

METHODS AND SYSTEMS FOR ISOLATING CULTURED BIOMASS AND INTACT CELLS UTILIZING ISOELECTRIC PRECIPITATION

FIELD OF THE INVENTION

[0001] Provided are methods and systems for isolating edible biomass from a suspension cell culture comprising metazoan cells, methods and systems of isolating intact cells from a cell culture, and cell-based food products comprising the edible biomass obtained from the methods described herein.

BACKGROUND OF THE INVENTION

[0002] Foods formulated from metazoan cells, cultured in vitro, have prospective advantages over their corporal-derived animal counterparts, including improved nutrition safety, and ethical implications. Production of these products have been projected to require fewer resources, convert biomass at a higher caloric efficiency, and result in reduced environmental impacts relative to conventional in vivo methods. Together, metazoan cells, and their extracellular products, constitute a biomass that can be isolated from a culture vessel for formulation of cell-based food products, such as cultured meat.

[0003] However, several challenges persist, including mass production of enough cells for formulating into a cell-based food product and obtaining a desired moisture content, texture profile, and flavor (i.e. organoleptic qualities) of the resulting cell-based food product formulations. Current biomass isolation techniques for biomass suspensions may include one or more centrifugation steps to reduce media volume followed by one or more wash buffer steps to rinse away salts and other media residuals that may impact, e.g., the organoleptic qualities of a finished product. Conventional applications of centrifugation concentrate a majority of the cells of the biomass in a heavy phase and residual media and other low density materials, such as amino acids, lysed cells, and free proteins, remain in the light phase and risk being discarded as waste. Although high centrifugation speeds improve separation, they also expose the cells to high pressures that can potentially rupture the cell wall. Materials escaping from ruptured cell walls, e.g., amino acids, peptides, and proteins, can result in lower yields through loss in the light phase during centrifugation and that of the ruptured cells which remains in the heavy phase, is more susceptible to quality degradation through accelerated oxidation. The fragility of the cells of the biomass

therefore places an upper limit on acceptable centrifugation speeds, which in turn limits the ability to sufficiently concentrate the valuable biomass to a moisture content within desired levels. Thus, a need remains for biomass isolation processes which yield a higher concentration of viable cells. A need also remains for biomass isolation processes with higher yield of cells, cell fragments, cell by-products, proteins, lipids, amino acids, or some combination thereof.

[0004] These needs and others are met by the disclosure presented herein.

SUMMARY OF THE INVENTION

[0005] Provided are methods of isolating cell biomass from a suspension culture of metazoan cells for production of a cell-based food product in which the isolated biomass has improved intact cell and protein yield and reduced moisture content. The resulting isolated biomass also has improved characteristics such as texture and color. The methods described herein also provide for more efficient isolation of edible biomass from suspension culture, as isoelectric precipitation via pH adjustment can streamline processing and eliminate the need for multiple centrifugation steps. The method involves adjusting the pH of the expanded suspension culture to at or around the isoelectric point of the proteins in the suspension culture, generally within pH 4.3 to pH 5.7, to promote precipitation of free proteins and cell-associated proteins, facilitating isolation of the same into the heavy phase even at low centrifugation speed. Use of the isoelectric point precipitation (IEP) can result in a reduction in the number of centrifugation and washing steps (for example, from two centrifugation and washing steps to only one centrifugation and washing step) to obtain the edible biomass for use in a cell-based food product with the improved characteristics. In embodiments, the method involves adjusting the pH of the expanded suspension culture material to at or around pH 11.0 to pH 12.5 for a period of time, for example, a period of about 30 min, before adjustment of the pH of the material to a pH from pH 4.3 to pH 5.7. Methods described herein may result in edible biomass which has a lower moisture content and potentially does not require vacuum drying, or other similar post centrifugation drying steps, to produce the cell-based food product for consumption. In embodiments, the edible biomass has sensory improvements compared to edible biomass processed without an acidic pH adjustment or IEP, such improvements include increased whiteness, improved

neutral taste, reduced off-notes, reduced aromas of sulphur, reduced aromas of rubber, reduced metallic taste, and improved hardness.

[0006] Accordingly, in one aspect, provided herein are methods of isolating edible biomass (including cells and protein) from a suspension cell culture comprising metazoan cells, wherein the method comprises a. culturing the metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture; b. adjusting the pH of the cell culture from about pH 4.3 to pH 5.7 to cause formation of a light phase and a heavy phase; and c. separating the light phase from the heavy phase, wherein the cell culture comprises free proteins and cell-associated proteins, wherein the heavy phase comprises a majority portion of the free proteins and cell-associated proteins from the cell culture, and wherein the heavy phase comprises the edible biomass to be isolated.

[0007] In one aspect, provided herein are cell-based food products comprising edible biomass from a suspension cell culture isolated according to the methods disclosed herein. The edible biomass concentrated by IEP as described herein has improved viable cell count, protein (both free and cell-associated) yield, protein integrity (e.g. as determined by SDS-PAGE), color, reduced moisture content, improved acceptability levels, decreased sulphur, rubber and metallic profiles, and improved hardness (g) compared to edible biomass isolated without IEP. The cell-based food products produced using the edible biomass obtained from the methods described herein also have similar or improved characteristics such as texture (e.g., springiness, hardness, cohesiveness) and color compared to cell-based food products produced using edible biomass isolated in other ways, such as without IEP.

EMBODIMENTS

[0008] Embodiment 1. A method of isolating an edible biomass from a suspension cell culture comprising metazoan cells, wherein the method comprises: (a) culturing the metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture; (b) adjusting the pH of the cell culture to a range of 4.3 to 5.7; (c) forming the cell culture into a light phase and a heavy phase; and (d) separating the light phase from the heavy phase, wherein the cell culture comprises intact cells, free proteins, and cell-associated proteins, wherein the heavy phase comprises a majority portion of the

intact cells, free proteins, and cell-associated proteins from the cell culture, together comprising the isolated edible biomass.

[0009] Embodiment 2. The method according to embodiment 1, wherein the metazoan cells are derived from poultry cells having phenotypes of fibroblasts, myofibroblasts, myogenic cells, or a combination thereof.

[0010] Embodiment 3. The method according to embodiment 2, wherein the myogenic cells are myoblasts, myocytes, satellite cells, muscle derived stem cells, myogenic pericytes, or mesoangioblasts.

[0011] Embodiment 4. The method according to any one of embodiments 1-3, wherein the poultry cells are derived from chicken, duck, or turkey.

[0012] Embodiment 5. The method according to any one of embodiments 1-4, wherein the cell culture comprises about 15×10^6 to about 25×10^6 cells per mL.

[0013] Embodiment 6. The method according to embodiment 5, wherein adjusting the pH of the cell culture comprises adjusting the pH to in the range of 4.5 to 5.5.

[0014] Embodiment 7. The method according to any one of embodiments 1-6, wherein adjusting the pH of the cell culture comprises adding an acidic component to the cell culture.

[0015] Embodiment 8. The method according to any one of embodiments 1-7, wherein adjusting the pH of the cell culture comprises adding hydrochloric acid, citric acid, glucono delta lactone (GDL), lactic acid, malic acid, ascorbic acid, caprylic acid, sorbic acid, caproic acid, benzoic acid, propionic acid, acetic acid, formic acid, fumaric acid, tartaric acid, or a combination thereof, to the cell culture.

[0016] Embodiment 9. The method according to embodiment 8, wherein adjusting the pH of the cell culture comprises adding citric acid, GDL, lactic acid, malic acid, ascorbic acid, or a combination thereof, to the cell culture.

[0017] Embodiment 10. The method according to embodiment 8 or 9, wherein adjusting the pH of the cell culture comprises adding citric acid, GDL, or a combination thereof, to the cell culture.

[0018] Embodiment 11. The method according to any one of embodiments 1 to 10, wherein prior to step (b) there is a first step of adjusting the pH of the cell culture to a range of about 11 to about 12.5.

[0019] Embodiment 12. The method according to embodiment 11, wherein the first step of adjusting the pH of the cell culture comprises adjusting the pH to in the range of 11.5 to 12.5.

[0020] Embodiment 13. The method according to embodiment 12, wherein the first step of adjusting the pH of the cell culture comprises adjusting the pH to about 12.

[0021] Embodiment 14. The method according to any one of embodiments 11 to 13, wherein the first step of adjusting the pH of the cell culture comprises adding a basic component to the cell culture.

[0022] Embodiment 15. The method according to embodiment 14, wherein the first step of adjusting the pH of the cell culture comprises adding sodium hydroxide (NaOH), potassium hydroxide (KOH), magnesium hydroxide (Mg(OH)₂), calcium hydroxide (Ca(OH)₂), or a combination thereof, to the cell culture.

[0023] Embodiment 16. The method according to embodiment 15, wherein the first step of adjusting the pH of the cell culture comprises adding sodium hydroxide (NaOH) to the cell culture.

[0024] Embodiment 17. The method according to any one of embodiments 1-16, wherein separating the light phase from the heavy phase comprises centrifuging the cell culture.

[0025] Embodiment 18. The method according to any one of embodiments 1-17, wherein the method comprises only one centrifugation step to separate the light phase from the heavy phase.

[0026] Embodiment 19. The method according to embodiment 17 or 18, wherein centrifuging the cell culture is performed from about 500 G to about 5000 G or 1000G to 25000G.

[0027] Embodiment 20. The method according to any one of embodiments 17-19, wherein adjusting pH of the cell culture comprises adding GDL to obtain a pH of about 4.5 and centrifuging the cell culture at about 2000 G to 3000 G or about 2500 G.

[0028] Embodiment 21. The method according to any one of embodiments 17-20, wherein adjusting pH of the cell culture comprises adding citric acid to obtain a pH of about 4.5 or about 4.5 to 5.5 and centrifuging the cell culture at about 4000 G to 5500 G or about 5000 G.

[0029] Embodiment 22. The method according to any one of embodiments 17-21, wherein centrifuging the cell culture is performed for from about 5 min to about 20 min.

[0030] Embodiment 23. The method according to any one of embodiments 17-19, wherein centrifuging the cell is performed at about 3000 G for about 10 min.

[0031] Embodiment 24. The method according to any one of embodiments 1-23, wherein the cell culture comprises a volume of from about 50 L to 100,000 L prior to adjusting the pH.

[0032] Embodiment 25. The method according to any one of embodiments 7-24, wherein adding the acidic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%.

[0033] Embodiment 26. The method according to any one of embodiments 14-25, wherein adding the basic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%.

[0034] Embodiment 27. The method according to any one of embodiments 1-26, wherein the cell culture is not homogenized.

[0035] Embodiment 28. The method according to any one of embodiments 1-26, wherein the cell culture is homogenized prior to step b).

[0036] Embodiment 29. The method according to any one of embodiments 1-28, wherein the cell culture after step c) comprises from about 0.3 g to about 10.0 g of the heavy phase per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase.

[0037] Embodiment 30. The method according to any one of embodiments 1-29, wherein the edible biomass comprises from about 0.01 g to about 0.5 g of total free proteins and cell-associated proteins per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase.

[0038] Embodiment 31. The method according to any one of embodiments 1-30, wherein the first step of adjusting the pH of the cell culture is to a pH of about 12 by addition of NaOH to the cell culture, wherein the second step of adjusting the pH of the cell culture is to a pH of about 4.5 by addition of citric acid to the cell culture, and wherein

separating the light phase from the heavy phase further comprises centrifuging the cell culture at 3000 G for about 10 min.

[0039] Embodiment 32. The method according to any one of embodiments 1-31, wherein a concentration of total intact cells in the heavy phase range from about 300×10^6 cells/mL to about 600×10^6 cells/mL.

[0040] Embodiment 33. The method according to any one of embodiments 1-32, wherein the method is performed at room temperature or at a temperature between 20° to 30° C.

[0041] Embodiment 34. The method according to any one of embodiments 1-33, wherein the method is performed at ambient pressure.

[0042] Embodiment 35. The method according to any one of embodiments 1-34, wherein the method is performed at a temperature of about 5 to 20 °C.

[0043] Embodiment 36. The method according to any one of embodiments 1-35, wherein the edible biomass comprises from about 5.0% to about 15.0% protein according to a bicinchoninic acid (BCA) assay.

[0044] Embodiment 37. The method according to any one of embodiments 1-36, wherein the edible biomass comprises from about 3.0% to about 15.0% protein according to the Kjeldahl method.

[0045] Embodiment 38. The method according to any one of embodiments 1-37, wherein at least a partial portion of the suspension cell culture is removed from a cell culture tank before the adjustment of the pH to initiate a new suspension cell culture for isolating edible biomass.

[0046] Embodiment 39. The method according to any one of embodiments 1-38, wherein at least a partial portion of the suspension cell culture is retained in a cell culture tank wherein the suspended cell culture was cultured to initiate a new suspension cell culture for isolating edible biomass and a remaining portion of the suspended cell culture is transferred to a second tank for pH adjustment.

[0047] Embodiment 40. The method according to any one of embodiments 1-39, wherein the cell culture has an initial pH of about 6.7.

[0048] Embodiment 41. The method according to any one of embodiments 1-40, wherein the suspension culture comprises no more than 10%, 7%, 5%, 1%, 0.5%, or no more than 0.01% animal-derived serum.

[0049] Embodiment 42. A method of isolating edible biomass from a suspension cell culture comprising metazoan cells, wherein the method comprises (a) culturing the metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture; (b) adjusting the pH of the cell culture to about 12, wherein adjusting the pH of the cell culture comprises adding NaOH to the cell culture; (c) adjusting the pH of the cell culture to about 4.5 to cause formation of a light phase and a heavy phase, wherein adjusting the pH of the cell culture comprises adding citric acid to the cell culture; and (d) separating the light phase from the heavy phase, wherein separating the light phase from the heavy phase further comprises centrifuging the cell culture at about 3000 G for about 10 min, wherein the cell culture comprises free proteins and cell-associated proteins, wherein the heavy phase comprises a majority portion of the free proteins and cell-associated proteins from the cell culture, and wherein the heavy phase comprises the isolated edible biomass.

[0050] Embodiment 43. An isolated edible biomass prepared from a method of any one of embodiments 1-42.

[0051] Embodiment 44. The isolated edible biomass according to embodiment 43, wherein a hardness of the isolated edible biomass is from about 1000 g to about 6000 g according to a texture profile analysis (TPA).

[0052] Embodiment 45. The isolated edible biomass according to embodiment 43 or 44, wherein a cohesiveness score of the isolated edible biomass is from about 4.0 to about 9.0 according to a texture profile analysis (TPA).

[0053] Embodiment 46. The isolated edible biomass according to any one of embodiments 43-45, wherein a springiness score of the cell isolated edible biomass is between about 4.0 and about 9.0 according to a texture profile analysis (TPA).

[0054] Embodiment 47. The isolated edible biomass according to any one of embodiments 43-46, wherein a gumminess score of the isolated edible biomass is between about 100 to about 1000 according to a texture profile analysis (TPA).

[0055] Embodiment 48. The isolated edible biomass according to any one of embodiments 43-47, wherein a chewiness score of the isolated edible biomass is between about 100 to about 500 according to a texture profile analysis (TPA).

[0056] Embodiment 49. The isolated edible biomass according to any one of embodiments 43-48, wherein a resilience of the cell-based food product is between about 4.0 and 9.0 according to a texture profile analysis (TPA).

[0057] Embodiment 50. The isolated edible biomass according to any one of embodiments 43-49, wherein a peak positive force value of the isolated edible biomass according to the Warner-Bratzler shear method is between about 100 g and about 3000 g.

[0058] Embodiment 51. The isolated edible biomass according to any one of embodiments 43-50, wherein the isolated edible biomass comprises a moisture content of from about 20% to about 90%.

[0059] Embodiment 52. The isolated edible biomass according to any one of embodiments 43-51, wherein the isolated edible biomass comprises a water activity (aw) of from about 0.7 aw to about 0.9 aw.

[0060] Embodiment 53. The isolated edible biomass according to any one of embodiments 43-52, wherein the cell-based food product comprises a water activity (aw) of about less than or equal to 0.9 aw.

[0061] Embodiment 54. The isolated edible biomass according to any one of embodiments 43-53, wherein the isolated edible biomass comprises a binding score of from about 5 to about 500 at 10 °C according to a texture profile analysis (TPA).

