PROTEIN COMPOSITIONS AND CONSUMABLE PRODUCTS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application Ser. No. 62/873,154, filed July 11, 2019 and US Provisional Patent Application Ser. No. 62/873,159, filed July 11, 2019. The entire contents of the aforementioned patent applications are incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 10, 2020, is named 49160-714.601_ST25.txt and is 95,504 bytes in size.

BACKGROUND

[0003] Proteins are important dietary nutrients. They can serve as a fuel source or as sources of amino acids, including the essential amino acids that cannot be synthesized by the body. The daily recommended intake of protein for healthy adults is 10% to 35% of a person's total calorie needs, and currently the majority of protein intake for most humans is from animal-based sources. In addition, athletes and bodybuilders may rely upon increased protein consumption to build muscle mass and improve performance. With the world population growth and the coinciding growth in global food demand, there is a need to provide alternative sustainable, non-animal-based sources of proteins as useful source of protein for daily diet, dietary supplementation and sports nutrition.

SUMMARY

[0003a] In one aspect, provided herein is a composition comprising a recombinant ovomucoid protein (rOVD), wherein the rOVD comprises at least one glycosylated asparagine residue and the rOVD is substantially devoid of N-linked mannosylation, wherein the rOVD forms a clear solution at a concentration of from about 26% to about 30% w/v in an aqueous solution, at a pH of from about 2.5 to about 6, and at room temperature; and/or wherein a solution comprising the rOVD shows substantially higher solution clarity as compared to a whey protein solution at a concentration of 9% (w/v) in an aqueous solution, at a pH of from about 2 to about 6, and at the same temperature.

[0003b] In one aspect, provided herein is a beverage composition comprising the composition as described herein and at least one consumable liquid, wherein the rOVD forms a clear solution in

the beverage composition, and wherein the concentration of rOVD is from about 0.1% w/v to about 30% w/v in an aqueous solution, at a pH of from about 2.5 to about 6, and at room temperature.

[0004] An ~~aspect~~example of the present disclosure is a composition comprising a recombinant ovomucoid protein (rOVD). The rOVD comprises at least one glycosylated asparagine residue and the rOVD is substantially devoid of N-linked mannosylation.

[0005] In some ~~embodiment~~examples, each glycosylated asparagine comprises a single N-acetylglucosamine. The rOVD may comprise at least three glycosylated asparagine residues. In some cases, the rOVD is a secreted form of the rOVD protein. In various ~~embodiment~~examples, the composition is a powder. The composition may have a protein content of at least 30% rOVD protein, at least 40% rOVD protein, at least 50% rOVD protein, at least 60% rOVD protein, at least 70% rOVD protein, at least 80% rOVD protein, at least 85% rOVD protein, at least 90%

rOVD protein, or at least 95% rOVD protein on a weight/weight basis and/or a weight per total volume of composition basis. In some cases, the powder is capable of being dissolved in a liquid.

[0006] Another ~~aspect~~example of the present disclosure is a composition comprising a recombinant ovomucoid protein (rOVD). The composition is a powder formulated for human or animal consumption and the composition has a protein content of at least 70% rOVD protein, at least 80% rOVD protein, at least 85% rOVD protein, at least 90% rOVD protein, or at least 95% rOVD protein on a weight/weight basis and/or a weight per total volume of composition basis. **[0007]** The powder may comprise less than 15%, 12%, 10%, 8%, 6%, 5%, 3%, 2% or 1% moisture on a weight/weight basis and/or a weight per total volume of composition basis. The powder may comprise less than 30%, 27%, 25%, 22%, 20%, 17%, 15%, 12%, 10%, 8%, 5%, 3% or 1% free carbohydrate content. In some ~~embodiment~~examples, the powder is capable of being dissolved in a liquid.

[0008] In ~~embodiment~~examples, a composition comprises one or more additional ingredients selected from the group consisting of a flavoring, a coloring agent, a sweetener, an amino acid, a protein, an acidulant, a preservative, and ash. In some cases, the composition comprises less than 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, 0.75%, 0.5%, 0.25% or 0.1% ash. The amino acid may be selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof.

