NON-ANIMAL BASED PROTEIN SOURCES WITH FUNCTIONAL PROPERTIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/888,674, filed August 19, 2019, the contents of which are incorporated by reference in its entirety.

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 August 18, 2020, is named 49160-717.601_ST25.txt and is 287,890 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Proteins are important dietary nutrients and food ingredients. 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, proteins are used in a wide variety of foods and food ingredients. In many cases, these proteins are sourced from animals. 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, food ingredients and food products.

SUMMARY OF THE DISCLOSURE

[0004] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

[0004a] In one aspect, provided herein is an ingredient composition for producing an egg-less food item, the ingredient composition comprising a recombinant ovalbumin (rOVA) and ash, wherein the egg-less food item does not comprise any egg-white proteins except the rOVA; wherein the pH of the rOVA when solubilized in an aqueous solution is above 3.5; wherein the ingredient composition comprises from about 1% to about 98% rOVA and at least 0.5% ash w/w or w/v; wherein when the ingredient composition is present in the egg-less food item in an amount

comprising between about 2% and about 15% (w/w) rOVA in the egg-less food item; and wherein the rOVA provides an equivalent or an improvement in a characteristic compared to an otherwise similar egg-less food item comprising native egg white, wherein the characteristic is selected from the group consisting of: gelling, foam capacity, foam stability, whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification, and cohesiveness.

[0005] In some examples, provided herein are ingredients for producing egg-less food items. The ingredient composition for producing an egg-less food item may comprise a recombinant ovalbumin (rOVA), wherein the pH of the rOVA may be between about 3.5 and about 7.0; wherein the rOVA when present in the egg-less food item in an amount between about 2% and about 15% (w/w); and wherein the rOVA provides to the egg-less food item at least one egg white characteristic selected from gelling, foaming, whipping, fluffing, binding, springiness, aeration,

solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above about 3.7 when solubilized.

[0011] In some cases, the pH when solubilized may be between about 6 and about 6.8. In some cases, the pH of the rOVA when solubilized may be less than about 6.1. In some cases, the rOVA may be present in the egg-less food item in an amount of less than about 8%. In some cases, the rOVA may be present in the egg-less food item in an amount of about 7% or less than 7%.

[0012] In some examples, provided herein are baked goods. A baked food product, may comprise: (i) a recombinant ovalbumin (rOVA), wherein the pH of the rOVA when solubilized may be between about 3.5 and about 7.0; (ii) at least one fat or oil; (iii) at least one grain starch; and (iv) at least one sweetener; wherein the rOVA provides the baked food product at least one egg white characteristic selected from binding, springiness, aeration, browning, texturizing, humectant, and cohesiveness, and the baked food product does not comprise any natural egg white proteins or a natural egg white.

[0013] In some cases, the rOVA may be present at about 2% to 15% in the product (w/w of total protein or w/w of total food product prior to baking). In some cases, the rOVA is present at about 2% to about 5% in the product (w/w). In some cases, the baked good may comprise a dairy component or a leavening agent, or a combination thereof. In some cases, the product may be a cake, a bread, a roll, a pastry, a cracker, a muffin, a scone, a biscuit, or a cookie. In some cases, the baked product may have a crumb structure equivalent to or better than a similar baked product made with a natural egg white or a natural whole egg. In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the percentage weight loss is lower in a baked product made with rOVA when compared to an equivalent baked product made with whole egg.

[0014] In some examples, provided herein are emulsified products. An emulsified product may comprise: (i) a recombinant ovalbumin (rOVA); (ii) at least one fat or oil; (iii) water; wherein the rOVA may be present in the product at about 2% to 15% (w/w). In some cases, the emulsified product may comprise an acidifying agent. In some cases, the product may be a salad dressing, a sauce, mayonnaise, sandwich spread or a gravy.

[0015] In some examples, described herein are food products comprising (i) a recombinant ovalbumin (rOVA), wherein the pH of the rOVA when solubilized may be between about 3.5 and about 7.0; (ii) at least one sweetener; and (iii) optionally, a consumable liquid; wherein the rOVA

may be present in the food product at about 2% to about 15% (w/w) and wherein the rOVA provides foaming, whipping, fluffing or aeration to the food product.

