

CLAIMS

What is claimed is:

1. 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.
2. The composition of claim 1, wherein each glycosylated asparagine comprises a single N-acetylglucosamine.
3. The composition of claim 1 or claim 2, wherein the rOVD comprises at least three glycosylated asparagine residues.
4. The composition according to any one of claims 1 to 3, wherein the rOVD is a secreted form of the rOVD protein.
5. The composition according to any one of claims 1 to 4, wherein the composition is a powder.
6. The composition of claim 5, wherein the composition has 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.
7. A composition comprising a recombinant ovomucoid protein (rOVD), wherein 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.
8. The composition of any one of claims 5 to 7, wherein the powder comprises 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.
9. The composition of any one of claims 5 to 8, wherein the powder comprises less than 30%, 27%, 25%, 22%, 20%, 17%, 15%, 12%, 10%, 8%, 5%, 3% or 1% free carbohydrate content.

10. The composition of any one of claims 1 to 9, wherein the 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.
11. The composition of any one of claims 1 to 10, wherein 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.
12. The composition of claim 10 or claim 11, wherein the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof.
13. The composition of any one of claims 5 to 12, wherein the powder is capable of being dissolved in a liquid.
14. A composition comprising a recombinant ovomucoid protein (rOVD), wherein 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.
15. The composition of claim 14, wherein the rOVD comprises at least one asparagine residue linked to N-acetyl glucosamine and the rOVD is substantially devoid of N-linked mannosylation.
16. The composition according to claim 14 or claim 15, wherein the concentration of rOVD is 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.
17. The composition of any one of claims 14 to 16, wherein 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.
18. The composition of any one of claims 14 to 17, wherein 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.

19. The composition of any one of claims 14 to 18, wherein the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a solid consumable composition lacking rOVD.
20. The composition of any one of claims 14 to 19, wherein 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.
21. The composition of any one of claims 14 to 20, wherein the solid consumable composition is a snack bar, a protein bar, a nutrition bar, an energy bar, or a protein supplement.
22. The composition of any one of claims 14 to 21, wherein 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.
23. A composition comprising a recombinant ovomucoid protein (rOVD), wherein 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.
24. The composition of claim 23, wherein 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.
25. The composition of claim 23 or claim 24, wherein the composition has a protein content comprising at least 5% rOVD, and wherein the liquid consumable composition is substantially optically clear.
26. The composition of any one of claims 23 to 25, wherein 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.
27. The composition of any one of claims 23 to 26, wherein 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.

28. The composition of any one of claims 23 to 27, wherein 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.
29. The composition of any one of claims 23 to 28, wherein the rOVD does not substantially affect a sensory rating for odor and/or for taste as compared to a liquid consumable composition lacking rOVD.
30. The composition of any one of claims 23 to 29, wherein 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.
31. The composition of any one of claims 23 to 30, wherein the rOVD remains substantially soluble after the liquid consumable composition has been heated to a temperature of between about 72°C and about 121°C.
32. The composition of any one of claims 23 to 31, wherein 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.
33. The composition of any one of claims 23 to 32, wherein the heat treatment comprises exposure of the liquid to a temperature of between about 72°C and about 121°C.
34. The composition of any one of claims 23 to 33, wherein the rOVD has a solubility in the liquid greater than the solubility of whey protein, soy protein, or pea protein at the same concentration as the rOVD.
35. The composition of any one of claims 23 to 34, wherein the liquid consumable composition has a pH of between about 2.0 and about 8.0.
36. The composition of any one of claim 14 to 35, wherein the solid form formulated for human or animal consumption or the liquid formulated for human or animal consumption 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 and a preservative.

37. The composition of claim 36, wherein the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof.
38. The composition of claim 36, wherein the protein is a lysozyme protein.
39. The composition of claim 38, wherein the lysozyme protein is an egg white lysozyme (OVL).
40. The composition of claim 39, wherein the ratio of rOVD to OVL is between about 60% rOVD:40% OVL and about 82% rOVD:18% OVL.
41. The composition of any one of claims 38 to 40, wherein the lysozyme is a recombinant lysozyme protein.
42. The composition of any one of claims 36 to 41, wherein the protein and/or the amino acid provides an improved amino acid balance to the solid form or the liquid.
43. The composition of any one of claims 36 to 42, wherein a protein digestibility corrected amino acid score (PDCAAS) is equal to or greater than about 0.75.
44. The composition of claim 43, wherein the PDCAAS is greater than or equal to about 0.8, 0.85, 0.90, 0.95 or the PDCAAS is about or is 1.0.
45. The composition of any one of claims 39 to 44, wherein the liquid comprising rOVD and OVL, wherein the proteins are soluble and composition is optically clear.
46. The composition of any one of claims 23 to 45, wherein 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.
47. The composition of claim 46, wherein the beverage comprises carbonation.
48. The composition of any one of claims 23 to 47, wherein the liquid consumable composition is 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.
49. The composition of any one of claims 23 to 48, wherein the liquid is an emulsion.

