

METHODS FOR PRODUCING A COMESTIBLE FOOD BASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/582,629, titled “METHODS FOR PRODUCING A COMESTIBLE FOOD BASE,” filed on September 14, 2023, which is incorporated by reference in its entirety.

BACKGROUND

[0002] As the world’s population continues to grow, cell-based or cultured animal products for consumption have emerged as an attractive alternative (or supplement) to conventional animal products, such as meat from animals and supplemental products derived therefrom. For instance, cell-based, cultivated, or cultured meat represents a technology that could address the specific dietary needs of humans. Cell-based animal food products can be prepared from one or more of cultivated adherent and suspension cells derived from a non-human animal. Cell-based meat products, for instance, are often formed and shaped to mimic familiar forms of conventional meat.

[0003] In addition to addressing dietary needs, cell-based animal food products help alleviate several drawbacks linked to conventional animal products for humans, livestock, and the environment. For instance, conventional meat production involves controversial practices associated with animal husbandry, slaughter, and harvesting. Other drawbacks associated with harvested or slaughtered meat production include low conversion of caloric input to edible nutrients, microbial contamination of the product, emergence and propagation of veterinary and zoonotic diseases, relative natural resource requirements, and resultant industrial pollutants, such as greenhouse gas emissions and nitrogen waste streams.

[0004] Despite advances in creating cell-based animal food products, existing methods or systems for cultivating and processing cell-based animal food products face several shortcomings, such as challenges with cost, efficiency, safety, and equipment maintenance. For example, existing methods for cultivating and processing cell-based animal food products often suffer from slow cellular growth, low yields of cell-based products, and an overall inefficient use of resources. In particular, existing methods require prohibitive amounts of time and resources to generate small amounts of cell-based animal food products. To compensate for slow times of growing cell-based tissue and lack of cellular tissue mass, for example, some manufacturers ramp up the same existing methods by increasing the number and size of machines and tools used to grow cellular tissue. However, such upscaling of cultivation equipment presents additional challenges with respect to overall equipment and supply costs, sterility and cleanability of bioreactors and support equipment, and harvestability of cultivated cells. Relatedly, many conventional systems and methods, particularly when upscaled for increased production, produce exorbitant amounts of process waste from which costs are not generally recovered.

[0005] These, along with additional problems and issues persist in existing methods for producing cell-based animal food products and derivatives thereof.

BRIEF SUMMARY

[0006] This disclosure generally describes systems, apparatuses, and methods for producing a comestible food base from cell culture media previously utilized during production of a cell-based animal food product. For example, the disclosed methods include growing animal cells within a bioreactor system by circulating cell culture media through the bioreactor system, extracting circulated cell culture media from the bioreactor system, and integrating one or more supplemental ingredients with the extracted cell culture media to produce a comestible food base.

[0007] Relatedly, the disclosed implementations include comestible food bases comprising expended cell culture media previously utilized during cultivation of one or more cell masses of a cell-based animal food product, proteins released from the one or more cell masses of the cell-based animal food product during the cultivation thereof, and one or more supplementary ingredients integrated with the expended cell culture media to enhance a flavor, a texture, or a nutritional content thereof.

[0008] Additional features and advantages of one or more implementations of the present disclosure are outlined in the description which follows, and in part will be obvious from the description, or may be learned by the practice of such example implementations.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Various implementations will be described and explained with additional specificity and detail through the use of the accompanying drawings, which are summarized below.

[0010] FIG. 1 illustrates an overview diagram of preparing a comestible food base in accordance with one or more implementations of the present disclosure.

[0011] FIG. 2 illustrates an overview diagram of collecting cells from an animal and proliferating the collected cells in accordance with one or more implementations of the present disclosure.

[0012] FIG. 3 illustrates an overview diagram of adapting proliferated animal cells to a suspension culture in accordance with one or more implementations of the present disclosure.

[0013] FIG. 4 illustrates an overview diagram of growing animal cells within a bioreactor system in accordance with one or more implementations of the present disclosure.

