (3) is expressed by a cell from a different species, or (4) does not occur in nature (e.g., it is a fragment of a polypeptide found in nature or it includes amino acid analogs or derivatives not found in nature or linkages other than standard peptide bonds). One or more proteins or fractions may be partially removed or separated from residual source materials and/or non-solid protein materials and, therefore, are non-naturally occurring and are not normally found in nature. A polypeptide or protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques known in the art and as described herein. A polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be “isolated” from its naturally associated components. As thus defined, “isolated” does not necessarily require that the protein, polypeptide, peptide or oligopeptide so described has been physically removed from its native environment.

[0045] Sequence homology for polypeptides, which is also referred to as percent sequence identity, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wis. 53705. Protein analysis software matches similar sequences using a measure of homology assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as “Gap” and “Bestfit” which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild-type protein and a mutein thereof. See, e.g., GCG Version 6.1.

[0046] A preferred algorithm when comparing a particular polypeptide sequence to a database containing a large number of sequences from different organisms is the computer program BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990); Gish and States, Nature Genet. 3:266-272 (1993); Madden et al., Meth. Enzymol. 266:131-141 (1996); Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997); Zhang and Madden, Genome Res. 7:649-656 (1997)), especially blastp or tblastn (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)).

[0047] Preferred parameters for BLASTp are: Expectation value: 10 (default); Filter: seg (default); Cost to open a gap: 11 (default); Cost to extend a gap: 1 (default); Max. alignments: 100 (default); Word size: 11 (default); No. of descriptions: 100 (default); Penalty Matrix: BLOWSUM62.

[0048] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences. Database searching using amino acid sequences can be measured by algorithms other than blastp known in the art. For instance, polypeptide sequences can be compared using FASTA, a program in GCG Version 6.1. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. Pearson, Methods Enzymol. 183:63-98 (1990) (incorporated by reference herein). For example, percent sequence identity between amino acid sequences can be determined using FASTA with its default parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

5.2 Adzuki Bean Protein Isolate Compositions

[0049] In preferred embodiments, the protein isolate provided herein is derived from adzuki bean. In some embodiments, the adzuki bean is *Vigna angularis*. In various aspects of the invention, the purified adzuki bean protein isolate described herein can be produced from any source of adzuki bean protein, including any varietal or cultivar of *V. angularis*. For example, the protein isolate can be prepared directly from whole plant material such as whole adzuki bean, or from an intermediate material made from the plant, for example, a dehulled bean, a flour, a powder, a meal, ground grains, a cake (such as, for example, a defatted or de-oiled cake), or any other intermediate material suitable to the processing techniques disclosed herein to produce a purified protein isolate. In some embodiments, the source of the plant protein may be a mixture of two or more intermediate materials. The examples of candidate intermediate materials provided herein are not intended to be limiting.

[0050] In preferred embodiments, provided herein are adzuki bean protein isolate compositions predominantly comprising a protein-based fraction. In preferred embodiments, the protein fraction is 50%-60%, 60%-70%, 70%-80%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even 100% of the adzuki bean isolate. In preferred embodiments, at least 60% by weight of the purified isolate is the protein fraction. In preferred embodiments, at least 65% by weight of the purified isolate is the protein fraction. In preferred embodiments, at least 70% by weight of the purified isolate is

the protein fraction. In some embodiments, at least 75% by weight of the purified isolate is the protein fraction. In some embodiments, at least 80% by weight of the purified isolate is the protein fraction. In some embodiments, up to about 95% by weight of the purified isolate is the protein fraction.

[0051] Preferred embodiments include high purity protein isolates from adzuki bean that comprise at least 10% by weight of protein consisting of or comprising at least one globulin-type protein. While not wishing to be bound by a particular theory, it is believed that the globulin fraction provides the basis for functionality. Accordingly, the purified protein isolates are enriched in globulin protein relative to whole adzuki bean. In some embodiments, the globulin-like protein is adzuki bean 8s globulin/beta-conglycinin. In some embodiments, the globulin-like protein is adzuki bean 11s globulin/glycinin. In some embodiments, at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% or greater than 20% by weight of the protein fraction of the isolate consists of or comprises adzuki bean 8s globulin/beta-conglycinin. In some embodiments, at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% or greater than 20% by weight of the protein fraction of the isolate consists of or comprises adzuki bean 11s globulin/glycinin.

[0052] In some embodiments, purified protein isolate is concentrated between 100-200 g/L or higher.

[0053] In some embodiments, proteins in the composition comprise nondenatured proteins. In other embodiments, proteins in the composition comprise denatured proteins.

[0054] In some embodiments, the adzuki bean protein isolate comprises about 1% to 10%, 2% to 9%, 3% to 8%, or 4% to 6% of carbohydrates (e.g., starch, polysaccharides, fiber) derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of carbohydrates derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or about 1% of carbohydrates derived from the plant source of the isolate. In some embodiments, practice of the methods of provided herein results in producing an adzuki bean protein isolate in which at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the carbohydrate originally found in the adzuki bean protein source has been reduced.

[0055] In some embodiments, the adzuki bean protein isolate comprises about 1% to 10%, 2% to 9%, 3% to 8%, or 4% to 6% of ash derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of ash derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises about 9%, 8%, 7%, 6%, 5%, 4%, 3%,

2%, or about 1% of ash derived from the plant source of the isolate. In some embodiments, practice of the methods of provided herein results in producing an adzuki bean protein isolate in which at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the ash originally found in the adzuki bean protein source has been reduced.

[0056] In some embodiments, the adzuki bean protein isolate comprises about 1% to 10%, 2% to 9%, 3% to 8%, or 4% to 6% of fats derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of fats derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or about 1% of fats derived from the plant source of the isolate. In some embodiments, practice of the methods of provided herein results in producing an adzuki bean protein isolate in which at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the fats originally found in the adzuki bean protein source has been reduced.

[0057] In some embodiments, the adzuki bean protein isolate comprises about 1% to 10% of moisture derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises less than about 10%, 9%, 8%, 7%, 6% or 5% of moisture derived from the plant source of the isolate. In some embodiments, the adzuki bean protein isolate comprises about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or about 1% of moisture derived from the plant source of the isolate.

[0058] In a particular embodiment, the adzuki bean protein isolate comprises less than 5% of carbohydrates, less than 5% of ash, less than 5% of fats, and less than 80% of moisture derived from the plant source of the isolate.

5.2.1 Adzuki Bean Proteins of the Isolate Compositions

[0059] Leguminous plants contain many types of proteins, two of which are globulins and albumins. Globulins and albumins are soluble proteins and make up the majority of the total proteins in adzuki bean. Globulins can be further classified as legumins, vicilins and convicilins.

[0060] The globulin-type protein making up the majority by weight of the protein fraction of the isolates provided herein may all be of the same type of globulin-type protein, or it may comprise more than one types of globulin-type protein. For example, the globulin-type protein may include 7S globulin, 8S globulin, and/or 11S globulin. The globulin-type protein may also or alternatively include protein(s) homologous to 7S, 8S, and/or 11S globulin.

[0061] In some embodiments, the adzuki bean protein isolate comprises a protein having sequence at least 75% identical to one or more globulin-type proteins from adzuki bean (e.g. 7S, 8S, 11S), wherein the protein is enriched in the isolate in relation to the amount of protein found

in the plant source of the isolate. In some embodiments, the enriched protein has at least 50%, 60, 70%, 80%, 85%, 90%, 95%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or even higher identity to one or more globulin-type proteins from adzuki bean (e.g. 7S, 8S, 11S).

[0062] In some embodiments, the adzuki bean protein isolate provided herein comprises at least one protein comprising an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, or 95% identity, or higher, to a sequence selected from the group of sequences consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6. In some embodiments, the adzuki bean protein isolate comprises at least two, three, four, five, or six, or more enriched proteins comprising an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, or 95% identity, or higher, to a sequence selected from the group of sequences consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

[0063] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:1. In some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:1, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the amount the protein having at least 90% identity to SEQ ID NO:1 is at least 5, 10, 15, or 20% of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:1, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 1.

[0064] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:2. In some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:2, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the amount the protein having at least 90% identity to SEQ ID NO:2 is at least 5, 10, 15, or 20% of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:2, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 2.

[0065] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:3. In some embodiments, the adzuki bean protein

isolate comprises a protein having at least 90% identity to SEQ ID NO:3, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:3, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 3.

[0066] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:4. In some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:4, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:4, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 4.

[0067] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:5. In some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:5, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:5, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 5.

[0068]

[0069] In, some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:6. In some embodiments, the adzuki bean protein isolate comprises a protein having at least 90% identity to SEQ ID NO:6, in an amount that is at least 1% of the total protein of the isolate. In some embodiments, the adzuki bean protein isolate is enriched for a protein having at least 90% identity to SEQ ID NO:6, in relation to the amount of the protein found in the plant source of the isolate. In some embodiments, the enriched protein is enriched by at least 5%, 10%, 15%, 20% or greater than 20%. In any of the preceding embodiments, the adzuki bean protein isolate can comprise a protein having at least 95% identity to SEQ ID NO: 6.

[0070] According to other embodiments, purified protein isolates comprising a fragment of the above-described proteins are provided. These fragments preferably include at least 20 contiguous amino acids, more preferably at least 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or even more contiguous amino acids.

5.3 Methods of Producing Adzuki Bean Protein Isolates

[0071] Also provided herein are methods for producing an adzuki bean protein isolate having high functionality for a broad range of food applications. In some embodiments, the methods for producing the isolate comprise one or more steps selected from:

[0072] (a) Extracting one or more adzuki bean proteins from an adzuki bean protein source in an aqueous solution. In some embodiments, the extraction is performed at a pH between about 6.5-10.0.

[0073] (b) Purifying protein from the extract using at least one of two methods:

[0074] (i) precipitating protein from the extract at a pH near the isoelectric point of a globulin-rich fraction, for example a pH between about 5.0-6.0; and/or

[0075] (ii) fractionating and concentrating protein from the extract using filtration such as microfiltration, ultrafiltration or ion-exchange chromatography.

[0076] (c) Recovering purified protein isolate.

[0077] In preferred embodiments, the methods provided herein produce adzuki bean protein isolates comprising one or more of the following features: a protein content of at least 60% by weight; a globulin-type protein content of at least 50% by weight of the protein content; a reduced oxidative enzyme activity relative to an otherwise unmodified source of the adzuki bean protein; and one or more modulated organoleptic properties that differ from the otherwise unmodified source of the adzuki bean protein.

[0078] In preferred embodiments, the adzuki bean protein isolate is produced using a series of mechanical processes, with the only chemicals used being pH adjusting agents, such as sodium hydroxide and citric acid, and ethylenediaminetetraacetic acid (EDTA) to prevent lipid oxidation activities that may affect the flavor of the isolate.

5.3.1 Dehulling and Milling

[0079] Although the adzuki bean protein isolates provided herein may be prepared from any suitable source of adzuki bean protein, where the starting material is whole plant material such as whole adzuki bean, a first step of the methods provided herein comprises dehulling the raw source material. In some such embodiments, raw adzuki beans may be de-hulled in one or more steps of pitting, soaking, and drying to remove the seed coat (husk) and pericarp (bran). The de-hulled adzuki beans are then milled to produce flour with a well-defined particle distribution size. In some embodiments, the particle distribution size is less than 1000, 900, 800,

700, 600, 500, 400, 300, 200 or 100 μm . In a particular embodiment, the particle distribution size is less than 300 μm to increase the rate and yield of protein during the extraction step. The types of mills employed may include one or a combination of a hammer, pin, knife, burr, and air classifying mills.

[0080] When feasible, air classification of the resultant flour may be deemed necessary to expedite the protein extraction process and enhance efficiency of the totality of the process. The method employed is to ensure the adzuki beans are milled to a particle size that is typically less than 45 μm , utilizing a fine-grinding mill, such as an air classifying mill. The resultant flour is then passed through an air classifier, which separates the flour into both a coarse and fine fraction. The act of passing the flour through the air classifier is intended to concentrate the majority of the available protein in the flour into a smaller portion of the total mass of the flour. Typical fine fraction (high-protein) yields may be 10-50%. The fine fraction tends to be of a particle size of less than 20 μm ; however, this may be influenced by growing season and region of the original adzuki bean. The high-protein fraction typically contains 150-220% of the protein in the original sample. The resultant starch-rich byproduct stream also becomes value added, and of viable, saleable interest as well.

5.3.2 Extraction

[0081] In preferred embodiments, the methods comprise an extraction step. In some embodiments of the extraction step, an intermediate starting material, for example, adzuki bean flour, is mixed with aqueous solution to form a slurry. In some embodiments, the aqueous solution is water, for example soft water. The aqueous extraction may include creating an aqueous solution comprising one part of the source of the plant protein (*e.g.*, flour) to about, for example, 3 to 15 parts aqueous extraction solution. In other embodiments, 5 to 10 volumes of aqueous extraction solution is used per one part of the source of the plant protein. Additional useful ratios of aqueous extraction solution to flour include 1:1, 2:1, 4:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1 or alternatively 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15.

[0082] Preferably, the aqueous extraction is performed at a desired temperature, for example, about 2-10 $^{\circ}\text{C}$ in a chilled mix tank to form the slurry. In some embodiments, the mixing is performed under moderate to high shear. In some embodiments, a food-grade de-foaming agent (*e.g.*, KFO 402 Polyglycol) is added to the slurry to reduce foaming during the mixing process.

[0083] The pH of the slurry may be adjusted with a food-grade 50% sodium hydroxide solution to reach the desired extraction pH for solubilization of the target protein into the aqueous solution. In some embodiments, the extraction is performed at a pH between about 6.5-

10.0. In some embodiments, the extraction is performed at a pH of about pH 5.5-pH 9, pH 6.0-pH 8.5 or more preferably pH 6.5-pH 8. In a particular embodiment, the extraction is performed at a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or 10.0. In a particular embodiment, the extraction is performed at a pH of about 7.0.

[0084] Following extraction, the solubilized protein extract is separated from the slurry, for example, in a solid/liquid separation unit, consisting of a decanter and a disc-stack centrifuge. The extract is centrifuged at a low temperature, preferably between 3-10°C. The extract is collected and the pellet is resuspended, preferably in 3:1 water-to-flour. The pH is adjusted again and centrifuged. Both extracts are combined and filtered through using a Nylon mesh.

5.3.3 Charcoal Treatment

[0085] Optionally, the protein extract may be subjected to a carbon adsorption step to remove non-protein, off-flavor components, and additional fibrous solids from the protein extraction. This carbon adsorption step leads to a clarified protein extract. In one embodiment of a carbon adsorption step, the protein extract is then sent through a food-grade granular charcoal-filled annular basket column (<5% w/w charcoal-to-protein extract ratio) at 4°C to 8°C. An illustrative carbon adsorption protocol is also provided in Example 1 below.

5.3.4 Acid Precipitation

[0086] In some embodiments, following extraction and optionally carbon adsorption, the clarified protein extract is acidified with a food-safe acidic solution to reach its isoelectric point under chilled conditions (*e.g.*, 2°C to 8°C). Under this condition, the target protein precipitates and becomes separable from the aqueous solution. In some embodiments, the pH of the aqueous solution is adjusted to approximately the isoelectric point of at least one of the one or more globulin-type proteins in the protein-rich fraction. In some embodiments, the pH is adjusted from an aqueous solution comprising the protein extract which has an initial pH of about 6.5-10.0 prior to the adjusting step. In some embodiments, the pH is adjusted to about 5.0 to 6.5. In some embodiments, the pH is adjusted to about 5.2-6.5, 5.3 to 6.5, 5.4 to 6.5, 5.5 to 6.5, or 5.6 to 6.5. In some embodiments, the pH is adjusted to about 5.2-6.0, 5.3 to 6.0, 5.4 to 6.0, 5.5 to 6.0, or 5.6 to 6.0. In certain embodiments, the pH is adjusted to about pH 5.4-5.8. In some embodiments, the pH is adjusted to about 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4 or 6.5. In a preferred embodiment of the methods provided herein, the pH is adjusted, and precipitation of desired adzuki bean proteins is achieved, at about pH 5.6.

[0087] Suitable food-grade acids to induce protein precipitation include but are not limited to malic, lactic, hydrochloric acid, and citric acid. In a particular embodiment, the

precipitation is performed with a 20% food-grade citric acid solution. In other embodiments, the precipitation is performed with a 40% food-grade citric acid solution.

[0088] In some embodiments, in addition to the pH adjustment, EDTA, for example, 2 mM of food-grade EDTA, may be added to the precipitation solution to inhibit lipid oxidation that may produce off-flavor compounds.

[0089] In alternative embodiments, the precipitation step comprises isoelectric precipitation at pH 5.6 combined with cryo-precipitation (at 1-4°C), wherein the pH is adjusted to 5.4-5.8.

[0090] In another alternative embodiment, low ionic strength precipitation at high flow rates is combined with cryo-precipitation (at 1-4°C). In some such embodiments, rapid dilution of the filtrate is performed in cold (1-4°C) 0.3% NaCl at a ratio of 1 volume of supernatant to 3 volumes of cold 0.3% NaCl. Additional resuspension and homogenization steps ensure production of desired protein isolates.

[0091] The precipitated protein slurry is then removed from the pH-adjusted aqueous solution and sent to a solid/liquid separation unit (for example, a one disc-stack centrifuge) with the addition of 0.3% (w/w) food-grade sodium chloride, and a protein curd is recovered in the heavy phase. In preferred embodiments the protein curd is washed with 4 volumes of soft water under chilled conditions (2 to 8°C), removing final residual impurities such as fibrous solids, salts, and carbohydrates.

5.3.5 Filtration

[0092] In some embodiments of the methods, filtration is used as an alternative, or an addition to, acid precipitation. Without being bound by theory, it is believed that while acid precipitation of the protein aids to remove small molecules, alternative methods such as ultra-filtration (UF) may be employed to avoid precipitation/protein aggregation events. Thus, in some embodiments, purifying the protein-rich fraction to obtain the adzuki bean protein isolate comprises performing a filtration, microfiltration or ultrafiltration procedure utilizing at least one selective membrane.

5.3.6 Pasteurization

[0093] In some embodiments, the washed protein curd solution resulting from acid precipitation and separation is pasteurized in a high temperature/short time pasteurization step to kill any pathogenic bacteria that may be present in the solution. In a particular embodiment, pasteurization is performed at 74°C for 20 to 23 seconds. In particular embodiments where a dry isolate is desired, the pasteurized solution is passed through a spray dryer to remove any residual water content. The typical spray drying conditions include an inlet temperature of

170°C and an outlet temperature of 70°C. The final dried protein isolate powder typically has less than 5% moisture content.

5.3.7 Particular Embodiments of the Isolation Methods

[0094] An exemplary embodiment of the methods for producing an adzuki bean protein isolate provided herein is as follows:

[0095] 1) Extraction with soft water at pH > 6.5 in one or more stages. Extraction involves contacting adzuki bean flour with the aqueous solution in a ratio of 1:3 - 1: 15 (flour:water) under moderate-to-high shear followed by solid-liquid separation step;

[0096] 2) Optional treatment with activated carbon;

[0097] 3) Isoelectric precipitation (pH 5.6 +/- 0.2) combined with cryo-precipitation method (at 1-4 °C) OR low ionic strength precipitation at very high flow rates combined with cryo-precipitation method (at 1-4 °C);

[0098] 4) Followed by solid-liquid separation step.

[0099] Typically, the separation step includes washing with low concentration NaCl solution, 0.1% to 0.9% NaCl, preferably 0.3% to 0.5% NaCl.

[00100] Additional details of protein isolate purification protocols are described in Examples 2 and 3 below.

5.3.8 Order Of Steps And Additional Steps

[00101] It is to be understood that the steps of the method described above may be performed in alternative orders. For example, in some embodiments, subjecting the source of the plant protein to the fractionation process occurs before reducing the at least one impurity and before purifying the protein-rich fraction to obtain the purified protein isolate. In such embodiments, reducing the at least one impurity may occur either before or after purifying the protein-rich fraction to obtain the purified protein isolate. In some embodiments, reducing an impurity occurs before subjecting the source of the plant protein to the fractionation process and before purifying the protein-rich fraction to obtain the purified protein isolate, and subjecting the source of the plant protein to the fractionation process occurs before purifying the protein-rich fraction to obtain the purified protein isolate.

[00102] In some embodiments, the process includes additional steps, including one or more selected from: recovering the purified protein isolate (e.g., using centrifugation), washing the purified protein isolate, making a paste using the purified protein isolate, or making a powder using the purified protein isolate. In some embodiments, the purified protein isolate is rehydrated (e.g., to about 80% moisture content), and the pH of the rehydrated purified protein isolate is adjusted to a pH of about 6.0.

[00103] In other aspects, the compositions and methods provided herein reduce or remove a fraction comprising carbohydrate (e.g., starch) or a carbohydrate-rich protein isolate, post extraction, and provides opportunities to utilize these streams as product streams for multiple food applications including noodles and multiple bakery applications. Accordingly, also provided herein is an adzuki bean-derived carbohydrate (e.g., starch) fraction or a carbohydrate-rich protein isolate produced by the methods of extraction described herein.

