protein compositions. In some examples, the methods described herein are food-safe, inexpensive, and scalable, while minimizing the development of undesirable odors and flavors in the purified protein and increasing protein yield. In some examples of methods of purifying protein provided herein in which the pH is less than 8.5 (e.g., 8.0 or less) during the purification process, increased off-flavors and/or off-odors can be present in the resulting protein composition and process yields can be reduced compared to otherwise corresponding methods of purifying protein in which the pH is greater than 8.5 (e.g., 9.0 or greater) during some or all of the purification process. In some examples, compositions described herein can be food-safe and inexpensive, with minimal undesirable odors and flavors. Total cellular protein, e.g., proteins that are isolated from throughout a cell or produced by it, including proteins from the cytoplasm, and nucleus and subcellular compartments (e.g., lysosomes, peroxisomes, mitochondria, endoplasmic reticulum, Golgi apparatus, periplasm, secretory vesicles, extracellular matrix, biofilm, chloroplast and nucleus) as applicable, can be purified using the methods described herein. In some examples, a protein composition as described herein can comprise total cellular protein, but the term "total" does not indicate that every cellular protein is present in the protein composition. In some examples, a protein composition as described herein can consist essentially of total cellular protein.

Furthermore, the protein in a protein composition can be functional. As described herein, functional proteins can have one or more of the following properties: non-denatured; capable of forming a gel upon heating (e.g., a suspension of about 25 to about 250 mg/mL (e.g., about 25 to about 50 mg/mL, about 25 to about 100 mg/mL, about 25 to about 150 mg/mL, about 25 to about 200 mg/mL, about 50 to about 250 mg/mL, about 100 to about 250 mg/mL, about 150 to about 250 mg/mL, or about 200 to about 250 mg/mL) at a pH of about 7.0) thermally transitions to a gel upon heating to about 65°C); thermally denatures during incubation between about 50°C and about 85°C, with greater than about 80% of the protein denaturing after about 20 minutes at about 85°C, as measured either by differential scanning calorimetry (DSC) or differential scanning fluorimetry (DSF); in a solution or suspension of purified protein at or above about 50 mg/mL (5% w/v), protein forms a freestanding gel (with, e.g., a 100 Pa storage modulus)

when heated at or above about 85°C for about 20 minutes; can denature and gel between about pH 5.5 and about pH 10.0; can denature and gel in solutions with ionic strength (I) below about 0.5M, when I is calculated based on the concentration of non-protein solutes; at a protein concentration of about 10 mg/mL, particle size distribution D10, D50 and D90 are less than about 0.1 µm, 1.0 µm and 5 µm, respectively; has enzymatic activity; has an emulsion activity index (EAI) of greater than or equal to about 50 m^2/g protein across a pH range of about 4.0 to about 8.0.

In some examples, a (w/v) suspension can refer to the amount of dry solids (in grams) per 100 mL of solution.

Non-limiting examples of functional proteins that can be present in a protein composition include proteins that have enzymatic activity such as, without limitation, cysteine synthase (Met17p, ED 2.5.1.47), cystathionine beta-synthase (Cys4p, EC 4.2.1.22), hexokinase, glucose oxidase, glutathione reductase, catalase, and lipase.

Enzymatic activity also can be described more generically and examples can include, for example amino acid catabolism (e.g., hydrogen sulfide (H_2S) present at less than about 0.1 ppm in the headspace when no L-cysteine (e.g., L-cysteine by itself, or provided in the form of a mixture of isomers) is added (e.g., to 5 mL of a 2% (w/v) suspension at pH 7.0), and H_2S present at greater than or equal to about 0.2 ppm (e.g., greater than or equal to about 0.3 ppm) after (e.g., after about 24 hours at 25 °C) L-cysteine is added to 25 mM final concentration (e.g., to 5 mL of a 2% (w/v) suspension)), glucose catabolism (e.g., generation of pyruvate from glucose, generation of glucose-6-phosphate, generation of lactate, production of D-glucono-δ-lactone), lipid catabolism (e.g., lipid hydrolysis), reduction of glutathione disulfide, and decomposition of hydrogen peroxide. For example, enzymatic activity can be illustrated using a single-enzyme reaction (e.g., generation of glucose-6-phosphate from glucose by hexokinase) or a multi-enzyme reaction, e.g., transformation of a starting material to a final product by more than one enzyme (e.g., generation of pyruvate from glucose by the enzymes of glycolysis or generation of glutathione from glutamate, cysteine and glycine by the cellular glutathione biosynthesis pathway).