[0014] FIG. 5 illustrates an overview diagram of extracting cell masses and expended cell culture media from a bioreactor system in accordance with one or more implementations of the present disclosure.

[0015] FIG. 6 illustrates an overview diagram of producing a comestible food base from an expended cell culture media according to one or more implementations of the present disclosure.

[0016] FIG. 7 illustrates a flowchart of a series of acts for producing a comestible food base in accordance with one or more implementations of the present disclosure.

[0017] FIG. 8 illustrates experimental results comparing conventional chicken broth and a comestible food base comprising expended cell culture media in accordance with one or more implementations of the present disclosure.

DETAILED DESCRIPTION

[0018] This disclosure describes one or more embodiments of a method for producing a comestible food base from expended cell culture media utilized in growing a cell-based animal food product. Generally, the disclosed methods comprise growing animal cells within a bioreactor system by circulating cell culture media through the bioreactor system, extracting circulated cell culture media from the bioreactor system, and producing a comestible food base by integrating supplementary ingredients with the extracted cell culture media.

[0019] To illustrate, in some implementations, a comestible food base comprises expended cell culture media previously utilized during cultivation of one or more cell masses of a cell-based animal food product, proteins released from the one or more cell masses of the cell-based animal food product during the cultivation, and one or more supplementary ingredients configured to enhance a flavor, a texture, or a nutritional content of the expended cell culture media. In various implementations, for example, the comestible food base comprises a liquid stock, broth, or related food base product. Alternatively, in some implementations, the comestible food base comprises a dehydrated food additive, flavoring, or nutritional supplement.

[0020] Moreover, in one or more implementations, the cell culture media utilized to produce the comestible food base comprises a plurality of edible ingredients, such as ingredients approved for use in food as Generally Recognized As Safe (GRAS). In some implementations, each ingredient of the plurality of edible ingredients is at or below a respective acceptable daily intake (ADI) value. In particular, in various implementations, the plurality of edible ingredients of the cell culture media comprises ingredients configured to stimulate and/or promote the growth of animal cells within a bioreactor system.

[0021] The disclosed methods provide several benefits relative to existing systems and methods for producing comestible cell-based animal food products. In particular, the disclosed methods utilize a cell culture media comprising edible ingredients to cultivate cell-based animal food products, such as but not limited to cell-based meats, then utilize the expended cell culture media to produce a comestible food base in addition to the cultivated cell-based animal food product. By implementing cultivation with a cell culture media comprised of edible ingredients, the disclosed methods enable efficient use of otherwise dispensable byproducts of animal cell

growth processes. Accordingly, the disclosed methods increase process efficiency and decrease costs over existing systems and methods for the production of cell-based animal food products.

[0022] Moreover, the disclosed methods provide for the production of a novel food product—a comestible food base derived from the production of cell-based animal food products. For example, in some implementations, the disclosed comestible food base comprises a unique nutritional profile relative to conventional food bases, such as stocks, broths, and related products derived from slaughtered animal meats. Further, the disclosed methods enable the production of cruelty-free food bases with a similar flavor and texture to such conventional animal products.

[0023] As illustrated by the foregoing discussion, the present disclosure utilizes a variety of terms to describe features and advantages of the disclosed systems and methods. Additional detail is now provided regarding the meaning of such terms.

[0024] As used herein, the term “cells” refers to cells that are useful for forming a cell-based meat product for consumption. Generally, cells may comprise non-human mesenchymal progeny. For instance, cells may comprise at least one of muscle cells, muscle progenitor cells, or muscle support cells. In particular, cells may comprise different cell types, such as one or more of myoblasts, mesangioblasts, myofibroblasts, mesenchymal stem cells, hepatocytes, fibroblasts, pericytes, adipocytes, epithelial, chondrocytes, osteoblasts, osteoclasts, pluripotent cells, somatic stem cells, endothelial cells, or other similar cell types. Furthermore, cells may comprise different types of progenitor cells, including myogenic progenitors, adipogenic progenitors, mesenchymal progenitors, or other types of progenitor cells. In some instances, the cells may comprise cells from distinct lineages, such as ectoderm or endoderm lineages, that have been transdifferentiated into cells useful for forming a cell-based meat product for consumption, such as those cell types described above.