[0062] Embodiment 55. The isolated edible biomass according to any one of embodiments 43-54, wherein the isolated edible biomass comprises a binding score of from about 5 to about 500 at 15 °C according to a texture profile analysis (TPA).

[0063] Embodiment 56. The isolated edible biomass according to any one of embodiments 43-55, wherein the isolated edible biomass comprises a whiteness score (L*) of from about 70 to about 80.

[0064] Embodiment 57. The isolated edible biomass according to any one of embodiments 43-55, wherein the isolated edible biomass comprises a redness score (a*) of about -3.3 to about -4.0.

[0065] Embodiment 58. The isolated edible biomass according to any one of embodiments 43-55, wherein the isolated edible biomass comprises b* score of about 6.5 to about 8.5.

[0066] Embodiment 59. A cell-based food product comprising an isolated edible biomass prepared from a method of any one of embodiments 1-42 and flavoring or aroma agents.

[0067] Embodiment 60. A system for culturing and isolating metazoan cells comprising: a first culture vessel and a second culture vessel; wherein the first vessel is in fluid communication with the second vessel to allow flow of the suspension culture between the first vessel and the second vessel, wherein each culture vessel is configured for culturing metazoan cells as a suspension culture, wherein each culture vessel comprises a first inlet configured for adding culture media and a second inlet configured for adding an acidic and/or basic component to the vessel to adjust the pH of the suspension culture to 11.0 or 12.5 or to 4.3 to 5.7 to form a light phase and a heavy phase, wherein each culture vessel further comprises a mixer configured to mix the acidic and/or basic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the culture vessel is at 75% capacity or more.

[0068] Embodiment 61. The system according to embodiment 60, further comprising a centrifuge, wherein each culture vessel comprises an outlet configured such that at least the heavy phase can be transferred to the centrifuge or the heavy phase and only a minor portion of the light phase can be transferred to the centrifuge.

[0069] Embodiment 62. The system according to embodiment 58, wherein the system is configured such that the heavy phase of the suspension culture is only centrifuged once.

[0070] Embodiment 63. A system for culturing and isolating edible biomass comprising: a culture vessel configured for culturing metazoan cells as a suspension culture and comprising an inlet for adding culture media; a second vessel in fluid communication with the culture vessel such that at least a major portion of the suspension culture can be transferred to the second vessel, wherein the second vessel comprises an inlet configured for adding an acidic and/or basic component to the second vessel to adjust the pH of the transferred suspension culture to 11.0 to 12.5 or to 4.3 to 5.7 to form a light phase and a

heavy phase, wherein the second vessel further comprises a mixer configured to mix the acidic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the second vessel is at 75% capacity or more.

[0071] Embodiment 64. The system according to embodiment 63 comprising a centrifuge, wherein the second vessel comprises an outlet configured such that at least the heavy phase can be transferred to the centrifuge or the heavy phase and only a minor portion of the light phase can be transferred to the centrifuge.

[0072] Embodiment 65. The system for culturing and isolating edible biomass of any one of embodiments 60 to 64 configured to complete the method according to any one of embodiments 1-43.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] FIG. 1A is a flow chart diagram outlining the steps to the methods of isolating edible biomass utilizing isoelectric precipitation.

[0074] FIGS. 1B and 1C are schematics of system embodiments for culturing and isolating edible biomass utilizing IEP.

[0075] FIG. 2 is a plot showing representative data for protein solubility curve results. The isoelectric point was found to be approximately in the range of pH 4.5-5.5.

[0076] FIG. 3 is flow chart diagram outlining the variables and conditions for isoelectric point experiments.

[0077] FIG. 4 is a plot showing representative data for the sediment yields per 100 g CCF for pH adjustment experiments in which the pH was adjusted using citric acid, GDL, or HCl.

[0078] FIG. 5 is a plot showing representative data for the protein yields as measured using BCA assays per 100 g CCF for pH adjustment experiments in which the pH was adjusted using citric acid, GDL, or HCl.

[0079] FIG. 6 is a plot showing representative data for the total protein yields per 100 g CCF as measured using Kjeldahl for pH adjustment experiments in which the pH was adjusted using citric acid or GDL.

[0080] FIG. 7 is a plot showing representative data showing the differences in dry solid and dry protein % recovery by the method disclosed herein utilizing isoelectric precipitation compared to a conventional method not using pH adjustment.

[0081] FIG. 8A-B are representative images for the cell suspension after pH adjustment with citric acid. FIG. 8A is an image of the sediment. FIG. 8B is an image of the supernatant.

[0082] FIG. 9A-B are representative images for the cell suspension after pH adjustment with GDL. FIG. 9A is an image of the sediment. FIG. 9B is an image of the supernatant.

[0083] FIG. 10A-B are representative images for the cell suspension after pH adjustment with HCl. FIG. 10A is an image of the sediment. FIG. 10B is an image of the supernatant.

[0084] FIG. 11 is a plot showing representative data for the estimated total intact cells/mL for each sediment sample.

[0085] FIG. 12 is a plot showing representative data for the heat-induced gel formation of recovered biomass using a temperature sweep test.

[0086] FIG. 13 is an image showing representative SDS-PAGE data for recovered biomass samples pH adjusted with HCl, GDL, and citric acid.

[0087] FIG. 14 is a plot showing representative data for color improvement of recovered biomass by IEP with HCl, GDL, and citric acid (CA) compared to conventional method (LSS). All IEP treatments showed higher efficiency in reducing a^* (redness) values compared to the conventional method. Also, IEP caused higher L^* (lightness) and subsequent whiteness values compared to the conventional method, specifically in case of GDL and CA treatments.

[0088] FIG. 15 is a graph showing average hardness levels (g) of material isolated from the two pH adjustment process (DR23 CERES, DR25 CERES, DR26 CERES, DR27 CERES and DR28 CERES) compared to minced chicken breast (left column) and the hardness of material isolated from suspension cells using the conventional process.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0089] Where a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term. As used in this specification and in the appended claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise, e.g., “a compound” includes a plurality of compounds. Thus, for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[0090] As used herein and unless otherwise specified, the term “about” means within plus or minus 5% of a given value or range.

[0091] As used herein, the term “biomass” refers to a combination of intact cells, cell fragments, cell by-products, free proteins, cell-associated proteins, and fluids associated with the cells and cell fragments.

[0092] As used herein, the term “dry weight” refers to weight with substantially no water, e.g., less than 5%, less than 1% or less than 0.1% water

[0093] As used herein, the term “isolated biomass” or “isolated edible biomass” refers to edible biomass separated from at least a portion of the cell culture media in which the biomass was cultured. This provides a concentrated biomass by reducing moisture content. The “IEP-isolated biomass” or “IEP-isolated edible biomass” refers to isolated biomass that was separated from at least a portion of the cell culture media by a method that comprised isoelectric precipitation. The “CERES-isolated biomass or CERES-isolated edible biomass” refers to isolated biomass that was separated from at least a portion of the cell culture media by a method that comprised a pH change to about 11.0 to about 12.5 followed by isoelectric precipitation.

[0094] As used herein, the term “heavy phase” refers to the heavy phase of centrifugation, e.g. the pellet, and/or to a precipitate that forms after pH adjustment of a cell culture.

[0095] As used herein, the term “ingredients” refers to, without limitation, biologically active substances, food safe ingredients, non-animal ingredients, animal ingredients,

nutritional supplements, flavoring agents, salts, sugars, vitamins, and minerals used in the cell-based food products described herein.

[0096] As used herein, the term “isoelectric point” refers to the solution pH at which the net overall charge of a protein or mixture of proteins is such that precipitation of at least a subset of the proteins occurs.

[0097] As used herein, the term “light phase” refers to the supernatant during centrifugation, after centrifugation, and/or after pH adjustment of the cell culture.

[0098] As used herein, the term “metazoan cells” refers to cells derived from animals having the body composed of cells differentiated into tissues and organs. In some embodiments, the metazoan cells are mammalian cells, or cells derived from mammalian animals. In some embodiments the mammalian cell is a poultry, bovine, sheep, or porcine cell.

[0099] As used herein, the term “moisture content” refers to the amount of water and other volatile substances in a food product calculated as percentage change in mass following the evaporation of water from a sample to dry weight. Variables that can be adjusted to modulate the moisture content of the cell-based food products provided herein include but are not limited to textured proteins with different water absorbing characteristics, lipid content, and/or binding agents with different water retention characteristics.

[00100] As used herein, the term “myogenic” refers to a cell that has characteristics of skeletal muscle tissue. Natively myogenic cells include, but are not limited to, myoblasts, myocytes, satellite cells, side population cells, muscle derived stem cells, mesenchymal stem cells, myogenic pericytes, or mesoangioblasts. In other instances, cells lacking myogenic phenotypes are transdifferentiated into cells having myogenic phenotypes.

[00101] As used herein, the term “fibroblastic” refers to a cell that has characteristics of skin tissue and/or a phenotype associated with fibroblasts. In some instances, fibroblastic cells may be transdifferentiated to exhibit other useful phenotypes, such as those of myoblasts or adipocytes.

[00102] As used herein, the term “adipogenic” refers to a cell that has the characteristics of fat tissue and/or a phenotype associated with adipocytes. In some instances, cells lacking adipocyte phenotypes are transdifferentiated into cells having adipogenic phenotypes.

[00103] As used herein, the term “peak positive force” refers to the greatest amount of force required to shear a sample in two as measured by a Warner-Bratzler shear force test as described herein.

[00104] As used herein, the term “substantially free of” or “substantially free from” means an amount that is largely but not necessarily wholly free of a component (e.g., cells or animal components (e.g., serum), or cell culture media reagents (e.g., glucose)). For example, when referring to being “substantially free of a component,” “substantially free of” refers to the component being 0.1% or less of by volume, weight/volume, or volume/volume of the final concentration of the cell culture media. For example, when referring to being “substantially free of a cell or cell type,” “substantially free of” is referring to an absolute number within a population or concentration/percentage within a population below a value where the cell or cell type, or any cell derived therefrom, could meaningfully contribute to the larger population. For example, a population substantially free of a cell (or type of cell) means that upon differentiation of the population the cell or type of cell (from which the population is substantially free of) does not significantly contribute progeny, e.g. 0.01%, to the differentiated population.

[00105] As used herein, “suspension culture” refers to a type of culture in which single cells and/or small aggregates of cells multiply (grow) while suspended in agitated liquid media.

[00106] As used herein, the term “texture” refers to mechanical characteristics of a cell-based food product that are correlated with sensory perceptions of the cell-based food product. Texture can be assessed by “texture profile analysis” or “TPA”.

[00107] As used herein, the terms “texture profile analysis” or “TPA” refers to a double compression test for determining the textural properties of a cell-based food product. A TPA test uses multiple rounds of compression as a texture analyzer to provide insight into how samples behave when chewed. TPA is performed using a TA.ET Express Texture Analyzer (Texture Technologies Corp.) and a polymethyl methacrylate cylinder probe of 25 mm diameter (Texture Technologies Corp., Hamilton, MA). The cylinder probe is used to compress each sample with a trigger force of 20 g to 40% compression in a 2-cycle analysis at a test speed of 0.5 mm/sec. The deformation curve of the sample is obtained, and from the deformation curve Force 1, Force2, Area FT 1:2, Time-diff 1:2, Area FT 1:3,

Area FT 2:3, Area FT 4:6, and Time-diff 4:5 are derived according to the manufacturer's protocol. From this raw data, the mechanical characteristics are calculated as follows: springiness = (time-diff 4:5 / time-diff 1:2); cohesiveness = (area FT 4:6 / area FT 1:3); hardness = force 1; gumminess = (hardness x cohesiveness); chewiness = (springiness x gumminess); and resilience = (area FT 2:3 / area FT 1:2); as described in Food Texture and Viscosity Second Edition: Concept and Measurement, Malcolm C. Bourne, April 2002, Academic Press, New York. A description of how to prepare a sample of isolated biomass for a TPA is provided in Example 12.

[00108] As used herein, the term "textured protein" refers to a protein used to provide a textural modification to cultured cells. A non-limiting example of a "textured protein" is a textured soy protein (TSP), which can be referred to as soy meat or soya chunks. Textured protein can be produced from any protein-rich seed meal.

[00109] As used herein, the term "water activity" or "(aw)" refers to the hygroscopic nature of a substance; or the tendency of a substance that absorbs water from its surroundings. The water activity of a solution is expressed as $A_w = P/P_o$, where P is the water vapor pressure of the solution and P_o is the vapor pressure of pure water at the same temperature; water activity is the partial vapor pressure of water in a solution divided by the standard state partial vapor pressure of water. In the field of food science, the standard state is most often defined as pure water at the same temperature. A water activity of 0.80 means the vapor pressure is 80 percent of that of pure water. The water activity increases with temperature. The moisture condition of a product can be measured as the equilibrium relative humidity (ERH) expressed in percentage or as the water activity expressed as a decimal.

[00110] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

1. METHODS OF ISOLATING EDIBLE BIOMASS OR INTACT CELLS UTILIZING IEP

[00111] Isolating edible biomass or intact cells from a suspension cell culture comprising metazoan cells can comprise: a.) culturing metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture; optionally adjusting the pH of the cell culture from 11.0 to 12.5 for a period of time before adjusting the pH downward; b.) adjusting the pH of the cell culture from 4.3 to 5.7 to cause formation of a light phase and a heavy phase; and c.) separating the light phase from the heavy phase, wherein the cell culture comprises free proteins and cell-associated proteins, wherein the heavy phase comprises a majority portion of the free proteins and cell-associated proteins from the cell culture, and wherein the heavy phase comprises the edible biomass. A further step can comprise washing the heavy phase to remove culture media components, such as salts and/or centrifuging the heavy phase, such as after washing. A non-limiting example for the steps in culturing and isolating edible biomass or intact cells is shown in FIG. 1A.

Advantages of the method include color, texture, improved sensory parameters, for example reduced off-notes, reduced aromas of sulphur and rubber, and reduced metallic taste, relative to preparation produced without the IEP

[00112] Metazoan cells are cultured in suspension, optionally, in a serum free media. After the suspended culture **110** reaches a desired cell titer, the pH of cell culture **110** is adjusted with an acidic reagent in a pH adjustment step **120** to yield a cell culture material pH between 4.3 to 5.7 or in some embodiments, 4.5 to 5.5. Optionally, prior to adjusting to the acidic pH, the pH of the culture material is adjusted to a pH from about 11.0 to about 12.5 and then held at that pH for an appropriate period of time, for example half an hour. A precipitate will form after adjusting the pH to the acidic pH. Thus, a heavy phase **160** and light phase **140** will form to facilitate separation of the culture media from the biomass. Next, the heavy phase **160** formed in the pH adjustment step **120** undergoes a centrifugation step **130** facilitating further separation of the culture media in the light phase, from the biomass in a heavy phase **160**. The heavy phase **160** comprising edible biomass can then undergo a rinse step **150** or otherwise processed further, for example, by centrifugation or any other method known in the art or described herein resulting in the isolated biomass **170**.