The protein composition can have food activity. As described herein, proteins with food activity can have one or more of the following properties (defined on a per-gram basis): capable of forming a gel; capable of emulsifying oil and water (oil-in water, water-in-oil); capable of emulsifying air and water (air-in-water).

In the description and Figures, the following abbreviations are used: CS (cell suspension); RN (cell wash); LY (lysate, e.g., obtained by bead milling, homogenizer, high shear mixer or microfluidizer); CN (centrate, e.g., supernatant of centrifugal spin to remove solids); MF (microfiltration, e.g., using a pore size of 0.2. 0.3, or 0.45 μm in diameter); DF (diafiltration, e.g., using pH 9.3 +/- 0.3 water); UF (ultrafiltration, e.g., using a molecular weight cutoff of 5, 10, 30, or 50 kDa); PZ (pasteurization, e.g., at 65 °C for 60 seconds); SD (spray drying, e.g., with an inlet temperature of 180 °C, an outlet temperature of 80 °C and a feed of 0.27 LPM).

The term "about" is used with respect to a particular value to account for experimental variation when measuring the value.

In one example, this document includes a method for purifying protein from a cell (e.g., a plurality of cells), the method including lysing an aqueous suspension of the plurality of cells to obtain a cell lysate; clarifying the cell lysate, optionally in the presence of one or more flocculants, to obtain a clarified lysate; filtering the clarified lysate to obtain a filtered lysate; concentrating the filtered lysate to obtain a protein composition; and optionally pasteurizing the protein composition of protein to obtain a pasteurized protein composition, wherein the lysing, clarifying, and filtering steps, independently, are performed at a pH between about 8.5 and about 12.0.

In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 10% by weight animal products. In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 5% by weight animal products. In some examples, a protein composition or food product described herein contains less than 1% by weight animal products. In some examples, a protein composition or food product described herein contains no animal products.

In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 10% by weight animal-derived products. In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 5% by weight animal-derived products. In some examples, a protein composition or a food product described herein contains less than 1% by weight animal-derived products. In some examples, a protein composition or food product described herein contains no animal-derived products.

In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 10% by weight animal meat. In some examples of a protein composition or a food product described herein, the protein composition or food product contains less than 5% by weight animal meat. In some examples, a protein composition or food product described herein contains less than 1% by weight animal meat. In some examples, a protein composition or food product described herein contains no animal meat.

In some examples of a protein composition or a food product described herein, the protein composition or food product is free of or is substantially free of lactose, *E. coli*, whey, casein, animal fat, soy proteins, nut proteins, ovalbumins, gelatin, dairy products, animal products, animal-derived products, agar, carrageenan, tofu, cholesterol, or two or more thereof.

As used herein, the term "animal products" refers to a material obtained from or produced by the body of an animal (e.g., a mammal, a bird, a fish, an amphibian, a reptile, an insect, a mollusk, a crustacean, a coral, an arachnid, or a horseshoe crab). Examples include, without limitation, meat, fat, flesh, blood, milk, eggs, isinglass, rennet, fur, skin, hair, bone, fibers, cartilage, casein, gelatin, and honey. The term "no animal products" means that the composition does not contain any animal products.

As used herein, the term "animal-derived products" refers to a material or compound derived from the body of an animal (e.g., a mammal, a bird, a fish, an amphibian, a reptile, an insect, a mollusk, a crustacean, a coral, an arachnid, or a horseshoe crab). Examples include, without limitation, a material or compound derived

in a bacterium, a yeast, an algae, a fungus, or a plant, independently of whether the protein is natively expressed in the bacterium, yeast, algae, fungus, or plant, respectively.

The term “not natively expressed” can refer to a protein that is produced in an organism that does not produce said protein in nature. Non-limiting examples of a protein that is not natively expressed include an animal protein expressed in bacteria, a plant protein expressed in yeast, and an animal protein expressed in algae.