[0025] As used herein, the term “cell mass” refers to a tissue or mass of cells. Cell mass refers to cells of cultivated meat gathered into a collective mass. In some implementations, the cell mass is comestible. Additionally, a cell mass can include grown cells that have been nourished by a growth medium to grow during a formation period within a cultivator. In some examples, a cell mass is grown from cells attached to a substrate in an adherent reactor.

[0026] As used herein, the term “cultivator” refers to a vessel used for culturing cells, such as a bioreactor. In particular, a cultivator refers to an environment that can hold cells and cell culture media. For example, a cultivator can include an adherent reactor system that contains a substrate for growing adherent cells. Additionally, a cultivator can include a substrate-free bioreactor system for growing cells in suspension.

[0027] As used herein, the term “suspension culture” (or “suspension”) refers to cells growing in an at least partially liquid growth medium in which cells grow, multiply, and/or

maintain nourishment. In particular, a suspension includes an agitated growth medium that is housed in a container in which single cells or small aggregates of cells grow, multiply, and/or maintain nourishment from the nutrients of the agitated growth medium. Cells grown in suspension are not attached to a substrate and therefore differ from a conventional adherent culture.

[0028] As used herein, the terms “closed environment” and “closed bioreactor system” refer to a space with regulated environmental factors. In particular, a closed environment or system includes a space in which certain parameters, such as pressure, temperature, segregation, and exposure to outside elements, are controlled. More specifically, external contaminants are limited, or nonexistent, within a closed environment or system. For instance, a closed environment or system is isolated from contaminants found in air (e.g., ambient air). Further, a closed environment or system comprises an area that is contained on all sides. In particular, a closed bioreactor system comprises an enclosed environment including one or more fluidly connected spaces that are at least substantially closed off from external contaminants. In another example, a closed environment or system comprises a transfer mechanism, such as a series of sterile containers including a cultivator and a harvest collector. The series of containers may be sterilized and sealed prior to receiving cells. In another example, a closed environment or system comprises a series of pipes for transporting and processing cells.

[0029] As used herein, the term “harvest” or “harvesting” refers to a process of removing cells from a closed environment, such as a closed bioreactor system. For example, harvesting refers to the process of removing cells from a closed environment and exposing them to air. Harvesting may comprise removing adherent cells or suspension cells from a closed environment. In one or more implementations, harvesting comprises opening a harvest collector holding cells, wherein the cells are exposed to air (e.g., ambient air, filtered air, and/or refrigerated air) and any contaminants therein.

[0030] More specifically, harvesting cells can occur, in one or more implementations, when the cells are removed from the cultivator (e.g., bioreactor). In particular, harvesting occurs when removing the cells from the cultivator involves removing the cells from a closed environment or exposing the cells to air. Alternatively, the cells are removed from the cultivator (e.g., bioreactor) without removing the cells from the closed environment such that harvesting takes place after the cells are removed from the cultivator.

[0031] Along similar lines, harvesting cells can occur, in one or more implementations, when the cells are separated from a cell culture media (e.g., growth or nutrient media). In particular, when separating the cells from the cell culture media involves removing the cells from a closed environment or exposing the cells to air, harvesting occurs. Alternatively, the cells are separated from a cell culture media before removing the cells from the closed environment such that

harvesting takes place after the cells are separated from a cell culture media (e.g., as described below in relation to FIG. 5).

[0032] Additional detail will now be provided regarding the disclosed systems, apparatuses, and methods in relation to illustrative figures portraying example implementations. FIG. 1 illustrates an example process for preparing comestible cell-based animal food products and a comestible food base in accordance with one or more implementations. By way of overview, FIG. 1 illustrates a series of acts 100 comprising an act 102 of cultivating animal cells within a bioreactor system, an act 104 of extracting animal cells from the bioreactor system, an act 106 of extracting circulated cell culture media from the bioreactor system, an act 108 of forming the animal cells into a comestible cell-based animal food product, and an act 110 of producing a comestible food base. FIGS. 2-6 and the corresponding paragraphs herein provide further detail with regard to cultivating animal cells, extracting circulated cell culture media, and producing a comestible food base in accordance with one or more implementations.