[0016] In some cases, the rOVA may further provide gelation to the food product. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of a chicken OVA and the pH is between about 6.5 and 7.0 when solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above about 3.7 when solubilized. In some cases, the food product may be a meringue, a whipped dessert, a whipped topping or a soufflé. In some cases, the rOVA may provide a foam capacity to the food product of at least 20%, 30%, 40%, or 50% greater than native egg white. In some cases, the rOVA may provide a time to foaming to the food product that may be at least 20%, 30%, 40%, or 50% faster than native egg white. In some cases, the pH of the rOVA when solubilized is between about 3.5 and about 4.5.

[0017] In some cases, the rOVA is present in the food product at about 5% to about 10% (w/w). In some cases, the rOVA is present in the food product at about 7% to about 8% (w/w). In some cases, the rOVA is present in the food product at about 4%, about 7%, or about 12% (w/w). In some cases, the pH of the rOVA when solubilized is about 6. In some cases, the rOVA is present in the food product at between about 9% and about 10% (w/w). In some cases, the pH of the rOVA when solubilized is about 7. In some cases, the product may be a beverage. In some cases, the beverage may be a consumable alcohol. In some cases, the rOVA provides foaming, whipping, fluffing or aeration to the consumable alcohol beverage. In some cases, the beverage is a coffee drink. In some cases, the rOVA provides foaming, whipping, fluffing or aeration to the coffee drink. In some cases, the coffee drink lacks a dairy component.

[0018] In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the rOVA does not contaminate the food product with Salmonella. In some cases, the food product is a protein bar, an energy bar, a nutrition bar or a granola bar. In some cases, the food product comprises between about 4% and about 8% (w/w) rOVA. In some cases, the bar is baked or is unbaked.

[0019] In some examples, described herein is a meat-analog food product. A meat-analog food product may comprise: (i) a recombinant ovalbumin (rOVA); (ii) at least one fat or oil; and (iii) a plant-derived protein; wherein the rOVA may be present in the food product between about 2% and about 15% (w/w); and wherein the rOVA acts as a binding agent or a gelling agent, or a combination thereof.

[0020] In some cases, the plant protein may be an extruded plant protein. In some cases, the plant protein may be a non-extruded plant protein. In some cases, the meat analog food product may be selected from a burger, patty, sausage, hot dog, sliced deli meat, jerky, bacon, nugget, a ground meat-like composition, and a formed meat-like composition. In some cases, the rOVA may provide a hardness to the food product that may be greater than native egg white. In some cases, the rOVA may provide a chewiness to the food product that may be greater than native egg white. In some cases, the rOVA may provide a springiness comparable to native egg white.

[0021] In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of a chicken OVA and the pH is between about 6.5 and 7.0 when solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above about 3.7 when solubilized. In some cases, the rOVA is present in the food product at about 4%, at about 5%, or at about 6% (w/w). In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA.

[0022] In some examples, provided herein are egg-white substitutes. An egg-white substitute may comprise: (i) a recombinant ovalbumin (rOVA); (ii) at least one fat or oil; and (iii) a polysaccharide or polysaccharide-containing ingredient; wherein the rOVA may be present in the composition at about 2% to 15% (ww); and wherein the composition may have one or more characteristics selected from hardness, adhesiveness, fracturability, cohesiveness, gumminess, and chewiness, and the one or more characteristics are equivalent to or improved as compared to natural egg white when the egg-white substitute may be cooked.

[0023] In some cases, the egg-white substitute may further comprise a flavoring agent or a coloring agent, or a combination thereof. In some cases, the polysaccharide or polysaccharide-containing ingredient may be a starch. In some cases, the polysaccharide or polysaccharide-containing ingredient may be selected from gellan gum, sodium alginate, and psyllium or any combination thereof. In some cases, the rOVA may provide a hardness to the food product that may be greater than native egg white.

[0024] In some cases, the rOVA may provide a chewiness to the food product that may be greater than native egg white. In some cases, the rOVA may provide a gumminess and/or springiness comparable to native egg white. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of a chicken OVA and the pH is between about 6.5 and 7.0 when solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above

about 3.7 when solubilized. In some cases, the rOVA is present in the food product between about 10% and about 12% (w/w).

[0025] In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA.