5.4 Scrambled Egg Analog Using Transglutaminase

[00104] In another aspect, provided herein is a plant-based scrambled egg analog comprising an adzuki bean protein isolate produced by the methods described herein, wherein the adzuki bean protein has been contacted with transglutaminase to provide advantageous textural, functional and organoleptic properties.

[00105] Food processing methods employing transglutaminases have been described in, for example, Japanese Patent 59059151, which discloses treating an emulsion containing proteins, oils or fats, and water with transglutaminase to produce gelatinous, crosslinked gel. Numerous patents disclose use of transglutaminase with milk or cheese such as U.S. Patent No. 6,093,424 and other references disclose transglutaminase with pea protein isolates, for example, Chinese Patent 101703147A. Even in view of these efforts, the need still exists for methods and compositions to produce an edible emulsion such as an egg substitute or a scrambled egg analog.

[00106] Thus, in some embodiments of the methods and compositions provided herein, transglutaminase is added to plant-based egg mimetic emulsions comprising adzuki bean protein isolate to achieve firmer and smoother gel textures upon heating of the emulsions.

[00107] In some embodiments, the transglutaminase is microencapsulated when utilized in the preparation of plant-based egg analogs provided herein. Microencapsulation of transglutaminase enzyme in plant-based egg mimetic emulsions maintains a stable emulsion by preventing contact of the protein substrate with the transglutaminase enzyme. Cross-linking reaction is initiated upon heating to melt the microencapsulating composition. Egg mimetic emulsions which include transglutaminase are inherently unstable in that the cross-linking reaction commences upon addition of the transglutaminase enzyme to the emulsion.

[00108] In addition to the preparation of plant-based egg analogs from adzuki bean protein isolates, this approach can be applied to other pulse relatives of the legume family which show similar functionality.

[00109] One advantage of transglutaminase is enhancing shelf-stable refrigerated or room temperature egg-mimetic emulsions which can be used to produce high-quality prepared food products exhibiting many of the characteristics of cooked or scrambled eggs or baked products, e.g. cakes and cookies, which normally contain eggs. Additional advantage includes producing

protein-rich ingredients with variable molecular weight and size creating a range of textures in finished food products. Accordingly, in various aspects, transglutaminase aids in functionality and texture of the final products.

[00110] In certain aspects of the invention, the method for producing egg substitute composition comprises contacting a legume protein with an amount of transglutaminase, preferably between 0.0001% to 0.1% to produce desired plant protein isolates. In some preferred embodiments, the method provides an amount of transglutaminase between 0.001% and 0.05%. In more preferred embodiments, the method provides an amount of transglutaminase between 0.001% and 0.0125%. In other embodiments, protein isolates produced outside of the preferred ranges produced scramble analog that were thicker or not readily homogenized into a formulation. Increased amount of transglutaminase to the protein does not appear to precipitate at pH 5.6 when the protein extract is treated with transglutaminase. Accordingly, the additional step of contacting the protein with the preferred amount of transglutaminase produced a desirable scramble analog.

[00111] Accordingly, in various aspects, the invention provides a scramble analog comprising the following formulation: Protein Solids: 11.3g, Water: 81.79g, Disodium phosphate: 0.4g, Oil: 6.2g, NaCl: 0.31g (based on total weight of 100g) wherein the protein solids are contacted with between 0.001% and 0.0125% of transglutaminase.

[00112] In additional embodiments, the methods and compositions lack lipoygenase.

[00113] Accordingly, the present invention provides compositions for egg replacement, said composition comprising a plant-based protein isolate modified by transglutaminase; wherein said composition is essentially egg-free and, wherein said composition comprises one or more functional properties of a natural egg. Preferably, composition comprises emulsifying properties of a natural egg. More preferably, the composition provides plant-based protein isolate modified by 0.0001% to 0.0125% transglutaminase and exhibits reduced or even significantly reduced lipoygenase activity or other enzymes which can oxidize lipids, as expressed on a volumetric basis relative to the whole plant extract. More preferably, the composition is essentially free of lipoygenase or enzymes that can oxidize lipids. In further embodiments, the plant-based protein isolate is stably cross-linked.

[00114] In some aspects, the transglutaminase reduces or does not cross-link to lipoygenase or enzymes that can oxidize lipids. Additional embodiments include encapsulating transglutaminase in microcapsules. The composition comprising the plant-based protein isolate is suitable for refrigeration or storage at room temperature and is shelf stable in emulsion. In further aspects, the transglutaminase is free, cross-linked and/or immobilized.

[00115] Additional aspects of the invention include a purified protein isolate comprising: a transglutaminase modified plant protein content of at least 60% by weight; a globulin-type protein content of at least 50% by weight of the plant protein; a reduced oxidative enzyme activity relative to an otherwise unmodified source of the plant protein; and one or more modulated organoleptic properties that differ from the otherwise unmodified source of the plant protein. Preferred embodiments include purified protein isolate modified by 0.0001% to 0.0125% transglutaminase.

[00116] In accordance with preferred methods of the invention, provided are methods for producing a purified protein isolate comprising: a. extracting one or more plant protein from a source in an aqueous solution at a pH between about 6.5-10.0; b. precipitating the plant protein at pH near its isoelectric point of a globulin-rich fraction or a pH between about 5.0-6.0; or fractionating and concentrating the plant protein using filtration, microfiltration or ultrafiltration or ion-exchange chromatography; c. recovering the purified protein isolate comprising a plant protein content of at least 60% by weight; a globulin-type protein content of at least 50% by weight of the plant protein; a reduced oxidative enzyme activity relative to an otherwise unmodified source of the plant protein; and one or more modulated organoleptic properties that differ from the otherwise unmodified source of the plant protein; and d. modifying the plant protein with transglutaminase in the extraction step a. or the recovery step b.

[00117] Preferred embodiments of the method to produce a purified protein isolate include step d. of modifying the plant protein with transglutaminase in the extraction step a. or the recovery step b. comprises 0.0001% to 0.0125% transglutaminase. Further embodiments provide compositions comprising purified protein isolate that is stably cross-linked and is essentially free of lipoxygenase or enzymes that can oxidize lipids. Additional embodiments include encapsulating and/or immobilizing the transglutaminase.

[00118] In accordance with preferred compositions of the invention, provided is an egg substitute composition comprising: plant protein solids, water, disodium phosphate, oil and salts such as NaCl, wherein said plant protein solid comprises a plant-based protein isolate modified by transglutaminase. Preferably, the plant-based protein isolate of the egg substitute composition is modified with 0.0001% to 0.0125% transglutaminase. The transglutaminase reduces or does not cross-link to lipoxygenase or enzymes that can oxidize lipids. In such embodiments, the egg substitute composition exhibits reduced or even significantly reduced lipoxygenase activity or other enzymes which can oxidize lipids. The transglutaminase cross-links proteins other than lipoxygenase and leaves the lipoxygenase free allowing it to stay in the supernatant in the isoelectric precipitation and centrifugation steps, away from the cross-linked protein. More preferably, the egg substitute composition is essentially free of lipoxygenase or

enzymes that can oxidize lipids. Additional embodiments include contacting the plant-based protein isolate with transglutaminase that are encapsulated in microcapsules. The resulting egg substitute composition is suitable for refrigeration or storage at room temperature and is shelf stable in emulsion. Upon heating the emulsion, the egg substitute composition forms a gel, e.g., a scrambled egg analog. The egg substitute composition of the invention exhibits one or more organoleptic properties similar to a natural egg. The composition has modulated organoleptic properties such as increased or decreased fluffiness, airiness and mealy texture.

5.4.1 Cross-Linking to Prepare Egg-like Textures

[00119] Adzuki bean protein isolates suitable for producing egg-like textures can be prepared by adding a cross-linking step to the methods of preparing adzuki bean isolates provided herein. In one example, the cross-linking step can be added to the extraction step of the procedure, as depicted in **FIG. 1B**. For instance, a homogenous aqueous solution combining one part of adzuki bean flour with three to fifteen parts of water is prepared and pH adjusted to 6.5 to 8 with a suitable inorganic or organic acid or base. This mixture is centrifuged and the protein rich supernatant is decanted from the carbohydrate rich heavy phase. Transglutaminase powder is added to the protein rich solution at a concentration of 0.001 to 0.5% (w/w), heated to roughly 50C (optimum reaction temperature for transglutaminase) and incubated for 15 to 90 minutes. The reaction mixture was quickly heated to >70C for 1 to 5 minutes to inactivate the transglutaminase enzyme. The pH of the solution is adjusted to or near the isoelectric point of the globulin-rich component of the protein mixture (pH of about 5.4 - 5.8), rapidly cooled to less than 50C and centrifuged at > 3,000 x g. The supernatant is decanted, leaving a protein-rich powder, white to light tan in appearance, which can then further processed by commonly-available methods into a dried powder. The protein-rich powder can be incorporated into plant-based egg-mimetic emulsions which produce an egg-like texture upon heating, either in an oven, pan, skillet or hot water bath.

[00120] In another example, a homogenous aqueous solution combining one part of adzuki bean flour with three to fifteen parts of water is prepared and pH adjusted to 6.5 to 8 with a suitable inorganic or organic acid or base. The solution is centrifuged at >3000 x g and the protein rich supernatant is separated from the carbohydrate rich heavy phase. Transglutaminase powder is added to the solution at a concentration of 0.001 to 0.5% (w/w), heated to roughly 50C (optimum reaction temperature for transglutaminase) and incubated for 15 to 90 minutes. After incubation, hydrogen peroxide solution is added to the solution to a final concentration of 0.01 to 0.1% (w/w). This oxidizes the cysteine residue on the transglutaminase, arresting activity. The solution is then brought to the PI point of the protein or protein fraction of interest (about 5.4-5.8 pH). The solution is then chilled and centrifuged. The supernatant is then

decanted leaving a globulin rich heavy fraction. The globulin-rich fraction is diluted with water to a solids concentration of roughly 5 - 20% solids (w/w) and then spray dried. This is then mixed with water for spray drying. Sodium hydroxide is a process aid in this process. It has the added benefit of being an anti-microbial agent as well as a bleaching agent. After spray drying, all remnants of the oxidizing agent should have fully decayed.

[00121] In another example, adzuki bean extract is contacted with transglutaminase, but the process does not include a step to stop transglutaminase activity. A homogenous aqueous solution combining one part of adzuki bean flour with three to fifteen parts of water is prepared and pH adjusted to 6.5 to 8 with a suitable inorganic or organic acid or base. This is centrifuged at $>3000 \times g$ and the protein rich supernatant is separated from the carbohydrate rich heavy phase. Transglutaminase powder is added to the solution at a concentration of 0.001 to 0.5% (w/w). The transglutaminase concentration would be chosen to create the desired texture for the finished product. The solution is heated to roughly 50C (optimum reaction temperature for transglutaminase) and incubated for 15 to 90 minutes. The solution is then brought to the PI point of the protein or protein fraction of interest (about 5.4-5.8 pH). The solution is then chilled and centrifuged. The supernatant is then decanted leaving a globulin rich heavy fraction. The resulting heavy fraction is quickly heated to $>70C$ for 1 to 5 minutes to inactivate the transglutaminase enzyme. The heavy fraction is mixed with water and spray dried.

[00122] Adzuki bean protein isolates suitable for producing egg-like textures can also be prepared by performing cross-linking after acid precipitation of the protein, as depicted in **FIG. 1C**. In one example, a homogenous aqueous solution combining one part of adzuki bean flour with three to fifteen parts of water is prepared and pH adjusted to 6.5 to 8 with a suitable inorganic or organic acid or base. The solution is then centrifuged at $>3,000 \times g$. The protein-rich supernatant is decanted leaving a carbohydrate rich heavy phase. The pH of the protein rich solution is adjusted to or near the isoelectric point of the globulin-rich component of the protein mixture (pH of about 5.4 - 5.8), resulting in the precipitation of the globulin-rich protein. The solution is centrifuged at $>3,000 \times g$. The supernatant is decanted, leaving a globulin-rich protein fraction. This globulin-rich protein fraction is re-diluted in water to achieve a protein concentration of 5 to 25%. Transglutaminase powder is added to the solution at a concentration of 0.001 to 0.5% (w/w), heated to roughly 50C (optimum reaction temperature for transglutaminase) and incubated for 15 to 90 minutes. The reaction mixture is quickly heated to $>70C$ for 1 to 5 minutes to inactivate the transglutaminase enzyme. The mixture is then rapidly cooled to less than 50C and centrifuged at $>3,000 \times g$. The supernatant is decanted, leaving a protein-rich powder, white to light tan in appearance, which can then further processed by commonly-available methods into a dried powder. The protein-rich powder can be

incorporated into plant-based egg-mimetic emulsions which produce an egg-like texture upon heating, either in an oven, pan, skillet or hot water bath.

[00123] In another embodiment in which cross-linking is applied after acid precipitation of the protein, dry fractionation is used in place of aqueous extraction to produce the concentrate, as depicted in **FIG. 1D**. De-hulled adzuki beans are passed through successive mills, e.g. roller mill, followed by pin mill, to develop a flour with very fine particle size. The flour is then passed through a high-speed cyclone to separate larger from smaller particles. The protein-rich particles, roughly 55-60% protein, are then diluted in water to achieve a solution of roughly 5 - 25% solids. The pH of the solution is adjusted to or near the isoelectric point of the globulin-rich component of the protein mixture (pH of about 5.4 - 5.8), resulting in the precipitation of the globulin-rich protein. The solution is centrifuged at $>3,000 \times g$. The supernatant is decanted, leaving a globulin-rich protein fraction. This globulin-rich protein fraction is re-diluted in water to achieve a protein concentration of 5 to 25%. Transglutaminase powder is added to the solution at a concentration of 0.001 to 0.5% (w/w), heated to roughly 50C (optimum reaction temperature for transglutaminase) and incubated for 15 to 90 minutes. The reaction mixture is quickly heated to $>70C$ for 1 to 5 minutes to inactivate the transglutaminase enzyme. The mixture is then rapidly cooled to less than 50C and centrifuged at $> 3,000 \times g$. The supernatant is decanted, leaving a protein-rich powder, white to light tan in appearance, which can then further processed by commonly-available methods into a dried powder. The protein-rich powder can be incorporated into plant-based egg-mimetic emulsions which produce an egg-like texture upon heating, either in an oven, pan, skillet or hot water bath.

5.4.2 Cross-linking with Immobilized Transglutaminase

[00124] As an alternative to using bulk, single-use transglutaminase enzyme, one may treat the process streams using transglutaminase enzyme immobilized on inert porous beads or polymer sheets, which may be used in either flat or spiral-wound configurations in a reactor. Typical immobilized enzyme supports for beads include silicon dioxide (perlite) or calcium alginate. The immobilized transglutaminase is prepared by contacting an aqueous solution of transglutaminase with the bead material and a cross-linking agent, such as glutaraldehyde which fixes the enzyme to the solid substrate. The enzyme-containing support is then dried and conditioned prior to use. The advantages of immobilized enzyme reactors include: 1) improved control of the enzymatic reaction exposure and temperature conditions leading to a more uniform outcome from batch-to-batch; and 2) improved economics enabled by reuse of the transglutaminase enzyme. The solid substrate reduces the potential and rate of denaturation of the transglutaminase enzyme.

5.4.3 Cross-linking with Microencapsulated Transglutaminase

[00125] In some embodiments of the methods provided herein, a microencapsulated transglutaminase enzyme is used for preparing a plant-based liquid egg-like emulsion. For example, an emulsion containing a fat, water and emulsifier is prepared with the fat having a melting point between 50 and 80 C. Representative fats include stearic acid, palm and coconut shortening. Transglutaminase enzyme is then dispersed in the emulsion using a high shear mixer or homogenizer to achieve a flowable solution of rough 5 - 20% solids. The emulsion is then spray dried under typical conditions (150 - 175 C), with short residence time. The transglutaminase enzyme-containing spray dried powder can then be incorporate into plant-based egg-mimetic emulsions as described herein.

5.5 Adzuki Bean Isolates With Modified Organoleptic Properties

[00126] Also provided herein is a process to produce an edible adzuki bean protein isolate from a source of an adzuki bean protein, the process comprising: subjecting the source of the adzuki bean protein to a fractionation process to obtain a protein-rich fraction, wherein at least 50% by weight of the protein-rich fraction comprises or consists of one or more globulin-type proteins; reducing at least one impurity, the at least one impurity associated with an off-odor or an off-flavor in the edible adzuki bean protein isolate; and purifying the protein-rich fraction to obtain the edible adzuki bean protein isolate. In some embodiments, at least 60% by weight of the edible adzuki bean protein isolate is plant protein. In some embodiments, an oxidative enzymatic activity of the edible protein isolate is lower than a corresponding oxidative enzymatic activity of the source of the plant protein. In some embodiments, an organoleptic property of the edible adzuki bean protein isolate differs from a corresponding organoleptic property of the source of the adzuki bean protein.

[00127] In certain aspects, the methods and compositions provide producing purified protein isolates having modulated organoleptic properties of one or more of the following characteristics: astringent, beany, bitter, burnt, buttery, nutty, sweet, sour, fruity, floral, woody, earthy, beany, spicy, metallic, sweet, musty, grassy, green, oily, vinegary, neutral and bland flavor or aromas. Preferably, the purified protein isolates exhibit modulated organoleptic properties such as a reduction or absence in one or more of the following: astringent, beany, bitter, burnt, buttery, nutty, sweet, sour, fruity, floral, woody, earthy, beany, spicy, metallic, sweet, musty, grassy, green, oily, vinegary neutral and bland flavor or aromas.

5.5.1 Methods for Modifying Organoleptic Properties

[00128] Preferably, the methods provided herein reduce or remove at least one impurity that may impart or is associated with an off-flavor or off-odor in the purified protein isolate. The one or more impurity may be a volatile or nonvolatile compound and may comprise, for

example, lipoxygenase (EC 1.13.11.-), which is known to catalyze oxidation of fatty acids. As other examples, the at least one impurity may comprise a phenol, an alcohol, an aldehyde, a sulfide, a peroxide, or a terpene. Other biologically active proteins classified as albumins are also removed including lectins and protease inhibitors such as serine protease inhibitors and tryptic inhibitors.

[00129] In some embodiments, reducing an impurity comprises reducing at least one enzyme that reacts with a lipid substrate. In such embodiments, reducing such impurity reduces at least one lipophilic off-flavor, lipophilic substrate, or a co-factor. In some embodiments, impurities are reduced by a solid absorption procedure using, for example, charcoal, a bentonite clay, or activated carbon.

[00130] In some embodiments, the purified adzuki bean protein isolate has a reduced oxidative enzymatic activity relative to the source of the adzuki bean protein. In some embodiments, the oxidative enzymatic activity is lipoxygenase activity. In some embodiments, the purified protein isolate has lower oxidation of lipids or residual lipids relative to the source of the plant protein due to reduced lipoxygenase activity.

[00131] In some embodiments, reduced lipoxygenase activity in the adzuki bean protein isolate is effected by contacting an adzuki bean protein extract or isolate with transglutaminase activity. Thus, also provided herein are adzuki bean protein isolates modified by transglutaminase, wherein the isolate exhibits reduced or even significantly reduced lipoxygenase activity (or other enzymes which can oxidize lipids) relative to the plant source of the isolate. For example, the adzuki bean protein isolates modified by transglutaminase can have at least about a 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% reduction in lipoxygenase activity (or activity of an enzyme that can oxidize lipids) relative to the plant source of the isolate. In some embodiments, the amount of transglutaminase used to modify the adzuki bean protein isolates is between about 0.0001% to 0.0125%), as expressed on a volumetric basis to the amount of isolate or extract being modified.

[00132] In additional embodiments, reducing the at least one impurity comprises removing a fibrous solid, a salt, or a carbohydrate. Reducing such impurity comprises removing at least one compound that may impart or is associated with the off-flavor or off-odor. Such compounds may be removed, for example, using an activated charcoal, carbon, or clay. As another example, the at least one compound may be removed using a chelating agent (e.g., EDTA, citric acid, or a phosphate) to inhibit at least one enzyme that oxidizes a lipid or a residual lipid. In a particular example, EDTA may be used to bind co-factor for lipoxygenase, an enzyme that can oxidize residual lipid to compounds, e.g. hexanal, which are known to leave to off-flavors.

[00133] In some embodiments, separation of the protein from the residual source materials removes the undesirable organoleptic properties associated with proteins such as beany flavors or any of the unsuitable flavor profiles associated with compounds from **Table 1**, below.