[0033] As shown in FIG. 1, in one or more implementations, animal cells are grown within a bioreactor 105 by performing the act 102 of cultivating the animal cells by circulating a cell culture media 103a through the bioreactor 105. In one or more implementations, animal cells (e.g., adherent meat cells) are prepared for cultivation by contacting cells to one or more cell culture substrates of a bioreactor 105 or bioreactor system. In one or more implementations, the cell culture substrates comprise a permeable substrate (e.g., permeable to physiological solutions) or an impermeable substrate (e.g., impermeable to physiological solutions). The substrate for the adherent cells can be flat, concave, or convex. Additionally, the substrate for the adherent cells can be textured so as to promote cell growth.

[0034] To illustrate, in one or more implementations, the adherent cells are prepared by contacting cell culture substrates to a cultivation infrastructure, including within a pipe-based bioreactor, a tank-based bioreactor, and so forth. In some implementations, the culturing of adherent cells can induce the production of extracellular matrix (ECM). Indeed, in one or more implementations, the ECM can act as an autologous scaffold to direct three-dimensional cellular growth. For example, in some implementations, the ECM can direct attachment, proliferation, and hypertrophy of cells on a plane perpendicular to the substrate. In addition, or in the alternative, in some implementations, the cultivation infrastructure may not comprise an exogenously added scaffold to promote self-assembly of a three-dimensional cellular biomass. In some implementations, the cultivation infrastructure may not comprise exogenous scaffolds such as a hydrogel or soft agar.

[0035] As mentioned above, adherent cells can be grown to form a cell sheet. Accordingly, an exemplary method of producing cell-based meat comprises: (a) providing fibroblasts and/or

myoblasts from a non-human organism; (b) culturing the fibroblasts and/or myoblasts in media under conditions under which the fibroblasts and/or myoblasts grow in either suspension culture or adherent culture, wherein the media is substantially free of serum and other components derived from an animal.

[0036] Additionally, as shown in FIG. 1, in one or more implementations, the adherent cells are prepared by performing an act 106 of growing cells on the substrate(s). In one or more implementations, the adherent cells are grown on a suitable substrate that is specifically treated to allow cell adhesion and spreading, such as a surface located within a sterile enclosure of a bioreactor. The culturing conditions for the generation of the animal cells for a comestible cell-based food product are generally aseptic and sterile. For example, cells are injected into a cultivation tank, such as an adherent bioreactor. The cultivator (i.e., the bioreactor 105) contains the substrate(s) (e.g., metal planks, sheets, or a lattice) to which cells can adhere. The cells are flowed through the enclosure of the bioreactor to allow cells to adhere to the substrate over time.

[0037] Before seeding the cells onto the substrate, in some implementations, the disclosed methods include preparing the substrate, such as by adding or flowing over adherent media to increase cell adherence to the substrate. As suggested above, in some implementations, the substrate is located within a bioreactor enclosure that is a sterile environment. To prepare the substrate in a bioreactor, the disclosed methods can include adding adherent media. The adherent media can be low in calcium to limit cellular clumping, so the cells spread out evenly across the substrate. The adherent media further facilitates attachment by the cells to the substrate. In some implementations, preparing the substrate further includes adding conditioning media and bringing the conditioning media up to temperature. The conditioning media further prepares the substrate by controlling pH, carbon dioxide, and oxygen levels within the cultivation tank. Additionally, the conditioning media may coat the substrates such that the adherence capability of the later seeded cells is enhanced.