[0026] In some examples, described herein are powdered ingredient compositions. A powdered ingredient composition may comprise a recombinant ovalbumin (rOVA), wherein the pH of the rOVA when solubilized may be between about 3.5 and about 7.0, wherein the rOVA may be at least 75% w/w of the composition, and wherein the rOVA may comprise one or more N-linked glycosylation sites having mannose linked to an N-acetyl glucosamine, and wherein the N-linked glycosylation sites lack galactose. In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the amino acid sequence of the rOVA lacks an N-terminal methionine. In some cases, the rOVA further includes an EAEA amino acid sequence (SEQ ID NO: 75) at its N-terminus. In some cases, the composition comprises at least at least about 80%, at least about 85%, or at least about 90% rOVA (w/w).

[0027] In some examples, a liquid composition may comprise a recombinant ovalbumin (rOVA) and the composition may comprise at least 50% rOVA (w/w of total protein or w/w of total composition). In some cases, the composition may comprise at least about 60%, at least about 65%, at least about 75%, at least about 80%, at least about 85%, or at least about 90% rOVA (w/w).

[0028] In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA.

[0029] In some cases, the amino acid sequence of the rOVA lacks an N-terminal methionine. In some cases, the rOVA further includes an EAEA amino acid sequence (SEQ ID NO: 75) at its N-terminus. In some cases, the pH of the solubilized rOVA may be between about 3.5 and about 7.0. In some cases, the pH of the solubilized rOVA may be between about 6 and about 6.8. In some cases, the pH of the solubilized rOVA may be less than about 6.1.

[0030] In some cases, the rOVA may provide to an egg-less food item at least one egg white characteristic selected from gelling, foaming, whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification, and cohesiveness. In some cases, the rOVA may provide an equivalent or an improvement in the

characteristic compared to native egg white in a similar egg-less food item. In some cases, the rOVA may provide to the egg-less food item a foam capacity of at least 20%, 30%, 40%, or 50% greater than native egg white.

[0031] In some cases, the rOVA may provide to the egg-less food item a time to foaming that may be at least 20%, 30%, 40%, or 50% faster than native egg white. In some cases, the rOVA may provide to the egg-less food item a hardness that may be greater than native egg white. In some cases, the pH of the rOVA when solubilized is between about 3.5 and about 4.5. In some cases, the rOVA is present in the egg-less food item at about 5% to about 10% (w/w). In some cases, the rOVA is present in the egg-less food item at about 7% to about 8% (w/w). In some cases, the rOVA is present the egg-less food item at about 4%, about 7%, or about 12% (w/w). In some cases, the pH of the rOVA when solubilized is about 6. In some cases, the rOVA may provide to the egg-less food item a chewiness that may be greater than native egg white. In some cases, the rOVA may provide to the egg-less food item a springiness comparable to native egg white.

[0032] In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of a chicken OVA and the pH is between about 6.5 and 7.0 when solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above about 3.7 when solubilized. In some cases, the rOVA does not contaminate the egg-less food item with Salmonella.

[0033] In some examples, described herein are dry or powdered compositions comprising a recombinant ovalbumin (rOVA), wherein the composition may comprise at least 50% rOVA (w/w of total protein or w/w of total composition). In some cases, the composition may comprise at least about 60%, at least about 65%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% rOVA (w/w). In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1.

[0034] In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the amino acid sequence of the rOVA lacks an N-terminal methionine. In some cases, the rOVA further includes an EAEA amino acid sequence (SEQ ID NO: 75) at its N-terminus. In some cases, the rOVA may provide to an egg-less food item at least one egg white characteristic selected from gelling, foaming, whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification, and cohesiveness. In some cases, the rOVA may provide an equivalent or an improvement in the characteristic compared to native egg white in a similar egg-less food item.

[0035] In some cases, the rOVA may provide to the egg-less food item a foam capacity of at least 20%, 30%, 40%, or 50% greater than native egg white. In some cases, the rOVA may provide to the egg-less food item a time to foaming that may be at least 20%, 30%, 40%, or 50% faster than native egg white. In some cases, the pH of the rOVA when solubilized is between about 3.5 and about 4.5. In some cases, the rOVA is present in the egg-less food at about 4%, about 7%, or about 12% (w/w). In some cases, the pH of the rOVA when solubilized is about 6.

[0036] In some cases, the rOVA may provide to the egg-less food item a hardness that may be greater than native egg white. In some cases, the rOVA may provide to the egg-less food item a chewiness that may be greater than native egg white. In some cases, the rOVA may provide to the egg-less food item a springiness comparable to native egg white. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of a chicken OVA and the pH is between about 6.5 and 7.0 when solubilized. In some cases, the rOVA provides improved gelation when the rOVA comprises an amino acid sequence of an ostrich OVA and the pH is less than about 6.0 and above about 3.7 when solubilized.