[00134] The methods and compositions disclosed herein provide protein isolate characterized by its capacity to exhibit good organoleptic properties, for example by reducing undesired characteristics such as a “beany” aroma and taste. In preferred embodiments, components that are associated with off-flavors are removed or substantially reduced. Removal of undesired compounds may improve aroma, flavor or taste or a combination thereof. In some embodiments, methods for producing proteins isolates reduced in off-flavors involve one or more of the following methods:

[00135] 1) isoelectric point (pH ~5.6 +/- 0.2) precipitation to significantly reduce the level of lipoxygenase, which can oxidize any residual lipid to compounds, e.g. hexanal, which are known to leave to off-flavors ;

[00136] 2) use of a chelating agent, e.g. EDTA, to bind co-factor for lipoxygenase; and/or

[00137] 3) use of immobilized activated charcoal after extraction to remove compounds which can contribute to off-flavors. In cases where lipid substrate is abundant, lipids may be collected in the supernatant and removed or reduced. The disclosed methods may provide improved functionality of a protein isolate through enrichment of a class of proteins and reduction of enzymes such as lipoxygenase, that catalyze oxidation primarily of unsaturated fatty acids or unsaturated fats. Accordingly, in some embodiments, methods for purifying a protein fraction or reducing a class of proteins to reduce off-flavors minimally affect the protein isolate composition's capacity to retain one or more desired functional properties.

[00138] Accordingly, in certain aspects, the methods and compositions disclosed herein modulate or improve the flavor profile of the protein isolate, which in turn modulates or improves the flavor profile of the food product comprising the protein isolate. In certain embodiments, the removal or reduction of certain non-protein fractions of the source of the plant protein, such as polysaccharides, especially those indigestible forms in legumes, may impart more desirable flavors. The removal or reduction of the non-protein fractions may result in the removal or reduction of undesirable small molecules, including cross-linked polyphenols, volatiles, and heavy metal ions. The disclosed methods and compositions may provide for the removal or reduction of target compounds including but not limited to cross-linked polyphenols, volatiles, heavy metal ions, p-coumaric acid (4-Hydroxycinnamic acid), ferulic acid (4-Hydroxy-3-methoxycinnamic acid) and 4-hydroxybenzoic acid (known polyphenols) in addition to various compounds that are attributed to undesired flavors or aroma. Accordingly, the methods and compositions may provide solubilized plant proteins characterized as having a

neutral flavor and/or aroma. In yet other embodiments, the methods and compositions provide for modulation of flavors or aroma wherein a select compound is removed, reduced, or even incorporated.

[00139] Accordingly, one or more desired flavors or aroma may be removed, reduced, or added by modulating one or more small molecules that are attributed to certain flavors. One such method involves precipitation of protein to remove small molecules that are commonly associated with imparting undesirable flavors.

[00140] One way to characterize the aroma of a food product sample includes use of a GC SNFR Olfactory Port (PerkinElmer). Volatilized compounds from samples are injected into the GC column and the resulting compounds are separated and identified using mass spectrum.

Table 1 provides an exemplary list of compounds and their effect on sensory properties.

Additional compounds can be separated and identified to associate their sensory properties.

[00141] **Table 1:**

<u>Compound Name</u>	<u>Sensory Properties</u>
1-heptanol	Green, solvent flavor
1-Hexanol	Sharp, green, fruity
1-pentanol	unpleasant aroma
1-propene, 1-(methylthio)	herbicide
11,4-pentadiene	hydrocarbon odor
2-heptenal	natural nematocyte
2-hexenal	leaf aldehyde
2-Octenal, (E)-	nutty, cooked flour
2-pentyl-furan	green, fruity
Benzaldehyde	almond
Benzyl Alcohol	floral
Butanedial	-
Diallyl disulphide	garlic-derived sulphur compound
Dodecanal	natural oil, citrus oil
nonanal	natural oil/Culex attractant/perfume
Hstragole	natural oil: anise, tarragon, basil etc.
Hexanal	fresh cut grass
Hydroperoxide, hexyl	-
Pentadecanal	waxy
pentanal	
Sulfoxide, methyl	valeraldehyde; flavoring
phenethyl	-
Tetradecanal	strong fatty oris odor
Trifluoroacetyl-a-terpineol	-

[00142] Accordingly, in some embodiments, the method provides for the removal or reduction of one or more of these and/or other compounds. For example, as shown in **Table 1**, the presence of hexanal may result in a food product having an aroma resembling fresh cut grass. In some embodiments, such odor is removed or reduced. Similarly, the method provides

for iterating, modifying or improving formulations by identifying certain compounds in the formulation, associating the one or more compounds to an olfactory sense and removing or reducing the compounds.

[00143] In some embodiments, the purified protein isolate has one or more organoleptic properties that differ from a corresponding organoleptic property of the source of the plant protein. Examples of organoleptic properties include, but are not limited to, astringent flavor or aroma, beany flavor or aroma, bitter flavor or aroma, burnt flavor or aroma, buttery flavor or aroma, nutty flavor or aroma, sweet flavor or aroma, sour flavor or aroma, fruity flavor or aroma, floral flavor or aroma, woody flavor or aroma, earthy flavor or aroma, beany flavor or aroma, spicy flavor or aroma, metallic flavor or aroma, sweet flavor or aroma, musty flavor or aroma, grassy flavor or aroma, green flavor or aroma, oily flavor or aroma, vinegary flavor or aroma, neutral flavor or aroma, or bland flavor or aroma. The source of the plant protein may have a flavor, an aroma, or a sensory impression (e.g., a beany flavor or smell) that makes the source of the plant protein undesirable or unsuitable for use in place of a reference food, such as, for example, an egg. Relative to the source of the plant protein, the purified protein isolate has a modified organoleptic property, and this modified organoleptic property may make the purified protein isolate more suitable for use in or as a substitute for the reference food. In other words, the purified protein isolate may have an organoleptic property that gives the purified protein isolate, or a composition incorporating the purified protein isolate, a flavor, aroma, or sensory impression that is similar or equivalent to the flavor, aroma, or sensory impression of the reference food. For example, the purified protein isolate may reduce or eliminate an organoleptic property of the source of the plant protein.

[00144] In some embodiments, an organoleptic property of the purified protein isolate may be similar or equivalent to the corresponding organoleptic property of an egg. In some embodiments, the purified protein isolate provides a flavor, an aroma, or a sensory impression that is similar or equivalent to the flavor, aroma, or sensory impression of a reference food product, such as, for example, an egg (liquid, scrambled, or in patty form), a cake (e.g., pound cake, yellow cake, or angel food cake), a cream cheese, a pasta, an emulsion, a confection, an ice cream, a custard, milk, a deli meat, chicken (e.g., chicken nuggets), or a coating.

5.6 Food Functionality of Adzuki Bean Protein Isolates

[00145] In certain aspects, the high purity adzuki bean protein isolates provided herein exhibit desirable functional characteristics such as emulsification, water binding, foaming and gelation properties as measured by standard methods in industry. In comparison to characteristics of an egg, such properties of purified protein isolates as measured are comparable to at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,

84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater. The methods provided herein produce high purity, preferably 50%, 60%, 70%, 80%, 90% or greater adzuki bean protein isolates that exhibit functional properties, e.g., emulsification and gelation consistent to a food product such as an egg. In preferred embodiments, the protein content is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater.

[00146] The functional properties may include but are not limited to crumb density, structure/texture, elasticity/springiness, coagulation, binding, moisturizing, mouthfeel, leavening, aeration/foaming, creaminess, and emulsification of the food product such as an egg. Mouthfeel is a concept used in the testing and description of food products. Products made using exemplary protein isolates can be assessed for mouthfeel. In some embodiments products, e.g., baked goods, made using exemplary protein isolates have mouthfeel that is similar to products made with natural eggs. In some embodiments the mouthfeel of the products made using the exemplary protein isolates is superior to the mouthfeel of previously known or attempted egg substitutes, e.g., bananas, modified whey proteins, or Egg Beaters™.

[00147] Examples of properties which may be included in a measure of mouthfeel include: Cohesiveness: Degree to which the sample deforms before rupturing when biting with molars; Density: Compactness of cross section of the sample after biting completely through with the molars; Dryness: Degree to which the sample feels dry in the mouth; Fracturability: Force with which the sample crumbles, cracks or shatters. Fracturability encompasses crumbliness, crispiness, crunchiness and brittleness; Graininess: Degree to which a sample contains small grainy particles, may be seen as the opposite of smoothness; Gumminess: Energy required to disintegrate a semi-solid food to a state ready for swallowing; Hardness: Force required to deform the product to given distance, i.e., force to compress between molars, bite through with incisors, compress between tongue and palate; Heaviness: Weight of product perceived when first placed on tongue; Moisture absorption: Amount of saliva absorbed by product; Moisture release: Amount of wetness/juiciness released from sample; Mouthcoating: Type and degree of coating in the mouth after mastication (for example, fat/oil); Roughness: Degree of abrasiveness of product's surface perceived by the tongue; Slipperiness: Degree to which the product slides over the tongue; Smoothness: Absence of any particles, lumps, bumps, etc., in the product; Uniformity: Degree to which the sample is even throughout; homogeneity; Uniformity of Bite: Evenness of force through bite; Uniformity of Chew: Degree to which the chewing characteristics of the product are even throughout mastication; Viscosity: Force

required to draw a liquid from a spoon over the tongue; and Wetness: Amount of moisture perceived on product's surface.

[00148] The purified protein isolate may also have one or more functional properties by itself or when incorporated into a composition. Such functional properties may include, but are not limited to, one or more of emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color.

[00149] In some embodiments, at least one functional property of the purified protein isolate differs from the corresponding functional property of the source of the plant protein. In some embodiments, at least one functional property of the purified protein isolate is similar or equivalent to the corresponding functional property of a reference food product, such as, for example, an egg (liquid, scrambled, or in patty form), a cake (e.g., pound cake, yellow cake, or angel food cake), a cream cheese, a pasta, an emulsion, a confection, an ice cream, a custard, milk, a deli meat, chicken (e.g., chicken nuggets), or a coating.

[00150] In some embodiments, when the purified protein isolate is included in a food composition, the food composition has at least one functional property that is similar or equivalent to the corresponding functional property of a reference food product, such as, for example, an egg, liquid egg, scrambled egg, an egg patty, a cake (e.g., pound cake, yellow cake, or angel food cake), cream cheese, pasta, an emulsion, a confection, an ice cream, a custard, milk, a deli meat, chicken (e.g., chicken nuggets), or a coating.

[00151] In some embodiments, the purified protein isolate, either alone or when incorporated into a composition, is capable of forming a gel under heat or at room temperature.

5.7 Food Applications of Adzuki Bean Isolates

[00152] In some embodiments, the adzuki bean protein isolate provided herein is used as a direct protein replacement of animal- or vegetable-based protein in a variety of conventional food and beverage products across multiple categories at use levels. In some embodiments, the adzuki bean protein isolate is also used as a supplement to existing protein in food products.

Table 2 provides exemplary uses of the adzuki protein a wide range of food categories.

Table 2. Exemplary Food-Uses and Use Levels for Adzuki Bean Protein Isolate in Conventional Food and Beverage Products
--

Food Category	Proposed Food-Uses	Highest Use Level of Adzuki Bean Protein Isolate (%) in Final Product
Baked Goods and Baking Mixes	Cereal and granola bars	10
	Crackers	5
	Meal replacement/nutritional bars/energy bars	30
Beverages and Beverage Bases	Fermented beverages made from rice/barley/grains/legumes/tea	8
	Non-milk based instant protein powders	90 (powder)
	Non-milk based nutritional beverages (RTD, and powdered types) including protein-enriched fruit smoothies	20 (as consumed)
	Non-milk based weight control beverages, instant shakes, and protein drinks (RTD and powdered types)	10 (as consumed)
Breakfast Cereals	Breakfast cereals (RTE)	3
Condiments and Relishes	Bean dips and spreads	5
	Seasoning sauces	3
Dairy Product Analogs	Non-dairy cheese	5
	Non-dairy cream cheese, spread, and dips	5
	Non-dairy cream or sour cream (liquid and powder)	3
	Non-dairy ice cream and frozen desserts	3
	Non-dairy milk	3
	Non-dairy coffee whiteners	3
	Non-dairy yogurt and non-dairy drinkable yogurts	8
	Whipped topping	3
Frozen Dairy Desserts and Mixes	Ice cream and other frozen dairy desserts	3
Fruit and Water Ices	Ice pops and sorbets	3
Gelatins, Puddings, and Fillings	Puddings and mousse	3
Grain Products and Pasta	Pasta	4
Milk Products	Milk-based instant protein powders	90 (powder)
	Milk-based nutritional beverages (RTD and powdered types)	5 (as consumed)
	Milk-based weight control beverages, instant milkshakes, protein drinks (RTD and powdered types), and milk-based smoothies	3 (as consumed)

Table 2. Exemplary Food-Uses and Use Levels for Adzuki Bean Protein Isolate in Conventional Food and Beverage Products		
Food Category	Proposed Food-Uses	Highest Use Level of Adzuki Bean Protein Isolate (%) in Final Product
Plant Protein Products	Egg product analogs (meringue)	5
	Egg product analogs (quiche, frittata)	8
	Egg product analogs (scrambled eggs, omelet, hard boiled, liquid)	20
	Vegetarian food products and meat analogues	20
Snack Foods	Snack chips, popcorn, extruded snacks	5

[00153] The purified adzuki bean protein isolates provided herein are suitable for various food applications and have been incorporated into, e.g., edible egg-free emulsion, egg analog, egg-free scrambled eggs, egg-free patty, egg-free pound cake, egg-free angel food cake, egg-free yellow cake, egg-free cream cheese, egg-free pasta dough, egg-free custard, egg-free ice cream, and dairy-free milk.

[00154] In various aspects, the compositions and methods incorporate one or more purified protein isolates in multiple food applications including liquid and patty scrambled egg substitute to a desired level of emulsification, water binding and gelation. In a preferred embodiment, a functional egg replacement product comprises purified protein isolate or extract (10 – 15%), oil (10%), hydrocolloid, preservative, and optionally flavors, water, lecithin, xanthan, sodium carbonate, black salt.

[00155] Accordingly, the methods and compositions enable ingredients to have desired functionalities from one or more purified protein isolates that are suitable as replacement ingredients in various food applications including but not limited to meat substitutes, egg substitutes, baked goods and fortified drinks.

[00156] In some embodiments, the purified protein isolate is incorporated in an egg substitute. In some such embodiments, the organoleptic property of the purified protein isolate (e.g., a flavor or an aroma) is similar or equivalent to a corresponding organoleptic property of an egg. The egg substitute may exhibit at least one functional property (e.g., emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color) that is similar or equivalent to a corresponding

functional property of an egg. In addition to the purified protein isolate, the egg substitute may include but are not limited to one or more of iota-carrageenan, gum arabic, konjac, xanthan gum, or gellan.

[00157] In some embodiments, the purified protein isolate is incorporated in an egg-free cake, such as a pound cake, a yellow cake, or an angel food cake. In some such embodiments, at least one organoleptic property (e.g., a flavor or an aroma) of the egg-free cake is similar or equivalent to a corresponding organoleptic property of a cake containing eggs. The egg-free cake may exhibit at least one functional property similar or equivalent to a corresponding functional property of a cake containing eggs. The at least one function property may be, for example, one or more of emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color.

[00158] In some embodiments, the purified protein isolate is incorporated into an egg-free cake mix or an egg-free cake batter. In some such embodiments, the egg-free cake mix or batter has at least one organoleptic property (e.g., a flavor or aroma) of the egg-free cake batter is similar or equivalent to a corresponding organoleptic property of a cake mix or batter containing eggs. The egg-free cake mix or batter may exhibit at least one functional property similar or equivalent to a corresponding functional property of a cake batter containing eggs. The at least one functional property may be, for example, one or more of emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color.

[00159] In some embodiments in which the purified protein isolate is included in an egg-free pound cake, a peak height of the egg-free pound cake is at least 90% of the peak height of a pound cake containing eggs. In some embodiments in which the purified protein isolate is included in an egg-free pound cake batter, a specific gravity of the egg-free pound cake batter is 0.95–0.99.

[00160] In some aspects, increased functionality is associated with the purified protein isolate in a food application. For instance, food products produced with the purified protein isolate may exhibit increased functionality in dome or crack, cake resilience, cake cohesiveness, cake springiness, cake peak height, specific gravity of batter, center doming, center crack, browning, mouthfeel, spring-back, off flavors or flavor.

[00161] In some embodiments, the purified protein isolate is included in a cream cheese, a pasta dough, a pasta, a milk, a custard, a frozen dessert (e.g., a frozen dessert comprising ice cream), a deli meat, or chicken (e.g., chicken nuggets).

[00162] In some embodiments, the purified protein isolate is incorporated in a food or beverage composition, such as, for example, an egg substitute, a cake (e.g., a pound cake, a yellow cake, or an angel food cake), a cake batter, a cake mix, a cream cheese, a pasta dough, a pasta, a custard, an ice cream, a milk, a deli meat, or a confection. The food or beverage composition may provide sensory impressions similar or equivalent to the texture and mouthfeel that replicates a reference food or beverage composition. In some embodiments, before being included in a food or beverage composition, the purified protein isolate is further processed in a manner that depends on a target application for the purified protein isolate. For example, the purified protein isolate may be diluted in a buffer to adjust the pH to a pH appropriate for the target application. As another example, the purified protein isolate may be concentrated for use in the target application. As yet another example, the purified protein isolate may be dried for use in the target application.

[00163] Various food applications incorporating high purity protein isolates from adzuki bean as the main functional ingredient were made including an egg-free emulsion (e.g. for an egg-free food product similar or equivalent to scrambled eggs), pound cake, yellow cake, angel food cake, a cream cheese, a pasta dough, a pasta, a custard, an ice cream, a milk, a deli meat, or a confection.

5.7.1 Vegan Patty

[00164] Various experiments provide evidence that adzuki bean protein isolate are suited for use as the sole gelling agent in a formulated vegan patty. In some embodiments, a hydrocolloid system comprised of iota-carrageenan and gum arabic enhances native gelling properties of adzuki bean isolate in a formulated patty. In other embodiments, a hydrocolloid system comprised of high-acyl and low-acyl gellan in a 1.5:1 ratio enhances native gelling properties of adzuki bean isolate in a formulated patty. In further embodiments, a hydrocolloid system comprised of konjac and xanthan gum enhances native gelling properties of adzuki bean isolate in a formulated patty.

5.7.2 Egg-free Emulsion

[00165] In another aspect, provided herein is an edible egg-free emulsion comprising an adzuki bean protein isolate described herein. In some embodiments, the emulsion comprises one or more additional components selected from water, oil, fat, hydrocolloid, and starch. In some embodiments, at least or about 60-85% of the edible egg-free emulsion is water. In some embodiments, at least or about 10-20% of the edible egg-free emulsion is the protein isolate. In

some embodiments, at least or about 5-15% of the edible egg-free emulsion is oil or fat. In some embodiments, at least or about 0.01-6% of the edible egg-free emulsion is the hydrocolloid fraction or starch. In some embodiments, the hydrocolloid fraction comprises high-acyl gellan gum, low-acyl gellan gum, iota-carrageenan, gum arabic, konjac, locust bean gum, guar gum, xanthan gum, or a combination of one or more gums thereof. In some embodiments, the emulsion further comprises one or more of: a flavoring, a coloring agent, an antimicrobial, a leavening agent, and salt.

[00166] In some embodiments, the emulsion further comprises phosphate. In some embodiments, the phosphate is selected from the group consisting of disodium phosphate (DSP), sodium hexamethaphosphate (SHMP), tetrasodium pyrophosphate (TSPP). In a particular embodiment, the emulsion comprises DSP. In another particular embodiment, the emulsion comprises DSP. In some embodiments, the amount of DSP in the emulsion is at least or about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.11%, 0.12%, 0.13%, 0.14% or 0.15%; or greater than 0.15%. In another particular embodiment, the emulsion comprises SHMP. In some embodiments, the SHMP is a short chain SHMC, regular chain SHMP or a long chain SHMP. In some embodiments, the amount of SHMP in the emulsion is at least or about 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, or 1%; or greater than 1%.

[00167] In a particular embodiment, provided herein is an edible egg-free emulsion having a pH of about 5.6 to 6.8. In some embodiments, the edible egg-free emulsion comprises water, an adzuki bean protein isolate described herein, an enzyme that modifies a structure of the protein isolate, and oil or fat. In some embodiments, the enzyme comprises a transglutaminase or proteolytic enzyme. In some embodiments, at least or about 70-85% of the edible egg-free emulsion is water. In some embodiments, at least or about 7-15% of the edible egg-free emulsion is the protein isolate. In some embodiments, at least or about 0.0005-0.0025% (5-25 parts per million) of the edible egg-free emulsion is the enzyme that modifies the structure of the protein isolate. In some embodiments, at least or about 5-15% of the edible egg-free emulsion is oil or fat.

[00168] Also provided herein is a patty made using any of the egg-free emulsions described above.

[00169] In some embodiments, a method provides an egg-free emulsion prepared using the protein isolate described herein, wherein the egg-free emulsion may be used to make for example an egg-free food product similar or equivalent to scrambled eggs, an omelet, or a quiche prepared using eggs. Accordingly, in some embodiments an egg-free emulsion comprises one or more of the exemplary protein isolates disclosed herein. The egg-free emulsion may further comprise for example a lipid, one or more carbohydrates, and optionally a

protein-modifying enzyme, salt, flavorings, and/or colors. The proportions of these ingredients may be selected to modulate the texture, flavor, and/or color of the resulting egg-free food product. The resulting egg-free food product may provide sensory impressions similar or equivalent to the texture and mouthfeel that replicates an egg. Sensory quality parameters of a liquid scramble and patty are characterized as soft, compact gel with clean bite and moderate chewiness similar to eggs.