[0038] The disclosed methods include growing the cells into a cellular tissue by performing the act 102 of cultivating the cells with a cell culture media 103a. Generally, the seeded cells (including the seeded initial cells and the previously unlabeled cells) are grown in conditions that allow the formation of cellular tissue for a formation period. In some cases, the formation period can equal 4-14 days or other periods. During the formation period, cells are provided with additional nutrients, media, growth factors, and other supplements to promote cellular growth, such as the cell culture media 103a. The cell culture media 103a can include growth factors and beneficial proteins. At nutrient intervals during the formation period, additional feeds, amino acids, proteins, vitamins, minerals, and growth factors may be added to the cell culture media 103a to support growth in the seeded cells. Additionally, or alternatively, the disclosed methods include

adding pre-harvest media before harvest. For instance, three days before harvest, a pre-harvest media including yeast concentrate may be added to the cultivation tank to supplement the cell culture media 103a.

[0039] As mentioned above, the cell culture media 103a can include a plurality of edible ingredients configured to stimulate and/or promote growth of the cells within the bioreactor 105. As mentioned above, for example, the edible ingredients can include a growth factor configured to stimulate cell growth. Further, in some implementations, the edible ingredients of the cell culture media 103a include additives configured for cultivation of the cell-based animal food product, the additives including one or more of an adhesion protein, amino acids, carbohydrates, vitamins, inorganic salts, trace metals, lipids, or food oils. Also, in some implementations, the edible ingredients include one or more of glycolysis intermediates, iron carriers, ferric maltol, ferrous gluconate, sheer protectants, polyethylene glycol, methylcellulose, simethicone, food-grade plant oils, or hydrolysates.

[0040] Indeed, as mentioned previously, the cell culture media 103a can include various edible ingredients approved for human consumption, such as ingredients that are Generally Recognized As Safe (GRAS). In some implementations, each ingredient is approved for use by one or more of the FDA, USDA, Codex Alimentarius, European Food Safety Authority, or the Food Chemicals Codex. Also, in some implementations, the cell culture media 103a comprises edible ingredients with concentrations at or below an acceptable daily intake (ADI) value for each respective ingredient.

[0041] In one or more implementations, the adherent cells include cellular tissue of cultured meat gathered into a collective or agglomerated mass, including via growth on a substrate. In one or more implementations, the cultivation infrastructure for cultivating the adherent cells has a three-dimensional structure or shape for cultivating a monolayer of adherent cells. Additionally, in some implementations, the cultivation infrastructure can promote the adherent cells to form a three-dimensional growth of metazoan cells in the cultivation infrastructure to provide a scaffold-less self-assembly of a three-dimensional cellular biomass.

[0042] In some implementations, adherent cells within the bioreactor 105 are grown on a three-dimensional cultivation infrastructure. The three-dimensional cultivation infrastructure may be sculpted into different sizes, shapes, and forms, as desired, to provide the shape and form for the adherent cells to grow and resemble different types of muscle tissues such as steak, tenderloin, shank, chicken breast, drumstick, lamb chops, fish filet, lobster tail, etc. The three-dimensional cultivation infrastructure may be made from natural or synthetic biomaterials that are non-toxic so that they may not be harmful if ingested. Natural biomaterials may include, for example, collagen, fibronectin, laminin, or other extracellular matrices. Synthetic biomaterials may include, for

example, hydroxyapatite, alginate, polyglycolic acid, polylactic acid, or their copolymers. The three-dimensional cultivation infrastructure may be formed as a solid or semisolid support.

[0043] Alternatively, in some implementations, animal cells are grown in a cultivation structure comprising a bioreactor without substrates, where the animal cells are proliferated in a suspension format. In the suspension format, cell culture media may be added in discrete batches, circulated between a bioreactor and media tank, continuously added and removed, or some combination thereof. In any case, bioreactors supporting suspension animal cells can further implement agitation (e.g., mixing) to reduce clumping and settling of animal cells and require that animal cells, which typically only proliferate in adherent conditions, are adapted to proliferate in suspension conditions.