[0009] Yet another ~~aspect~~example of the present disclosure is a composition comprising a recombinant ovomucoid protein (rOVD). The composition is in a solid form formulated for human or animal consumption, wherein the rOVD provides protein fortification to the composition and at least one additional feature selected from the group consisting of mouthfeel, texture, hardness, stability to heat treatment, and stability to pH.

[0010] In various ~~embodiment~~examples, the rOVD comprises at least one asparagine residue linked to N-acetyl glucosamine and the rOVD is substantially devoid of N-linked mannosylation. The concentration of rOVD may be greater than about 5%, about 10%, about 15%, about 20%, or about 25% on a weight/weight basis and/or a weight per total volume of composition basis and/or a weight per total volume of composition basis. In some cases, the rOVD does not substantially alter the visible appearance or mouthfeel of the solid consumable composition as compared to a solid consumable composition lacking rOVD; the rOVD does not substantially alter the visible appearance or mouthfeel of the solid consumable composition as compared to a solid consumable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD; the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a solid consumable composition lacking rOVD; and/or the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the

rOVD. In some ~~embodiment~~examples, the solid consumable composition is a snack bar, a protein bar, a nutrition bar, an energy bar, or a protein supplement. In some cases, the solid consumable composition comprises one or more additional ingredients selected from the group consisting of a flavoring, a coloring agent, a sweetener, an amino acid, a protein, an acidulant, a preservative, and ash.

[0011] In an ~~aspect~~example, the present disclosure provides a composition comprising a recombinant ovomucoid protein (rOVD). The composition is a liquid formulated for human or animal consumption, wherein the rOVD provides protein fortification to the composition and at least one additional feature selected from the group consisting of solubility, mouthfeel, stability to heat treatment, and stability to pH.

[0012] In some cases, the composition has a protein content comprising at least 15% rOVD, at least 20% rOVD protein, at least 30% rOVD protein, or at least 40% rOVD protein on a weight/weight basis and/or a weight per total volume of composition basis. The composition may have a protein content comprising at least 5% rOVD, and in which the liquid consumable composition is substantially optically clear. In ~~embodiment~~examples, the composition has an optical clarity greater than a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD. In some cases, the rOVD does not substantially alter the visible appearance or mouthfeel of the liquid consumable composition as compared to a liquid consumable composition lacking rOVD; the rOVD does not substantially alter the visible appearance or mouthfeel of the liquid consumable composition as compared to a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD; the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a liquid consumable composition lacking rOVD; and/or the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD. The rOVD may remain substantially soluble after the liquid consumable composition has been heated to a temperature of between about 72°C and about 121°C. In some cases, the rOVD has a greater solubility, optical clarity or both solubility and optical clarity in the liquid following a heat treatment than the stability of whey protein, soy protein, or pea protein at the same concentration as the rOVD. In some ~~embodiment~~examples, the heat treatment comprises exposure of the liquid to a temperature of between about 72°C and about 121°C. The rOVD may have a solubility in the liquid greater than the solubility of whey protein, soy protein, or pea protein at the same concentration as the rOVD. In some cases, the liquid consumable composition has a pH of between about 2.0 and about 8.0.

[0013] In some ~~embodiment~~examples, a solid form formulated for human or animal consumption or a liquid formulated for human or animal consumption may comprise one or more additional ingredients selected from the group consisting of a flavoring, a coloring agent, a sweetener, an amino acid, a protein, an acidulant and a preservative. In various ~~embodiment~~examples, the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof. In some cases, the protein is a lysozyme protein, e.g., an egg white lysozyme (OVL). The ratio of rOVD to OVL may between about 60% rOVD:40% OVL and about 82% rOVD:18% OVL. The lysozyme may be a recombinant lysozyme protein. In some cases, the protein and/or the amino acid provides an improved amino acid balance to the solid form or the liquid. In ~~embodiment~~examples, a protein digestibility corrected amino acid score (PDCAAS) is equal to or greater than about 0.75, e.g., greater than or equal to about 0.8, 0.85, 0.90, 0.95 or the PDCAAS is about or is 1.0. The liquid consumable composition may comprise rOVD and OVL and the proteins are soluble and composition is optically clear.