5.7.3 Baked Cakes

[00170] In another aspect, provided herein are one or more egg-free cake mixes, suitable for preparing one or more egg-free cake batters, from which one or more egg-free cakes can be made. In some embodiments, the egg-free cake mix comprises flour, sugar, and an adzuki bean protein isolate described herein. In some embodiments, the egg-free cake mix further comprises one or more additional components selected from: cream of tartar, disodium phosphate, baking soda, and a pH stabilizing agent. In some embodiments, the flour comprises cake flour.

[00171] Also provided herein is an egg-free cake batter comprising an egg-free cake mix described above, and water. In some embodiments, the egg-free cake batter is an egg-free pound cake batter, an egg-free angel food cake batter, or an egg-free yellow cake batter. In some embodiments, the egg-free cake batter has a specific gravity of 0.95-0.99.

[00172] Also provided herein is an egg-free cake made from an egg-free cake batters described above. In some embodiments, a peak height of the egg-free cake is at least 90% of a peak height of a pound cake containing eggs. In some embodiments, one or more characteristics of the egg-free cake is similar or equivalent to one or more corresponding characteristics of a cake containing eggs. In some embodiments, the one or more characteristics comprise resilience, cohesiveness, springiness, peak height, center doming, center crack, browning, mouthfeel, spring-back, or flavor. In some embodiments, the one or more characteristics comprise hardness, resilience, cohesiveness, springiness, or chewiness. In some embodiments, the one or more characteristics comprise a functional property or an organoleptic property. In some embodiments, the functional property comprises one or more of: emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color.

[00173] In a particular embodiment, provided herein is an egg-free pound cake mix, comprising flour, sugar, and an adzuki bean protein isolate described herein. In some embodiments, the flour comprises cake flour. In some embodiments, the egg-free pound cake mix further comprises oil or fat. In some embodiments, the oil or fat comprises butter or

shortening. In some embodiments, at least or about 25-31% of the egg-free pound cake batter is flour. In some embodiments, at least or about 25-31% of the egg-free pound cake batter is oil or fat. In some embodiments, at least or about 25-31% of the egg-free pound cake batter is sugar. In some embodiments, at least or about 6-12% of the egg-free pound cake batter is the protein isolate. In some embodiments, the batter further comprises disodium phosphate or baking soda.

[00174] Also provided herein is an egg-free pound cake batter comprising an egg-free pound cake mix described above, and further comprising water. In some embodiments, the egg-free pound cake batter comprises about four parts of the egg-free pound cake mix; and about one part water. In some embodiments, at least or about 20-25% of the egg-free pound cake batter is flour. In some embodiments, at least or about 20-25% of the egg-free pound cake batter is oil or fat. In some embodiments, at least or about 20-25% of the egg-free pound cake batter is sugar. In some embodiments, at least or about 5-8% of the egg-free pound cake batter is the protein isolate. In some embodiments, at least or about 18-20% of the egg-free pound cake batter is water.

[00175] In another particular embodiment, provided herein is an egg-free angel food cake mix comprising flour, sugar, and an adzuki bean protein isolate described herein. In some embodiments, at least or about 8-16% of the egg-free angel food cake mix is flour. In some embodiments, at least or about 29-42% of the egg-free angel food cake mix is sugar. In some embodiments, at least or about 7-10% of the egg-free angel food cake mix is the protein isolate. In some embodiments, the egg-free angel food cake mix further comprises cream of tartar, disodium phosphate, baking soda, or a pH stabilizing agent. In some embodiments, the flour comprises cake flour. Also provided herein is an egg-free angel food cake batter comprising an egg-free angel food cake mix described above, and water.

[00176] In another particular embodiment, provided herein is an egg-free yellow cake mix, comprising flour, sugar, and an adzuki bean protein isolate described herein. In some embodiments, at least or about 20-33% of the egg-free yellow cake mix is flour. In some embodiments, at least or about 19-39% of the egg-free yellow cake mix is sugar. In some embodiments, at least or about 4-7% of the egg-free yellow cake mix is the protein isolate. In some embodiments, the egg-free yellow cake mix further comprises one or more of baking powder, salt, dry milk, and shortening. Also provided herein is an egg-free yellow cake batter comprising an egg-free yellow cake mix described above, and water.

[00177] Some embodiments provide methods to produce an egg-free pound cake using a protein isolate. For instance, a batter is created by mixing liquid solution comprising adzuki bean protein isolate with sugar, cake flour, and butter at 17°C to 20°C in 1:1:1:1 w/w ratio. The ingredients are blended together using single stage mixing on Hobart mixer for 6 minutes at

22°C. The batter is baked in pound cake pan for 45 minutes in 150°C and cooled in pan for 2 hours at 22°C.

[00178] Sensory quality parameters of cakes made with the protein isolates are characterized as fluffy, soft, airy texture. The peak height was measured to be 90-110% of pound cake containing eggs. The specific gravity of cake batter with the purified adzuki bean protein isolate was 0.95-0.99 which was similar to that of cake batter with whole eggs of 0.95-0.96.

5.7.4 Cream Cheese Analog

[00179] In another aspect, provided herein is an egg-free cream cheese comprising an adzuki bean protein isolate described herein. In some embodiments, the egg-free cream cheese comprises one or more additional components selected from water, oil or fat, and hydrocolloid. In some embodiments, at least or about 75-85% of the egg-free cream cheese is water. In some embodiments, at least or about 10-15% of the egg-free cream cheese is the protein isolate. In some embodiments, at least or about 5-10% of the egg-free cream cheese is oil or fat. In some embodiments, at least or about 0.1-3% of the egg-free cream cheese is hydrocolloid. In some embodiments, the hydrocolloid comprises xanthan gum or a low-methoxy pectin and calcium chloride system. In some embodiments, the egg-free cream cheese further comprises a flavoring or salt. In some embodiments, one or more characteristics of the egg-free cream cheese is similar or equivalent to one or more corresponding characteristics of a cream cheese containing eggs. In some embodiments, the characteristic is a taste, a viscosity, a creaminess, a consistency, a smell, a spreadability, a color, a thermal stability, or a melting property. In some embodiments, the characteristic comprises a functional property or an organoleptic property. In some embodiments, the functional property comprises: emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color. In some embodiments, the organoleptic property comprises a flavor or an odor.

[00180] Example 31 provides an exemplary cream cheese analog using a hydrocolloid system comprised of low-methoxy pectin with CaCl₂ forms a continuous, soft gel with textural organoleptic properties reminiscent of cream cheese. Additional results provide a hydrocolloid system comprised of xanthan gum forms a continuous, soft gel with textural organoleptic properties reminiscent of cream cheese.

5.7.5 Egg-free pasta dough

[00181] In another aspect, provided herein is an egg-free pasta dough comprising an adzuki bean protein isolate described herein. In some embodiments, the egg-free pasta dough comprises one or more additional components selected from flour, oil or fat, and water. In some embodiments, the flour comprises semolina flour. In some embodiments, the oil or fat comprises extra virgin oil. In some embodiments, the egg-free pasta dough further comprises salt. Also provided herein is an egg-free pasta made from an egg-free pasta dough described above. In some embodiments, the egg-free pasta is fresh. In some embodiments, the egg-free pasta is dried. In some embodiments, one or more characteristics of the egg-free pasta is similar or equivalent to one or more corresponding characteristics of a pasta containing eggs. In some embodiments, the one or more characteristics comprise chewiness, density, taste, cooking time, shelf life, cohesiveness, or stickiness. In some embodiments, the one or more characteristics comprise a functional property or an organoleptic property. In some embodiments, the functional property comprises: emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color. In some embodiments, the organoleptic property comprises a flavor or an odor.

5.7.6 Plant-based milk

[00182] In another aspect, provided herein is a plant-based milk comprising an adzuki bean protein isolate described herein. In some embodiments, the plant-based milk comprises one or more additional components selected from water, oil or fat, and sugar. In some embodiments, at least or about 5% of the plant-based milk is the protein isolate. In some embodiments, at least or about 70% of the plant-based milk is water. In some embodiments, at least or about 2% of the plant-based milk is oil or fat. In some embodiments, the plant-based milk further comprises one or more of: disodium phosphate, soy lecithin, and trace minerals. In particular embodiments, the plant-based milk is lactose-free. In other particular embodiments, the plant-based milk does not comprise gums or stabilizers.

5.7.7 Egg-free custard

[00183] In another aspect, provided herein is an egg-free custard comprising an adzuki bean protein isolate described herein. In some embodiments, the egg-free custard comprises one or more additional components selected from cream and sugar. In some embodiments, at least or about 5% of the egg-free custard is the protein isolate. In some embodiments, at least or about 81% of the egg-free custard is cream. In some embodiments, at least or about 13% of the egg-free custard is sugar. In some embodiments, the egg-free custard further comprises one or more

of: iota-carrageenan, kappa-carrageenan, vanilla, and salt. In some embodiments, the cream is heavy cream.

5.7.8 Egg-free ice cream

[00184] In another aspect, provided herein is an egg-free ice cream comprising an adzuki bean protein isolate described herein. In some embodiments, the egg-free ice cream is a soft-serve ice cream or a regular ice cream. In some embodiments, the egg-free ice cream comprises one or more additional components selected from cream, milk, and sugar. In some embodiments, at least or about 5% of the egg-free ice cream is the protein isolate. In some embodiments, at least or about 41% of the egg-free ice cream is cream. In some embodiments, at least or about 40% of the egg-free ice cream is milk. In some embodiments, at least or about 13% of the egg-free ice cream is sugar. In some embodiments, the egg-free ice cream further comprises one or more of iota-carrageenan, kappa-carrageenan, vanilla, and salt. In some embodiments, the cream is heavy cream. In some embodiments, the milk is whole milk. In particular embodiments, the egg-free ice cream is lactose-free. In other particular embodiments, the egg-free ice cream does not comprise gums or stabilizers. In other embodiments, the egg-free ice provides a traditional mouthfeel and texture of an egg-based ice cream but melts at a slower rate relative to an egg-based ice cream.

[00185] .

5.7.9 Food Applications: Co-Ingredients

5.7.9.1 Gums

[00186] Various gums useful for formulating food products include, e.g., gum acacia, Versawhip, Guar+Xanthan, Q-extract, CMC 6000 (Carboxymethylcellulose), Citri-Fi 200 (citrus fiber), Apple fiber, Fenugreek fiber.

5.7.9.2 Starches

[00187] Starch is one of the most prevalent food ingredients, and has been shown to have useful emulsifying properties. Starch and starch granules are known to stabilize emulsions. Consequently, one or more starches are produced from plant compositions, such as, for example, arrowroot starch, cornstarch, tapioca starch, adzuki bean starch, potato starch, sweet potato starch, rice starch, sago starch, wheat starch. The hydrophobicity allows starch to be adsorbed at the oil-water interface, which prevents re-coalescence and hence droplet stability.

5.7.9.3 Preservatives

[00188] In certain embodiments, the methods and compositions comprise an effective amount of an added preservative in combination with the protein isolate.

[00189] Preservatives prevent food spoilage from bacteria, molds, fungi, or yeast (antimicrobials); slow or prevent changes in color, flavor, or texture and delay rancidity (antioxidants); maintain freshness. They include but are not limited to the following: ascorbic acid, citric acid, sodium benzoate, calcium propionate, sodium erythorbate, sodium nitrite, calcium sorbate, potassium sorbate, BHA, BHT, EDTA, tocopherols (Vitamin E) and antioxidants, which prevent fats and oils and the foods containing them from becoming rancid or developing an off-flavor. See **Table 3**.

[00190] **Table 3:**

Substance/class	Some foodstuffs in which they are used
Sorbic acid and sorbate compounds	Cheese, wines, dried fruit, fruit sauces, toppings
Benzoic acid, and benzoate	Pickled vegetables, low sugar jams and jellies, candied fruits, semipreserved fish products, sauces
Sulphur dioxide and sulphite compounds	Dried fruits, fruit preserves, potato products, wine
Natamycin	Surface treatment of cheese and sausage
Nitrite and nitrate compounds	Sausage, bacon, ham, foie gras, cheese, pickled herring

5.8 Storage And Shelf Life of Compositions

[00191] In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable in storage at room temperature for up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks. In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable for storage at room temperature for months, e.g. greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 months. In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable for refrigerated or freezer storage for months, e.g. greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 months. In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable for refrigerated or freezer storage for years, e.g. greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 years. In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable for storage at room temperature for months, e.g. greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 months. In some embodiments, the protein isolate or compositions comprising the protein isolate may be stable for storage at room temperature for years, e.g. greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 years.

[00192] In some embodiments, storage as a dry material can increase the shelf-life of the protein isolate or a composition comprising the protein isolate. In some embodiments protein isolate or a composition comprising the protein isolate is stored as a dry material for later

reconstitution with a liquid, e.g. water. In some embodiments, the purified protein isolate is in powdered form, which may be less expensive to ship, lowers risk for spoilage and increases shelf-life (due to greatly reduced water content and water activity).

[00193] In other embodiments the purified protein isolate or a composition comprising the protein isolate is reconstituted with a liquid, e.g. water, milk, or other liquid suitable for consumption. In one example, 36-45 grams of liquid can be added to 12-15 grams dry weight of the composition to produce a liquid scramble. The amount of liquid can be varied to suit a particular purpose for the reconstituted composition.

[00194] In additional embodiments, food composition comprising the purified protein isolate or a formulation thereof in an emulsion, under heat, under refrigeration or colder conditions or not ambient conditions.

6. EXAMPLES

6.1 EXAMPLE 1: Charcoal Treatment of Protein Extracts

[00195] This example provides an exemplary protocol for performing a carbon adsorption step to remove non-protein, off-flavor components (such as beany flavor) in the protein extraction process. Typical starting weights for input legume flour or material range from 1-12 kg, and typical yield is about 25% with a moisture content around 78%.

[00196] Charcoal specifications: ~500um-1500um particle size, 12x30 mesh size, acid washed.

[00197] Charcoal preparation: 100g charcoal is mixed in 3kg of water, poured through a filter and collected. This wash step is repeated for a total of 2 washes.

[00198] Preparation of extract: water is combined with flour in a 3:1 water-to-flour ratio, then centrifuged at 6000 x g for 20 minutes at 4°C. Supernatant is collected and filtered through 100um Nylon mesh.

[00199] Charcoal treatment: prepared charcoal is mixed with 1L of extract and stirred for 15 minutes. The extract-charcoal mixture is then filtered through a 100um filter to remove large charcoal particles, and centrifuged at 10,000 x g for 15 minutes at 4°C to remove remaining ash. 500 mM CaNa₂EDTA is added to the extract to a final concentration of 2 mM CaNa₂ EDTA, mixed, then incubated at 4°C for 60 minutes. Rapid dilution of the filtrate is performed in cold (4°C) 0.3% NaCl at a ratio of 1 volume of supernatant to 3 volumes of cold 0.3% NaCl. The filtrate is then centrifuged at 10,000 x g for 15 minutes at 4°C, and the pellet collected.

[00200] The pellet is suspended and homogenized 1:4 (w/w) with 0.3% NaCl + 0.7 mM CaNa₂ EDTA (4°C), and centrifuged at 10,000 x g for 15 minutes at 4°C. The resulting pellet is washed 1:4 (w/w) with 0.3% NaCl (4°C), then centrifuged at 10,000 x g for 15 minutes at 4°C.

[00201] The final pellet is homogenized and the moisture content is recorded.

6.2 EXAMPLE 2: Protein Isolate Purification Protocol

[00202] This example provides an exemplary protocol for preparing adzuki bean protein isolate.

[00203] A. Multistage extraction. Water is mixed with adzuki bean flour in a 5:1 tap water-to-flour ratio. The pH of the mixture is adjusted to pH 6.5-pH 8 with NaOH. The mixture is centrifuged at 6000 x g for 15 minutes at 4°C. The extract is collected and the pellet is resuspended in 3:1 water-to-flour. The pH is adjusted to pH 6.5-pH 8 with NaOH, and centrifuged again at 6000 x g for 15 minutes at 4°C. Both extracts are combined and filtered through 100um Nylon mesh.

[00204] B. Charcoal filtration or off-flavor removal (optional). Charcoal specifications: ~500um-1500um particle size, 12x30 mesh size, acid washed. Charcoal preparation: 100g of charcoal is mixed in 3kg of water, poured through a filter, and the charcoal is collected. 1g of charcoal is then added to 10g of extract and incubated for ~15min. The mixture is then centrifuged at 10000 x g for 15 minutes, 4°C. The mixture can also be treated with a chelating agent, for example, 2 mM CaNa₂ EDTA.

[00205] C. Acid Precipitations. Isoelectric precipitation at pH 5.6 +/- 0.2 is combined with a cryo-precipitation method at 1-4 °C. pH is brought down to pH 5.4-5.8 with 20% Citric Acid. Cool on ice for 1h. Alternatively, low ionic strength precipitation can be performed at very high flow rates combined with cryo-precipitation method (at 1-4°C). Rapid dilution of the filtrate is performed in cold (4°C) 0.3% NaCl at a ratio of 1 volume of supernatant to 3 volumes of cold 0.3% NaCl. The filtrate is then centrifuged at 10,000 x g for 15 minutes at 4°C.

[00206] D. Recovery. The pellet is collected, resuspended and homogenized 1:4 (w/w) with 0.3% NaCl (4°C). The pH is maintained at 5.6 +/- 0.1 with citric acid. The suspension is centrifuged at 10,000 x g for 15 minutes at 4°C, and the final pellet is homogenized.

[00207] The final pellet is homogenized and the moisture content is recorded.

6.3 EXAMPLE 3: Pilot Scale Protein Isolation Method

[00208] This example provides an exemplary protocol for pilot scale preparation of adzuki bean protein isolates. A general process block flow diagram is shown in **FIG. 3**. The process starts with a protein extraction stage, where milled adzuki bean flour is mixed with 5 - 10 volumes of soft water to form a slurry in a chilled mix tank (2°C - 8°C). The pH of the slurry is adjusted with a food-grade 50% NaOH solution to reach pH 7 for solubilization of target protein into the aqueous solution. The slurry is then sent to a solid/liquid separation unit operation (typically a combination of one decanter and one disc-stack centrifuge), and the solubilized protein extract is separated from the fibrous starch fraction of the flour.

[00209] Optionally, the protein extract is pumped to pass through a food-grade charcoal filled annular basket column (at < 5% charcoal to protein extract ratio, w/w) at 4°C. The primary function of this carbon adsorption step is to remove non-protein, off-flavor components (such as beany flavor) in the protein extraction. It also removes some fibrous solids and therefore leads to a clarified protein extract.

[00210] The clarified protein extract is acidified with a 20% food-grade citric acid solution to reach its isoelectric point (pH 5.6) under a Cryo condition (2°C). Under this condition, the target protein precipitates and becomes separable from the aqueous solution. In addition to the pH adjustment, 2 mM of food-grade EDTA is added during this step to inhibit lipoxygenases activities that may incur off-flavor compounds generation. The precipitated protein slurry is then sent to a solid/liquid separation unit operation (typically one disc-stack centrifuge), and the protein curd is recovered in the heavy phase of the centrifuge step.

[00211] The protein curd is then washed with 4 volumes of soft water during the washing step under a Cryo condition (2°C). Washing is regarded as a polishing step to remove impurities (e.g. fibrous solids, salts, carbohydrates) in the protein curd. In this step, 0.3% (w/w) food-grade sodium chloride is typically added to facilitate solid/liquid separation during centrifugation.

[00212] The washed protein curd solution is then pasteurized through a High Temperature/Short Time (HTST) pasteurization step. Similar to milk pasteurization, the primary function of this step is to kill any pathogenic bacteria that may be present in the washed protein curd solution. An exemplary HTST condition is 74°C for 20 - 23 seconds.

[00213] The final step in the processing is spray drying, where the pasteurized protein solution is passed through a spray dryer to remove the water content. A typical spray drying condition has a dryer inlet temperature of 170°C, and a dryer outlet temperature of 72°C. The dried protein isolate powder typically has < 5% moisture content.

6.4 EXAMPLE 4: Egg-Like Patty

[00214] A representative formulation of an egg-like patty includes: water (75 - 85%); adzuki bean protein isolate (10 - 15%); oil (5 - 10%); hydrocolloid (0.1 - 3%) (which includes either one of the following combinations: (1) high-acyl & low-acyl gellan gum; (2) iota-carrageenan & gum arabic; (3) konjac & xanthan gum); starches (0.1-6%); flavors (1-2%); and salt (<1%). The emulsion mixture is at pH 5.6-6.8.

[00215] High purity isolates from adzuki bean are rehydrated to 80% moisture content and adjusted to pH 6.0 with 1M NaOH. Emulsion of plant protein isolate, oil, hydrocolloid, salt and other ingredients were prepared using a Pro Scientific shear mixer operated at 5000 rpm for 4 min at room temperature. Emulsion is deposited in round molds (3 in diameter), and the amount deposited per mold is 50g. Convection oven is set at 220°F for 55 min.