[0044] A cultivation infrastructure can be of any scale and support any volume of cellular biomass and culturing reagents. In some implementations, the cultivation infrastructure ranges from about 10 μ L to about 100,000 L. In exemplary implementations, the cultivation infrastructure is about 10 μ L, about 100 μ L, about 1 mL, about 10 mL, about 100 mL, about 1 L, about 10 L, about 100 L, about 1000 L, about 10,000 L, or even about 100,000L.

[0045] In one or more implementations, the comestible cell-based animal food product, unless otherwise manipulated to include, does not include vascular tissues, such as veins and arteries, whereas conventional meat does contain such vasculature, and contains the blood found in the vasculature. Accordingly, in some implementations, the comestible cell-based animal food product does not comprise any vasculature. Likewise, comestible cell-based animal food product, although composed of muscle or muscle-like tissues, unless otherwise manipulated to include, does not comprise functioning muscle tissue. Accordingly, in some implementations, the cell-based meat does not comprise functioning muscle tissue. It is noted that features such as vasculature and functional muscle tissue can be further engineered into the cell-based meat, should there be a desire to do so.

[0046] As further shown in FIG. 1, the cultivated animal cells, whether adherent or suspended, can be removed from the bioreactor 105 by performing the act 104 of extracting the animal cells from the bioreactor 105. Also, the expended cell culture media 103b (i.e., the cell culture media 103a after cultivation of the animal cells) can be removed by performing the act 106 of extracting circulated cell culture media from the bioreactor 105. In some implementations, for example, the cultivated animal cells and the expended cell culture media 103b are jointly removed from the bioreactor 105 and subsequently separated (e.g., as described below in relation to FIG. 5). Alternatively, in some implementations, the expended cell culture media 103b is drained or otherwise removed from the bioreactor 105 prior to extracting the animal cells therefrom.

[0047] In one or more implementations, adherent cells are harvested from the bioreactor 105 by detaching a cell sheet from the substrate(s) disposed therein. In particular, the adherent cells are harvested based on various factors. The adherent tissue may be harvested after a proliferation time period. For example, the adherent cells are harvested after the cells have been growing for anywhere between 4 and 14 days. In another example, the adherent cells are harvested based on completing a proliferation phase. More specifically, the adherent cells may be harvested when the cell sheet starts contracting and stops growing. For instance, the cell sheet may begin to detach from the substrate. In one or more implementations, the cell sheet is detached from the substrate after adding pre-harvest media before harvest.

[0048] As shown in FIG. 1, the series of acts 100 can optionally involve reducing the moisture and/or size of an agglomeration (e.g., the cell sheet) of adherent cells. For example, the act 108 can involve reducing a moisture content in the adherent cells by vacuum drying the adherent cells. In addition, in some implementations, the act 108 involves reducing a size of the meat cells via chopping, or other technique. Furthermore, the act 108 can involve forming the cells into the shape of a meat product (e.g., chicken breast, steak).

[0049] As also shown in FIG. 1, the series of acts 100 includes an act 110 of producing a comestible food base from the extracted cell culture media 103b. In some implementations, for example, the act 110 includes removing heavy phase (e.g., solids or sediments) from the extracted cell culture media 103b, such as by filtering, centrifugation, or other separation methods. Also, in some implementations, the act 100 includes heating the extracted cell culture media 103b to release volatiles, which may reduce undesirable odors, incorporate additional flavors or supplements, and/or sterilize the comestible food base. In one or more implementations, the act 110 can include mixing one or more flavoring ingredients (e.g., vegetables, herbs, spices, and other seasonings) prior to, during, or after heating the comestible food product. Further, in some embodiments, additional supplementary ingredients are added to the extracted cell culture media 103b, such as nutritional supplements, thickening agents, emulsifiers, and so forth. Indeed, according to the foregoing methods and the various implementations disclosed herein, the comestible food base produced from the extracted cell culture media 103b can comprise a liquid stock or broth, gravy, soup, sauce, dip, or other liquid additive, and/or can be further processed to produce a dehydrated food base, additive, or supplement, such as bouillon, seasoning, protein powder, and so forth. All such products are derived from what would otherwise be a waste material, i.e., the expended cell culture media. As such, reusing the expended cell culture media both reduces waste and simultaneously provides a useful slaughter-free food or cooking material that is traditionally derived from slaughter.