[0037] In some examples, provided herein are methods of making a food product. A method of making a food product may comprise: (i) providing a recombinant ovalbumin (rOVA) at a pH when solubilized of between about 3.5 and about 7.0; (ii) combining the rOVA in an amount between 2% and 15% (w/w) with one or more consumable ingredients to form a food product, wherein the rOVA may provide at least one egg white characteristic to the food product selected from gelling, foaming, whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification and cohesiveness.

[0038] In some examples, provided herein are methods of making an ingredient. A method of producing an ingredient composition may comprise: (i) expressing a recombinant ovalbumin (rOVA) in a microbial cell, wherein the rOVA may be secreted by the microbial cell into a liquid media; (ii) harvesting the liquid media containing secreted rOVA; (iii) performing a separation step at a pH of about 3.5; (iv) solubilizing the rOVA at a pH of about 12; (v) adjusting the final pH of the rOVA to between about 3.5 and about 7.0 to generate the ingredient composition.

[0039] In some cases, the separation step may comprise ion exchange chromatography or ammonium sulfate precipitation. In some cases, the ion exchange chromatography may be cation exchange chromatography or anion exchange chromatography, or a combination thereof. In some cases, the method further may comprise a filtration step following the solubilizing step. In some cases, the microbial cell may be a fungal cell. In some cases, the fungal cell may be a Pichia sp. In some cases, the microbial cell expresses a recombinant helper factor; wherein the helper factor enhances the level of expression or accumulation of rOVA.

[0040] In some cases, the rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. In some cases, the rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the amino acid sequence of the secreted rOVA lacks an N-terminal methionine. In some cases, the secreted rOVA further includes an EAEA amino acid sequence (SEQ ID NO: 75) at its N-terminus.

[0041] In some examples, an egg-less food product may comprise a recombinant ovalbumin (rOVA) in an amount of between about 15% and about 25% (w/w of total protein or w/w of food product). In some cases, the egg-less food product may comprise the rOVA) in an amount of up to about 23% (w/w).

[0042] In some examples, provided herein are uses of recombinant ovalbumin (rOVA). The recombinant ovalbumin (rOVA) may be used as an ingredient in making a baked good. rOVA may be used as an ingredient in making an egg-less food product. rOVA may be used as an ingredient in making a meat-analog food product. rOVA may be used as an ingredient in making an egg-white substitute. rOVA may be used as a substitute egg-wash for a baked product; wherein the substitute egg-wash may provide film formation equivalent to or better than an egg-wash may comprise a natural egg white or a natural whole egg.

[0043] rOVA may comprise an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1 or an amino acid sequence with at least 70% identity to SEQ ID NO: 2 or SEQ ID NO: 1. rOVA may comprise an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA. In some cases, the rOVA is present in the egg-wash in an amount between 8% and 9% (w/w).

[0044] In some examples, described herein are large-scale production of recombinant ovalbumin (rOVA). A large-scale production of rOVA, may comprise an at least 1-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production may comprise an at least 10-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production may comprise an at least 100-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production may comprise an at least 1000-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production comprises an at least 10,000-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production comprises an at least 100,000-liter liquid culture of microbial cells expressing the rOVA. In some cases, the large-scale production comprises about a 200,000-liter liquid culture of microbial cells expressing the rOVA.

[0045] In some examples, provided herein may be an ingredient composition for producing an egg-less food item comprising a recombinant ovalbumin. The recombinant ovalbumin may provide at least one egg white characteristic selected from the group consisting of gelling, foaming,

whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification and cohesiveness.

[0046] The egg white characteristic provided by the recombinant ovalbumin may be substantially the same or better than the same characteristic provided by a native egg white. The composition may not contain any native egg white protein. The composition may not contain any animal products.

[0047] The composition may not contain any protein extracted from an egg. The color of the composition may be improved in whiteness or colorlessness as compared to a native egg white. The recombinant ovalbumin may comprise a polypeptide sequence derived from the group consisting of chicken, goose, quail, ostrich, and duck.