[0014] In some cases, the liquid consumable composition is a beverage selected from the group consisting of a juice, a broth, a soup, a soda, a soft drink, a flavored water, a protein water, a fortified water, a carbonated water, a nutritional drink, an energy drink, a sports drink, a recovery drink, a heated drink, a coffee-based drink, a tea-based drink, a plant-based milk, a milk based drink, a non-dairy, plant based mild drink, infant formula drink, a meal replacement drink. In some ~~embodiment~~examples, the beverage comprises carbonation.

[0015] A liquid consumable composition may be a syrup comprising between 20% rOVD protein and at least 60% rOVD protein on a weight/weight basis and/or a weight per total volume of composition basis.

[0016] In some cases, the liquid consumable composition is an emulsion, e.g., a sauce, a gravy, or a salad dressing.

[0017] In another ~~aspect~~example, the present disclosure provides a composition comprising a recombinant ovomucoid protein (rOVD). The composition is in a semi-solid form formulated for human or animal consumption, in which the rOVD provides at least one additional feature selected from the group consisting of mouthfeel, texture, hardness, stability to heat treatment, and stability to pH.

[0018] In various ~~embodiment~~examples, the semi-solid consumable composition is a gummy, candy, jelly, syrup, gel, a gelled preparation. In some cases, the rOVD does not substantially alter the visible appearance or mouthfeel of the semi-solid consumable composition as compared to a semi-solid consumable composition lacking rOVD; the rOVD does not substantially alter the visible appearance or mouthfeel of the semi-solid consumable composition as compared to a semi-solid consumable composition containing whey protein, soy protein, or pea protein at the

same concentration as the rOVD; the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a semi-solid consumable composition lacking rOVD; and/or the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD. The semi-solid consumable composition may have an optical clarity greater than a comparable composition containing whey protein, soy protein, or pea protein at the same concentration as the rOVD.

[0019] The semi-solid consumable composition may comprise one or more additional ingredients selected from the group consisting of a flavoring, a coloring agent, a sweetener, an amino acid, a protein, an acidulant, and a preservative. The protein and/or the amino acid may provide an improved amino acid balance to the semi-solid consumable composition. In some cases, the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof. In some ~~embodiment~~examples, the protein and/or the amino acid provides an improved amino acid balance to the semi-solid consumable composition. The protein and/or the amino acid may provide an improved amino acid balance to the semi-solid consumable composition. In some cases, the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof. In ~~embodiment~~examples, the protein is a lysozyme protein, e.g., the lysozyme protein is an egg white lysozyme (OVL). The ratio of rOVD to OVL may be between about 60% rOVD:40% OVL and about 82% rOVD:18% OVL. The lysozyme may be a recombinant lysozyme protein. In various ~~embodiment~~examples, a protein digestibility corrected amino acid score (PDCAAS) is equal to or greater than about 0.75, 0.8, 0.85, 0.90, 0.95 or the PDCAAS is about or is 1.0.

[0020] In some cases, the rOVD comprises an rOVD that has been exposed to an oxidizing agent or an oxygen-generating agent. In various ~~embodiment~~examples, the oxygen-generating agent is hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone.

[0021] In some cases, the rOVD comprises an amino acid sequence that is naturally found in an avian species, e.g., chicken, quail, turkey, turkey vulture, hummingbird, duck, ostrich, goose, gull, guineafowl, pheasant, or emu, and any combination thereof.

[0022] The rOVD may comprise an amino acid sequence of one of SEQ ID No. 1-44 or an amino acid sequence having at least 85% sequence identity with one of SEQ ID No. 1-44.

[0023] In ~~embodiment~~examples, the rOVD is substantially a full-length rOVD amino acid sequence. **[0024]** In some cases, the rOVD provides protein fortification to the composition.

[0025] In some ~~embodiment~~examples, the rOVD is produced by a microbial host cell, e.g., In some cases, the microbial host cell is a yeast, a filamentous fungus, or a bacterium. The microbial host cell may be a Pichia species, a Saccharomyces species, a Trichoderma species, a Pseudomonas

species or an E. coli species. The microbial host cell may be *Pichia pastoris* or *Komagataella phaffii*.