[00216] Adzuki bean protein isolate was prepared in accordance with the procedure described in Example 2. Yield by wet mass was 37% and moisture was 85.67%.

[00217] The adzuki bean isolate was formulated into a vegan patty using the egg-free patty representative formulation described in Example 4 using gellan gum (**FIG. 4**). Trace minerals were added.

[00218] The purified mung bean protein isolate was prepared in parallel and used as a positive control. In the patty system, adzuki behaved similarly to mung bean but with a creamier and smoother mouthfeel. The vegan patty made with adzuki was relatively neutral in flavor but with a slightly beanier taste than mung bean control.

[00219] Instrumental texture profile parameters were measured using a Brookfield Texture Analyzer. Results are presented below in **Table 4**.

[00220] **Table 4. Texture analysis profile** (mean value, n=3)

Sample ID	Mung bean	Adzuki bean
Moisture content (%)	77.09%	85.67%
Hardness cycle 1 (g)	2362.2	882.67
Hardness cycle 2 (g)	21.47	8.44
Cohesiveness	0.26	0.27
Springiness (mm)	4.98	5.03
Springiness index	0.74	0.75
Chewiness (mJ)	30.77	11.79

6.5 EXAMPLE 5: Particle Size Distribution

[00221] The efficiency of seed milling is reflected in the particle size distribution of the flours, and influences the composition of isolated materials and their functionality. Particle size of adzuki bean flours was characterized using Mastersizer AeroS (Malvern). The flour particle size distribution was analyzed and the size mode, 10th (Size.quantile.10), 50th (Size.quantile.50) and 90th (Size.quantile.90) percentile values are provided in **Table 5** below. Materials used for isolation showed particle size distribution in a range of ~9-190 μm .

[00222] **Table 5**

Size Mode	Size.quantile.10 (μm)	Size.quantile.50 (μm)	Size.quantile.90 (μm)	Size.median.abs.dev (μm)
144	9.86	58.90	186	28.66

6.6 EXAMPLE 6: Protein Composition of Adzuki Bean Protein Isolates

[00223] Molecular analyses of adzuki bean protein isolates prepared in accordance with the methods described herein were undertaken to determine their compositional make-up. **Table 6** provides a proximate analysis of protein, carbohydrate, fat, moisture and ash content in adzuki

bean protein isolate prepared in accordance with the methods described herein, compared to the starting material, adzuki bean flour. Protein content was determined using the Dumas method of total nitrogen quantitation and a correction factor of 6.25.

[00224] **Table 6.**

Sample	Protein (%)	Carbohydrates (%)	Fats (%)	Moisture (%)	Ash (%)
Adzuki bean flour	24.1	59.6	1.53	11.4	3.41
Adzuki bean protein isolate	29	0.25	0.41	69.1	1.24

[00225] The identity of proteins from the adzuki bean isolate was investigated by two-dimensional-liquid chromatography-tandem mass spectrometry (2D-LC-MS/MS). Raw MS/MS spectra were searched against the *Vigna angularis* genome (legumeinfo.org/organism/Vigna/angularis) and a decoy sequence database. 564 Proteins in total were identified. Spectrum counting was used to calculate the relative amount of each protein in reference to the total protein amount. **Table 7** provides the predicted identities and relative abundance of proteins whose abundance was >1% of total protein in the sample (6 proteins representing ~57% of the total proteins).

[00226] **Table 7.**

<u>SEQ ID</u>	<u>Spectrum count</u>	<u>relative abundance (%)</u>	<u>Unique Peptide</u>	<u>Protein Coverage</u>	<u>Accession</u>	<u>Predicted function /Putative protein</u>	<u>MW</u>
SEQ ID NO: 1	5711	27.79	10	0.21	Vang06g13860.1	Glycinin, putative / 11-S seed storage protein	56.6
SEQ ID NO: 2	4378	21.30	9	0.20	Vang06g05170.1	beta-conglycinin, beta chain-like/ allergen gly M Bd 28 kDa protein	46.2
SEQ ID NO: 3	778	3.79	13	0.25	Vang03g11940.2	basic 7S globulin-like	46.1
SEQ ID NO: 4	420	2.04	20	0.32	Vang0032ss01820.1	NAD ⁺ :PROTEIN(ADP-ribosyl)-transferase	87

<u>SEQ ID</u>	<u>Spectrum count</u>	<u>relative abundance (%)</u>	<u>Unique Peptide</u>	<u>Protein Coverage</u>	<u>Accession</u>	<u>Predicted function /Putative protein</u>	<u>MW</u>
SEQ ID NO: 5	297	1.45	22	0.35	Vang02g13530.1	formate dehydrogenase	80.7
SEQ ID NO: 6	224	1.09	19	0.35	Vang0053ss00110.1	GTP-binding elongation factor Tu family protein	54.1

[00227] The protein profile of the isolate was also assessed via capillary gel electrophoresis using the Perkin Elmer LabChip GXII Touch HT system. As shown in **FIG. 5**, the sample chromatogram consist of 16 different peaks, with a major family of proteins having molecular weights between 45-70 kDa, which overlaps with a molecular weight range of the most abundant proteins determined by 2D-LC-MS/MS.

6.7 EXAMPLE 7: Thermal Stability

[00228] Adzuki bean protein isolate samples were run on a Malvern MicroCal VP Capillary Differential Scanning Calorimeter (DSC) with autosampler at a scan rate of 4° C/min from 4 to 100° C. Samples were resuspended in 100 mM Hepes, pH 8.6 and diluted to 0.5 mg/ml as determined by Pierce660™ in 10 mM Na₂HPO₄-NaH₂PO₄, pH 8.0 buffer. Melting temperatures were determined by the Malvern MicroCal VP Capillary MicroCal Origin 7 software routine using automatic buffer subtraction and automatic baseline arguments. As indicated in **FIG. 6**, the thermal denaturation temperature for the adzuki bean protein isolate is 81.2° C.

6.8 EXAMPLE 8: Characterization of Solubility and Turbidity Studies

[00229] Solubility of adzuki bean isolates was measured using the technique of nephelometry. Nephelometry measures the amount of light scattered within a liquid sample, and quantifies the turbidity with high sensitivity. A NEPHELOstar Plus plate reader (BMG Labtech) was used to perform solubility measurements in a 96 well plate based format. The NEPHELOstar uses a polarized helium-neon laser at 632.8 nm. The nephelometer settings that were used include a beam focus of 2.5 mm and intensity of 10 %. Orbital averaging was used, with a diameter of 3 mm. The measurement time per well was 0.26 s with a positioning delay (settling time) of 0.1 s. Before measurement each plate was subject to 10 s of double-orbital shaking at 500 rpm to homogenize the sample solutions within the wells. Solubility measurements were performed at room temperature. Clear, flat bottom 96 Well Greiner

Microplates were used. Solubility of the adzuki bean isolate was studied under various solvent conditions, including at pH 3, 5, and 7 as well as in the presence of 0, 0.4, 0.8, and 1 wt % NaCl. Citrate and disodium phosphate buffers were used to control the pH of the aqueous fractions. A concentration gradient of isolate ranging from 0 - 8.9 wt % protein was used to determine the protein solubility at all 12 solvent conditions (3 pHs x 4 NaCl concentrations). Each measurement was run in triplicate to ensure reproducibility.

[00230] Solubility was determined by performing a linear fit of the data of Relative Nephelometer Units (RNUs) versus protein concentration. Two lines were fit and the linear regression was optimized to obtain two lines of best fit for the increase in RNU that occurs as protein concentration increases. The solubility value was determined to be the protein concentration at which these two lines intersected one another. Data provided in **FIGS. 7, 8 and 9** corresponds to these values of solubility.

6.9 EXAMPLE 9: Characterization of Foam Stability

[00231] Foam stability measurements were performed on a Dynamic Foam Analyzer (DFA100) instrument from Krüss. Three measurement modes were used to collect the maximal amount of information on foam stability: foam and liquid level height (measured via light scattering), structure (measured via image analysis of time lapse images of foam), and liquid content (measured via conductivity measured along a string of electrodes placed at different heights throughout the sample). Advance software was used for data analysis. The following instrument settings were used: air flow rate of 0.2 L/min, with light illumination of 40 % for height measurements and light illumination of 75% for structure measurements. 45 mL of aqueous protein solution was used and twice that volume (90 mL) of air was mechanically purged through the liquid. Air was passed through a 16 - 40 um sintered glass frit in the DFA100. The stability of the foam was evaluated over a time period of 10 minutes, which began immediately after the purging of air through the sample. A data sampling interval of 6 frames per minute (fpm), resulting in 60 images that were analyzed for bubble structure. The camera was positioned at a height of 55 mm above the bottom of the measurement vessel.

[00232] Aqueous protein samples were prepared under various solvent conditions, including at pH 3, 5, and 7 and protein concentrations of 4.2 and 8.9 wt % protein. Citrate and disodium phosphate buffers were used to control the pH of the aqueous fractions. Each sample was run in triplicate.

[00233] The stability of the foam was calculated as the foam index. It is a measure of the maximum foam height and the decay of the foam height over time. The total height of the foam and solution was integrated in time from the end of the air purge until the end of the experiment (10 minutes). This integration incorporates the effects of both the maximum foaming capacity

(as described by the maximum height) and the stability (determined by the loss in height of the foam and solution). A high foam index indicates a well-performing material for foaming. Data in **FIG. 10** reports the foam quality of Adzuki Bean Isolates and several reference materials.

6.10 EXAMPLE 10: Characterization of Emulsion Stability

[00234] Emulsion stability measurements were performed on oil in water emulsions containing adzuki bean protein isolate in order to study the emulsification properties of the isolates. First, aqueous solutions of isolate under various solvent conditions were prepared. Final protein concentrations in the emulsions were 4.2 and 8.9 wt % protein, and aqueous fractions were prepared at pH 3, 5, and 7 as well as in the absence and presence of NaCl (1 wt %). Citrate and disodium phosphate buffers were used to control the pH of the aqueous fractions. Aqueous solutions were mixed by vortexing at high speed for about 10 seconds. Canola oil was added at a mass ratio of $\frac{1}{3}$ of the total volume via serological pipette and vortexed at high speed for 10 seconds. The total volume (about 15 mL) was homogenized at 5000 rpm using a 20 mm saw tooth probe with a Pro Scientific PRO25D homogenizer for 4 minutes. The emulsion was then distributed into 4 mL glass vials using a positive displacement pipette, dispensing 3 mL of sample per glass vial. Each sample was run in triplicate. Directly prior to measurement the sample was homogenized for 4 min at 5000 rpm using a 7 mm diameter saw tooth probe with the Pro Scientific PRO25D homogenizer.

[00235] A Formulation Turbiscan Lab instrument was used to study emulsion stability. This instrument uses light scattering to characterize phase separation within an emulsion. Raw data collected by the instrument includes values of transmittance and backscattering as a function of the height of the sample and time. Each sample was measured over a period of 10 minutes, with the backscattering of incident light on the sample measured at an interval of 25 s.

[00236] Backscattering data from Turbiscan measurements was processed in order to evaluate overall emulsion stability as a function of time. Backscattering (BS) at the final time point of 10 minutes was examined after subtracting a baseline of backscattering measured at time point 0 minutes (Equation 1). This change in backscattering (ΔBS) at 10 minutes over the entire height of the sample was used to extract an index of emulsion stability, referred to as the Stability index (SI) (Equation 2, **FIG. 11**). The stability index is essentially the area under the curve of ΔBS versus height, and is defined by the equations shown below.

$$\Delta BS_{h,t} = BS_{h,t} - BS_{h,0} \quad (1)$$

$$SI = \frac{\sum_{h=0}^H |\Delta BS_{h,t} - \Delta BS_{h,t-1}|}{H} + SI_{t-1} \quad (2)$$

The higher the stability index, the less stable the emulsion. Lower stability indices indicate higher emulsion stability.

6.11 Example 11: Gelation: Structure Building Properties and Study of Effect of Increasing Temperature

[00237] Gelation of high purity protein isolates of adzuki bean were characterized with dynamic oscillatory rheology. A discovery hybrid rheometer (DHR-1, TA Instruments) equipped with a flat parallel plate geometry (40 mm diameter) was used to monitor the material's viscoelastic properties as a result of increasing temperature. Samples were prepared at 16.7 % protein concentration at pH values of 3, 5, and 7 and in the absence and presence of sodium chloride (1 wt %). Citrate and disodium phosphate buffers were used to control the pH of the aqueous fractions. About 1.7 mL of sample was loaded onto the lower plate of the rheometer and was trimmed according to standard procedures. A solvent trap was loaded with 1 mL of distilled H₂O to prevent evaporation of water within the sample as a result of the increase in temperature during the measurement.

[00238] The storage (G') and loss (G'') modulus were continuously recorded during a temperature ramp from 30 to 95 °C at a heating rate of 2 °C/min under small deformation conditions (0.1 % strain) at a constant angular frequency of 10 rad/s followed by a 5 minute hold at 95 °C. After this hold, the temperature of the material was reduced to 50 °C and an amplitude sweep test from 0.01 to 100 % strain was carried out at a constant frequency of 10 rad/s in order to characterize the gelled material's linear viscoelastic region. Each sample was run in triplicate.

[00239] Rheology data was analyzed to extract certain features which are pertinent to characterizing the behavior of gels produced by the isolate under the conditions defined above. For simplicity, three such features will be discussed herein: onset gelation temperature, gel strength, and gel elasticity.

[00240] Onset gelation temperature was extracted from the raw data and interpreted as the inflection point of the phase angle versus temperature over the range examined. Upon gelation events the phase angle decreases markedly as a function of temperature and can be used as a precise measure of the temperature at which the material underwent a distinct transition in its internal structure. See **FIG. 12**.

[00241] Gel strength was extracted from the raw data and defined as the oscillation strain (%) at which the storage modulus exceeded the linear viscoelastic range as a function of oscillation strain. Gel elasticity was likewise extracted from the raw data of the storage modulus versus oscillation strain, and was defined as the stress (Pa) at which the storage modulus exceeded the linear viscoelastic range as a function of oscillation strain. See **FIG. 13**.

6.12 EXAMPLE 12: Viscosity: Flow Properties of Isolates from Adzuki Bean

[00242] Viscosity measurements were performed on a TA Instruments DHR-1 rheometer. Samples were prepared at 16.7 % protein concentration at pH values of 3, 5, and 7 and in the

absence and presence of sodium chloride (1 wt %). Citrate and disodium phosphate buffers were used to control the pH of the aqueous fractions. A flat parallel plate geometry was used for these rheological measurements. About 1.7 mL of sample was loaded onto the lower plate of the rheometer and was trimmed according to standard procedures.

[00243] The procedure used for the measurement of viscoelastic properties of the isolate consisted of a preshear step at 0.1 1/s for 30 seconds followed by an increasing flow ramp at 25 °C for a duration of 100 s varying the shear rate from 0.1 to 50 1/s and then a decreasing flow ramp at the same temperature and for the same duration decreasing the shear rate from 50 to 0.1 1/s. Each sample was run in triplicate.

[00244] A spline smoothed model was fit to viscosity as a function of shear rate for each of the two flow ramps (increasing and decreasing strain rate). Viscosity at zero shear rate was then extracted by extrapolating the spline model of the increasing flow ramp to zero shear rate. See **FIG. 14**. For extracting the yield stress, another linear model was fit to shear stress as a function of shear rate for each of the two flow ramps. The yield stress shown in **FIG. 15** is the intercept of the fit line of the increasing flow ramp.

[00245] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED:

1. A purified adzuki bean protein isolate comprising
an adzuki bean protein content of at least 60% by weight;
a globulin-type protein content of at least 50% by weight of the plant protein;
a reduced oxidative enzyme activity relative to an otherwise unmodified source of the plant protein; and
one or more modulated organoleptic properties that differ from the otherwise unmodified source of the adzuki bean protein.
2. The purified adzuki bean protein isolate of claim 1, characterized in that its isoelectric point ranges from about pH 5.2 to about pH 6.0.
3. The purified adzuki bean protein isolate of claim 1, characterized in that its isoelectric point ranges from about pH 5.4 to about pH 6.0.
4. The purified adzuki bean protein isolate of claim 1, characterized in that its isoelectric point ranges from about pH 5.6 to about pH 6.0.
5. The purified adzuki bean protein isolate of claim 1, characterized in that its isoelectric point is pH 5.6, 5.7, 5.8, 5.9 or 6.0.
6. The purified adzuki bean protein isolate of any one of claims 1 to 5, wherein the modulated organoleptic properties comprise a reduction or absence in one or more of the following: astringent, beany, bitter, burnt, buttery, nutty, sweet, sour, fruity, floral, woody, earthy, beany, spicy, metallic, sweet, musty, grassy, green, oily, vinegary neutral and bland flavor or aromas.
7. The purified adzuki bean protein isolate of any one of claims 1 to 6, wherein the reduced oxidative enzymatic activity comprises a reduction of oxidization of lipids or residual lipids relative to an otherwise unmodified source of the adzuki bean protein.
8. The purified adzuki bean protein isolate of any one of claims 1 to 7, wherein the reduced oxidative enzymatic activity comprises a reduction in the amount of lipids or residual lipids in

the isolate relative to the amount of lipids in an otherwise unmodified source of the adzuki bean protein.

9. The purified adzuki bean protein isolate of any one of claims 1 to 8, wherein the reduced oxidative enzymatic activity comprises a reduction in the amount of lipoxygenase (EC 1.13.11.-) relative to an otherwise unmodified source of the adzuki bean protein.

10. The purified adzuki bean protein isolate of any one of claims 1 to 9, wherein the purified protein isolate exhibits one or more functional properties selected from emulsification, water binding, foaming, gelation, crumb density, structure forming, texture building, cohesion/adhesion, elasticity/springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen/shine addition, freeze/thaw stability, and color.

11. An edible composition comprising the purified adzuki bean protein isolate of any one of claims 1 to 10, wherein the composition is selected from the group consisting of a gel, a foam, an emulsion, and an aqueous solution.

12. The purified adzuki bean protein isolate of any one of claims 1 to 11, wherein the isolate comprises greater than 75% adzuki bean protein.

13. The purified adzuki bean protein isolate of any one of claims 1 to 12, wherein the adzuki bean protein comprises at least 60% globulin-type protein.

14. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein comprises 11S globulin.

15. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein comprises 8S globulin.

16. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein comprises 7S globulin.

17. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein is a protein having at least 50% identity to SEQ ID NO. 1.
18. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein is a protein having at least 50% identity to SEQ ID NO. 2.
19. The purified adzuki bean protein isolate of any one of claims 1 to 13, wherein the globulin-type protein is a protein having at least 50% identity to SEQ ID NO. 3.
20. The purified adzuki bean protein isolate of any one of claims 1 to 19, wherein the globulin-type protein has been enriched in the isolate in relation to the amount of protein found in the plant source of the isolate.
21. The purified adzuki bean protein isolate of claim 20, wherein the enrichment is at least 5%, 10%, 15%, 20% or greater than 20%, in relation to the amount of protein found in the plant source of the isolate.
22. The purified adzuki bean protein isolate of any one of claims 1 to 21, wherein one or more compounds selected from the group consisting of allergens, anti-nutritional factors, and environmental contaminants has been reduced in the isolate in relation to the amount of the one or more compounds found in the plant source of the isolate.
23. The purified adzuki bean protein isolate of any one of claims 1 to 22, contacted with one or more co-ingredients selected from the group consisting of a hydrocolloid, a gum, a phosphate, and transglutaminase.
24. The purified adzuki bean protein isolate of any one of claims 1 to 23, wherein the phosphate is disodium phosphate.
25. An egg substitute comprising the purified adzuki bean protein isolate of any one of claims 1 to 23, wherein the egg substitute is characterized as having one or more organoleptic properties similar to an egg.

26. The egg substitute of claim 25, wherein the egg substitute exhibits at least one functional property similar or equivalent to a corresponding functional property of an egg.

27. The egg substitute recited in claim 25, wherein the at least one functional property comprises emulsification, water binding capacity, foaming, gelation, crumb density, structure forming, texture building, cohesion, adhesion, elasticity, springiness, solubility, viscosity, fat absorption, flavor binding, coagulation, leavening, aeration, creaminess, film forming property, sheen addition, shine addition, freeze stability, thaw stability, or color.

28. A food or beverage composition comprising the purified adzuki bean protein isolate of any one of claims 1 to 27.

29. The food or beverage composition of claim 28, wherein the food or beverage composition is selected from the group consisting of an edible egg-free emulsion, egg analog, egg-free scrambled eggs, egg-free patty, egg-free pound cake, egg-free angel food cake, egg-free yellow cake, egg- and dairy-free cream cheese, egg-free pasta dough, egg-free custard, egg-free ice cream, and dairy-free milk, cream cheese, pasta dough, pasta, milk or milk-like beverage, a food product comprising said milk or milk-like beverage, custard, ice cream, frozen desert, meat replicas, deli meat replicas, emulsified extruded meats, sausages, fish cake replicas, dips, fillings, spreads, chips, and crackers.