[0048] The recombinant ovalbumin may be sensory neutral with regard to taste, smell, mouthfeel or any combination thereof. The recombinant ovalbumin may provide the features of foaming and coagulation to the composition.

[0049] In some examples, provided herein are baked products comprising the ingredient composition provided herein. The recombinant ovalbumin may provide structure, texture or both structure and texture to the baked product. The recombinant ovalbumin may provide a protein fortification to the baked product. The recombinant ovalbumin may be at a concentration of between about 1% and about 20% (weight ovalbumin/weight product) in a baked product. The recombinant ovalbumin may be at a concentration of between about 0.1% and about 5% (weight ovalbumin/weight product) in a baked product.

[0050] The recombinant ovalbumin may be compatible with gluten formation. The baked product may be selected from the group consisting of cake, cookie, bagel, biscuit, bread, muffin, cupcake, scone, pancake, macaroon, meringue, choux pastry and soufflé. The cake made using such an ingredient may be pound cake, sponge cake, yellow cake, or angel food cake. The composition may further comprise one or more components selected from the group consisting of a sweetening agent, a gum, a hydrocolloid, a starch, a fiber, a plant protein, algal protein, a coloring agent and a flavoring extract.

[0051] The composition may provide one or more characteristics suitable for an egg-like dish, and wherein the characteristic may be selected from the group consisting of foaming, coagulation, binding, structure, texture, film-formation, nutritional profile, cholesterol free and protein fortification. In some examples, provided herein are egg-like dishes comprising the ingredient composition described herein. The egg-like dish may be selected from the group consisting of scramble, omelet, patty, soufflé, quiche and frittata. The egg-like dish may be vegan, vegetarian, halal or kosher.

[0052] The composition may provide one or more characteristics suitable for a processed meat product or meat-like product, and wherein the characteristic may be selected from the group consisting of high protein content, binding, and sensory neutrality. In some examples, provided herein are meat-like products, comprising the ingredient compositions provided herein.

[0053] The meat-like product may be selected from the group consisting of a burger, patty, sausage, hot dog, sliced deli meat, jerky, bacon, nugget and ground meat-like mixture or formed meat or meat-like composition. Ovalbumin may be present in an amount between about 0.1% and 30% in the meat-like product (weight ovalbumin/weight product).

[0054] The recombinant ovalbumin may provide the characteristic of binding suitable for adhesion of a food coating. A food coating may comprise the ingredients described herein. The food coating may be a batter or a breading. The recombinant ovalbumin may further provide the characteristic of crunchy texture to the food coating when cooked, baked or fried.

[0055] The recombinant ovalbumin may provide the characteristic suitable for a confectionary selected from the group consisting of odor neutrality, flavor, mouthfeel, texture, nutritional value and protein fortification. A confectionary product may comprise the ingredient compositions described herein. The confectionary may not contain egg or egg white. The confectionary may not contain any proteins extracted from egg or egg white. The recombinant ovalbumin may provide a firm or chewy texture to the confectionary. The recombinant ovalbumin may be present in an amount between about 0.1% and 15% (weight ovalbumin/weight confectionary). The confectionary may be a gummy, a taffy or a nougat.

[0056] The recombinant ovalbumin may provide a characteristic suitable for a dairy-like beverage selected from the group consisting of odor neutrality, flavor, mouthfeel, foaming, frothiness, texture, and nutritional value. A dairy-like beverage may comprise the ingredient compositions described herein. The dairy-like beverage may not contain egg or egg white. The beverage may be selected from the group consisting of smoothie, milkshake, “egg-nog”, and coffee beverage. The recombinant ovalbumin may be present in an amount between about 0.1% and 20% (weight ovalbumin/volume beverage).

[0057] Recombinant ovalbumin may provide a characteristic suitable for a dessert product selected from the group consisting of creamy texture, low fat content, odor neutrality, flavor, mouthfeel, texture, binding, and nutritional value. A dessert product may comprise the ingredient compositions described herein. The dessert product may be selected from the group consisting of a mousse, a cheesecake, a custard, a pudding, a popsicle, a frozen dessert, and an ice cream. The dessert product may be vegan, vegetarian or dairy-free. The recombinant ovalbumin may be present in an amount between about 0.1% and 10% (weight ovalbumin/weight dessert product).