[0026] Another ~~aspect~~example is a consumable composition comprising a recombinant ovomucoid protein (rOVD). The rOVD provides protein fortification to the composition; in which the rOVD provides a solubility that is comparable or higher than a native ovomucoid protein.

[0027] An ~~aspect~~example of the present disclosure is a consumable powder protein composition comprising a recombinant ovomucoid protein (rOVD). The protein content of the composition is greater than 70%; in which the composition comprises less than 2% ash, less than 20% carbohydrates, and less than 1% fat by acid hydrolysis on a weight/weight basis and/or a weight per total volume of composition basis.

[0028] Another ~~aspect~~example of the present disclosure is a consumable composition comprising a recombinant ovomucoid protein (rOVD). The composition has a protein content comprising at least 15% rOVD protein on a weight/weight basis and/or a weight per total volume of composition basis.

[0029] In an ~~aspect~~example, the present disclosure provides a consumable composition comprising a recombinant ovomucoid protein (rOVD). The rOVD provides protein fortification to the composition; in which the rOVD provides a water retention capacity higher than a native ovomucoid protein.

[0030] Yet another ~~aspect~~example of the present disclosure is a beverage composition comprising a recombinant ovomucoid protein (rOVD) and at least one consumable liquid, in which the rOVD is substantially soluble in the composition, in which the beverage composition is substantially optically clear, and in which the concentration of rOVD is greater than about 5% on a weight/weight basis and/or a weight per total volume of composition basis. The beverage is selected from the group consisting of a juice, a broth, a soup, a soda, a soft drink, a flavored water, a protein water, a fortified water, a carbonated water, a nutritional drink, an energy drink, a sports drink, a recovery drink, a heated drink, a coffee-based drink, a tea-based drink, a plant-based milk, a milk based drink, a non-dairy, plant based mild drink, infant formula drink, a meal replacement drink. The beverage may comprise carbonation.

[0031] In an ~~aspect~~example, the present disclosure provides a method of preparing a consumable food preparation. The method comprising the steps of: providing a recombinant OVD (rOVD) produced by a microbial host; in which the rOVD comprises N-linked glycosylation and in which rOVD is substantially devoid of N-linked mannosylation; producing a preparation by combining or mixing the rOVD with at least one consumable ingredient; in which the rOVD provides protein fortification to the composition and at least one additional feature selected from the group

consisting of solubility, optical clarity, mouthfeel, texture, hardness, stability to heat treatment and stability to pH.

[0032] In various ~~embodiment~~examples, the rOVD comprises one or more glycosylated asparagine residues, in which each glycosylated asparagine residue comprises a single N-acetylglucosamine. In some cases, the rOVD is present in the consumable food preparation in or in about 1%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39% or 40% on a weight/weight basis and/or a weight per total volume of the preparation.

[0033] The method may further comprise heat-treating the preparation, e.g., exposing the preparation to a temperature between about 72°C and about 121°C. The heat-treating may comprise hot fill, pasteurization, retort, boiling, baking, broiling or grilling. In ~~embodiment~~examples, the preparation has a pH between about 2 and about 6.

[0034] In some cases, the method further comprises expressing rOVD protein in the microbial host., e.g., a yeast, a filamentous fungus, or a bacterium. In some ~~embodiment~~examples, the microbial host is a pichia species, a saccharomyces species, a Trichoderma species, a pseudomonas species or an E. coli species. In some cases, the microbial host is *Pichia pastoris* or *Komagataella phaffii*.

[0035] In some ~~embodiment~~examples, the method further comprises expressing an enzyme in the microbial host having an activity to remove a glycan by cleaving within a chitobiose core of high mannose and hybrid oligosaccharides on an N-linked glycoprotein. In various ~~embodiment~~examples, the enzyme comprises EndoH, an OCH1-EndoH fusion or an active fragment of EndoH.

[0036] In some cases, the rOVD is secreted from the microbial host, and in which the method further comprises isolating the secreted rOVD prior to combining or mixing the rOVD with the at least one consumable ingredient.

[0037] The method may further comprise separating the secreted rOVD from the microbial host and exposing the rOVD to an oxidizing agent or an oxygen-generating agent, e.g., hydrogen peroxide, sodium percarbonate, activated chlorine dioxide, bubbled oxygen, or ozone.