30. The purified adzuki bean protein isolate of any one of claims 1 to 24, wherein the adzuki bean protein isolate is produced by a method comprising:

- (a) extracting one or more adzuki bean proteins from a source of adzuki bean protein in an aqueous solution at a pH between about 6.5 and 10.0; and
- (b) precipitating the extracted adzuki bean protein at a pH in a range of pH 5.2 to pH 6.0.

31. A method for producing a purified adzuki bean protein isolate comprising:

- (a) extracting one or more adzuki bean proteins from a source of adzuki bean protein in an aqueous solution at a pH between about 6.5-10.0;
- (b) precipitating the adzuki bean protein at a pH near its isoelectric point of a globulin-rich fraction, or a pH between about 5.0-6.0; or fractionating and concentrating the plant protein using microfiltration or ultrafiltration; and

(c) recovering a purified adzuki bean protein isolate comprising
an adzuki bean protein content of at least 60% by weight;
a globulin-type protein content of at least 50% by weight of the adzuki bean protein;
a reduced oxidative enzyme activity relative to an otherwise unmodified source of the
adzuki bean protein; and
one or more modulated organoleptic properties that differ from the otherwise unmodified
source of the adzuki bean protein.

32. The method of claim 31, wherein the extraction step is performed within a pH range of about 6.5-8.0.

33. The method of claim 31, wherein the precipitating step is performed within a pH range of about pH 5.2 to pH 6.0.

34. The method of claim 31, wherein the precipitating step is performed within a pH range of about pH 5.4 to pH 6.0.

35. The method of claim 31, wherein the precipitating step is performed within a pH range of about pH 5.6 to pH 6.0.

36. The method of claim 31, wherein the precipitating step is performed at pH 5.6, 5.7, 5.8, 5.9 or 6.0.

37. The method of any one of claims 31 to 36, further comprising the step of treating the plant protein with activated carbon, charcoal or clay.

38. The method of any one of claims 31 to 37, further comprising the step of cryo-precipitating at a temperature of about 1 to 4°C.

39. The method of any one of claims 31 to 38, further comprising the recovery of the purified protein isolate by centrifugation.

40. The method of any one of claims 31 to 39, further comprising rehydrating the purified protein isolate to 80% moisture content and adjusting to about pH 6.0.

41. The method of any one of claims 31 to 40, wherein the method further comprises one or more additional steps selected from the group consisting of: diluting aqueous protein in calcium salt solution; filtering protein via heat treatment; diafiltering via selective membranes; diluting protein in buffer to adjust pH per target application; concentrating protein depending upon application; and drying protein depending upon application.
42. The method of one of claims 31 to 41, wherein the method comprises reducing lipophilic off-flavors, substrates or co-factors.
43. The method of one of claims 31 to 41, wherein the method modulates off-flavors or aromas; modulates desired flavors; or provides sensory impressions similar or equivalent to a reference food product.
44. The method of one of claims 31 to 43, further comprising the step of formulating the purified adzuki bean protein isolate in a food or beverage composition.
45. A purified adzuki bean protein isolate produced by the method of any one of claims 31-44.
46. A food or beverage composition produced by a method comprising the method of any one of claims 31-44.
47. An egg substitute produced by the method of any one of claims 31-44.
48. A purified adzuki bean protein isolate comprising:
a transglutaminase-modified adzuki bean protein content of at least 60% by weight;
a globulin-type protein content of at least 50% by weight of the plant protein;
a reduced oxidative enzyme activity relative to an otherwise unmodified source of the plant protein; and
one or more modulated organoleptic properties that differ from the otherwise unmodified source of the plant protein.

49. The purified protein isolate of claim 48, wherein said purified adzuki bean protein isolate is modified by 0.0001% to 0.0125% transglutaminase.

50. A method for producing a purified protein isolate comprising:

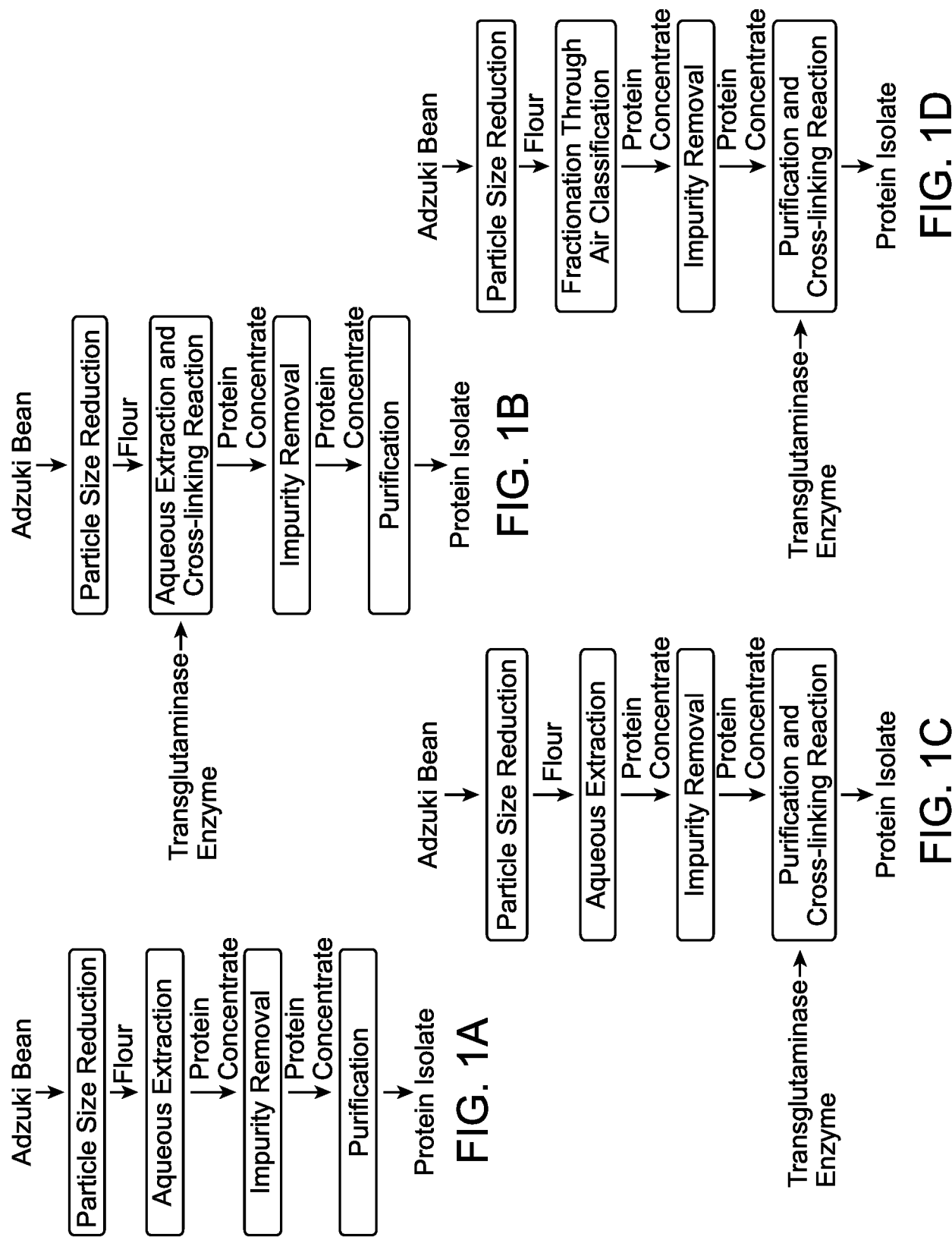
- a. extracting one or more plant protein from a source in an aqueous solution at a pH between about 6.5-10.0;
- b. precipitating the plant protein at pH near its isoelectric point of a globulin-rich fraction or a pH between about 5.2-6.0; or fractionating and concentrating the plant protein using microfiltration or ultrafiltration;
- c. recovering the purified protein isolate comprising
 - a plant protein content of at least 60% by weight;
 - a globulin-type protein content of at least 50% by weight of the plant protein;
 - a reduced oxidative enzyme activity relative to an otherwise unmodified source of the plant protein; and
 - one or more modulated organoleptic properties that differ from the otherwise unmodified source of the adzuki bean protein; and
- d. modifying the adzuki protein with transglutaminase in the extraction step a. or the recovery step b.

51. The method for producing a purified protein isolate of claim 50, wherein said step d. of modifying the plant protein with transglutaminase in the extraction step a. or the recovery step b. comprises contacting the adzuki bean protein with 0.0001% to 0.0125% transglutaminase.

52. The method for producing a purified protein isolate of claim 50 or 51, wherein said purified protein isolate is stably cross-linked.

53. The method for producing a purified protein isolate of any one of claims 50 to 52, wherein said purified protein isolate is essentially free of lipooxygenase or enzymes that can oxidize lipids.

54. The method for producing a purified protein isolate of any one of claims 50 to 53, wherein said transglutaminase is encapsulated or immobilized on a solid substrate.