[0058] The recombinant ovalbumin may provide a characteristic suitable for a sauce or dressing selected from the group consisting of binding, emulsifying, odor neutrality, and mouthfeel. A sauce or dressing may comprise the ingredient compositions described herein. The sauce or dressing may be selected from the group consisting of salad dressing, mayonnaise, commercial mayonnaise substitutes, alfredo sauce, and hollandaise sauce. The sauce or dressing may not contain egg, egg white, or any protein extracted from egg.

[0059] The recombinant ovalbumin may provide a characteristic suitable for a snack food selected from the group consisting of binding, protein supplementation, flavor neutrality, odor neutrality, and mouth feel. A snack food may comprise the ingredient compositions described herein. The snack food may be a protein bar, a nutrition bar or a granola bar. The ingredient composition may further comprise one or more additional components selected from the group consisting of a sweetener, a gum, a plant protein, algal protein, a flavoring, a colorant, a thickener, an acidulant and an emulsifier.

[0060] In some examples, provided herein are methods of producing an egg white replacer. The egg-white replacer may comprise providing a recombinant ovalbumin; mixing the recombinant ovalbumin with at least one additional component to form the egg white replacer. The recombinant ovalbumin may provide at least one egg white characteristic selected from the group consisting of gelling, foaming, whipping, fluffing, binding, springiness, aeration, creaminess and cohesiveness to the egg white replacer. The egg white replacer may not contain any egg, egg white, protein extracted or isolated from egg. The at least one egg white characteristic may be the same or better than a native egg provided in the same amount or concentration (weight/volume).

[0061] The method may further comprise producing the recombinant ovalbumin in a heterologous host cell, wherein the host cell may be E.coli, yeast, filamentous fungus, or Trichoderma. The yeast or filamentous fungus may be selected from the group consisting of a Saccharomyces species and a Pichia species. The recombinant ovalbumin may be secreted from the host cell. The recombinant ovalbumin may be glycosylated by the host cell and wherein the glycosylation of the ovalbumin may be not identical to ovalbumin isolated from chicken egg.

[0062] The method may further comprise treating the secreted ovalbumin with a deglycosylation enzyme. The deglycosylation enzyme may be expressed by the host cell.

[0063] The host may comprise a nucleic acid sequence encoding the recombinant ovalbumin, and the recombinant ovalbumin has an amino acid sequence of an ovalbumin from an avian species. The host may comprise a nucleic acid sequence encoding the recombinant ovalbumin, and the recombinant ovalbumin has an amino acid sequence of an ovalbumin that has at least 95% sequence identity with an ovalbumin from an avian species. The avian species may be chicken, duck, goose, ostrich, or quail.

[0064] The ovalbumin from the avian species may be selected from the group consisting of SEQ ID NO. 1-74.

[0065] In some examples, provided herein is a recombinant protein composition for use as an egg-white replacer. The composition can comprise a recombinant ovalbumin and at least one additional component. The recombinant ovalbumin may provide at least one egg white characteristic selected from the group consisting of gelling, foaming, whipping, fluffing, binding, springiness, aeration, creaminess and cohesiveness to the composition. The composition may not contain any egg, egg white, protein extracted or isolated from egg. The at least one egg white characteristic may be the same or better than a native egg compared at the same amount or concentration (weight/volume).

[0066] The recombinant ovalbumin may have an amino acid sequence of an ovalbumin from an avian species. The recombinant ovalbumin may have an amino acid sequence of an ovalbumin that has at least 95% sequence identity with an ovalbumin from an avian species.

[0067] The avian species may be chicken, duck, goose, ostrich, or quail. The ovalbumin from the avian species may be selected from the group consisting of SEQ ID NO. 1-74.

[0068] An animal nutrition composition may comprise a recombinant ovalbumin (rOVA). The rOVA may be in a form selected from whole cell extract, fractionated cell extract and isolated protein. The composition may be comprised within a pet food, an animal feed, a chewy treat, bone broth, smoothie or other liquid for animal nutrition and a solid nutritional supplement suitable for animal consumption.

[0069] Additionally, any composition, food product, ingredient, use, or method disclosed herein is applicable to any herein-disclosed composition, food product, ingredient, use, or method. In other words, any aspect or example described herein can be combined with any other aspect or example as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0070] 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 of which:

[0071] **FIGs. 1A-B** illustrate glycosylation patterns of native OVA and rOVA produced in P. pastoris respectively.

[0072] **FIG. 2** illustrates pound cakes and their cross-sections made using rOVA compared to cakes made using eggs.