The term “pasteurized” can mean any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage.

The term “intact”, as it pertains to cells, as used herein includes cells that are perforated, but not cells that are burst or lysed. In cells that have subcellular compartments, intact cells typically retain the subcellular compartments within the cellular membrane. Exemplary methods that result in the lysis of cells include mechanical lysis (e.g., bead beating, bead milling, grinding, or rotator-shaker homogenizers), cryopulverization, high pressure cell disruption (e.g., by French press, or microfluidizer), sonication, and nitrogen decompression. In some examples when intact cells are specified, none of mechanical lysis (e.g., bead beating, bead milling, grinding, or rotator-shaker homogenizers), cryopulverization, high pressure cell disruption (e.g., by French press, or microfluidizer), sonication, or nitrogen decompression were performed. In some examples, intact cells can be determined by particle size distribution. In some examples, the particle size distribution of intact cells (e.g., that have undergone a treatment, such as any of the treatments described herein), can be significantly unchanged compared to a suspension of single cells. For example, the particle size distribution median for intact yeast cells can be about 3 μm. In some examples, the particle size distribution of intact yeast cells can lack a peak smaller than about 3 μm, which can be indicative of cell fragments.

As used herein, “low flavor” with respect to a protein composition means that the protein composition has less flavor than the source of the protein composition (e.g., yeast, if a yeast protein composition is described). For example, less of one or more flavor

compounds that give rise to a distinguishing flavor associated with the source of the protein. In some examples, a low flavor protein composition can have little flavor of its own. In some instances, a low flavor protein composition has less flavor than a known protein composition (e.g., a commercial soy protein isolate). Having less flavor can be determined, for example, by a trained human panelist, or, for example, by measurement of one or more volatile compounds commonly understood to impart flavor and/or aroma.

The term "flavor compound" as used herein refers to a compound that imparts to a protein composition as described herein a taste and/or aroma detectable, for example, by a trained human panelist. In some examples, a flavor compound can be a positive aspect, e.g., imparting a flavor that is expected in a use of the protein composition, e.g., a flavor compound suggestive of an animal meat flavor in a meat replica product. In some examples, a flavor compound can be a negative aspect, e.g., imparting a flavor that is not expected in a use of the protein composition, e.g., a flavor compound suggestive of soy in a meat replica product.

The term "aromaome" can mean the totality of aromas associated by the ordinary human observer with a particular food, ingredient, or cooking process. Non-limiting examples of aromaomes are a poultry aromaome, a chicken aromaome, a beef aromaome, a pork aromaome, a seafood aromaome, a game meat aromaome, a cinnamon aromaome, a chocolate aromaome, a deep frying aromaome, and a grilling aromaome.

In one example, this disclosure includes a method for purifying protein from a plurality of cells, the method including lysing an aqueous suspension of the plurality of cells to obtain a cell lysate; clarifying the cell lysate, optionally in the presence of one or more flocculants, to obtain a clarified lysate; filtering the clarified lysate to obtain a protein composition; and optionally pasteurizing the protein composition, to obtain a pasteurized protein composition, wherein steps a), b), c), and d) independently, are performed at a pH between about 8.5 and about 12.0.

In another example, this disclosure includes a method for purifying protein from a plurality of cells, the method including lysing an aqueous suspension of the plurality of cells to obtain a cell lysate; filtering the cell lysate to obtain a protein composition; and optionally pasteurizing the protein composition to obtain a pasteurized protein

composition, wherein steps a), b), and c) independently, are performed at a pH between about 8.5 and about 12.0.

These and other examples can optionally include any of the following. Filtering can include microfiltration, ultrafiltration, diafiltration, or a combination thereof. A clarifying step can be performed by centrifugation to less than about 20% dry solids. A plurality of cells can include microbial cells. A method can further include washing an aqueous suspension of a plurality of cells at a pH between about 8.5 and about 12.0 before step a). A protein composition can include at least about 35%, on a dry weight basis, of compounds larger than 5 kDa. At least about 50% of the protein in a protein composition can fall between about 10 kDa and about 200 kDa.