1.1 Cell Culture

[00113] In some embodiments, the cell culture comprises a volume of from about 1 L to about 500,000 L prior to adjusting the pH. Prior to adjusting the pH, the cell culture can comprise, for example, a volume of 50 mL, 100 mL, 500 mL, 1 liters (L), 2L, 3L, 4L, 5L, 10L, 15L, 20L, 25L, 50L, 100L, 200L, 250L, 350L, 500 liters (L), 1000L, 2,500L, 3,000L, 5,000L, 10,000L, 25,000L, 50,000L, 100,000L, 200,000L, 250,000L, or 500,000L. Prior to adjusting the pH, the cell culture can comprise, for example, a volume of from about 50 mL to about 100 mL, about 50 mL to about 500 mL, about 50 mL to about 1 L, about 50 mL to about 5 L, about 50 mL to about 20 L, about 50 mL to about 100 L, about 50 mL to about 500 L, about 50 mL to about 10,000 L, about 50 mL to about 50,000 L, about 50 mL to about 100,000 L, about 50 mL to about 500,000 L, about 100 mL to about 500 mL, about 100 mL to about 1 L, about 100 mL to about 5 L, about 100 mL to about 10 L, about 100 mL to about 50 L, about 100 mL to about 100 L, about 100 mL to about 250 L, about 100 mL to about 1000 L, about 100 mL to about 10,000 L, about 100 mL to about 100,000 L, about 100 mL to about 500,000 L, about 500 mL to about 1 L, about 500 mL to about 5 L, about 500 mL to about 10 L, about 500 mL to about 50 L, about 500 mL to about 100 L, about 500 mL to about 250 L, about 500 mL to about 1000 L, about 500 mL to about 10,000 L, about 500 mL to about 100,000 L, about 500 mL to about 500,000 L, about 1 L to about 10 L, from about 1 L to about 50 L, from about 1 L to about 100 L, from about 10 L to about 100 L, from about 10 L to about 200 L, from about 10 L to about 350 L, from about 10 L to about 500 L, from about 10 L to about 1000 L, from about 50 L to about 250 L, from about 50 L to about 500 L, from about 50 L to about 1000 L, from about 100 L to about 500 L, from about 100 L to about 1000 L, from about 100 L to about 2,500 L, from about 100 L to about 3,000 L, from about 100 L to about 10,000 L, from about 500 L to about 1000 L, from about 500 L to about 2,500 L, from about 500 L to about 5,000 L, from about 500 L to about 10,000 L, from about 500 L to about 25,000 L, from about 1,000 L to about 1,500 L, from about 1,000 L to about 5,000 L, from about 1,000 L to about 10,000 L, from about 1,000 L to about 50,000 L, from about 1,000 L to about 100,000 L, from about 5,000 L to about 10,000 L, from about 5,000 L to about 25,000 L, from about 5,000 L to about 50,000 L, from about 5,000 L to about 100,000 L, from about 5,000 L to about 250,000 L, from about 10,000 L to about 50,000 L, from about 10,000 L to about 100,000

L, from about 10,000 L to about 250,000 L, from about 10,000 L to about 500,000 L, from about 25,000 L to about 50,000 L, from about 25,000 L to about 100,000 L, from about 25,000 L to about 250,000 L, from about 25,000 L to about 500,000 L, from about 50,000 L to about 100,000 L, from about 50,000 L to about 250,000 L, from about 50,000 L to about 500,000 L, from about 100,000 L to about 200,000 L, from about 100,000 L to about 250,000 L, from about 200,000 L to about 250,000 L, from about 200,000 L to about 500,000 L, or from about 250,000 to about 500,000 L.

[00114] Prior to adjusting the pH, the cell culture can comprise, for example, a volume of about 50 L, about 100 L, about 150 L, about 250 L, about 500 L, about 750 L, about 1,000 L, about 1,300 L, about 1,500 L, about 1,700 L, about 2,000 L, about 2,200 L, about 2,500 L, about 2,700 L, or about 3,000 L.

[00115] In some embodiments, the cell culture comprises 15×10^6 to 25×10^6 cells per mL.

[00116] In some embodiments, the cell culture is homogenized or subjected to cell lysis prior to adjusting the pH. Homogenization can comprise subjecting the cell culture to chemical and/or mechanical cell lysis techniques such as sonication, as well known in the art. In some embodiments, the cell culture is not homogenized prior to adjusting the pH.

[00117] In some embodiments, the initial pH of the suspension cell culture prior to pH adjustment is about 6.0 to about 7.0. The initial pH of the suspension cell culture prior to pH adjustment can be, for example, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, or about 7.0. In some embodiments, the initial pH of the suspension cell culture is about 6.7.

[00118] In some embodiments, the suspension culture comprises no more than about 10%, no more than about 7%, no more than about 5%, no more than about 1%, no more than about 0.5%, or no more than about 0.01% animal-derived serum.

[00119] The pH adjustment and/or separation are performed at ambient temperature or alternatively, controlled temperatures suitable for cell type. In embodiments, the pH adjustment and/or separation are performed at a temperature between 10° to 45°C , such as between 10° to 20° , 20° to 30° , 30° to 40° , or at about 25°C . In some embodiments, the pH adjustment and separation are performed at a temperature substantially similar to the temperature of the culture during expansion phase or thereabout. In embodiments, the acid component, when introduced into the culture, has a temperature substantially similar to the

temperature of the culture during expansion. Similarly, the wash liquid is at a temperature similar to the temperature of the culture during expansion. Alternatively, the ambient temperature of the room in which pH adjustment and separation is performed may also be at the temperature of the culture during expansion.

[00120] In some embodiments, at least a partial portion of the suspension cell culture is reserved before the adjustment of the pH, wherein the reserved portion is not pH adjusted and is used to initiate a new suspension cell culture for expansion, e.g. fed-batch. In some embodiments, about 1% to about 95 % by weight of the suspension cell culture is reserved, e.g. from a cell culture tank before the adjustment of the pH to initiate a new suspension cell culture for isolating edible biomass, or left behind in the cell culture tank while the portion to be pH adjusted is transferred to a separate tank. For example, about 1% to about 5% by weight, about 1% to about 10% by weight, about 1% to about 15% by weight, about 5% to about 10% by weight, about 5% to about 15% by weight, about 5% to about 20% by weight, about 10% to about 15% by weight, or about 15% to about 20% by weight can be removed from a cell culture tank before the adjustment of the pH to initiate a new suspension cell culture for isolating edible biomass. In fed-batch embodiments, the reserved amount may be between about 5% to 20%, while example processes approaching a chemostat-like cadence may reserve about 85% to about 95%.

[00121] In some embodiments, at least a partial portion of the suspension cell culture is retained in a cell culture tank and a greater portion is transferred to a second vessel configured to adjust the pH of the cell culture and optionally, separate a heavy phase from a light phase. In the cell culture tank, culture media can be mixed with the retained cell culture to initiate a new suspension cell culture for expansion. In some embodiments, about 1% to about 20% by weight of the suspension cell culture is retained in the cell culture tank wherein the suspended cell culture was cultured to initiate a new suspension cell culture for isolating edible biomass and a remaining portion of the suspended cell culture is transferred to a second tank for pH adjustment. For example, about 1% to about 5% by weight, about 1% to about 10% by weight, about 1% to about 15% by weight, about 5% to about 10% by weight, about 5% to about 15% by weight, about 5% to about 20% by weight, about 10% to about 15% by weight, or about 15% to about 20% by weight can be retained in a cell culture tank wherein the suspended cell culture was cultured to initiate a new suspension

cell culture for isolating edible biomass and a remaining portion of the suspended cell culture is, for example, transferred to a second tank for pH adjustment and/or otherwise pH adjusted in an inline method, e.g. pH adjusted while flowing through a pipe.

[00122] In some embodiments, the method of isolated edible biomass from a suspension cell culture comprising metazoan cells comprises a. culturing metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture; b. adjusting the pH of the cell culture to about 4.5 to cause formation of a light phase and a heavy phase, wherein adjusting the pH of the cell culture comprises adding citric acid to the cell culture; and c. separating the light phase from the heavy phase, wherein separating the light phase from the heavy phase comprises centrifuging the cell culture at about 2500 G to about 5000 G for about 10 min, wherein the cell culture comprises cells, free proteins and cell-associated proteins, wherein the heavy phase comprises a majority portion of the cells, free proteins, and cell-associated proteins from the cell culture.

1.2 Cooling the Biomass

[00123] In order to preserve the biomass, the biomass's temperature can be adjusted to and/or maintained at refrigeration temperatures at a point in the method of isolating the biomass that is after the culture phase is complete. In some embodiments, the methods described herein comprise adjusting the temperature of the biomass (e.g., the internal temperature of the biomass) from a growth temperature, which can depend on the cell type, to a refrigeration temperature, such as 33 to 40 degrees fahrenheit. In some embodiments, adjusting the temperature comprises cooling the biomass from a growth temperature to a temperature below the growth temperature that is a refrigeration temperature. In embodiments, adjusting the temperature to a refrigeration temperature is performed prior to pH adjusting the suspension cell culture, after pH adjusting the cell suspension and prior to centrifugation, or after centrifugation the edible biomass. After the temperature adjustment, the biomass is maintained at the refrigeration temperature.

1.3 Cells

[00124] The suspension culture used in disclosed embodiments can comprise naturally occurring, genetically engineered, or modified metazoan cells. Generally, the cells are from any metazoan species whose tissues are suitable for dietary consumption. In some embodiments, the cells may demonstrate a capacity for differentiation into tissue, such as

skeletal muscle tissue, other muscle tissues, skin tissue, or fat tissue, or any tissue-like association of cells. The cells used in the methods of the present disclosure can be primary cells or obtained from cell lines.

[00125] In some embodiments, the metazoan cells are derived from any non-human animal species intended for human or non-human dietary consumption for example, cells of avian, ovine, caprine, porcine, bovine, piscine origin, or for example, cells of livestock, poultry, avian, game, or aquatic species.

[00126] In some embodiments, the metazoan cells are from livestock such as domestic cattle, pigs, sheep, goats, camels, water buffalo, rabbits, and the like. In some embodiments, the metazoan cells are from poultry such as domestic chicken, turkeys, ducks, geese, quail, pigeons, and the like. In some embodiments, the cells are derived from chicken, duck, turkey, or a combination thereof. In further embodiments, the metazoan cells are derived from poultry. In some embodiments, the poultry cells are derived from chicken, duck, or turkey. In a yet further embodiment, the metazoan cells are poultry cells derived from chicken. In some embodiments, the metazoan cells are derived from game species such as wild deer, gallinaceous fowl, waterfowl, hare, and the like. In some embodiments, the metazoan cells are from aquatic species or semi-aquatic species harvested commercially from wild fisheries or aquaculture operations, or for sport, including certain fish, crustaceans, mollusks, cephalopods, cetaceans, crocodilians, turtles, frogs, and the like.

[00127] In some embodiments, the metazoan cells are from exotic, conserved or extinct animal species. In some embodiments, the cells are from *Gallus gallus*, *Gallus domesticus*, *Bos taurus*, *Sus scrofa*, *Meleagris gallopavo*, *Anas platyrhynchos*, *Salmo salar*, *Thymus thymus*, *Ovis aries*, *Coturnix coturnix*, *Capra aegagrus hircus*, or *Homarus americanus*. Accordingly, exemplary cell-based food products of the present disclosure include chicken products, duck products, turkey products, bovine products, and porcine products.

[00128] In some embodiments, the population of cells are fibroblasts, myofibroblasts, or myogenic cells, or a combination thereof.

[00129] In some embodiments, the cells are not natively myogenic (e.g., are non-myogenic cells such as fibroblasts or non-myogenic stem cells that are cultured to become myogenic cells (e.g., in suspension culture)).

[00130] In some embodiments, the cells are non-myogenic, and such non-myogenic cells can be programmed to be myogenic; for example, the cells may comprise fibroblasts modified to express one or more myogenic transcription factors. In some embodiments, the myogenic transcription factors include MYOD1, MYOG, MYF5, MYF6, PAX3, PAX7, paralogs, orthologs, and genetic variants thereof. In some embodiments, the cells are modified to express one or more myogenic transcription factors as described in a PCT publication, WO/2015/066377, which is herein incorporated by reference in its entirety.

[00131] In some embodiments, the cells are genetically modified to inhibit a pathway, e.g. the HIPPO signaling pathway. Exemplary methods to inhibit the HIPPO signaling pathway as described in a PCT Application No. PCT/US2018/031276, which is herein incorporated by reference in its entirety.

[00132] In some embodiments, the cells are modified to express telomerase reverse transcriptase (TERT) and/or inhibit cyclin-dependent kinase inhibitors (CKI). In some embodiments, the cells are modified to express TERT and/or inhibit cyclin-dependent kinase inhibitors as described in a PCT publication, WO 2017/124100, which is herein incorporated by reference in its entirety.

[00133] In some embodiments, the cells are modified to express glutamine synthetase (GS), insulin-like growth factor (IGF), and/or albumin. Exemplary methods of modifying cells to express GS, IGF, and/or albumin are described in a PCT Application No. PCT/US2018/042187, which is herein incorporated by reference in its entirety.

[00134] In some embodiments, the cell-based food product has various characteristics. Exemplary characteristics of the cell-based food are described in PCT Application No. PCT/US2021/016681, which is herein incorporated by reference in its entirety.

[00135] In some embodiments the cells are genetically edited, modified, or adapted to grow without the need of specific ingredients including specific amino acids, carbohydrates, vitamins, inorganic salts, trace metals, TCA cycle intermediates, lipids, fatty acids, supplementary compounds, growth factors, adhesion proteins, and recombinant proteins.

[00136] In some embodiments, the cells may comprise any combinations of the modifications described herein.

1.4 Cell Culture Media Formulation

[00137] The cells are grown in a nutrient medium in suspension culture. Once the desired cell concentration is achieved, the cell culture is then processed by the isoelectric point precipitations as described herein. A suitable nutrient medium is described in U.S. Patent Publication No. 2022/0073870, which is herein incorporated by reference in its entirety.

[00138] In some embodiments, the cell culture media is itself edible, for example, it does not cause any deleterious effects if ingested. The media formulations provided herein generally comprise ingredients that are each individually or in combination edible, e.g., safe to consume, food-grade, food-safe, food-quality, food-acceptable, food-compatible, food-category, foodstuff, for food use, eatable, comestible.

[00139] In some embodiment, a nutrient medium for the production of cell-based food products comprises a plurality of ingredients, wherein each ingredient is approved for use in food and/or is at or below its average daily intake (ADI) value. In some embodiments, an intermediate version of the cell culture medium may or may not be edible. In some embodiments, the cell culture medium comprises a plurality of ingredients wherein one or more ingredients is approved for use in food. In some embodiments, the cell culture medium comprises a plurality of ingredients wherein each ingredient is approved for use in food. As used in the present disclosure, an ingredient “approved for use in food” refers to an ingredient that is approved for use in food by a widely accepted standard, e.g., a nationally or internationally accepted standard. These standards include, but are not limited to, standards set forth by regulatory bodies such as the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), the World Health Organization (WHO), the United Nations Food and Agriculture Organization (FAO), and the European Food Safety Authority (EFSA).

[00140] Accordingly, in some embodiments, the cell culture media of the present disclosure may comprise ingredients approved for use in food that are recognized by the FDA as Generally Recognized As Safe (GRAS). In some embodiments, the cell culture media comprises ingredients that are GRAS certified ingredients, Non-GRAS certified ingredients, or mixtures thereof. In certain embodiments the cell culture media comprises only GRAS certified ingredients. As the list of GRAS substances is updated by the FDA, the GRAS certified ingredients that may be used in the formulation of the cell culture medium may be modified. In some embodiments, the cell culture media comprise

ingredients with a currently unknown GRAS status. It is contemplated that the media provided herein may comprise an ingredient that may currently have an unknown GRAS status or a Non-GRAS status, but in the future may be GRAS certified.

[00141] In some embodiments, the cell culture media may comprise ingredients approved for use in food as recognized by the FDA, the USDA, the FAO, or the WHO.

[00142] In some embodiments, the cells are grown in media that contains no more than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or no more than 0.01% animal-derived serum. In some embodiments, the media is substantially free of animal-derived serum or free of animal-derived serum. In some embodiments, the cell or population of cells are grown in an ACF or serum-free media. In some embodiments, the media contains no more than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or no more than 0.01% animal-derived components. In some embodiments, the media is substantially free of animal-derived components or free of animal-derived components. In some embodiments, the medium is chemically defined.

1.5 pH Adjustment

[00143] In the context of proteins, at a solution pH that is above the isoelectric point (pI), the surface of proteins is predominantly negatively charged and therefore like-charged molecules will exhibit repulsive forces. In other words, individual proteins will repel, and there will be less protein aggregation. Likewise, at a solution pH that is below the pI, the surface of the protein is predominantly positively charged and repulsion between proteins occurs. Again, such conditions are conducive to decreased protein aggregation. However, at the pI of the protein the negative and positive charges substantially cancel, repulsive electrostatic forces are reduced, and the attraction forces among the protein particles dominate. Such attractive forces lead to increased protein aggregation, and will thus facilitate the precipitation of proteins out of solution. In a solution of cells, Applicant has discovered that a similar effect can be achieved with IEP while keeping at least a majority of the cells intact. In the context of creating a cell-based food product from an isolated biomass, the components of the biomass, e.g., the amount of intact cells, as well as the manner in which it is isolated, e.g., pH and acidic reagent, with an optional step of increasing the pH prior to adjusting the pH downward to IEP, can affect texture, taste, and appearance.