2 / 15

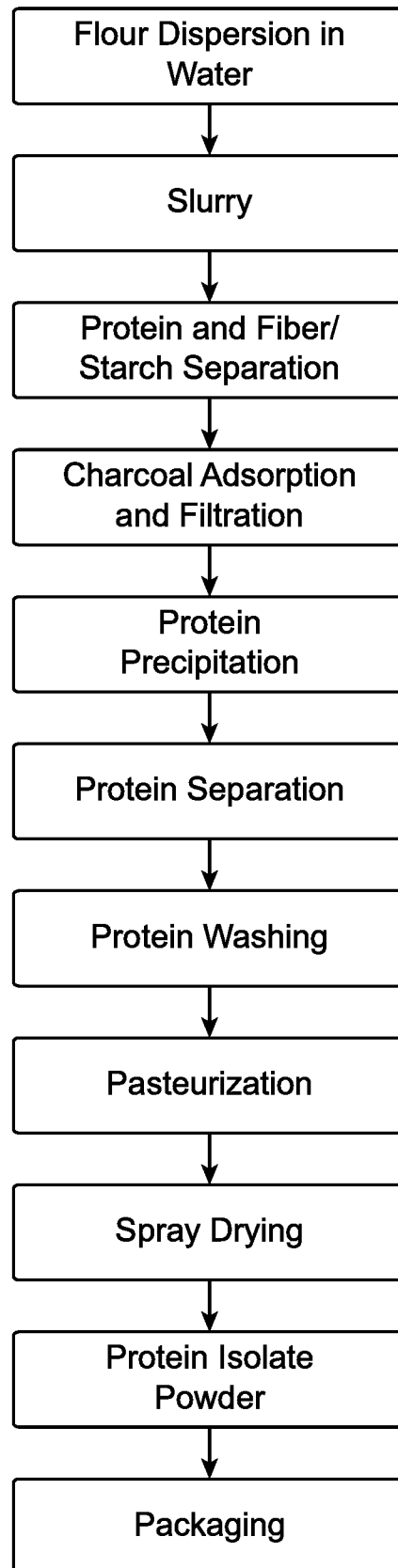


FIG. 2

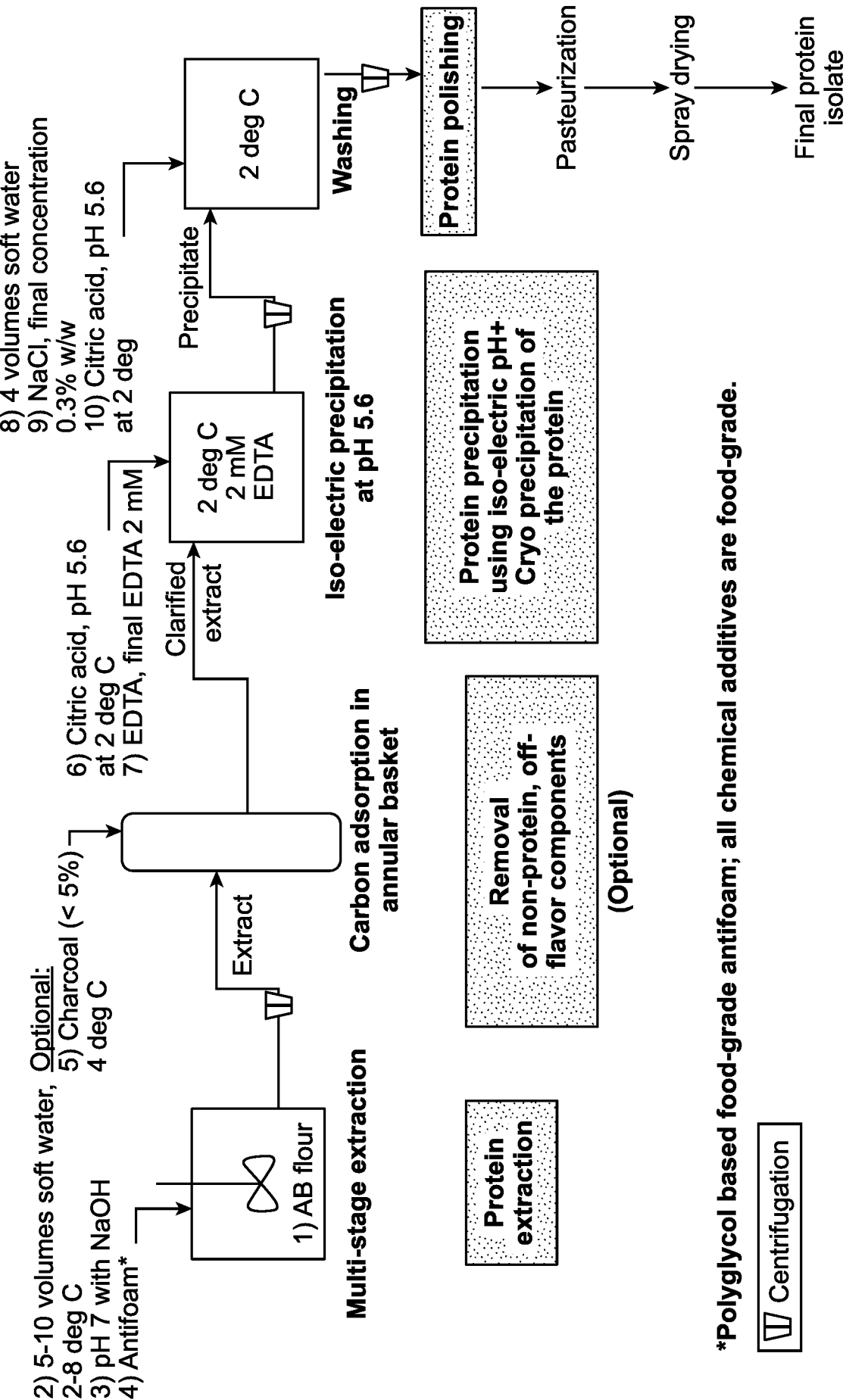


FIG. 3

4 / 15

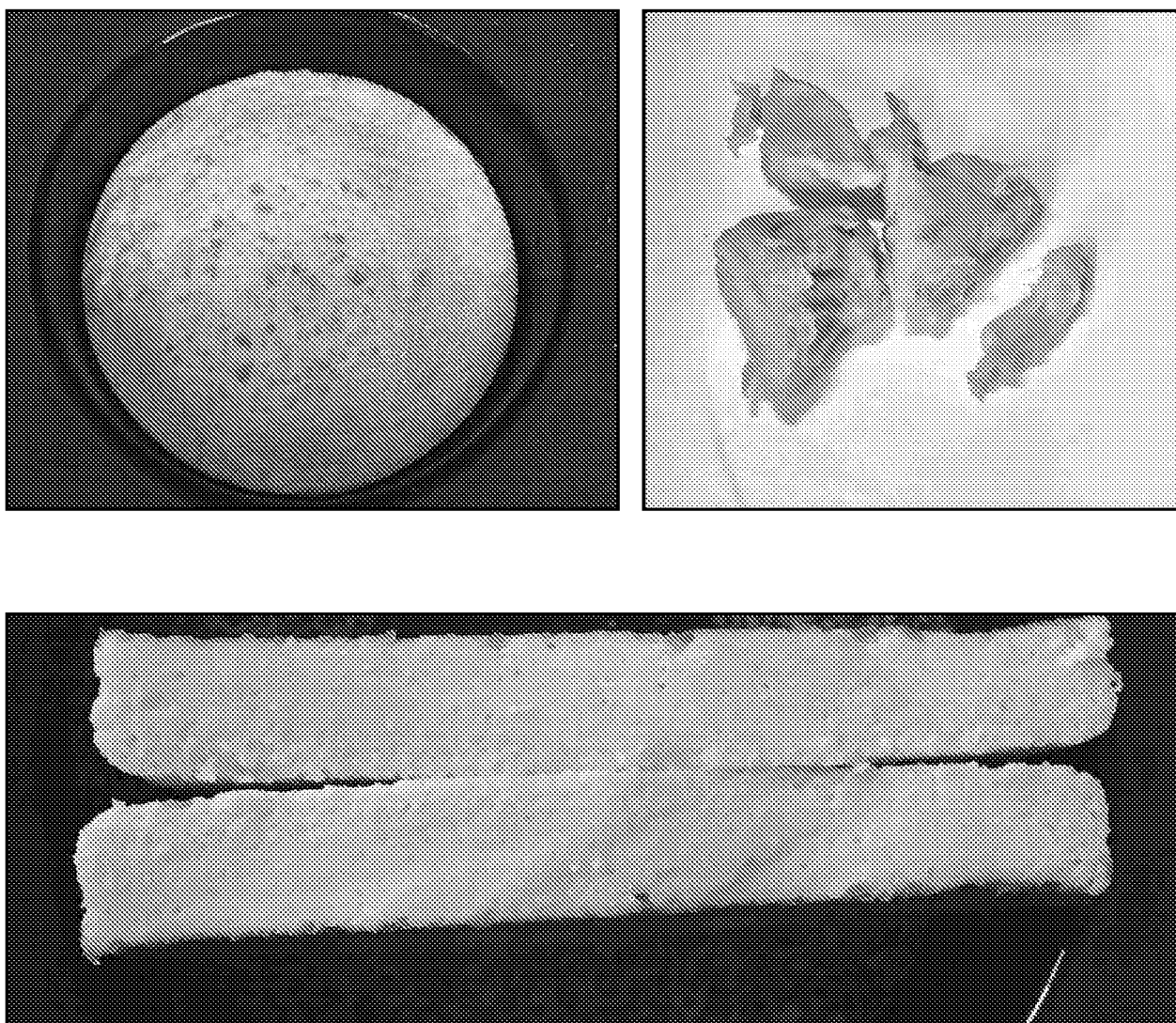


FIG. 4

5 / 15

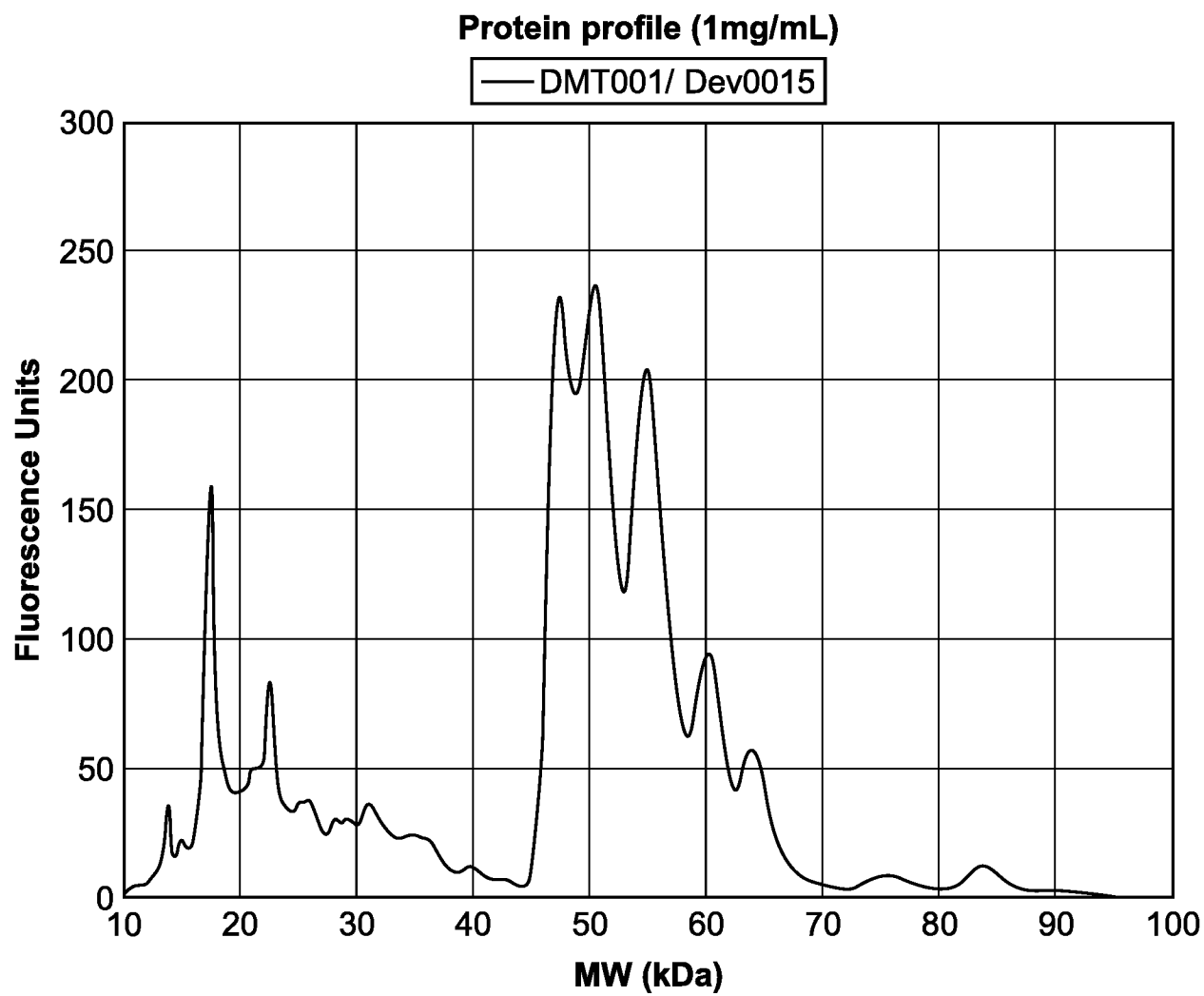


FIG. 5

6 / 15

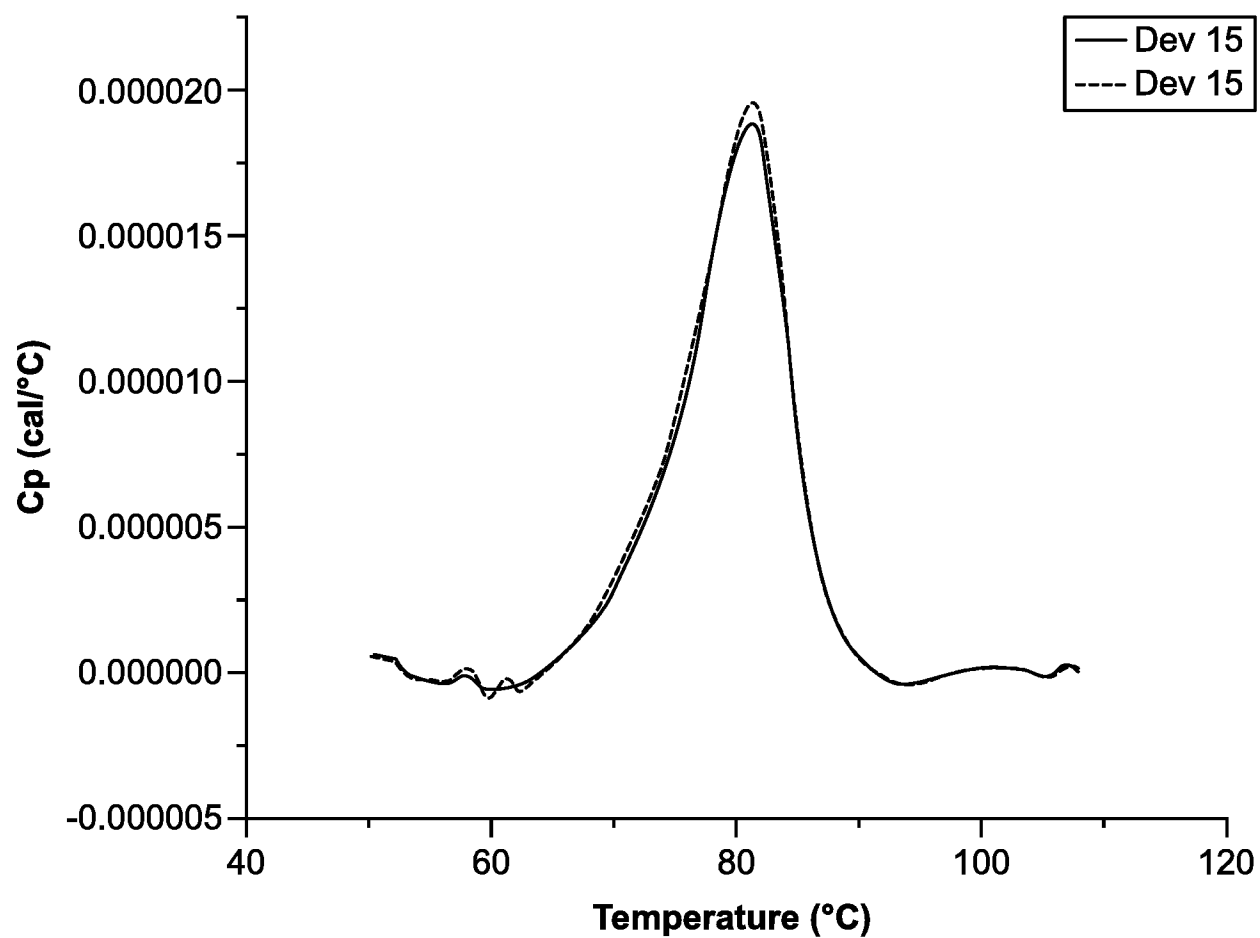


FIG. 6

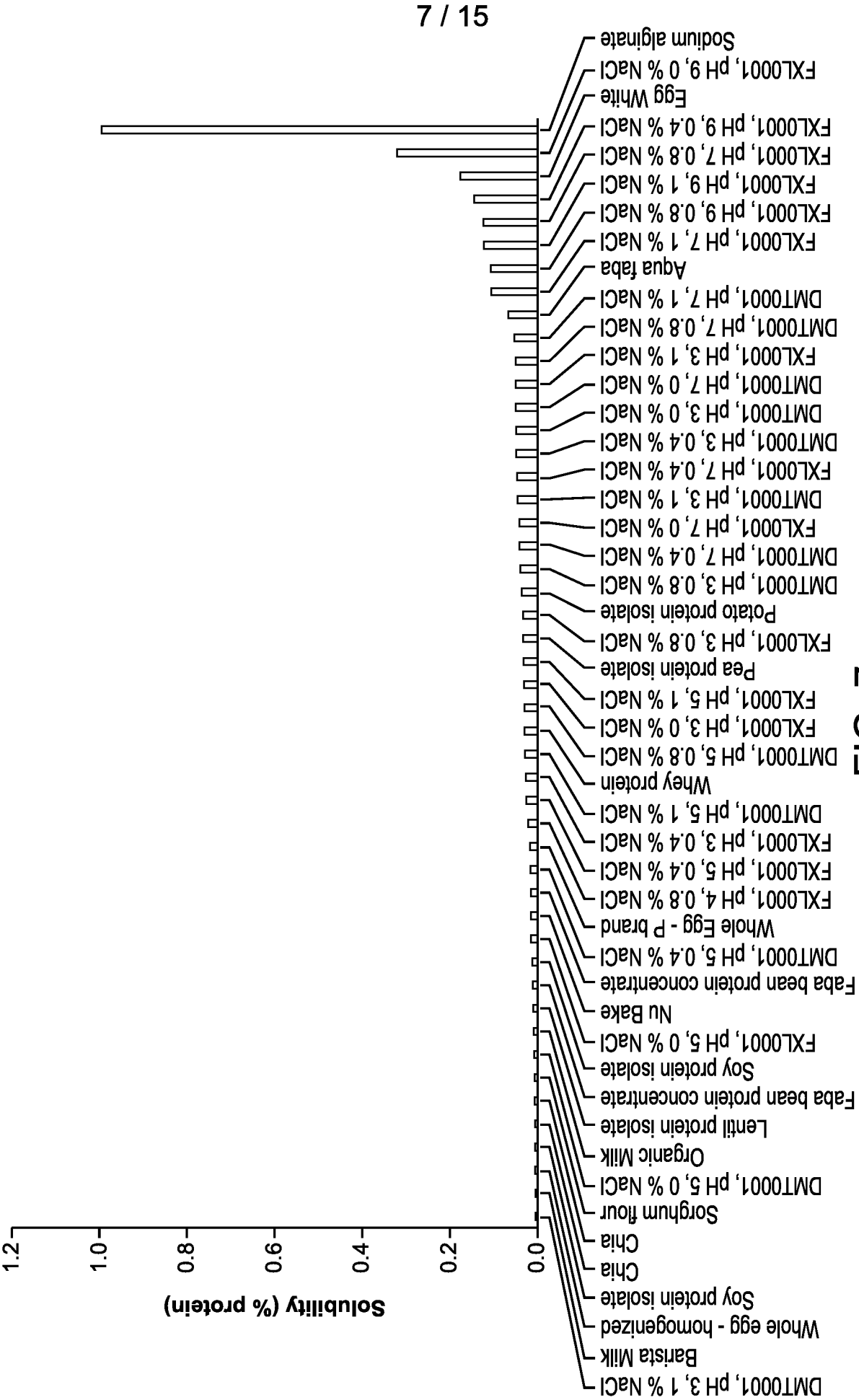
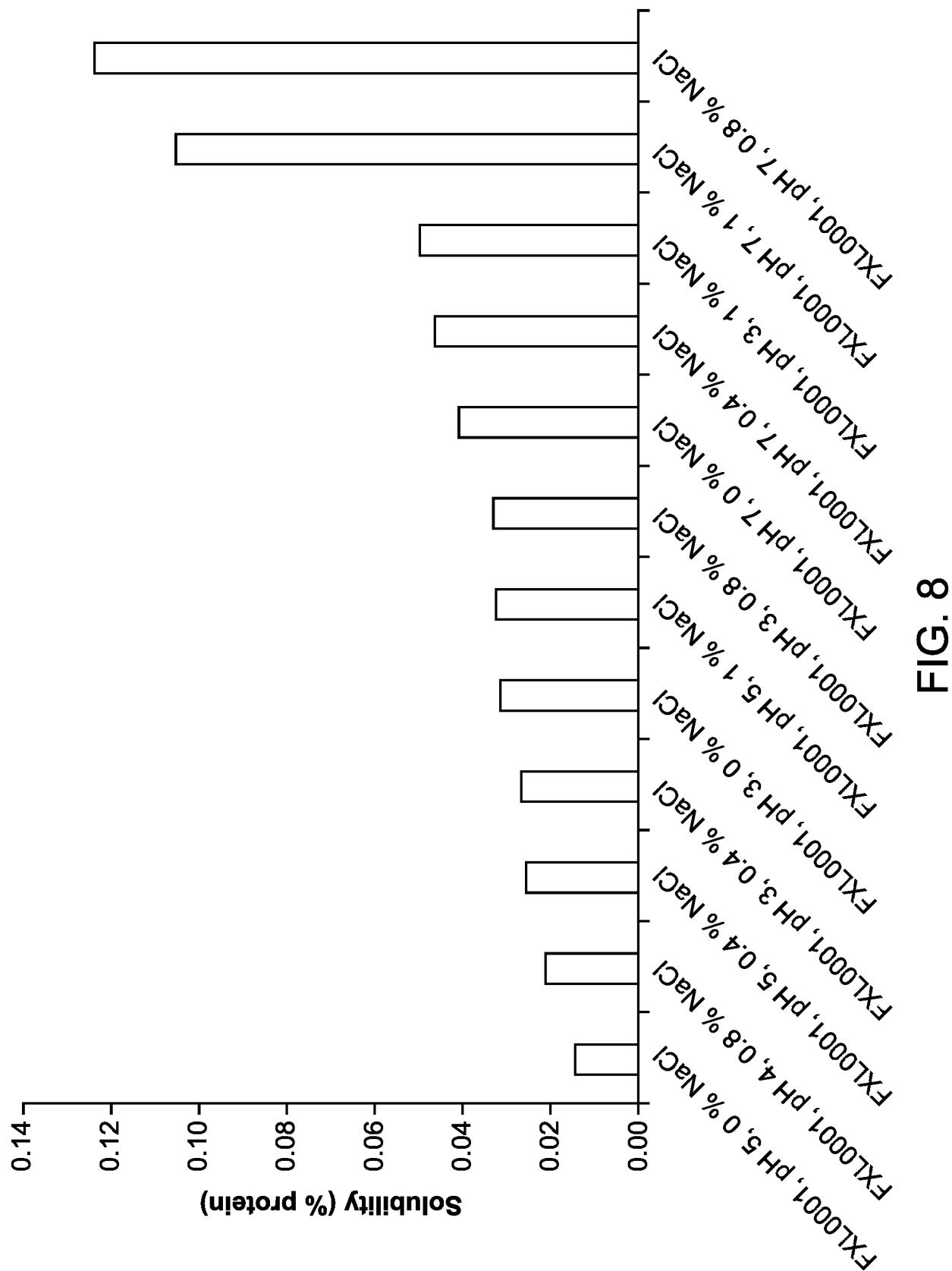
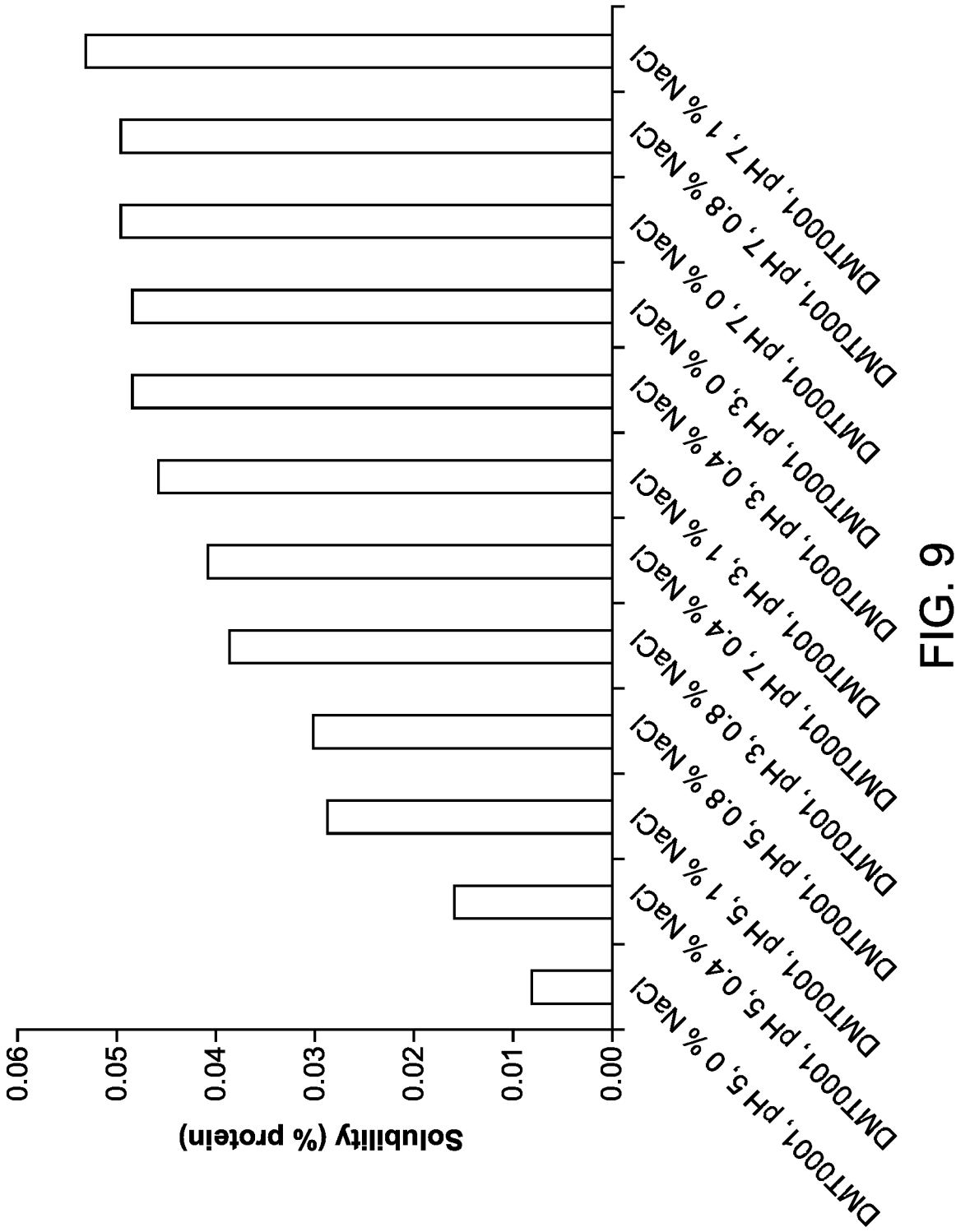