50. The composition of claim 49, wherein the emulsion is a sauce, a gravy, or a salad dressing.

51. A composition comprising a recombinant ovomucoid protein (rOVD), wherein the composition is in a semi-solid form formulated for human or animal consumption, wherein 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.

52. The composition of claim 51, wherein the semi-solid consumable composition is a gummy, candy, jelly, syrup, gel, a gelled preparation.

53. The composition of claim 51 or claim 52, wherein 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.

54. The composition of any one of claims 51 to 53, wherein 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.

55. The composition of any one of claims 51 to 54, wherein the semi-solid consumable 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.

56. The composition of any one of claims 51 to 55, wherein 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.

57. The composition of any one of claims 51 to 56, wherein 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.

58. The composition of any one of claims 51 to 57, wherein the semi-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, and a preservative.

59. The composition of claim 58, wherein the protein and/or the amino acid provides an improved amino acid balance to the semi-solid consumable composition.
60. The composition of claim 59, wherein the amino acid is selected from tryptophan, isoleucine, leucine, and valine, or a combination thereof.
61. The composition of claim 59, wherein the protein is a lysozyme protein.
62. The composition of claim 61, wherein the lysozyme protein is an egg white lysozyme (OVL).
63. The composition of claim 62, wherein the ratio of rOVD to OVL is between about 60% rOVD:40% OVL and about 82% rOVD:18% OVL.
64. The composition of any one of claims 61 to 63, wherein the lysozyme is a recombinant lysozyme protein.
65. The composition of any one of claims 61 to 64, wherein 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.
66. The composition of any one of claims 1 to 65, wherein the rOVD comprises an rOVD that has been exposed to an oxidizing agent or an oxygen-generating agent.
67. The composition of claim 66, wherein the oxygen-generating agent is hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone.
68. The composition of any one of claims 1 to 67, wherein the rOVD comprises an amino acid sequence that is naturally found in an avian species.
69. The composition of claim 68, wherein the avian species is chicken, quail, turkey, turkey vulture, hummingbird, duck, ostrich, goose, gull, guineafowl, pheasant, or emu, and any combination thereof.
70. The composition of any one of claims 1 to 69, wherein rOVD comprises 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.
71. The composition of any one of claims 1 to 70, wherein the rOVD is substantially a full-length rOVD amino acid sequence.

72. The composition of any one of claims 1 to 71, wherein the rOVD provides protein fortification to the composition.

73. The composition of any one of claims 1 to 72, wherein the rOVD is produced by a microbial host cell.

74. The composition of claim 73, wherein the microbial host cell is a yeast, a filamentous fungus, or a bacterium.

75. The composition of claim 73 or claim 74, wherein the microbial host cell is a *Pichia* species, a *Saccharomyces* species, a *Trichoderma* species, a *Pseudomonas* species or an *E. coli* species.

76. The composition of any one of claims 73 to 75, wherein the microbial host cell is *Pichia pastoris* or *Komagataella phaffii*.

77. A consumable powder protein composition comprising a recombinant ovomucoid protein (rOVD), wherein the protein content of the composition is greater than 70%; wherein 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.

78. A consumable composition comprising a recombinant ovomucoid protein (rOVD), wherein 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.

79. A beverage composition comprising a recombinant ovomucoid protein (rOVD) and at least one consumable liquid, wherein the rOVD is substantially soluble in the composition, wherein the beverage composition is substantially optically clear, and wherein the concentration of rOVD is greater than about 5% on a weight/weight basis and/or a weight per total volume of composition basis.

80. The beverage composition of claim 79, wherein the a 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.

81. The beverage composition of claim 79 or claim 80, wherein the beverage comprises carbonation.
82. A method of preparing a consumable food preparation comprising the steps of:
providing a recombinant OVD (rOVD) produced by a microbial host;
wherein the rOVD comprises N-linked glycosylation and wherein rOVD is substantially devoid of N-linked mannosylation;
producing a preparation by combining or mixing the rOVD with at least one consumable ingredient;
wherein 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.
83. The method of claim 82, wherein the rOVD comprises one or more glycosylated asparagine residues, wherein each glycosylated asparagine residue comprises a single N-acetylglucosamine.
84. The method of claim 82 or claim 83, wherein 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.
85. The method of any one of claims 82 to 84, further comprising heat-treating the preparation.
86. The method of claim 85, wherein heat-treating comprises exposure to a temperature between about 72°C and about 121°C.
87. The method of claim 85 or claim 86, wherein heat-treating comprises hot fill, pasteurization, retort, boiling, baking, broiling or grilling.
88. The method according to any one of claims 82 to 87, wherein the preparation has a pH between about 2 and about 6.
89. The method of one of claims 82 to 88, wherein the method further comprises expressing rOVD protein in the microbial host.