[00144] In embodiments, the pH of the suspended culture is first adjusted to be from 11.0 to 12.5. In some embodiments, the pH of the suspension cell culture can be adjusted to be, for example, about 11.0, about 11.1, about 11.2, about 11.3, about 11.4, about 11.5, about 11.6, about 11.7, about 11.8, about 11.9, about 12.0, about 12.1, about 12.2, about 12.3, about 12.4, or about 12.5. Without wishing to be bound to any theory, it is believed that a pH adjustment to a pH of about 11.0 to about 12.5 solubilizes the suspension cell protein. The pH of the culture can be increased by addition of a basic reagent (such as, e.g., NaOH) and then incubating, including with stirring, the suspension culture material for a period of time, including, for example, 30 minutes.

[00145] In some embodiments, the method comprises adjusting the pH of the cell culture from about 11.0 to about 12.5 comprises incubating the cell culture after the addition of a basic reagent, including in embodiments with mild stirring. In some embodiments, the cell culture can be incubated for a time of, for example, from about 5 min to about 3 h. The cell culture at pH 11 to 12.5 can be incubated, including, in embodiments, with mild stirring, for a time period of, for example, from about 5 min to about 10 min, about 5 min to about 20 min, about 5 min to about 30 min, about 5 min to about 45 min, about 5 min to about 60 min, about 5 min to about 1.5 h, about 5 min to about 2 h, about 5 min to about 2.5 h, about 5 min to about 3 h, about 10 min to about 20 min, about 10 min to about 30 min, about 10 min to about 45 min, about 10 min to about 60 min, about 10 min to about 2 h, about 10 min to about 3 h, about 20 min to about 30 min, about 20 min to about 45 min, about 20 min to about 60 min, about 20 min to about 1.5 h, about 20 min to about 2 h, about 20 min to about 2.5 h, about 20 min to about 3 h, about 30 min to about 45 min, about 30 min to about 60 min, about 30 min to about 1.5 h, about 30 min to about 2 h, about 30 min to about 2.5 h, about 30 min to about 3 h, about 45 min to about 60 min, about 45 min to about 1.5 h, about 45 min to about 2 h, about 45 min to about 3 h, about 60 min to about 1.5 h, about 60 min to about 2 h, about 60 min to about 2.5 h, about 60 min to about 3 h, about 1.5 h to about 2 h, about 1.5 h to about 2.5 h, about 1.5 h to about 3 h, about 2 h to about 2.5 h, or about 2.5 h to about 3 h. The cell culture can be incubated for a time period of, for example, about 5 min, about 10 min, about 15 min, about 30 min, about 45 min, about 60 min, about 1.5 h, about 2 h, about 2.5 h, or about 3 h.

[00146] In accordance with the present disclosure, the pH of the suspended culture material is adjusted, in some embodiments optionally after an initial adjustment of the pH to basic pH, to be about the average pI of the plurality of cells and proteins thereby causing precipitation. Specifically, the pH of the suspension culture of metazoan cells is adjusted to be from 4.3 to 5.7. In some embodiments, the pH of the suspension cell culture can be adjusted to be, for example, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, or about 5.7.

[00147] It is understood that in some embodiments, the pH value within a cell culture may not be the exact same throughout the entire volume of culture, i.e. non-homogenous pH, particularly in the case of volumes greater than 1000 L. Instead, with systems and methods described herein, a significant portion of the suspended culture would fall within a pH range. For example, 75%, 80%, 85%, 90%, 95%, 98%, or 100% of the suspended culture can be pH adjusted to fall within a desired pH range. An example of how to determine what percentage of the culture is titrated to within a range can involve collecting samples at various locations with a culture vessel, such as different depths and testing pH and calculating what percentage of these measurements fall within a desired range. The pH of a significant portion of the suspension cell culture can be adjusted to be from 4.3 to 5.5, 4.5 to 5.5, 4.7 to 5.5, 4.9 to 5.5, 5.0 to 5.5, 4.5 to 5.7, 4.5 to 5.5, 4.5 to 5.3, 4.5 to 5.0, 4.7 to 5.7, 4.7 to 5.5, or 4.7 to 5.3. In embodiments, prior to adjusting the pH to an acidic pH, the pH of a significant portion of the suspension cell culture can be adjusted to be from 11.0 to 12.5, 11.0 to 12.2, 11.0 to 12.0, 11.5 to 12.5, 11.5 to 12.2, 11.5 to 12.0, 11.7 to 12.5, 11.7 to 12.2, 11.7 to 12.0, 11.9 to 12.5, 11.9 to 12.2, to 11.9 to 12.0.

[00148] Adjusting the pH of the cell culture to be within the desired range or target comprises adding an acidic component or a basic component to the cell culture. The acidic component or basic component can be any acid or base suitable for use in food manufacturing or consumption. In some embodiments, the acidic and/or basic component is recognized Generally Recognized As Safe (GRAS) Type 1, 2, or 3 as determined in a final rule by the FDA. In some embodiments, the acidic and/or basic component can be any acid known in the art which is considered edible, e.g., safe to consume, food-grade, food-safe, food-quality, food-acceptable, food-compatible, food-category, food-stuff, for food use,

and/or comestible. The acidic component can be an acidic solution that is added to the cell culture as an aqueous solution. Alternatively or in addition thereto, the acidic component can also be added to the cell culture as a solid salt or as a slurry. The basic component can be a basic solution that is added to the cell culture as an aqueous solution. Alternatively or in addition thereto, the basic component can also be added to the cell culture as a solid salt or as a slurry.

[00149] In some embodiments, adjusting the pH of the cell culture comprises adding an organic acid to the cell culture. In some embodiments, adjusting the pH of the cell culture comprises adding an inorganic acid to the cell culture. In some embodiments, the acidic component can be a mixture of two or more different acids.

[00150] In some embodiments, adjusting the pH of the cell culture comprises adding hydrochloric acid, citric acid, glucono delta lactone (GDL), lactic acid, malic acid, ascorbic acid, caprylic acid, sorbic acid, caproic acid, benzoic acid, propionic acid, acetic acid, formic acid, fumaric acid, tartaric acid, or a combination thereof, to the cell culture. In some embodiments, adjusting the pH of the cell culture comprises adding citric acid, GDL, lactic acid, malic acid, ascorbic acid, or a combination thereof, to the cell culture.

[00151] In embodiments, in which adjusting the pH of the cell culture comprises adding an aqueous solution, the concentration of acid in the aqueous solution can depend on the strength of the acid. The acid concentration can be from about 10 mM to about 2M. For citric acid or HCl, the concentration can be about 1 M to 2 M to adjust the pH to be from 4.3 to 5.7 or to be about 4.5, about 5.0, or about 5.5. For GDL, the concentration can be about 0.5 M to 1.5 M to adjust the pH to be from 4.3 to 5.7 or to be about 4.5, about 5.0, or about 5.5. In some embodiments, the concentration is such that adding an acidic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%, of the initial cell culture volume.

[00152] Adjusting the pH of the cell culture can comprise mixing the cell culture during and/or after the addition of the acidic component to distribute the acidic component throughout at least a significant portion of the volume of the culture. The culture can be mixed by, for example, stirring (such as with stirring impellers or the like) or shaking. Alternatively or in addition thereto, the acid component can be introduced via a sparger,

which can also serve to mix the acidic component with the culture. Alternatively or in addition thereto, after or during the addition of the acidic component, the suspended culture can be pumped from the culture vessel through a circulation pipe and back to the culture vessel to cause mixing.

[00153] In some embodiments prior to adjusting the pH to an acidic pH, the pH of the culture material is adjusted to a basic pH by adding an organic base to the cell culture. In some embodiments, adjusting the pH of the cell culture comprises adding an inorganic base to the cell culture. In some embodiments, the basic component can be a mixture of two or more different bases.

[00154] In some embodiments, adjusting the pH of the cell culture comprises adding sodium hydroxide (NaOH), potassium hydroxide (KOH), magnesium hydroxide ($\text{Mg}(\text{OH})_2$), calcium hydroxide ($\text{Ca}(\text{OH})_2$), or a combination thereof, to the cell culture. In some embodiments, adjusting the pH of the cell culture comprises adding NaOH to the cell culture.

[00155] In embodiments, in which adjusting the pH of the cell culture comprises adding an aqueous solution, the concentration of base in the aqueous solution can depend on the strength of the base. The base concentration can be from about 10 mM to about 5M. For NaOH, the concentration can be about 1 M to 5 M to adjust the pH to be from about 11.0 to 12.5 or to be about 11.5, about 12, or about 12.2. In some embodiments, the concentration is such that adding a basic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%, of the initial cell culture volume.

[00156] Adjusting the pH of the cell culture can comprise mixing the cell culture during and/or after the addition of the basic component to distribute the basic component throughout at least a significant portion of the volume of the culture. The culture can be mixed by, for example, stirring (such as with stirring impellers or the like) or shaking. Alternatively or in addition thereto, the basic component can be introduced via a sparger, which can also serve to mix the basic component with the culture. Alternatively or in addition thereto, after or during the addition of the basic component, the suspended culture can be pumped from the culture vessel through a circulation pipe and back to the culture vessel to cause mixing.

[00157] In some embodiments, adjusting the pH of the cell culture comprises incubating the cell culture after the addition and prior to separation of the light phase from the heavy phase. In some embodiments, the cell culture can be incubated for a time until a precipitate or a sediment forms. In some embodiments, the cell culture can be incubated for a time of, for example, from about 5 min to about 3 h, including a time period that results in precipitation of the protein. The cell culture can be incubated for a time period of, for example, from about 5 min to about 10 min, about 5 min to about 20 min, about 5 min to about 30 min, about 5 min to about 45 min, about 5 min to about 60 min, about 5 min to about 1.5 h, about 5 min to about 2 h, about 5 min to about 2.5 h, about 5 min to about 3 h, about 10 min to about 20 min, about 10 min to about 30 min, about 10 min to about 45 min, about 10 min to about 60 min, about 10 min to about 2 h, about 10 min to about 3 h, about 20 min to about 30 min, about 20 min to about 45 min, about 20 min to about 60 min, about 20 min to about 1.5 h, about 20 min to about 2 h, about 20 min to about 2.5 h, about 20 min to about 3 h, about 30 min to about 45 min, about 30 min to about 60 min, about 30 min to about 1.5 h, about 30 min to about 2 h, about 30 min to about 2.5 h, about 30 min to about 3 h, about 45 min to about 60 min, about 45 min to about 1.5 h, about 45 min to about 2 h, about 45 min to about 3 h, about 60 min to about 1.5 h, about 60 min to about 2 h, about 60 min to about 2.5 h, about 60 min to about 3 h, about 1.5 h to about 2 h, about 1.5 h to about 2.5 h, about 1.5 h to about 3 h, about 2 h to about 2.5 h, or about 2.5 h to about 3 h. The cell culture can be incubated for a time period of, for example, about 5 min, about 10 min, about 15 min, about 30 min, about 45 min, about 60 min, about 1.5 h, about 2 h, about 2.5 h, or about 3 h.

[00158] The incubation period may overlap with a period of mixing. For example, for an initial portion of the incubation period, the culture is mixed. The later portion of the incubation period can comprise a dwell period wherein the precipitated biomass can settle to a bottom of the vessel in which it is contained, whether it be the culture vessel or a second vessel to which the culture was transferred prior to pH adjustment.

[00159] In some embodiments, adjusting the pH of the cell culture is performed at ambient temperature or at about 20 °C, about 30 °C, about 35 °C, about 40 °C, about 45 °C, or between 20° to 45° C, about 20 °C to about 40 °C, about 20 °C to about 35 °C, about 20 °C to about 30 °C, about 25 °C to about 45 °C, about 25 °C to about 40 °C, about 25 °C to

about 35 °C, about 25 °C to about 30 °C, about 30 °C to about 45 °C, or about 30 °C to about 40 °C. In embodiments, the pH adjustment is performed at a temperature that is the same as the temperature at which the biomass is expanded.

1.6 Separation

[00160] As mentioned previously, when the cell culture is pH adjusted to a desired pH range or target pH that is at or near the pI, a heavy phase comprising biomass and a light phase is formed. Some separation may occur by gravity separation during the incubation period. However, to make the separation of the two phases more distinct and thereby facilitate more efficient harvesting of the biomass, a separation step is performed. Separation, in accordance with the present disclosure, can comprise centrifuging, continuous centrifuging, and/or microfiltration. The methods disclosed herein may require only a single centrifugation step as opposed to two or more to obtain a percent water composition that is less than 90%, 85%, or 80%. Optionally, in other embodiments, a second centrifugation step may be performed.

[00161] The heavy phase and some or all of the light phase may be placed in a third vessel configured for centrifuging, or centrifuging may be completed in the same vessel as the vessel in which the pH adjustment is performed. In some embodiments, at least a portion of the light phase may be separated from the pH adjusted culture before performing the separation step, e.g., centrifuging, of the remaining light phase and the heavy phase.

[00162] In embodiments, centrifuging the pH-adjusted cell culture is performed from about 500 G to about 25000 G. Centrifuging the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture can be, for example, from about 500 G to about 4500, from about 500 G to about 4000 G, from about 500 G to about 3500 G, from about 500 G to about 3000 G, from about 500 G to about 2500 G, from about 500 G to about 2000 G, from about 500 G to about 1500 G, from about 500 G to about 1000 G, from about 750 G to about 5000 G, from about 750 G to about 4500, from about 750 G to about 4000 G, from about 750 G to about 3500 G, from about 750 G to about 3000 G, from about 750 G to about 2500 G, from about 750 G to about 2000 G, from about 750 G to about 1500 G, from about 750 G to about 1000 G, from about 1000 G to about 5000 G, from about 1000 G to about 4500, from about 1000 G to about 4000 G, from about 1000 G to about 3500 G, from about 1000 G to about 3000 G, from about 1000 G to about 2500 G,

from about 1000 G to about 2000 G, from about 1000 G to about 1500 G, from about 1500 G to about 5000 G, from about 1500 G to about 4500, from about 1500 G to about 4000 G, from about 1500 G to about 3500 G, from about 1500 G to about 3000 G, from about 1500 G to about 2500 G, from about 1500 G to about 2000 G, from about 2000 G to about 5000 G, from about 2000 G to about 4500, from about 2000 G to about 4000 G, from about 2000 G to about 3500 G, from about 2000 G to about 3000 G, from about 2000 G to about 2500 G, from about 3000 G to about 5000 G, from about 3500 G to about 5000 G, from about 4000 G to about 5000 G, from about 600 G to about 4000 G, from about 600 G to about 3500 G, from about 600 G to about 3000 G, from about 600 G to about 2000 G, from about 750 G to about 4000 G, or from about 1000 G to about 4000 G. For example, centrifuging the pH-adjusted cell culture is performed at about 500 G, about 750 G, about 800 G, about 1000 G, about 1200 G, about 1400 G, about 1500 G, about 1700 G, about 1800 G, about 2000 G, about 2200 G, about 2500 G, about 2800 G, about 3000 G, about 3200 G, about 3500 G, about 3800 G, about 4000 G, about 4200 G, about 4500 G, about 4800 G, or about 5000 G. In other embodiments, centrifuging the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture can be performed, for example, from about 20000 G to about 25000G, from about 15000 G to about 20000 G, from about 10000 G to about 15000 G, from about 5000 G to about 10000 G, from about 5000 G to about 8000 G, from about 5000 G to about 7000 G, or from about 5000 G to about 6000 G.

[00163] The centrifugation time of the light phase or at least a portion thereof and the heavy phase can be between 5 minutes and 20 minutes, such as for about 5 min to about 15 min, about 5 min to about 10 min, about 5 min to about 7 min, about 10 min to about 20 min, about 10 min to about 15 min, or about 15 min to about 20 min. Centrifuging the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture can be performed, for example, for about 5 min, about 7 min, about 9 min, about 10 min, about 12 min, about 15 min, about 17 min, or about 20 min.