In another example, this disclosure includes protein composition produced by a method comprising: lysing an aqueous suspension of a plurality of cells to obtain a cell lysate; filtering the cell lysate to obtain a protein composition; and optionally pasteurizing the protein composition to obtain a pasteurized protein composition, wherein steps a), b), and c) independently, are performed at a pH between about 8.5 and about 12.0.

This and other examples can optionally include any of the following. Filtering can include microfiltration, ultrafiltration, diafiltration, or a combination thereof. A process can further include washing an aqueous suspension of a plurality of cells at a pH between about 8.5 and about 12.0 before step a). At least about 50% of the protein in a protein composition can fall between about 10 kDa and about 200 kDa. A protein composition can have a buffering capacity of less than about 2.5 mmol NaOH per gram dry solids. Hydrogen sulfide (H_2S) can be detectable in an amount of less than about 0.1 ppm after about 24 hours at 25 °C when L-cysteine is not added to 5 mL of a 2% (w/v) suspension of a protein composition at pH 7.0. Hydrogen sulfide can be detectable an amount of at least about 0.2 ppm about 24 hours at 25 °C after 5 mL of a 2% (w/v) suspension of a protein composition is brought to about 25 mM final concentration of L-cysteine.

In some examples, provided herein are methods for purifying proteins from a plurality of cells having cell walls. In some examples, provided is a method for purifying proteins from a plurality of cells having cell walls, the method including: a)

perforating the cell walls of the plurality of cells, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition, and e) optionally pasteurizing the protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for purifying proteins from a plurality of cells, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition, and e) optionally pasteurizing the protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 10% by weight of the cytoplasmic proteins of the plurality of cells.In some examples, provided isa method for purifying proteins from a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a protein composition, and f) optionally pasteurizing the protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for purifying proteins from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion,

d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a protein composition, and f) optionally pasteurizing the protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells.

In one aspect, the present disclosure refers to a method for purifying proteins from a plurality of cells having cell walls, the method comprising:

a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0;

b) optionally perforating the cell walls of the plurality of cells;

c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion;

d) filtering the liquid portion to form a filtrate and a retentate;

e) concentrating the retentate to form a protein composition; and

f) optionally pasteurizing the protein composition,

wherein each of a)-e), independently, are performed at a pH of about 8.5 to about 12.0, and/or wherein the method results in the liquid portion comprising at least about 10% by weight of the cytoplasmic proteins of the plurality of cells.

In one aspect, the present disclosure refers to a method for purifying proteins from a plurality of cells having cell walls, the method comprising:

a) perforating the cell walls of the plurality of cells;

b) optionally treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0;

c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion;

d) filtering the liquid portion to form a filtrate and a retentate;

e) concentrating the retentate to form a protein composition; and

f) optionally pasteurizing the protein composition,

wherein each of a)-e), independently, are performed at a pH of about 8.5 to about 12.0, and/or wherein the method results in the liquid portion comprising at least about 10% by weight of the cytoplasmic proteins of the plurality of cells.

In some examples, provided herein are methods for purifying a plurality in proteins from a plurality of cells. In some examples, provided is a method for purifying proteins from a plurality of cells, the method including: a) heating the plurality of cells to a temperature of about 50°C to about 85°C, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition, and e) optionally pasteurizing the protein

composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells.

In one aspect, the present disclosure refers to a protein composition prepared by any one of the methods disclosed herein.

In one aspect, the present disclosure refers to use of a protein composition prepared by any one of the methods disclosed herein in a food, a beverage, or a supplement.

In one aspect, the present disclosure refers to a protein composition comprising:

a plurality of functional proteins,

wherein the protein composition has one or more of the following features:

wherein the protein composition has a buffering capacity of less than about 3.0 mmol NaOH per gram dry solids;

wherein heating a 10% (w/v) suspension of the protein composition to at least about 95 °C results in a gel with a storage modulus of at least about 100 Pa; and

wherein H_2S is detectable in an amount of less than about 0.1 ppm in the headspace after about 24 hours at 25 °C when L-cysteine is not added to 5 mL of a 2% (w/v) suspension of the protein composition at pH 7.0 and/or wherein H_2S is detectable in an amount of at least about 0.2 ppm in the headspace after about 24 hours at 25 °C and after about 25 mM L-cysteine is added to 5 mL of a 2% (w/v) suspension of the protein composition at pH 7.0.