[0050] The paragraphs above describe methods for forming a cell mass into a cell-based animal food product, such as a comestible cell-based meat, and for producing a comestible food base from cell culture media utilized during cultivation of the cell-based animal food product. FIGS. 2-6 and the following accompanying paragraphs describe procurement of cells, growth of cells into a cell mass, and subsequent production of cell-based animal food products and a comestible food base in accordance with one or more implementations. Generally, FIGS. 2-5 illustrate a process of collecting cells from an animal, growing cells in a favorable environment, banking successful cells, and collecting cells into a cell mass followed by de-wetting and/or other treatments, and FIG. 6 illustrates producing a comestible food base from expended cell culture media utilized during one or more of the procedures illustrated by FIGS. 2-5.

[0051] As illustrated by step 202 in FIG. 2, tissue is collected from a living animal via biopsy. In particular, stem cells, mesenchymal progeny, ectoderm lineage, and/or endoderm lineages can be isolated from the removed tissue. In some implementations of the present disclosure, tissue, such as fat and others, are processed to isolate stem cells, mesenchymal, ectoderm, and/or endoderm progeny or lineage cells. As illustrated, tissue 204 is removed from an animal. In some examples, the tissue 204 is removed from a living animal by taking a skin sample from the living animal. For instance, skin or muscle samples may be taken from a chicken, cow, fish, shellfish or another animal.

[0052] Cells may be extracted from the tissue 204 that was removed from the animal. More specifically, the tissue 204 is broken down by enzymatic and/or mechanical means. To illustrate, FIG. 2 includes digested tissue 206 that comprises the cells to be grown in cultivation.

[0053] Cells in the digested tissue 206 may be proliferated under appropriate conditions to begin a primary culture. As illustrated in FIG. 2, cells 208 from the digested tissue 206 are spread on a surface or substrate and proliferated until they reach confluence. As shown in FIG. 2, in some cases, cells 212 have reached confluence when they start contacting other cells in the vessel, and/or have occupied all the available surface or substrate.

[0054] In some examples, cells are stored and frozen (i.e., banked) at different steps along the cell culture process. Cryopreservation generally comprises freezing cells for preservation and long-term storage. In some implementations, tissue and/or cells are removed from a surface or substrate, centrifuged to remove moisture content, and treated with a protective agent for cryopreservation. For example, as part of cryopreservation, tissues and cells are stored at temperatures at or below -80C. The protective agent may comprise dimethyl sulfoxide (DMSO) or glycerol.

[0055] Cells stored through cryopreservation may be used to replenish working cell stock. For instance, while a portion of the digested tissue 206 is used as the cells 208 spread on a surface

or substrate, the remaining or excess digested tissue 206 is transferred to cryovials 210 for storage. Furthermore, the cells 212 may be banked once reaching confluence and stored in cryovials 214.

[0056] Once the cells 212 have reached confluence, or just before the cells 212 have reached confluence (e.g., occupation of about 80% of the substrate), the disclosed process comprises a series of cell passage steps. During cell passage, the cells 212 are divided into one or more new culture vessels for continued proliferation. To illustrate, the cells 212 may be diluted or spread on one or more surfaces or substrates to form the cells 218. The cells 218 are then grown 216 to confluence, or just before confluence.

[0057] The cycle of dividing the cells 212 into the cells 218 for continued proliferation in new culture vessels may be repeated for a determined number of cycles. Typically, cell lines derived from primary cultures have a finite life span. Passaging the cells allows cells with the highest growth capacity to predominate. In one example, cells are passaged for five cycles to meet a desired genotypic and phenotypic uniformity in the cell population.