[00164] In some embodiments, continuous centrifuging of the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture can be performed from about 100 G to about 5000 G. Continuous centrifuging the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture can be, for example, from about 100 G to about 1500G, from about 500 G to about 1000 G, from about 1000 G to about

1500 G, from about 1500 G to about 2000 G, from about 2500 G to about 3000 G, from about 3500 G to about 4000 G, or from about 4500 G to about 5000 G.

[00165] In some embodiments, wherein adjusting the pH of the cell culture comprises adding GDL to obtain a pH of about 4.5, the light and heavy phases of the pH-adjusted cell culture are centrifuged from about 2000 G to 3000 G. In some embodiments, wherein adjusting the pH of the cell culture comprises adding GDL to obtain a pH of about 4.5, the light and heavy phases of the pH-adjusted cell culture are centrifuged at about 2500 G. The centrifuging time can be about 10 minutes or between about 7 to 20 minutes.

[00166] In some embodiments, wherein adjusting the pH of the cell culture comprises adding citric acid to obtain a pH of about 4.5 or from 4.5 to 5.5, the light phase or at least a portion thereof and the heavy phase of the pH-adjusted cell culture are centrifuged from 4000 G to 5500 G, such as 5000 G. The centrifuging time can be about 10 minutes or between about 7 to 20 minutes.

[00167] Upon centrifugation, the heavy phase comprises a sediment. The light phase is decanted away or the sediment is drained away to isolate the edible biomass, i.e. to obtain the IEP-isolated or CERES-isolated edible biomass.

1.7 Yields of Isolated Biomass

[00168] The yield of the edible biomass obtained from the IEP-comprising method described above is from about 0.3 g to about 10.0 g of edible biomass per 100 g of a total cell culture fluid (CCF) having a concentration of 15 to 25 million cells per milliliter, wherein the CCF comprises the combined weight of the heavy phase and the light phase. More specifically, the yield can be from about 0.3 g to about 1.7 g of IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 1.7 g to about 2 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 2 g to about 3 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 3 g to about 4 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 4 g to about 5 g of edible biomass per 100 g of a total cell culture fluid (CCF), from about 5 g to about 6 g of edible biomass per 100 g of a total cell culture fluid (CCF), from about 6 g to about 7 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 7 g to about 8 g of the IEP-isolated

or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), from about 8 g to about 9 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF), or from about 9 g to about 10 g of the IEP-isolated or CERES-isolated edible biomass per 100 g of a total cell culture fluid (CCF). The IEP-isolated or CERES-isolated edible biomass yield can be for example, about 0.3 g, about 0.5 g, about 0.7 g, about 1.0 g, about 1.3 g, about 1.5 g, about 1.7 g, about 2 g, about 3 g, about 4 g, about 5 g, about 6 g, about 7 g, about 8 g, about 9 g, or about 9.5 g per 100 g of a total cell culture fluid (CCF).

[00169] Another measure of yield is that of the amount of protein in the IEP-isolated or CERES-isolated biomass. The protein content of the edible biomass can be measured using any method known in the art for quantifying protein content. The protein content can be measured by any scientifically-accepted method, such as a bicinchoninic acid (BCA) protein assay kit or the Kjeldahl method.

[00170] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises from about 0.01 g to about 0.5 g of total proteins per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase. In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises from about 0.01 g to about 0.4 g, about 0.01 g to about 0.3 g, about 0.01 g to about 0.2 g, about 0.01 g to about 0.1 g, about 0.01 to about 0.05 g, about 0.05 g to about 0.1 g, about 0.05 g to about 0.2 g, about 0.05 g to about 0.3 g, about 0.05 g to about 0.4 g, about 0.05 g to about 0.5 g, about 0.1 g to about 0.5 g, about 0.1 g to about 0.4 g, about 0.1 g to about 0.3 g, about 0.1 g to about 0.2 g, about 0.2 g to about 0.5 g, about 0.2 g to about 0.4 g, about 0.2 g to about 0.3 g, about 0.3 g to about 0.5 g, about 0.3 g to about 0.4 g, or about 0.4 g to about 0.5 g of total free proteins and cell-associated proteins per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase. In some embodiments, the isolated edible biomass comprises about 0.01 g, about 0.1 g, about 0.2 g, about 0.3 g, about 0.4 g, or about 0.5 g of total free proteins and cell-associated proteins per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase.

[00171] In some embodiments, the isolated edible biomass obtained from the IEP comprising methods described herein comprises a mass protein yield that is from about 10% to about 80% higher than the mass protein yield obtained for edible biomass isolated

after multiple centrifugation steps and without IEP. The isolated edible biomass obtained from the IEP-comprising methods described herein can comprise, for example, a protein yield that is from about 20% to about 70%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, about 30% to about 50%, about 30% to about 40%, about 40% to about 80%, about 40% to about 70%, about 40% to about 60%, about 40% to about 50%, about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 60% to about 80%, about 60% to about 70%, or about 70% to about 80% higher than the mass protein yield obtained for edible biomass isolated after multiple centrifugation steps and without IEP. The isolated edible biomass can comprise, for example, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, or about 80% higher than the mass protein yield obtained for edible biomass isolated after multiple centrifugation steps and without IEP.

[00172] In some embodiments, the isolated edible biomass obtained from the IEP comprising methods described herein comprises from about 5.0% to about 15.0% protein according to a bicinchoninic acid (BCA) assay. The isolated edible biomass can comprise, for example, from about 5.0% to about 13% protein, from about 5.0% to about 10% protein, from about 5.0% to about 8% protein, from about 7% to about 15% protein, from about 7% to about 13% protein, from about 7% to about 10% protein, from about 10% to about 15% protein, or from about 13% to about 15% protein according to a BCA assay. The isolated edible biomass can comprise, for example, about 5%, about 7%, about 10%, about 13%, or about 15% protein according to a BCA assay.

[00173] In some embodiments, the IEP-isolated or CERES-isolated edible biomass obtained according to the methods described herein comprises from about 3.0% to about 15% protein according to the Kjeldahl method. The isolated edible biomass can comprise, for example, from about 3.0% to about 13% protein, from about 3.0% to about 10% protein, from about 3.0% to about 8% protein, from about 5.0% to about 15% protein, from about 7% to about 13% protein, from about 7% to about 10% protein, from about 10% to about 15% protein, or from about 13% to about 15% protein according to a BCA assay. The isolated edible biomass can comprise, for example, about 3%, about 5%, about 7%, about 10%, about 13%, or about 15% protein according to the Kjeldahl method. This

percent protein yield can be with a method comprising a single centrifuge step and no other drying step.

1.8 Characteristics of the Isolated Biomass

[00174] The isolated edible biomass obtained from the method described herein can have different properties compared to isolated edible biomasses obtained from prior art methods. Such properties include the size distribution of the proteins in the biomass, the color of the isolated biomass, TPA parameters, the percent water content of the isolated biomass, the amount of intact cells per gram or mL of the isolated biomass, and the storage modulus of the isolated biomass.

[00175] In some embodiments, the isolated edible biomass obtained from the methods described herein exhibits similar or decreased concentrations of degraded proteins as measured by SDS-PAGE compared to the biomass isolated after multiple centrifugation steps and /or without IEP.

[00176] Cell viability can be determined using ViCell packed cell volume (PCV) measurements. In some embodiments, the concentration of total intact cells in the IEP-isolated or CERES-isolated biomass ranges from about 3×10^6 cells/mL to about 600×10^6 cells/mL. The concentration of total intact cells in the isolated biomass can range, for example, from about 300×10^6 cells/mL to about 500×10^6 cells/mL, from about 400×10^6 cells/mL to about 600×10^6 cells/mL, or from about 500×10^6 cells/mL to about 600×10^6 cells/mL as measured by ViCell PCV. The concentration of total intact cells in the IEP-isolated or CERES-isolated biomass can be, for example, about 300×10^6 cells/mL, about 350×10^6 cells/mL, about 400×10^6 cells/mL, about 450×10^6 cells/mL, about 500×10^6 cells/mL, about 550×10^6 cells/mL, or about 600×10^6 cells/mL. A description of a PCV assay is provided in Example 10, which can be used to determine the concentration of total intact cells in the isolated biomass.

[00177] In some embodiments, the edible biomass obtained from the methods disclosed herein exhibits a higher initial and final storage modulus during heat-induced gel formation compared to edible biomass produced without pH adjustment. See Example 7 describing the temperature sweep test that can be used to determine this variable. In embodiments, the biomass obtained from the methods described herein is not vacuum dried to produce the cell-based food product.

[00178] In some embodiments, the initial storage modulus of the edible biomass obtained from the methods described herein is from about 10 Pa to about 1000 Pa. In some embodiments, the initial storage modulus of the edible biomass obtained from the methods described herein is from about 10 Pa to about 900 Pa, from about 10 Pa to about 800 Pa, from about 10 Pa to about 700 Pa, from about 10 Pa to about 500 Pa, from about 10 Pa to about 300 Pa, from about 10 Pa to about 200 Pa, from about 10 Pa to about 100 Pa, from about 100 Pa to about 1000 Pa, from about 200 Pa to about 1000 Pa, from about 500 Pa to about 1000 Pa, from about 700 Pa to about 1000 Pa, from about 800 Pa to about 1000 Pa, from about 500 Pa to about 1000 Pa, from about 500 Pa to about 900 Pa, or from about 500 Pa to about 800 Pa. In some embodiments, the initial storage modulus of the edible biomass obtained from the methods described herein is about 10 Pa, about 50 Pa, about 100 Pa, about 200 Pa, about 300 Pa, about 400 Pa, about 500 Pa, about 600 Pa, about 700 Pa, about 800 Pa, about 900 Pa, or about 1000 Pa.

[00179] In some embodiments, the final storage modulus of the IEP-isolated or CERES-isolated edible biomass is from about 10 Pa to about 1000 Pa. In some embodiments, the final storage modulus of the IEP-isolated or CERES-isolated edible biomass is from about 10 Pa to about 900 Pa, from about 10 Pa to about 800 Pa, from about 10 Pa to about 700 Pa, from about 10 Pa to about 500 Pa, from about 10 Pa to about 300 Pa, from about 10 Pa to about 200 Pa, from about 10 Pa to about 100 Pa, from about 100 Pa to about 1000 Pa, from about 200 Pa to about 1000 Pa, from about 500 Pa to about 1000 Pa, from about 700 Pa to about 1000 Pa, from about 800 Pa to about 1000 Pa, from about 500 Pa to about 1000 Pa, from about 500 Pa to about 900 Pa, or from about 500 Pa to about 800 Pa. In some embodiments, the final storage modulus of the IEP-isolated or CERES-isolated edible biomass is about 10 Pa, about 50 Pa, about 100 Pa, about 200 Pa, about 300 Pa, about 400 Pa, about 500 Pa, about 600 Pa, about 700 Pa, about 800 Pa, about 900 Pa, or about 1000 Pa.

[00180] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a moisture content that is less than the moisture content of edible biomass isolated after multiple centrifugation steps and without IEP.

1.9 Moisture Content and Water Activity

[00181] In some embodiments, an IEP-isolated or CERES-isolated edible biomass obtained from the methods described herein comprises a moisture content about 60% to about 95% (e.g., to about 60%, about 60% to about 90%, about 60% to about 80%, about 60% to about 80%, about 60% to about 70%, about 70% to about 90%, about 70% to about 80%, or about 80% to about 90%) by weight of solution.

[00182] In some embodiments, an IEP-isolated or CERES-isolated edible biomass obtained from the methods described herein comprises a water activity (aw) of about 0.7 aw to about 0.9 aw (e.g., about 0.7 aw to about 0.85 aw, about 0.7 aw to about 0.8 aw, about 0.7 aw to about 0.75, about 0.75 aw to about 0.9 aw, about 0.75 aw to about 0.85 aw, about 0.75 aw to about 0.8 aw, about 0.8 aw to about 0.9 aw, about 0.8 aw to about 0.8 aw 5, or about 0.8 aw 5 to about 0.9 aw).

[00183] In some embodiments, an IEP-isolated or CERES-isolated edible biomass obtained from the methods described herein comprises a water activity (aw) less than or equal to 0.9 aw, less than or equal to 0.8 aw, less than or equal to 0.7 aw, less than or equal to 0.6 aw, less than or equal to 0.5 aw, less than or equal to 0.4 aw, less than or equal to 0.3 aw, less than or equal to about 0.2 aw, or less than or equal to about 0.1 aw.

1.10 Hardness

[00184] In some embodiments, the IEP-isolated or CERES-isolated edible biomass obtained from the methods described herein comprises a hardness of about 6000 grams (g) of force or less (e.g., about 5500 g, about 5000 g or less, about 4500 g or less, about 4000 g or less, about 3500 g or less, about 3000 g or less, about 2500 g or less, about 2000 g or less, or about 1500 g or less; between about 1500 g or less and about 6000 g or less, about 5500 g or less, about 5000 g or less, about 4500 g or less, about 4000 g or less, about 3500 g or less, about 3000 g or less, about 2500 g or less, about 2000 g or less; about 1500 g or less, about 1000 g or less, or about 500 g or less).

[00185] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises between about between about 1000 g to about 6000 g (e.g., about 1000 g to about 5500 g, about 1000 g to about 5000 g, about 1000 g to about 4500 g, about 1000 g to about 4000 g, about 1000 g to about 3500 g, about 1000 g to about 3000 g, about 1000 g to about 2500 g, about 1000 g to about 2000 g, about 1000 g to about 1500 g, about 1500 g to about 6000 g, about 1500 g to about 5500 g, about 1500 g to about 5000 g, about 1500 g to

about 4500 g, about 1500 g to about 4000 g, about 1500 g to about 3500 g, about 1500 g to about 3000 g, about 1500 g to about 2500 g, about 1500 g to about 2000 g, about 2000 g to about 6000 g, about 2000 g to about 5500 g, about 2000 g to about 5000 g, about 2000 g to about 4500 g, about 2000 g to about 4000 g, about 2000 g to about 3500 g, about 2000 g to about 3000 g, about 2000 g to about 2500 g, about 2500 g to about 6000 g, about 2500 g to about 5500 g, about 2500 g to about 5000 g, about 2500 g to about 4500 g, about 2500 g to about 4000 g, about 2500 g to about 3500 g, about 2500 g to about 3000 g, about 2500 g to about 2500 g, about 3000 g to about 6000 g, about 3000 g to about 5500 g, about 3000 g to about 5000 g, about 3000 g to about 4500 g, about 3000 g to about 4000 g, about 3000 g to about 3500 g, about 3500 g to about 6000 g, about 3500 g to about 5500 g, about 3500 g to about 5000 g, about 3500 g to about 4500 g, about 3500 g to about 4000 g, about 4000 g to about 6000 g, about 4000 g to about 5500 g, about 4000 g to about 5000 g, about 4000 g to about 4500 g, about 4500 g to about 6000 g, about 4500 g to about 5500 g, about 4500 g to about 5000 g, about 5000 g to about 6000 g, about 5000 g to about 5500 g, or between about 5500 g to about 5500 g of force.

[00186] In some embodiments, hardness testing loads are expressed in Newton (N). In some embodiments, hardness can be expressed in kilogram-force (kgf), gram-force (gf) or (g), or pond (p). The correlation between kgf, kp, and N is: 1.0 kgf = 1,000 gf (g) = 1.0 kp = 9.81 N.

1.11 Resilience

[00187] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a resilience score from a TPA analysis (see, e.g., Example 3) of about 9, about 8.5, about 8, about 7.5, about 7, about 6.5, about 6, about 5.5, about 5, about 4.5, about 4, or about 3.5, or about 3.0, or about 2.5, or about 2.0, about 1.5 or about 1.0.