[0073] **FIG. 3** illustrates meringues made using rOVA compared to meringues made using eggs.

CLAIMS

WHAT IS CLAIMED IS:

1. An ingredient composition for producing an egg-less food item, the ingredient composition comprising a recombinant ovalbumin (rOVA) and ash,

wherein the egg-less food item does not comprise any egg-white proteins except the rOVA;

wherein the pH of the rOVA when solubilized in an aqueous solution is above 3.5;

wherein the ingredient composition comprises from about 1% to about 98% rOVA and at least 0.5% ash w/w or w/v;

wherein when the ingredient composition is present in the egg-less food item in an amount comprising between about 2% and about 15% (w/w) rOVA in the egg-less food item; and

wherein the rOVA provides an equivalent or an improvement in a characteristic compared to an otherwise similar egg-less food item comprising native egg white, wherein the characteristic is selected from the group consisting of: gelling, foam capacity, foam stability, whipping, fluffing, binding, springiness, aeration, coating, film forming, emulsification, browning, thickening, texturizing, humectant, clarification, and cohesiveness.

2. The ingredient composition of claim 1, wherein the rOVA comprises a polypeptide represented by an amino acid sequence selected from the group consisting of SEQ ID NO: 1-74 or an amino acid sequence with at least 97% identity with one of SEQ ID NO: 1-74.

3. The ingredient composition of claim 1 or 2, wherein the rOVA comprises an amino acid sequence of a duck OVA, an ostrich OVA, or a chicken OVA.

4. The ingredient composition of claim 2 or claim 3, wherein the amino acid sequence of the rOVA lacks an N-terminal methionine.

5. The ingredient composition of any one of claims 1 to 4, wherein the ingredient composition is a powder.

6. The ingredient composition of claim 5, wherein the powder comprises at least 50% rOVA w/w or w/v or at least 75% rOVA w/w or w/v.

7. The ingredient composition of any one of claims 1 to 6, wherein the rOVA provides to the egg-less food item a foam capacity higher than a foam capacity provided by native egg white in a similar egg-less food item.

8. The ingredient composition of any one of claims 1 to 7, wherein the rOVA provides to the egg-less food item a hardness higher than a hardness provided by native egg white in a similar egg-less food item.

9. The ingredient composition of any one of claims 1 to 8, wherein rOVA is present in the egg-less food item in an amount of less than about 8%.

10. The ingredient composition of any one of claims 1 to 9, wherein the rOVA is expressed by a yeast or fungal host cell.

11. The ingredient composition of claim 10, wherein the host cell is selected from a Pichia species, a Saccharomyces species, a Trichoderma species, a Pseudomonas species and an Aspergillus species.

12. The ingredient composition of any one of claims 1 to 11, wherein a glycosylation pattern of the rOVA is devoid of N-linked galactose units.

13. The ingredient composition of any one of claims 1 to 12, wherein the pH of the rOVA when solubilized is between about 3.5 and about 4.5.

14. The ingredient composition of any one of claims 1 to 13, wherein the ingredient composition further comprises one or more consumable additives.

15. The ingredient composition of claim 14, wherein the one or more consumable additives comprise a flavoring agent, a coloring agent, an oil, a polysaccharide or a combination thereof.

16. The ingredient composition of any one of claims 1 to 15, wherein the egg-less food item is a baked product.

17. The ingredient composition of claim 16, wherein the baked product has a crumb structure equivalent to or better than a similar baked product made with a natural egg white.

18. The ingredient composition of claim 16 or claim 17, wherein the baked product is selected from the group consisting of cake, cookie, bagel, biscuit, bread, cracker, muffin, cupcake, scone, roll, pastry, pancake, macaroon, meringue, choux pastry, and soufflé

19. The ingredient composition of any one of claims 16 to 18, wherein the baked product has a crumb structure equivalent to or better than a similar baked product made with a natural egg white.

20. The ingredient composition any one of claims 1 to 15, wherein the egg-less food item is a liquid or semi-solid food product.

21. The ingredient composition of claim 20, wherein the liquid composition further comprises one or more solvents.

22. The ingredient composition of any one of claims 1 to 15, wherein the egg-less food item is an emulsified food product.

23. The ingredient composition any one of claims 1 to 15, wherein the egg-less food item is a meringue, a whipped dessert, a whipped topping or a soufflé.