In one aspect, the present disclosure refers to a food, a beverage, or a supplement comprising any one of the protein compositions disclosed herein.

In some examples, provided herein are methods for preparing a cytosolic protein-enriched protein composition from a plurality of cells having cell walls. In some examples, provided is a method for preparing a cytosolic protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a cytosolic protein-enriched protein composition, and e) optionally pasteurizing the cytosolic protein-enriched protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a cytosolic protein-enriched protein composition from a plurality of cells, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) separating the aqueous suspension of

the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a cytosolic protein-enriched protein composition, and e) optionally pasteurizing the cytosolic protein-enriched protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 10% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a cytosolic protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a cytosolic protein-enriched protein composition, and f) optionally pasteurizing the cytosolic protein-enriched protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a cytosolic protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a cytosolic protein-enriched protein composition, and f) optionally pasteurizing the cytosolic protein-enriched protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a cytosolic protein-enriched protein composition from a plurality of cells, the method including: a) heating the plurality of

cells to a temperature of about 50°C to about 85°C, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a cytosolic protein-enriched protein composition, and e) optionally pasteurizing the cytosolic protein-enriched protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells.

In some examples, provided herein are methods for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells having cell walls. In some examples, provided is a method for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) extracting protein from the solids portion to form a membrane-bound and/or subcellular compartment protein-enriched protein composition, and d) optionally pasteurizing the membrane-bound and/or subcellular compartment protein-enriched protein composition, wherein each of a)-b), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) extracting protein from the solids portion to form a membrane-bound and/or subcellular compartment protein-enriched protein composition, and d) optionally pasteurizing the membrane-bound and/or subcellular compartment protein-enriched protein composition, wherein each of a)-b), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the

method results in the liquid portion including at least about 10% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) extracting protein from the solids portion to form a membrane-bound and/or subcellular compartment protein-enriched protein composition, and e) optionally pasteurizing the membrane-bound and/or subcellular compartment protein-enriched protein composition, wherein each of a)-c), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) extracting protein from the solids portion to form a membrane-bound and/or subcellular compartment protein-enriched protein composition, and e) optionally pasteurizing the membrane-bound and/or subcellular compartment protein-enriched protein composition, wherein each of a)-c), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for preparing a membrane-bound and/or subcellular compartment protein-enriched protein composition from a plurality of cells, the method including: a) heating the plurality of cells to a temperature of about 50°C to about 85°C, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) extracting protein from the solids portion to form

a membrane-bound and/or subcellular compartment protein-enriched protein composition, and e) optionally pasteurizing the membrane-bound and/or subcellular compartment protein-enriched protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, the extracting protein from the solids portion comprises mechanical lysis of the solids portion.

In some examples, provided herein are methods for purifying a soluble protein from a plurality of cells having cell walls. In some examples, provided is a method for purifying a soluble protein from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition including the soluble protein, and e) optionally pasteurizing the protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for purifying a soluble protein from a plurality of cells, the method including: a) treating an aqueous suspension of the plurality of cells expressing the soluble protein with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition including the soluble protein, and e) optionally pasteurizing the protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 10% by weight of the cytoplasmic proteins of the plurality of cells.In some examples, provided is a method for purifying a soluble protein from a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells expressing the soluble protein with a base until the pH of the aqueous

suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a protein composition including the soluble protein, and f) optionally pasteurizing the protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for purifying a soluble protein from a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, c) separating the aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, d) filtering the liquid portion to form a filtrate and a retentate, e) concentrating the retentate to form a protein composition including the soluble protein, and f) optionally pasteurizing the protein composition, wherein each of a)-e), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, provided is a method for purifying a soluble protein from a plurality of cells, the method including: a) heating the plurality of cells expressing the soluble protein to a temperature of about 50°C to about 85°C, b) separating an aqueous suspension of the plurality of cells to form a solids portion and a liquid portion, c) filtering the liquid portion to form a filtrate and a retentate, d) concentrating the retentate to form a protein composition including the soluble protein, and e) optionally pasteurizing the protein composition, wherein each of a)-d), independently, are performed at a pH of about 8.5 and about 12.0, and/or wherein the method results in the liquid portion including at least about 50% by weight of the cytoplasmic proteins of the plurality of cells. In some examples, the soluble protein is a heme-containing protein. In some examples, the method comprises treating the plurality of cells with about 5 mM to about 500 mM reducing equivalents of includes reductant. In some examples, treatment with the reductant comprises treatment with