[00188] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a resilience score from a TPA analysis of between about 4.0 to about 9.0 (e.g., about 4.0 to about 8.5, about 4.0 to about 8.0, about 4.0 to about 7.5, about 4.0 to about 7.0, about 4.0 to about 6.5, about 4.0 to about 6.0, about 4.0 to about 5.5, about 4.0 to about 5.0, about 4.0 to about 4.5, about 4.5 to about 9.0, about 4.5 to about 8.5, about 4.5 to about 8.0, about 4.5 to about 7.5, about 4.5 to about 7.0, about 4.5 to about 6.5, about 4.5 to about 6.0, about 4.5 to about 5.5, about 4.5 to about 5.0, about 5.0 to about 9.0, about 5.0 to about 8.5,

about 5.0 to about 8.0, about 5.0 to about 7.5, about 5.0 to about 7.0, about 5.0 to about 6.5, about 5.0 to about 6.0, about 5.5 to about 9.0, about 5.5 to about 8.5, about 5.5 to about 8.0, about 5.5 to about 7.5, about 5.5 to about 7.0, about 5.5 to about 6.5, about 5.5 to about 6.0, about 6.0 to about 9.0, about 6.0 to about 8.5, about 6.0 to about 8.0, about 6.0 to about 7.5, about 6.0 to about 7.0, about 6.0 to about 6.5, about 6.5 to about 9.0, about 6.5 to about 8.5, about 6.5 to about 8.0, about 6.5 to about 7.5, about 6.5 to about 7.0, about 7.0 to about 9.0, about 7.0 to about 8.5, about 7.0 to about 8.0, about 7.0 to about 7.5, about 7.5 to about 9.0, about 7.5 to about 8.5, about 7.5 to about 8.0, about 8.0 to about 9.0, about 8.0 to about 8.5, or about 8.5 to about 9.0).

1.12 Cohesiveness

[00189] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a cohesiveness score from a TPA analysis of between about 0.1 to about 0.8 (e.g., about 0.2 to about 0.75, about 0.2 to about 0.7, about 0.2 to about 0.65, about 0.2 to about 0.6, about 0.2 to about 0.55, about 0.2 to about 0.5, about 0.2 to about 0.45, about 0.2 to about 0.4, about 0.2 to about 0.35, about 0.2 to about 0.3, about 0.2 to about 0.25, about 0.25 to about 0.8, about 0.25 to about 0.75, about 0.25 to about 0.7, about 0.25 to about 0.65, about 0.25 to about 0.6, about 0.25 to about 0.55, about 0.25 to about 0.5, about 0.25 to about 0.45, about 0.25 to about 0.4, about 0.25 to about 0.35, about 0.25 to about 0.3, about 0.3 to about 0.8, about 0.3 to about 0.75, about 0.3 to about 0.7, about 0.3 to about 0.65, about 0.3 to about 0.6, about 0.3 to about 0.55, about 0.3 to about 0.5, about 0.3 to about 0.45, about 0.3 to about 0.4, about 0.3 to about 0.35, about 0.35 to about 0.8, about 0.35 to about 0.75, about 0.35 to about 0.7, about 0.35 to about 0.65, about 0.35 to about 0.6, about 0.35 to about 0.55, about 0.35 to about 0.5, about 0.35 to about 0.45, about 0.35 to about 0.4, about 0.4 to about 0.8, about 0.4 to about 0.75, about 0.4 to about 0.7, about 0.4 to about 0.65, about 0.4 to about 0.6, about 0.4 to about 0.55, about 0.4 to about 0.5, about 0.4 to about 0.45, about 0.45 to about 0.8, about 0.45 to about 0.75, about 0.45 to about 0.7, about 0.45 to about 0.65, about 0.45 to about 0.6, about 0.45 to about 0.55, about 0.45 to about 0.5, about 0.5 to about 0.8, about 0.5 to about 0.75, about 0.5 to about 0.7, about 0.5 to about 0.65, about 0.5 to about 0.6, about 0.5 to about 0.55, about 0.55 to about 0.8, about 0.55 to about 0.75, about 0.55 to about 0.7, about 0.55 to about 0.65, about 0.55 to about 0.6, about 0.6 to about 0.8, about 0.6 to about 0.75, about 0.6 to about 0.7, about

0.6 to about 0.65, about 0.65 to about 0.8, about 0.65 to about 0.75, about 0.65 to about 0.7, about 0.7 to about 0.8, about 0.7 to about 0.75, or about 0.75 to about 0.8.

[00190] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a cohesiveness score from a TPA analysis (see, e.g., Example 3) of about 0.80, about 0.75, about 0.7, about 0.65, about 0.6, about 0.55, about 0.5, about 0.45, about 0.4, about 0.35, about 0.3, about 0.25, about 0.2, about 0.15, about 0.1, or about 0.05.

1.13 Springiness

[00191] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a springiness score from a TPA analysis of between about 20 and about 80, e.g., about 20 to about 70, about 20 to about 60, about 20 to about 50, about 20 to about 40, about 20 to about 30, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 30 to about 40, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 80, about 60 to about 70, or about 70 to about 80.

[00192] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a springiness score from a TPA analysis of about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 15, about 10, about 5, about 1, or about 0.1.

1.14 Gumminess

[00193] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a gumminess score from a TPA analysis of between about 100 to about 1000 (e.g., about 100 to about 900, about 100 to about 800, about 100 to about 700, about 100 to about 600, about 100 to about 500, about 100 to about 400, about 100 to about 300, about 100 to about 200, about 200 to about 1000, about 200 to about 900, about 200 to about 800, about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 1000, about 300 to about 900, about 300 to about 800, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 1000, about 400 to about 900, about 400 to about 800, about 400 to about 700, about 400 to about 600, about 400 to about 500, about 500 to about 1000, about 500 to about 900, about 500 to about 800, about 500 to about 700, about 500 to about 600, about 600 to about 1000, about 600 to about 900, about 600 to about 800, about

600 to about 700, about 700 to about 1000, about 700 to about 900, about 700 to about 800, about 800 to about 1000, about 800 to about 900, or about 900 to about 1000.

[00194] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a gumminess score from a TPA analysis of about 5000, about 2500, about 2000, about 1500, about 1250, about 1100, about 1000, about 900, about 800, about 700, about 600, about 500, about 400, about 300, about 200, or about 100.

1.15 Chewiness

[00195] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a chewiness score from a TPA analysis of between about 100 to about 500 (e.g., about 100 to about 400, about 100 to about 300, about 100 to about 200, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 500, about 300 to about 400, or about 500 to about 500).

[00196] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a chewiness score from a TPA analysis of about 5000, about 2500, about 1500, about 1250, about 1000, about 900, about 800 about 700, about 600, about 550, about 500, about 450, about 400, about 350, about 300, about 250, about 200, about 150, about 100, about 50 or about 25.

1.16 Binding

[00197] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a binding score from a TPA analysis (in N*mm) at 10°C of about 500, about 400, about 300, about 200, about 190, about 180, about 170, about 160, about 150, about 140, about 130, about 120, about 110, about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, about 10 or about 5.

[00198] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a binding score at 15°C of about 500, about 400, about 300, about 200, about 190, about 180, about 170, about 160, about 150, about 140, about 130, about 120, about 110, about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, about 10 or about 5.

1.17 Shear

[00199] In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a peak positive force as measured using the modified Warner-Bratzler (WB)

shear method of from about 100 g to about 3000 g. Example 13 describes the method for performing the WB shear method in accordance with the present disclosure. In some embodiments, the IEP-isolated edible biomass comprises a peak positive force of from about 100 g to about 2500 g, about 100 g to about 2000 g, about 100 g to about 1500 g, about 100 g to about 1000 g, about 100 g to about 750 g, about 100 g to about 500 g, about 300 g to about 3000 g, about 300 g to about 2500 g, about 300 g to about 2000 g, about 300 g to about 1500 g, about 300 g to about 1000 g, about 300 g to about 750 g, about 500 g to about 3000 g, about 500 g to about 2500 g, about 500 g to about 2000 g, about 500 g to about 1500 g, about 500 g to about 1000 g, about 1000 g to about 3000 g, about 1000 g to about 2500 g, about 1000 g to about 2000 g, about 1000 g to about 1500 g, about 1500 g to about 3000 g, about 1500 g to about 2000 g, or about 2000 g to about 3000 g. In some embodiments, the IEP-isolated or CERES-isolated edible biomass comprises a peak positive force of about 100 g, about 200 g, about 300 g, about 400 g, about 500 g, about 600 g, about 700 g, about 800 g, about 900 g, about 1000 g, about 1300 g, about 1500 g, about 1700 g, about 2000 g, about 2300 g, about 2500 g, or about 3000 g.

1.18 Color

[00200] The color of the IEP-isolated or CERES-isolated edible biomass can vary with the type of acidic component utilized for IEP. For example, citric acid results in a whiter biomass than HCl or GDL. A color can be measured by a colorimeter as described in Example 11. In some embodiments, the color of the IEP-isolated edible biomass is characterized as having less red and higher lightness values as compared to biomass obtained without IEP.

[00201] In some embodiments, the IEP-isolated or CERES-isolated edible biomass has an average L* value of about 70 to about 80. In some embodiments, the average L* value is about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80. In some embodiments, the biomass has an average a* value of about -3.3, -3.4, -3.5, -3.6, -3.7, -3.8, -3.9, or -4.0. In some embodiments, the biomass has an average b* value of about 6.5 to about 8.5, about 6.5, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.2, or 8.5.

[00202] In some embodiments, the color of isolated biomass can be measured using a ColorFlex EZ Colorimeter according to the method described in Example 11.

1.19 Aroma / Taste

[00203] In some embodiments, the IEP-isolated or CERES-isolated biomass has an improved neutral taste and reduced off-notes. In some embodiments, the IEP-isolated or CERES-isolated biomass has reduced aromas of sulphur and rubber and a lower sulphur / rubber aroma score. In some embodiments, the isolated biomass has a sulphur / rubber aroma score of below about 10, 9, 8, 7, or 6. In some embodiments, the IEP-isolated or CERES-isolated biomass has a sulphur / rubber aroma score of about 6 to about 10 or about 6 to about 8.

[00204] In some embodiments, the IEP-isolated or CERES-isolated biomass has reduced metallic taste and a lower metallic taste score. In some embodiments, the isolated biomass has a metallic taste score of below about 15, or about 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14. In some embodiments, the isolated biomass has a metallic taste score of from about 5 to about 12, or from about 5 to about 10 or from about 5 to about 8.

2. SYSTEMS FOR ISOLATING EDIBLE BIOMASS OR INTACT CELLS USING IPE

[00205] Another embodiment of the present disclosure is a system for culturing and isolating metazoan cells according to the methods described herein.

[00206] In some embodiments and with reference to FIG. 1B, the system **100** can comprise a first culture vessel **10** and a second culture vessel **20**. The first vessel **10** is in fluid communication with the second vessel **20** to allow flow of the suspension culture between the first vessel **10** and the second vessel **20**. Each culture vessel **10**, **20** is configured for culturing metazoan cells as a suspension culture. Each culture vessel **10**, **20** comprises a first inlet **30** configured for adding culture media and a second inlet **40** configured for adding, optionally a basic component, and then an acidic component to the vessel to adjust the pH of the suspension culture, optionally to a pH of 11.0 to 12.5, then, after a period of time, to a pH of 4.3 to 5.7 to form a light phase and a heavy phase. Each culture vessel further comprises a mixer **50** configured to mix the acidic and/or basic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the culture vessel is at 75% capacity or more.

[00207] The system **100** can also comprise a centrifuge **60** and each culture vessel comprises an outlet **61** configured such that the pH-adjusted suspension culture or at least

the heavy phase can be transferred to the centrifuge. In some embodiments, the system **100** is configured such that the suspension culture or at least the heavy phase of the suspension culture is only centrifuged once.

[00208] In embodiments, the system **100** comprises a cooler and is configured such that the cooler cools the pH-adjusted suspension or at least the heavy phase when it is transferred to centrifuge **60** and maintains the downstream product thereof at the cooler temperature. In embodiments, the system **100** comprises a cooler and is configured such that the cooler cools the heavy phase as it exits centrifuge **60** and maintains the downstream product thereof at the cooler temperature.

[00209] In some embodiments and with reference to FIG. 1C, a system **200** for culturing and isolating edible biomass can comprise a culture vessel **70** configured for culturing metazoan cells as a suspension culture and comprising an inlet **30** for adding culture media and a second vessel **80** in fluid communication with the culture vessel such that at least a major portion of the suspension culture can be transferred to the second vessel. The second vessel **80** comprises an inlet **40** configured for adding, optionally a basic component, and after a period of time, an acidic component to the second vessel to adjust the pH of the transferred suspension culture, optionally to a pH of from 11.0 to 12.5, then, after a period of time, to a pH of 4.3 to 5.7 to form a light phase and a heavy phase. The second vessel **80** further comprises a mixer **50** configured to mix the acidic and/or basic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the second vessel is at 75% capacity or more.

[00210] The system **200** can also comprise a centrifuge **60**. The second vessel **80** comprises an outlet **61** configured such that the pH-adjusted suspension is transferred to the centrifuge. In some embodiments, the system **200** is configured such that the heavy phase of the suspension culture is only centrifuged once.

[00211] In embodiments, the system **200** comprises a cooler and is configured such that the cooler cools suspension culture as it is transferred to vessel **80** and maintains the downstream product thereof at the cooler temperature. In other embodiments, the system **200** comprises a cooler and is configured such that the cooler cools the pH-adjusted suspension or at least the heavy phase from vessel **80** as it is transferred to centrifuge **60** and maintains the downstream product thereof at the cooler temperature. In embodiments,

the system **200** comprises a cooler and is configured such that the cooler cools the heavy phase as it exits centrifuge **60** and maintains the downstream product thereof at the cooler temperature.

2.1 Culture Vessel and Second Vessels for pH Adjustment

[00212] Vessels **10**, **20**, **70** and **80** described above may be any container configured to hold a suspension culture, such as a tube, a flask, a multi-well plate, a dish, a vat, a bioreactor, and the like. The portion of the vessel in contact with the suspension culture is composed of a material suitable for use with food preparation, such as stainless steel.

[00213] In terms of scale, the vessels described herein are configured to hold a suspension culture volume that ranges from about 10 μ L to about 500,000 L. In some embodiments, the vessel can hold a volume of suspension culture that is about 50 mL, about 100 mL, about 500 mL, about 1 L, about 10 L, about 100 L, about 1000 L, about 3,000 L, about 5,000 L, about 10,000 L, 25,000L, 50,000L, 100,000L, 200,000L, 250,000L, or 500,000L. The culture vessel can hold a volume of suspension culture, for example and without limitation, from about 50 mL to about 100 mL, about 50 mL to about 500 mL, about 50 mL to about 1 L, about 50 mL to about 5 L, about 50 mL to about 20 L, about 50 mL to about 100 L, about 50 mL to about 500 L, about 50 mL to about 10,000 L, about 50 mL to about 50,000 L, about 50 mL to about 100,000 L, about 50 mL to about 500,000 L, about 100 mL to about 500 mL, about 100 mL to about 1 L, about 100 mL to about 5 L, about 100 mL to about 10 L, about 100 mL to about 50 L, about 100 mL to about 100 L, about 100 mL to about 250 L, about 100 mL to about 1000 L, about 100 mL to about 10,000 L, about 100 mL to about 100,000 L, about 100 mL to about 500,000 L, about 500 mL to about 1 L, about 500 mL to about 5 L, about 500 mL to about 10 L, about 500 mL to about 50 L, about 500 mL to about 100 L, about 500 mL to about 250 L, about 500 mL to about 1000 L, about 500 mL to about 10,000 L, about 500 mL to about 100,000 L, about 500 mL to about 500,000 L, about 1 L to about 10 L, from about 1 L to about 50 L, from about 1 L to about 100 L, from about 10 L to about 100 L, from about 10 L to about 200 L, from about 10 L to about 350 L, from about 10 L to about 500 L, from about 10 L to about 1000 L, from about 50 L to about 250 L, from about 50 L to about 500 L, from about 50 L to about 1000 L, from about 100 L to about 500 L, from about 100 L to about 1000 L, from about 100 L to about 2,500 L, from about 100 L to about 3,000 L, from about 100 L to about

10,000 L, from about 500 L to about 1000 L, from about 500 L to about 2,500 L, from about 500 L to about 5,000 L, from about 500 L to about 10,000 L, from about 500 L to about 25,000 L, from about 1,000 L to about 1,500 L, from about 1,000 L to about 5,000 L, from about 1,000 L to about 10,000 L, from about 1,000 L to about 50,000 L, from about 1,000 L to about 100,000 L, from about 5,000 L to about 10,000 L, from about 5,000 L to about 25,000 L, from about 5,000 L to about 50,000 L, from about 5,000 L to about 100,000 L, from about 5,000 L to about 250,000 L, from about 10,000 L to about 50,000 L, from about 10,000 L to about 100,000 L, from about 10,000 L to about 250,000 L, from about 10,000 L to about 500,000 L, from about 25,000 L to about 50,000 L, from about 25,000 L to about 100,000 L, from about 25,000 L to about 250,000 L, from about 25,000 L to about 500,000 L, from about 50,000 L to about 100,000 L, from about 50,000 L to about 250,000 L, from about 50,000 L to about 500,000 L, from about 100,000 L to about 250,000 L, from about 100,000 L to about 500,000 L, from about 200,000 L to about 250,000 L, from about 200,000 L to about 500,000 L, or from about 250,000 to about 500,000 L.