[0038] In some cases, the method further comprises drying, powdering, and/or spray-drying the rOVD.

[0039] In various ~~embodiment~~examples, preparation is suitable for human consumption and/or for animal consumption.

[0040] An ~~aspect~~example of the present disclosure is a consumable composition produced by a herein-disclosed method.

[0041] Another ~~aspect~~example of the present disclosure is a recombinant ovomucoid (rOVD) protein comprising N-linked glycosylation, in which the N-linked glycosylation comprises N-acetyl glucosamine and substantially lacks mannose residues.

[0042] In some cases, the rOVD further comprises O-linked glycosylation. At least one asparagine residue of the OVD is glycosylated and has a single N-acetyl glucosamine residue. In ~~embodiment~~examples, at least three asparagine residues of rOVD have a single N-acetyl glucosamine residue. The rOVD protein may comprise an rOVD that has been exposed to an oxidizing agent or an oxygen-generating agent, e.g., hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone.

[0043] Yet another ~~aspect~~example is a composition comprising the rOVD protein according to any herein disclosed ~~aspect~~example or ~~embodiment~~example.

[0044] In some ~~embodiment~~examples, the composition is in powdered form and in which the protein content of the composition is about 70% or greater on a weight/weight basis and/or a weight per total volume of composition basis. In some cases, the rOVD protein is present in the composition at about 80% or greater on a weight/weight basis and/or a weight per total volume of composition basis.

[0045] In an ~~aspect~~example, the present disclosure provides a method of making an rOVD protein. The method comprising: producing rOVD protein in a eukaryotic host cell, in which the rOVD protein is secreted from the host cell and in which the host cell expresses an enzyme having an activity that removes mannose residues from N-acetyl glucosamine linkage; separating the rOVD protein from the host cell; exposing the rOVD protein to an oxidizing agent or an oxygen-generating agent; and separating rOVD from the oxidizing agent or oxygen-generating agent. **[0046]** In some ~~embodiment~~examples, the enzyme comprises EndoH, an OCH1-EndoH fusion, or an active fragment of EndoH. In some cases, the oxidizing agent or oxygen-generating agent comprises hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone. In some ~~embodiment~~examples, the host cell is a yeast or fungal cell.

[0047] In some cases, the host cell is a Pichia sp.

[0048] In any of the herein disclosed methods or compositions, the rOVD may be derived from an avian species.

[0049] In any of the herein disclosed methods or compositions, the rOVD may comprise an amino acid sequence of a chicken OVD, a goose OVD protein, a hummingbird OVD, or a turkey vulture OVD.

[0050] In any of the herein disclosed methods or compositions, the rOVD may comprise an amino acid sequence selected from the group consisting of SEQ ID No. 1-44 and an amino acid sequence having at least 85% sequence identity with SEQ ID No. 1-44.

[0051] Additional ~~aspect~~examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative ~~embodiment~~examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different ~~embodiment~~examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. The drawings and description are to be regarded as illustrative in nature, and not as restrictive. Any description herein concerning a specific composition and/or method apply to and may be used for any other specific composition and/or method as disclosed herein. Additionally, any composition disclosed herein is applicable to any herein-disclosed method. In other words, any ~~aspect~~example or ~~embodiment~~example described herein can be combined with any other ~~aspect~~example or ~~embodiment~~example as disclosed herein.

INCORPORATION BY REFERENCE

[0052] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "figure" and "FIG." herein), of which:

[0054] FIG. 1A illustrates the vector constructs used for the expression of rOVD.

[0055] FIG. 1B illustrates a comparison in the glycosylation pattern of native ovomucoid and a recombinant ovomucoid produced in *P. pastoris* and according to the present disclosure. Shown is a lack of the complex branched glycosylation (including a lack of mannose residues) on the recombinant ovomucoid when produced in a strain of *P. pastoris* comprising endoglycosidases.

[0056] FIG. 1C illustrates the glycosylation patterns of the recombinant OVD produced by *P. pastoris* without an endoglycosidase treatment. rOVD thus produced have complex branched glycosylation patterns.