about 20 mM to about 80 mM reducing equivalents of the reductant. In some examples, the reductant is selected from the group consisting of cysteine, glutathione, bisulfite, and a combination thereof. In some examples, the reductant is a food safe reductant. In some examples, the soluble protein has a melting point, and method further comprises, before a), heating the plurality of cells to a temperature of about 10°C or about 5°C below the melting point of the soluble protein. In some examples, the soluble protein has a melting point of at least about 60°C. In some examples, the soluble protein is heterologous to the plurality of cells.

In some examples, provided herein are methods of treating a plurality of cells (e.g., a plurality of cells having cell walls). In some examples, provided is a method for treating a plurality of cells having cell walls, the method including: perforating the cell walls of the plurality of cells, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 μg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a method for treating a plurality of cells, the method including: treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 μg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a method for treating a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 μg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a method for treating a plurality of cells having cell walls, the method

including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a method for treating a plurality of cells, the method including: heating the plurality of cells to a temperature of about 50°C to about 85°C, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a method for treating a plurality of cells having cell walls, the method including: perforating the cell walls of the plurality of cells, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0. In some examples, provided is a method for treating a plurality of cells, the method including: treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0. In some examples, provided is a method for treating a plurality of cells having cell walls, the method including: a) treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, b) perforating the cell walls of the plurality of cells, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0. In some examples, provided is a method for treating a plurality of cells having cell walls, the method including: a) perforating the cell walls of the plurality of cells, b) treating an aqueous suspension of the

plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0. In some examples, provided is a method for treating a plurality of cells, the method including: heating the plurality of cells to a temperature of about 50°C to about 85°C, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0.

In one aspect, the present disclosure refers to a method for treating a plurality of cells having cell walls, the method comprising:

perforating the cell walls of the plurality of cells, optionally wherein the perforating is performed at a pH of about 8.5 to about 12.0., optionally wherein the perforating is performed at a temperature of less than or equal to about 12 °C; optionally wherein the method further comprises, after the perforating, heating the plurality of cells to at least about 60 °C

wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids.

In one aspect, the present disclosure refers to a method for treating a plurality of cells, the method comprising:

treating an aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, optionally wherein the treating is performed at a temperature of less than or equal to about 12 °C;

wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids.

In one aspect, the present disclosure refers to a method for treating a plurality of cells, the method comprising:

heating the plurality of cells to a temperature of about 50°C to about 85°C, optionally wherein the heating is performed at a pH of about 8.5 to about 12.0, optionally wherein the method further comprises, after heating the plurality of cells, treating a aqueous suspension of the plurality of cells with a base until the pH of the aqueous suspension is about 8.5 to about 12.0, optionally wherein when the plurality of cells has cell walls, the method further comprises, after heating the plurality of cells, perforating the cell walls of the plurality of cells;

wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids.

In one aspect, the present disclosure refers to a composition comprising the plurality of cells prepared by any one of the methods disclosed herein.

Also provided herein are compositions of cells, such as those that have been treated as described herein. In some examples, provided is a composition including: a plurality of cells, wherein treatment of a supernatant of the plurality of cells with a mannosidase yields less than about 30 µg/mL detectable mannose in the supernatant, wherein the supernatant is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50°C for 10 minutes at pH 10.5 and centrifuged to remove solids. In some examples, provided is a composition including: a plurality of cells, wherein less than about 200 µg/mL beta glucan is detectable in a soluble phase, wherein the soluble phase is prepared using a 10% (w/v) suspension of the plurality of cells, after being incubated at 50 °C for 10 minutes at pH 12.0.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more examples of the disclosure are set forth in the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims. The word