[00214] Other features that culture vessels **10**, **20**, and **70** can comprise are described in U.S. Patent Publication No. 2022/0056394, which are herein incorporated by reference in their entireties.

3. CELL-BASED FOOD PRODUCTS

[00215] Another embodiment of the present disclosure is a cell-based food product comprising the isolated edible biomass obtained from a suspension cell culture isolated according to the methods described herein using IEP or CERES.

[00216] In some embodiments, isolated biomass is mixed with further ingredients to form the cell-based food products. Such ingredients are also edible or may be recognized by the FDA as Generally Recognized As Safe (GRAS). In some embodiments, the cell-based food products comprises ingredients that are GRAS certified ingredients, non-GRAS certified ingredients, or mixtures thereof. In some embodiments, the cell-based food product comprises only GRAS certified ingredients. As the list of GRAS substances is updated by the FDA, the GRAS certified ingredients that may be used in the cell-based food product may be modified.

4. EXAMPLES

[00217] The examples provided below are offered by way of illustration, and not by way of limitation.

EXAMPLE 1: Determination of pI

[00218] In this experiment, a pH-dependent protein solubility curve was obtained to determine the isoelectric point. 14 samples of solutions of LSS containing 1% w/w protein were provided. Each solution was homogenized for 1 min, followed by cooling. Next, the pH was adjusted for each solution using HCl/NaOH according to Table 1 below.

Table 1.

Sample	Adjusted pH
1	2
2	3
3	4
4	4.5
5	5
6	5.5
7	6
8	7
9	8
10	9
11	10
12	11
13	12
14	No Adjustment (Control)

[00219] Next, each sample was centrifuged at 4200 RPM for 15 min, and the supernatant was collected. Kjeldahl testing (AOAC protocol - #991.20-1994(1996), Nitrogen (total) in milk, Kjeldahl method) was used to determine the protein solubility, and a solubility curve (**FIG. 2**) was plotted, where the curve displays the amount of protein relative to the control in the supernatant. Accordingly, a low protein solubility value indicates that the protein has precipitated out of solution and is more readily available for collection. The results showed that the isoelectric point is approximately in the range of pH 4.5-5.5. pH 2, 3, and 10 yielded the highest protein% in the supernatant.

EXAMPLE 2: Study of Impact of Acid Type, pH, and Centrifugation Speed

[00220] Experiments were designed to study several conditions for cell isolating. The overall workflow is depicted in FIG. 3. The sample conditions are shown in Table 2 below. Briefly, samples of suspension culture were adjusted to pH 4.5, 5.0, or 5.5 using 1M citric acid, 0.5M glucono delta lactone, or 1M HCl, and stirred for 60 min at 300 RPM at 30 °C.

[00221] Next, the samples were centrifuged for 20 min at 500 RPM (38 xg), 2500 RPM (957 xg), or 5000 RPM (3829 xg), which resulted in a light phase (supernatant) and a heavy phase (sediment). The heavy phase was collected, and the pH was adjusted to 7 prior to analysis.

Table 2.

Sample No.	Acid	Adjusted pH	Centrifugation Speed (RPM)
1	HCl	4.5	500
2		5.0	2500
3		5.5	5000
4		4.5	2500
5		5.0	5000
6		5.5	500
7		4.5	5000
8		5.0	500
9		5.5	2500
10	GDL	4.5	500
11		5.0	2500
12		5.5	5000
13		4.5	2500
14		5.0	5000

Sample No.	Acid	Adjusted pH	Centrifugation Speed (RPM)
15		5.5	500
16		4.5	5000
17		5.0	500
18		5.5	2500
19	Citric acid	4.5	500
20		5.0	2500
21		5.5	5000
22		4.5	2500
23		5.0	5000
24		5.5	500
25		4.5	5000
26		5.0	500
27		5.5	2500

EXAMPLE 3: Sediment Yield

[00222] In this experiment, the sediment yield for samples 1-27 was determined. The results are shown in FIG. 4. The grams of sediment obtained in the heavy phase per 100 g of total cell culture fluid were measured. The samples pH adjusted with citric acid consistently had the highest sediment yield, followed by samples treated with GDL. Sample 26 (pH adjusted with citric acid to 5.0, centrifuged at 500 RPM) resulted in the highest overall sediment yield of ~1.8 g/100 g CCF. Some samples treated with HCl (sample 1, sample 6) did not yield any discernible sediment.

EXAMPLE 4: Protein Yield as Determined by BCA Assay

[00223] After collection of the sediment phases, a BCA protein assay was employed to determine the protein yields in each sediment sample. The results are shown in FIG. 5 as

grams of protein detected per 100 g of collected sediment. Notably, most samples that were treated with HCl were not able to be analyzed due to insufficient mass or liquid consistency. Those that were able to be analyzed, samples 3 and 8, had extremely low protein yields.

[00224] Furthermore, while centrifugation speed did not appear to have a significant effect on sediment mass, it did affect the protein yield, with 2500 RPM and 5000 RPM obtaining higher protein yields when compared to 500 RPM. Comparable protein yields were obtained by GDL and citric acid, with the highest overall protein yield of ~9% being obtained by sample 16 (GDL, pH. 4.5, 5000 RPM).

[00225] FIG. 6 shows a plot of the ratio of total protein that is precipitated and collected per 100 g of cell culture fluid input.

EXAMPLE 5. Scaling Metrics and Comparison to Conventional Method

[00226] As shown in FIG. 7, the zero-electric charge method yielded 65% higher protein per 100 g of cell culture fluid when compared to the conventional method, in which the pH was not adjusted to the pI, and two centrifugation steps were performed.

EXAMPLE 6: Cell Viability

[00227] Cell viability was analyzed using ViCell packed cell volume measurements. FIG. 8A shows an image of the sediment phase of a sample pH adjusted to 4.5 with citric acid and centrifuged at 5000 rpm (3829 xg), and FIG. 8B shows an image of the supernatant for the same sample. FIG. 9A shows an image of the sediment phase of a sample pH adjusted with GDL, and FIG. 9B shows an image of the supernatant for the same sample. the GDL sediment, some cells were observed to have a non-spherical morphology. Little to no debris was observed for the supernatant. Finally, FIG. 10A shows an image of the sediment phase of a sample pH adjusted with HCl, and FIG. 10B shows an image of the supernatant for the same sample. Some cells were elongated, similar to the GDL sediment sample. The estimated intact cell density for each sediment sample is shown in FIG. 11, and the numerical estimation is shown in Table 4 below. According to the ViCell results, the HCl treatment resulted in the highest cell “viability” in the sediment as measured by intact cells and trypan blue exclusion at ~52.5% viability. These are unlikely to be actually alive, as

the ViCell measurement looks at the cell's ability to exclude trypan blue. GDL and citric acid sediment samples had a viability of 18.9% and 24% viability, respectively.

Table 3.

Sediment Sample	Estimated Total Intact 10 ⁶ cells/mL
GDL	462
HCl	326
Citric acid	460
Typical cell density of LSS	400-600

EXAMPLE 7: Heat-Induced Gel Formation

[00228] A temperature sweep test was performed to monitor the heat-induced gel formation of isolated biomass. Measurements of temperature sweep tests were performed on an HR-20 Rheometer (TA Instruments).

[00229] The instrument was set up with the base plate. Material ran using this protocol preferentially uses a solids content of 25-30 % w/w confirmed by moisture analysis (see Example 9).

[00230] For each sample, 4-5 g of isolated biomass was portioned out into a centrifuge tube. Next, 2 % w/w (of weighed sample material) salt, *e.g.*, sodium chloride, and 0.5 % w/w (of weighed sample material) phosphate (Wenda Phos3000) were weighed out and added to the sample material. The sample was homogenized with a 7 mm probe, on ice, for 1 minute, or until the material is visibly homogenous and in a paste-like consistency. The homogenized material was rested in the refrigerator for a minimum of 30 minutes before measurement.

[00231] Prior to the measurement, the 40 mm crosshatched upper geometry of the rheometer was installed, and the lower cross hatched 40 mm plate and its attachments were installed on the lower plate base. After the loading gap action, about 3.5-4.5 g of sample was arranged in the center of the crosshatched lower geometry. During the "trim gap" action, if there was an excess of sample that was squeezed out from the two cross hatched plates, it was lightly trimmed away, taking care to avoid pulling material out from the gap between the two plates. The temperature sweep protocol was performed either at 1 Pa, 0.1

Hz for vacuum dried suspension material or similar, or 50 Pa, 1 Hz for minced chicken material or similar.

[00232] The isolated biomass by IEP contained half solid content but double protein%, compared to vacuum dried LSS (i.e., isolated biomass after centrifugation without IEP). The results are shown in FIG. 12. HCl-derived biomass has a comparable gel formation trend with biomass derived using the conventional method that does not include IEP and used two centrifugation steps. Citric acid and GDL-derived biomass showed higher initial and final storage modulus during the gel formation process.

EXAMPLE 8: Protein Degradation

[00233] FIG. 13 shows SDS-PAGE data for the biomass obtained from samples pH adjusted with citric acid (pH 4.5), GDL (pH 4.5), and HCl (pH 5.5) and centrifuged at 5000 rpm (3829 xg). According to the SDS-PAGE data, the highest degree of protein degradation was caused by adjustment using HCl, indicated by the higher concentration of polypeptides in a wide range of molecular weights, particularly at 66 kDa. Both GDL and citric acid resulted in lower protein degradation, indicated by lower concentration of low molecular weight peptides, but higher protein precipitation efficiency (based on biomass and protein percentage in the sediment).

EXAMPLE 9: Loss-on-Drying (LOD) Moisture Assay

[00234] Moisture content in isolated edible biomass is an important parameter to inform composition and identity. Traditional thermogravimetric analysis of moisture content is performed by heating samples in an oven and assessing the mass loss of the sample due to evaporation of moisture. For this assay, the term moisture refers to all volatile species that will evaporate from the product when heated. Much of the liquid in the isolated edible biomass is water.

[00235] Samples of edible biomass having a mass of 50-100 mg (Wet Sample Mass) were placed in a disposable aluminum crinkle dishes (VWR International). A calibrated, 4-decimal point balance (0.0000 g) was used to weigh the samples throughout the assay. Samples were prepared in triplicate. Steps were taken in sample handling to prevent moisture from a source other than the sample being transferred to the dish. The samples

were then transferred to a pre-heated 70 °C oven and dried for > 10 hours to ensure complete drying.

[00236] Samples were removed from the oven, allowed to cool to room temperature, and weighed at different time points. Complete drying was confirmed by no change in mass of the sample at different time points. The final mass (Dry Sample Mass) of each sample was recorded. The w/w% of moisture per sample was determined according to the following formula: $[(\text{Wet sample mass}) - (\text{Dry sample mass})] / (\text{Wet sample mass}) * 100 \%$

EXAMPLE 10: Packed Cell Volume (PCV)

[00237] The Packed Cell Volume assay can be used to ascertain the amount of intact cells per mL of isolated biomass. To perform the assay, a 5 mL sample of isolated biomass is added to a clean 10 mL PCV tube. The PCV tube is then centrifuged in a 15 mL conical holder for 5 min at 1000 g. Following centrifugation, the cell pellet volume is measured in the PCV tube stem. The percentage PCV is calculated according to the following equation:

[00238] $\% \text{ PCV} = [(\text{cell pellet volume, mL}) / (\text{sample volume, mL})] / \text{sample volume, mL} * 100 \%$

EXAMPLE 11: Colorimetry

[00239] Color of isolated biomass can be measured using a ColorFlex EZ Colorimeter according to the following method.

[00240] Sample Preparation: A 5 g sample of isolated biomass was obtained. If the sample exhibited visible heterogeneity, the sample was homogenized. The ~5 g sample was placed in a petri dish and if the sample was stiff and did not evenly cover the bottom of the dish, the sample was gently pressed with the lid of a 50 mL Falcon tube by slowly twisting the lid to obtain generally even coverage with no visible air pockets.

[00241] Analysis: The ColorFlex EZ Colorimeter was first standardized using white and black tiles. The petri dish containing the sample was placed in the insert port, and the sample cup cover was placed over the sample to prevent stray light from interfering with the measurement. Each measurement was performed in triplicate, with the sample cup being rotated to a different position for each replicate.

[00242] The color values are reported in CIELAB coordinates, or $L^*a^*b^*$. CIELAB was intended as a perceptually uniform space, where a given numerical change corresponds to a similar perceived change in color. Uniform changes of components in the $L^*a^*b^*$ color space aim to correspond to uniform changes in perceived color, so the relative perceptual differences between any two colors in $L^*a^*b^*$ can be approximated by treating each color as a point in a three-dimensional space (with three components: L^* , a^* , b^*) and taking the Euclidean distance between them. The three coordinates of CIELAB represent the perceptual lightness (L^* , from 0 to 100), its position between green and red (a^* , from -100 (green) to 100 (red)), and its position between blue and yellow (b^* , from -100 (blue) to 100 (yellow)).

[00243] This test was used to measure color by IEP with HCl, GDL, and citric acid (CA) as well as the conventional method (LSS). **FIG. 14** shows representative colorimetry data showing color improvement of recovered biomass by IEP with HCl, GDL, and citric acid (CA) compared to the conventional method (LSS). All IEP treatments showed higher efficiency in reducing a^* (redness) values compared to the conventional method. IEP also yielded higher L^* (lightness) and subsequent whiteness values compared to the conventional method, specifically in the case of GDL and CA treatments.

Example 12: Sample Preparation for Texture Profile Analysis (TPA)

[00244] Sample preparation of isolated biomass for TPA involves weighing $0.4\text{g} \pm 0.04\text{g}$ of the biomass into a weigh-boat and subsequently placing the sample into one well of a 48-well plate. Five samples are prepared per one batch of isolated biomass for a more robust dataset. The isolated biomass sample is pressed into the well and evenly distributed along the bottom, thereby forming a disc. A thin metal spatula or metal cylinder can be used to press and distribute the biomass. Steps are taken to ensure that as much of the 0.4 g sample is transferred into the well as possible.

Example 13: Modified WB Shear Test

[00245] The modified Warner-Bratzler method measures the force necessary to shear a sample in two. The resultant value is used as a representation of tenderness. According to the WB shear method, the peak positive force is defined as the greatest amount of force required to shear the sample in two. The method described herein utilizes a smaller sample size than the traditional WB shear method.

[00246] For isolated biomass, the isolated biomass to be used for the shear test is first mixed with Transglutaminase (TG) at 0.98% of material mass. The mixture is pressed into a mold, lightly vacuum sealed in the mold, and allowed to enzymatically set overnight at 4 C. The mold is shaped such that the sample will have a cylindrical-shape that is about 25-26 mm in height and 9 mm wide. Average measurements are obtained from the analysis of 3 to 5 independent samples per batch of isolated biomass.

[00247] Instrument set up: A TA.XT plus Texture Analyzer can be used. For the WB shear test, a 30kg load cell is used, as is a TA-07 Warner Bratzler knife with v-cut probe and the guillotine base plate, which is affixed to the TA-50 platform.

[00248] A sample is placed underneath the v-cut probe and the WB shear test is performed.