90. The method of claim 89, wherein the microbial host is a yeast, a filamentous fungus, or a bacterium.
91. The method of claim 89 or claim 90, wherein the microbial host is a *Pichia* species, a *saccharomyces* species, a *Trichoderma* species, a *pseudomonas* species or an *E. coli* species.
92. The method of one of claims 89 to 91, wherein the microbial host is *Pichia pastoris* or *Komagataella phaffii*.
93. The method of claim one of claims 89 to 92, further comprising expressing in the microbial host an enzyme having an activity to remove a glycan by cleaving within a chitobiose core of high mannose and hybrid oligosaccharides on an N-linked glycoprotein.
94. The method of claim 93, wherein the enzyme comprises EndoH, an OCH1-EndoH fusion or an active fragment of EndoH.
95. The method of one of claims 89 to 94, wherein the rOVD is secreted from the microbial host, and wherein the method further comprises isolating the secreted rOVD prior to combining or mixing the rOVD with the at least one consumable ingredient.
96. The method of claim 95, wherein the method further comprises separating the secreted rOVD from the microbial host and exposing the rOVD to an oxidizing agent or an oxygen-generating agent.
97. The method of 97, wherein the oxidizing agent or oxygen-generating agent comprises hydrogen peroxide, sodium percarbonate, activated chlorine dioxide, bubbled oxygen, or ozone.
98. The method according to any one of claims one of claims 82 to 97, further comprising drying, powdering, and/or spray-drying the rOVD.
99. The method according to any one of claims 82 to 98, wherein preparation is suitable for human consumption.
100. The method according to any one of claims 82 to 98, wherein preparation is suitable for animal consumption.
101. A consumable composition produced by the method according to any one of claims 82 to 100.

102. A recombinant ovomucoid (rOVD) protein comprising N-linked glycosylation, wherein the N-linked glycosylation comprises N-acetyl glucosamine and substantially lacks mannose residues.

103. The rOVD protein of claim 102, wherein the rOVD further comprises O-linked glycosylation.

104. The rOVD protein of claim 102 or claim 103, comprising at least one asparagine residue which is glycosylated and has a single N-acetyl glucosamine residue.

105. The rOVD protein of claim 104, wherein at least three asparagine residues of rOVD have a single N-acetyl glucosamine residue.

106. The rOVD protein according to any one of claims 102 to 105, wherein the rOVD protein comprises an rOVD that has been exposed to an oxidizing agent or an oxygen-generating agent.

107. The rOVD protein of claim 106, wherein the oxidizing agent or the oxygen-generating agent comprises hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone.

108. A composition comprising the rOVD protein according to any one of claims 102 to 107, wherein the composition is in powdered form and wherein 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.

109. The composition of claim 108, wherein 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.

110. A method of making an rOVD protein comprising:
producing rOVD protein in a eukaryotic host cell, wherein the rOVD protein is secreted from the host cell and wherein 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.

111. The method of claim 110, wherein the enzyme comprises EndoH, an OCH1-EndoH fusion, or an active fragment of EndoH.

112. The method of claim 110 or claim 111, wherein the oxidizing agent or oxygen-generating agent comprises hydrogen peroxide, sodium percarbonate, bubbled oxygen, activated chlorine dioxide, or ozone.

113. The method of any one of claims 110 to 112, wherein the host cell is a yeast or fungal cell.

114. The method of any one of claims 110 to 113, wherein the host cell is a *Pichia* sp.

115. The method according to any one of claims 82 to 100 or claims 110 to 114, the rOVD protein according to any one of claims 102 to 107 or a composition according to any one of claims 77 to 81, 101, 108, or 109, wherein the rOVD is derived from an avian species.

116. The method according to any one of claims 82 to 100 or claims 110 to 114, the rOVD protein according to any one of claims 102 to 107 or a composition according to any one of claims 77 to 81, 101, 108, or 109, wherein the rOVD comprises an amino acid sequence of a chicken OVD, a goose OVD protein, a hummingbird OVD, or a turkey vulture OVD.

117. The method according to any one of claims 82 to 100 or claims 110 to 114, the rOVD protein according to any one of claims 102 to 107 or a composition according to any one of claims 77 to 81, 101, 108, or 109, wherein the rOVD comprises 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.