FIG. 7

8 / 15





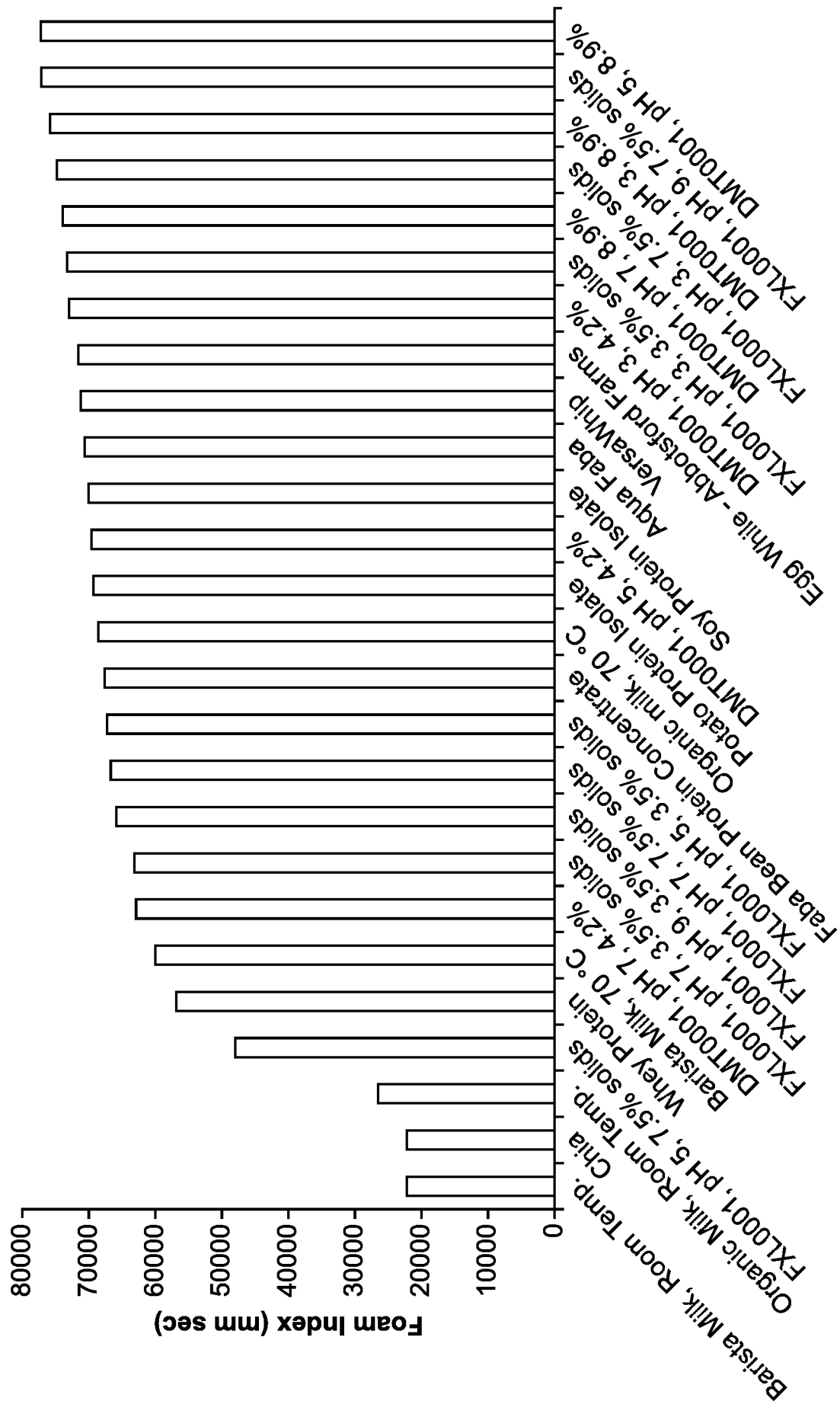


FIG. 10

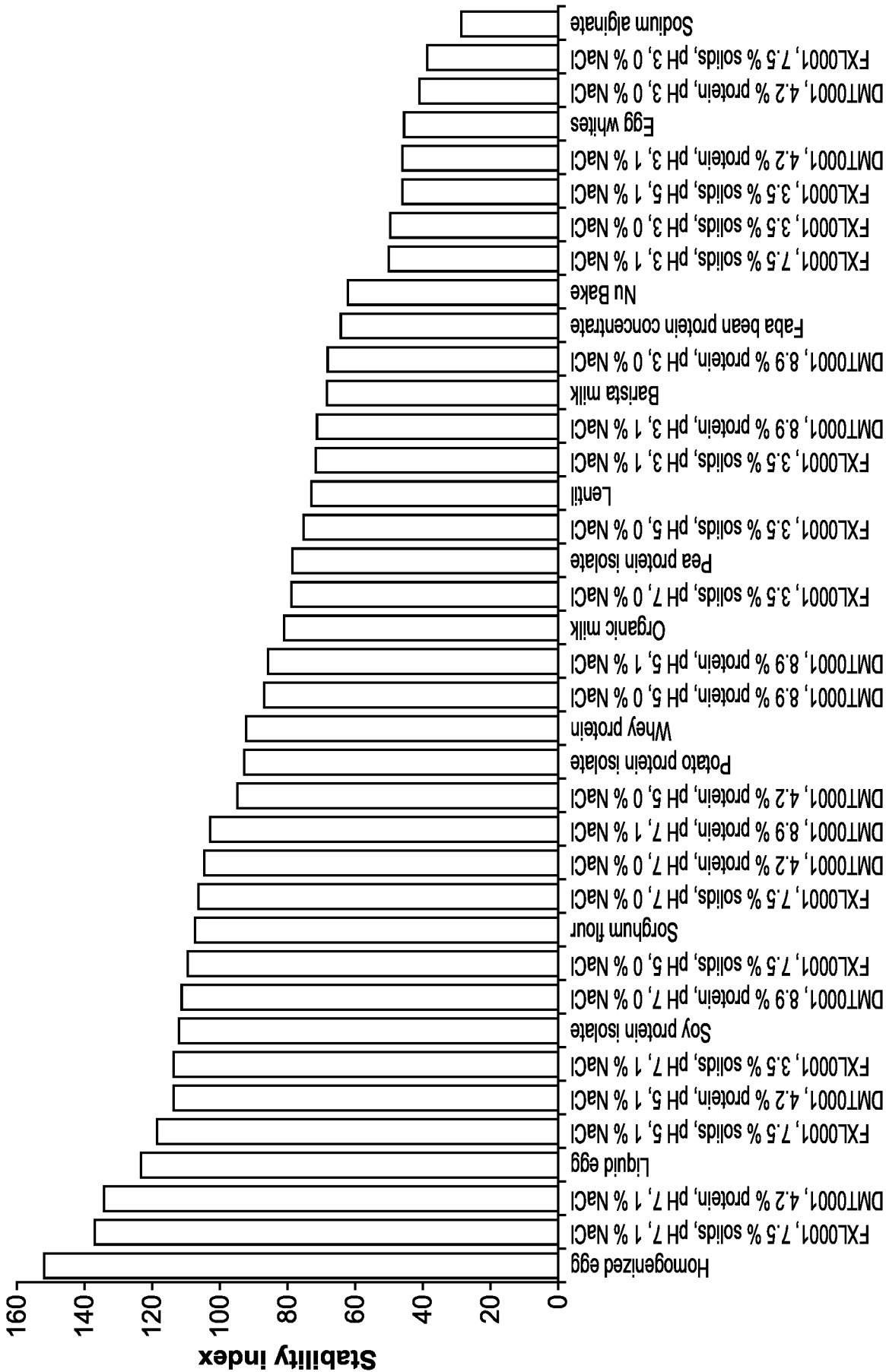


FIG. 11

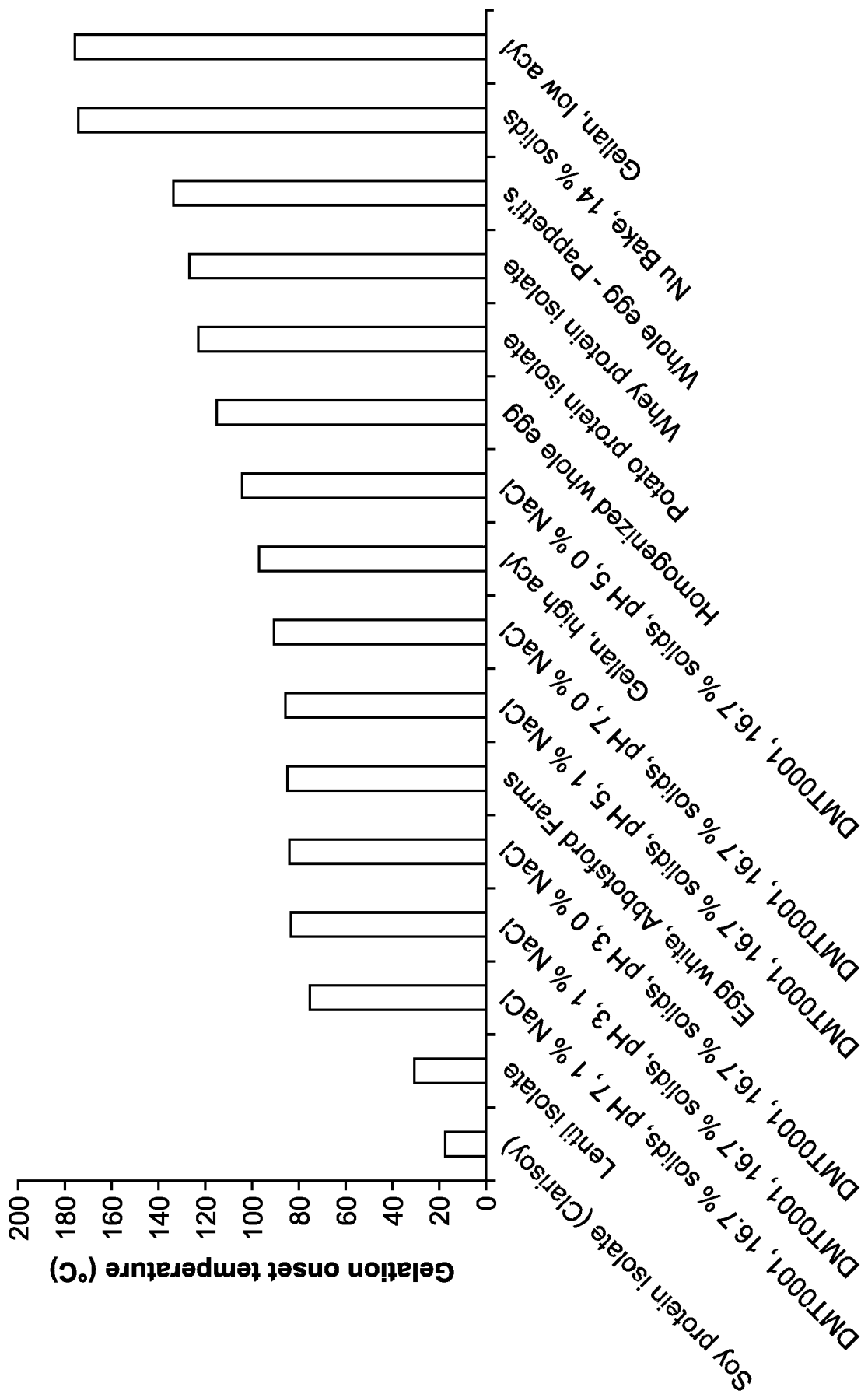


FIG. 12

13 / 15

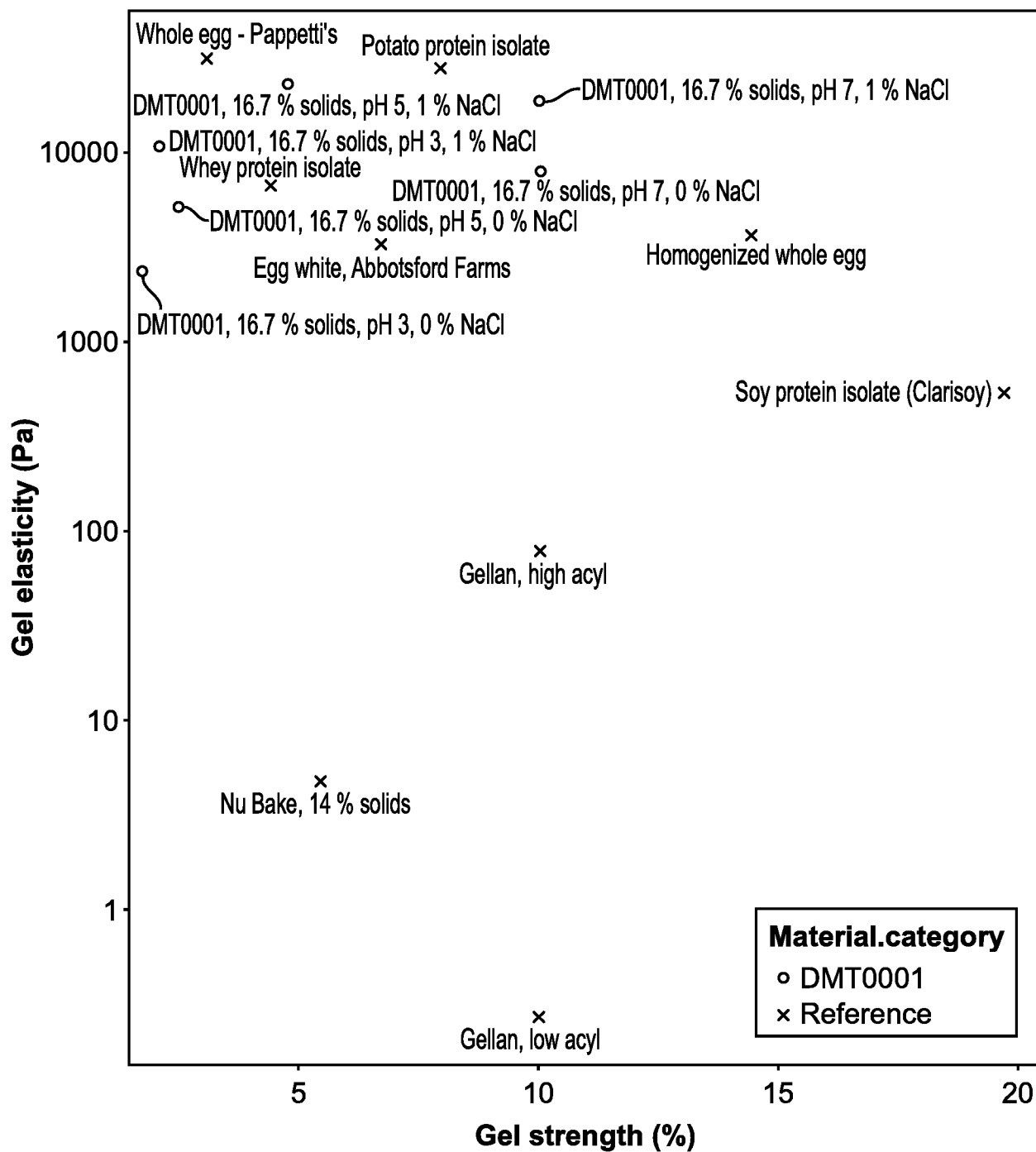
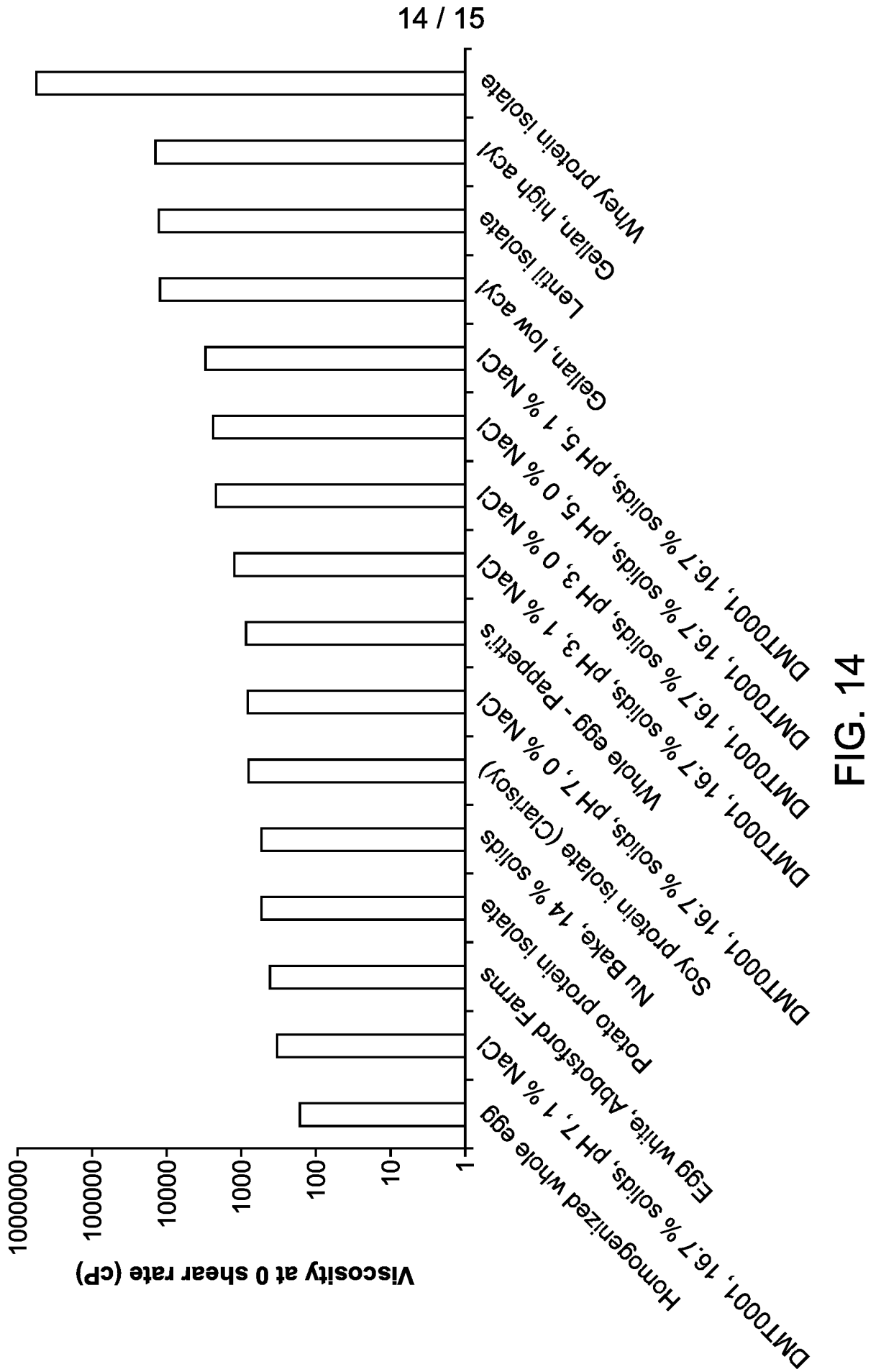
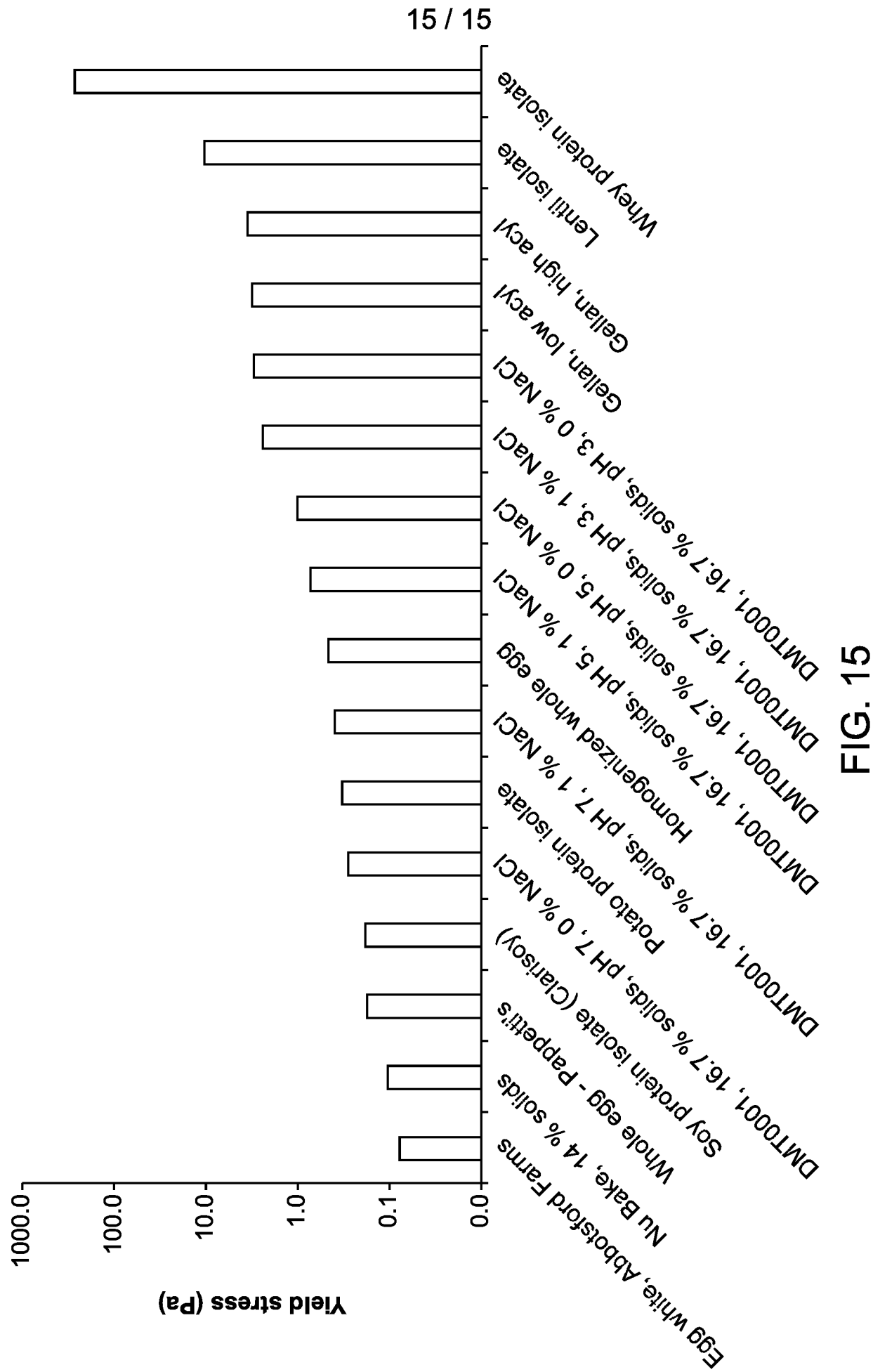


FIG. 13





INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/018523

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A23J1/14
 ADD.

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)
 A23J

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BARAC MIROLJUB B ET AL: "Comparative study of the functional properties of three legume seed isolates: adzuki, pea and soy bean", JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, SPRINGER (INDIA) PRIVATE LTD, INDIA, vol. 52, no. 5, 5 March 2014 (2014-03-05), pages 2779-2787, XP035483426, ISSN: 0022-1155, DOI: 10.1007/S13197-014-1298-6 [retrieved on 2014-03-05]	1-24, 30-46
Y	abstract page 2779, column 2 page 2780 page 2787, column 2 ----- -/-	25-29, 48-54



Further documents are listed in the continuation of Box C.



See patent family annex.

* 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

15 May 2017

Date of mailing of the international search report

07/06/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040,
 Fax: (+31-70) 340-3016

Authorized officer

Chavanne, Franz

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/018523

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/067453 A1 (BEYOND EGGS INC [US]; TETRICK JOSHUA [US]; BOOT JOHANNNES HENDRIKUS AN) 10 May 2013 (2013-05-10)	46,47
Y	abstract paragraphs [0072], [0073] -----	25-29
Y	WO 03/017777 A1 (FRAUNHOFER GES FORSCHUNG [DE]; SCHAEFER CHRISTIAN [DE]; FUNDA ELGER [D]) 6 March 2003 (2003-03-06) the whole document -----	48-54
A	TANG C H ET AL: "Properties of cast films of vicilin-rich protein isolates from Phaseolus legumes: Influence of heat curing", LWT- FOOD SCIENCE AND TECHNOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 42, no. 10, 1 December 2009 (2009-12-01), pages 1659-1666, XP026502423, ISSN: 0023-6438, DOI: 10.1016/J.LWT.2009.05.020 [retrieved on 2009-05-27] the whole document -----	31-36,50
A	EP 2 984 938 A1 (FUJI OIL CO LTD [JP]) 17 February 2016 (2016-02-17) abstract paragraphs [0021] - [0023], [0054], [0056] -----	31-36,50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/018523

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013067453	A1	10-05-2013	
		AU 2012332180 A1	12-06-2014
		AU 2017200379 A1	09-02-2017
		CA 2873413 A1	10-05-2013
		CN 104203000 A	10-12-2014
		EP 2773223 A1	10-09-2014
		HK 1202230 A1	25-09-2015
		US 2014356507 A1	04-12-2014
		US 2017042204 A1	16-02-2017
		WO 2013067453 A1	10-05-2013

WO 03017777	A1	06-03-2003	
		CA 2457132 A1	06-03-2003
		DE 10141166 A1	13-03-2003
		EP 1418818 A1	19-05-2004
		US 2004241284 A1	02-12-2004
		WO 03017777 A1	06-03-2003

EP 2984938	A1	17-02-2016	NONE