EXAMPLE 14: TWO pH ADJUSTMENT PROCESS (CERES)

[00249] In this experiment, prior to adjusting the pH of the cell culture material to the pI, the pH of the cell culture material is adjusted to a basic pH (also called the CERES process). Briefly, suspension cell biomass is obtained and diluted 1:1 with deionized (DI) water. 5M NaOH is added in 2-3% w/w quantity to adjust the diluted biomass to a pH of about 12 while mixing. The material is held at a pH of about 12 for about 30 minutes with mild stirring / agitation (75 RPM using a mechanical overhead mixture). Next, Food Grade Citric Acid Solution (1:1 citric acid: DI water) is added in 2-3% w/w quantity to adjust the diluted, pH adjusted biomass material to a pH of about 4.5. The material is held at a pH of about 4.5 for about 30 minutes with mild stirring / agitation (75 RPM using a mechanical overhead mixture). The material is transferred into 1L centrifuge inserts and centrifuges on the benchtop at 3829xg for 10 minutes. The sediment layer (heavy phase) is collected. Table 4 shows the characteristics of the resulting material.

Table 4.

Material	Solids Content Range (LOD)	Protein Content Range (DUMA)
Two-pH adjustment process (CERES)	12-14%	10-12%

[00250] The characteristics of the isolated biomass were assessed for color (Tables 5 and 6) and sensory characteristics (Table 7). The biomass resulting from the two-pH adjustment

process consistently had higher whiteness values (76.36 and 75.19 in Table 5 and 77.7 and 72.92 in Table 6) than biomass resulting from the conventional process (65.2 and 68.06 in Table 5 and 61.87 in Table 6). Table 7 shows that the biomass resulting from the two pH adjustment process has better levels of acceptability, decreased sulphur notes, decreased rubbery texture, and decreased metallic flavor relative to biomass resulting from the convention process. *See* Table 7.

Table 5. Color Analysis

Sample Name	Average L*	Average a*	Average b*	Average Whiteness
DR22_CONV (Conventional Process)	65.2	-3.23	10.95	63.4
BFG-HP_EPIC_SED (Two pH Adjustment Process)	76.36	-3.36	8.23	74.7
BFG-HP_BENCH_SED (Two pH Adjustment Process)	75.19	-3.92	7.62	73.8
CCF_BENCH_SED (Conventional Process)	68.06	-3.90	6.52	67.2

Table 6. Color Analysis

Material Type	Average L* (32 mm)	Average Whiteness (32 mm)	Solids Content (%)
DR13, Control (Conventional Process)	61.87	60.0	7.1
DR13 - CERES, Solids Corrected (Two pH Adjustment Process)	77.7	75.3	7.11
DR13 - CERES, Solids and pH Corrected (Two pH Adjustment Process)	72.92	70.6	7.48

Table 7. Sensory Analysis

Material Type	Sensory Summary	Sulphur / Rubber Aroma Score	Metallic Taste Score
DR23 Conventional (Conventional Process)	Off notes without further processing	13.5	19
DR23 CERES, Neutralised (Two pH Adjustment Process)	Neutral	10	12
DR27 Conventional	Off notes without further processing	14.2	22.3

DR27 CERES, Neutralised (Two pH Adjustment Process)	Neutral	6	5
DR28 Conventional	Neutral	11.5	15
DR28 CERES, Neutralised (Two pH Adjustment Process)	Neutral	6	5
Perfusion Material CERES R.1 (Two pH Adjustment Process)	Neutral	7	5
Perfusion Material CERES R.2 (Two pH Adjustment Process)	Neutral	7	11

[00251] The hardness (g) of the biomass from the two-pH adjustment process was also analyzed and compared to minced chicken breast (left column). DR23 CERES, DR25 CERES, DR26 CERES, DR27 CERES and DR28 CERES all had levels of hardness more similar to minced chicken breast than biomass isolated from suspension cells using the conventional process. *See* Fig. 15.

EQUIVALENTS

[00252] Although the invention is described in detail with reference to specific embodiments thereof, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00253] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated herein by reference in their entireties.

What is claimed is:

1. A method of isolating an edible biomass from a suspension cell culture comprising metazoan cells, wherein the method comprises:
 - a) culturing the metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture;
 - b) adjusting the pH of the cell culture to a range of 4.3 to 5.7;
 - c) forming the cell culture into a light phase and a heavy phase; and
 - d) separating the light phase from the heavy phase,wherein the cell culture comprises intact cells, free proteins, and cell-associated proteins,
wherein the heavy phase comprises a majority portion of the intact cells, free proteins, and cell-associated proteins from the cell culture, together comprising the isolated edible biomass.
2. The method according to claim 1, wherein the metazoan cells are derived from poultry cells having phenotypes of fibroblasts, myofibroblasts, myogenic cells, or a combination thereof.
3. The method according to claim 2, wherein the myogenic cells are myoblasts, myocytes, satellite cells, muscle derived stem cells, myogenic pericytes, or mesoangioblasts.
4. The method according to any one of claims 1-3, wherein the poultry cells are derived from chicken, duck, or turkey.
5. The method according to any one of claims 1-4, wherein the cell culture comprises about 15×10^6 to about 25×10^6 cells per mL .
6. The method according to claim 5, wherein adjusting the pH of the cell culture comprises adjusting the pH to in the range of about 4.3 to 5.5.
7. The method according to any one of claims 1-6, wherein adjusting the pH of the cell culture comprises adding an acidic component to the cell culture.
8. The method according to any one of claims 1-7, wherein adjusting the pH of the cell culture comprises adding hydrochloric acid, citric acid, glucono delta lactone (GDL), lactic

acid, malic acid, ascorbic acid, caprylic acid, sorbic acid, caproic acid, benzoic acid, propionic acid, acetic acid, formic acid, fumaric acid, tartaric acid, or a combination thereof, to the cell culture.

9. The method according to claim 8, wherein adjusting the pH of the cell culture comprises adding citric acid, GDL, lactic acid, malic acid, ascorbic acid, or a combination thereof, to the cell culture.

10. The method according to claim 8 or 9, wherein adjusting the pH of the cell culture comprises adding citric acid, GDL, or a combination thereof, to the cell culture.

11. The method according to any one of claims 1 to 10, wherein prior to step (b) there is a first step of adjusting the pH of the cell culture to a range of about 11 to about 12.5.

12. The method according to claim 11, wherein the first step of adjusting the pH of the cell culture comprises adjusting the pH to in the range of 11.5 to 12.5.

13. The method according to claim 12, wherein the first step of adjusting the pH of the cell culture comprises adjusting the pH to about 12.

14. The method according to any one of claims 11 to 13, wherein the first step of adjusting the pH of the cell culture comprises adding a basic component to the cell culture.

15. The method according to claim 14, wherein the first step of adjusting the pH of the cell culture comprises adding sodium hydroxide (NaOH), potassium hydroxide (KOH), magnesium hydroxide (Mg(OH)₂), calcium hydroxide (Ca(OH)₂), or a combination thereof, to the cell culture.

16. The method according to claim 15, wherein the first step of adjusting the pH of the cell culture comprises adding sodium hydroxide (NaOH) to the cell culture.

17. The method according to any one of claims 1-16, wherein separating the light phase from the heavy phase comprises centrifuging the cell culture.

18. The method according to any one of claims 1-17, wherein the method comprises only one centrifugation step to separate the light phase from the heavy phase.

19. The method according to claim 17 or 18, wherein centrifuging the cell culture is performed from about 500 G to about 5000 G or 1000G to 25000G.

20. The method according to any one of claims 17-19, wherein adjusting pH of the cell culture comprises adding GDL to obtain a pH of about 4.5 and centrifuging the cell culture at about 2000 G to 3000 G or about 2500 G.

21. The method according to any one of claims 17-20, wherein adjusting pH of the cell culture comprises adding citric acid to obtain a pH of about 4.5 or about 4.5 to 5.5 and centrifuging the cell culture at about 4000 G to 5500 G or about 5000 G.

22. The method according to any one of claims 17-21, wherein centrifuging the cell culture is performed for from about 5 min to about 20 min.

23. The method according to any one of claims 17-19, wherein centrifuging the cell is performed at about 3000 G for about 10 min.

24. The method according to any one of claims 1-23, wherein the cell culture comprises a volume of from about 50 L to 100,000 L prior to adjusting the pH.

25. The method according to any one of claims 7-24, wherein adding the acidic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%.

26. The method according to any one of claims 14-25, wherein adding the basic component to the cell culture increases the total volume of the cell culture by no more than about 20% or no more than 15% or no more than 10% or no more than 5%.

27. The method according to any one of claims 1-26, wherein the cell culture is not homogenized.

28. The method according to any one of claims 1-26, wherein the cell culture is homogenized prior to step b).

29. The method according to any one of claims 1-28, wherein the cell culture after step c) comprises from about 0.3 g to about 10.0 g of the heavy phase per 100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase.

30. The method according to any one of claims 1-29, wherein the edible biomass comprises from about 0.01 g to about 0.5 g of total free proteins and cell-associated proteins per

100 g of a total cell culture fluid (CCF), wherein the CCF comprises the heavy phase and the light phase.

31. The method according to any one of claims 1-30, wherein the first step of adjusting the pH of the cell culture is to a pH of about 12 by addition of NaOH to the cell culture, wherein the second step of adjusting the pH of the cell culture is to a pH of about 4.5 by addition of citric acid to the cell culture, and wherein separating the light phase from the heavy phase further comprises centrifuging the cell culture at 3000 G for about 10 min.

32. The method according to any one of claims 1-31, wherein a concentration of total intact cells in the heavy phase range from about 300×10^6 cells/mL to about 600×10^6 cells/mL.

33. The method according to any one of claims 1-32, wherein the method is performed at room temperature or at a temperature between 20° to 30° C.

34. The method according to any one of claims 1-33, wherein the method is performed at ambient pressure.

35. The method according to any one of claims 1-34, wherein the method is performed at a temperature of about 5 to 20 °C.

36. The method according to any one of claims 1-35, wherein the edible biomass comprises from about 5.0% to about 15.0% protein according to a bicinchoninic acid (BCA) assay.

37. The method according to any one of claims 1-36, wherein the edible biomass comprises from about 3.0% to about 15.0% protein according to the Kjeldahl method.

38. The method according to any one of claims 1-37, wherein at least a partial portion of the suspension cell culture is removed from a cell culture tank before the adjustment of the pH to initiate a new suspension cell culture for isolating edible biomass.

39. The method according to any one of claims 1-38, wherein at least a partial portion of the suspension cell culture is retained in a cell culture tank wherein the suspended cell culture was cultured to initiate a new suspension cell culture for isolating edible biomass and a remaining portion of the suspended cell culture is transferred to a second tank for pH adjustment.

40. The method according to any one of claims 1-39, wherein the cell culture has an initial pH of about 6.7.

41. The method according to any one of claims 1-40, wherein the suspension culture comprises no more than 10%, 7%, 5%, 1%, 0.5%, or no more than 0.01% animal-derived serum.

42. A method of isolating edible biomass from a suspension cell culture comprising metazoan cells, wherein the method comprises

a) culturing the metazoan cells as a suspension culture under conditions for cell expansion to form a cell culture;

b) adjusting the pH of the cell culture to about 12, wherein adjusting the pH of the cell culture comprises adding NaOH to the cell culture;

c) adjusting the pH of the cell culture to about 4.5 to cause formation of a light phase and a heavy phase, wherein adjusting the pH of the cell culture comprises adding citric acid to the cell culture; and

d) separating the light phase from the heavy phase, wherein separating the light phase from the heavy phase further comprises centrifuging the cell culture at about 3000 G for about 10 min,

wherein the cell culture comprises free proteins and cell-associated proteins, wherein the heavy phase comprises a majority portion of the free proteins and cell-associated proteins from the cell culture, and wherein the heavy phase comprises the isolated edible biomass.

43. An isolated edible biomass prepared from a method of any one of claims 1-42.

44. The isolated edible biomass according to claim 43, wherein a hardness of the isolated edible biomass is from about 1000 g to about 6000 g according to a texture profile analysis (TPA).

45. The isolated edible biomass according to claim 43 or 44, wherein a cohesiveness score of the isolated edible biomass is from about 4.0 to about 9.0 according to a texture profile analysis (TPA).

46. The isolated edible biomass according to any one of claims 43-45, wherein a springiness score of the cell isolated edible biomass is between about 4.0 and about 9.0 according to a texture profile analysis (TPA).

47. The isolated edible biomass according to any one of claims 43-46, wherein a gumminess score of the isolated edible biomass is between about 100 to about 1000 according to a texture profile analysis (TPA).

48. The isolated edible biomass according to any one of claims 43-47, wherein a chewiness score of the isolated edible biomass is between about 100 to about 500 according to a texture profile analysis (TPA).

49. The isolated edible biomass according to any one of claims 43-48, wherein a resilience of the cell-based food product is between about 4.0 and 9.0 according to a texture profile analysis (TPA).

50. The isolated edible biomass according to any one of claims 43-49, wherein a peak positive force value of the isolated edible biomass according to the Warner-Bratzler shear method is between about 100 g and about 3000 g.

51. The isolated edible biomass according to any one of claims 43-50, wherein the isolated edible biomass comprises a moisture content of from about 20% to about 90%.

52. The isolated edible biomass according to any one of claims 43-51, wherein the isolated edible biomass comprises a water activity (aw) of from about 0.7 aw to about 0.9 aw.

53. The isolated edible biomass according to any one of claims 43-52, wherein the cell-based food product comprises a water activity (aw) of about less than or equal to 0.9 aw.

54. The isolated edible biomass according to any one of claims 43-53, wherein the isolated edible biomass comprises a binding score of from about 5 to about 500 at 10 °C according to a texture profile analysis (TPA).

55. The isolated edible biomass according to any one of claims 43-54, wherein the isolated edible biomass comprises a binding score of from about 5 to about 500 at 15 °C according to a texture profile analysis (TPA).

56. The isolated edible biomass according to any one of claims 43-55, wherein the isolated edible biomass comprises a whiteness score (L^*) of from about 70 to about 80.
57. The isolated edible biomass according to any one of claims 43-55, wherein the isolated edible biomass comprises a redness score (a^*) of about -3.3 to about -4.0.
58. The isolated edible biomass according to any one of claims 43-55, wherein the isolated edible biomass comprises b^* score of about 6.5 to about 8.5.
59. A cell-based food product comprising an isolated edible biomass prepared from a method of any one of claims 1-42 and flavoring or aroma agents.
60. A system for culturing and isolating metazoan cells comprising:
a first culture vessel and a second culture vessel;
wherein the first vessel is in fluid communication with the second vessel to allow flow of the suspension culture between the first vessel and the second vessel,
wherein each culture vessel is configured for culturing metazoan cells as a suspension culture,
wherein each culture vessel comprises a first inlet configured for adding culture media and a second inlet configured for adding an acidic and/or basic component to the vessel to adjust the pH of the suspension culture to 11.0 or 12.5 or to 4.3 to 5.7 to form a light phase and a heavy phase,
wherein each culture vessel further comprises a mixer configured to mix the acidic and/or basic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the culture vessel is at 75% capacity or more.
61. The system according to claim 60, further comprising a centrifuge, wherein each culture vessel comprises an outlet configured such that at least the heavy phase can be transferred to the centrifuge or the heavy phase and only a minor portion of the light phase can be transferred to the centrifuge.
62. The system according to claim 58, wherein the system is configured such that the heavy phase of the suspension culture is only centrifuged once.
63. A system for culturing and isolating edible biomass comprising:

a culture vessel configured for culturing metazoan cells as a suspension culture and comprising an inlet for adding culture media;

a second vessel in fluid communication with the culture vessel such that at least a major portion of the suspension culture can be transferred to the second vessel,

wherein the second vessel comprises an inlet configured for adding an acidic and/or basic component to the second vessel to adjust the pH of the transferred suspension culture to 11.0 to 12.5 or to 4.3 to 5.7 to form a light phase and a heavy phase,

wherein the second vessel further comprises a mixer configured to mix the acidic component with the suspension culture such that at least 75% of the suspension culture is mixed with the acidic and/or basic component when the second vessel is at 75% capacity or more

64. The system according to claim 63 comprising a centrifuge, wherein the second vessel comprises an outlet configured such that at least the heavy phase can be transferred to the centrifuge or the heavy phase and only a minor portion of the light phase can be transferred to the centrifuge.

65. The system for culturing and isolating edible biomass of any one of claims 60 to 64 configured to complete the method according to any one of claims 1-43.