[0058] In some implementations, the disclosed method comprises immortalizing cells that have been grown and passaged for the determined number of cycles. For instance, the cells 218 may be immortalized. As shown in FIG. 3, for example, cells 302 have demonstrated a preferred growth capacity to proceed to immortalization. To achieve immortalization, the disclosed process transfects the cells 302 with genes of interest. In one example telomerase reverse transcriptase (TERT) is introduced to the cells 302. In some implementations, the cells may be subjected to a selection process as known by those skilled in the art. The cells 302 may then be passaged for a predetermined set of passaging cycles. In one example passaging cycle, the cells 302 are grown to (or near) confluence 306, then they are reseeded in new growth vessels, preserved in vials 304, or some combination of both. The disclosed process may include any number of passaging cycles to ensure that the cells have reached immortality (e.g., can passage 60+ times without senescing), a target growth capacity, and/or a target quantity for banking. For example, cells may be passaged until they have reached a passage level of 100 (e.g., have been passaged for 100 passaging cycles). In another example, cells are passaged until they reach a population doubling level of 100.

[0059] Cells that have reached immortality or a target growth capacity by living through a target passage level may be adapted to suspension culture. In one example, a suspension culture media and agitation of cells in this suspension environment help cells to adapt and start proliferating in the new growth environment. The cells adapted to suspension 308 may be stored in cryovials 310 for cryopreservation and banking. Cells in suspension 308 will begin to proliferate and the process begins a series of dilute and expand steps.

[0060] During dilution and expansion, cells are moved from growth vessels into newer, and progressively larger, growth vessels. For example, cells in suspension 308 may begin in a single

tube. The cells will proliferate and increase in cellular density. Once the cells have reached a target cell number (i.e., viable cell density (VCD) at desired volume), they are diluted and moved to a larger growth vessel. Optionally, the cells are banked in cryovials throughout expansion. For example, once cells in suspension reach a maximum VCD, the cells may begin to leave exponential growth due to overcrowding. After reaching a target density, the suspension cells may be transferred to a larger vessel 312 and diluted with additional media. The dilute-and-expand steps are repeated using progressively larger vessels (e.g., the vessel 314 and the vessel 316) and/or progressive dilution until the cells reach a production-ready volume. For example, cells may be production ready at about a 1,000 - 100,000 liter scale at 5 million cells per mL. The cells may be banked in cryovials at any of the dilution and expansion cycles.

[0061] As part of preparing cells to form cell-based-meat products, the disclosed process comprises growing the cells as an adherent culture. Generally, cells that are grown attached to a substrate form a texture that more closely resembles tissue found in conventional meat. Thus, the cells may be transferred from growth in suspension to growth in an adherent reactor. For example, the cells grown in suspension in the vessel 316 may be transferred to growth on a substrate. For example, FIG. 4 illustrates a bioreactor system comprising a plurality of adherent bioreactors 410 connecting in parallel to a media vessel 402. The media vessel 402 holds the cells grown in suspension media. In some implementations, cells from the vessel 316 are transferred directly to a cell culture media (or just “media”) vessel 402. In one example, the media vessel 402 comprises the vessel 316. The adherent bioreactors 410 may comprise pipe-based bioreactors. As shown, a plurality of valves 406 is secured to the plurality of adherent bioreactors 410 to enable individual use and access of each of the adherent bioreactors 410. For instance, to limit flow to only a first bioreactor of the plurality of adherent bioreactors 410, the valve 406 of the first bioreactor is opened while the remaining valves 406 are closed. Furthermore, the bioreactor system can include a directional valve 404 for changing between flow directions.

[0062] In some implementations, and as illustrated in FIG. 4, cells (e.g., adherent cells or suspension adapted cells) are prepared by flowing cells suspended in media (e.g., cell culture media) across substrates in the plurality of adherent bioreactors 410. More particularly, cells from the media vessel 402 may contact or land on the substrates in the plurality of adherent bioreactors 410. Cells and media that flowed through the adherent bioreactors 410 are cycled back to the media vessel 402. The media and cells can be cycled through the adherent bioreactors 410 until a target adherent cell density is reached. For instance, in some implementations, the disclosed method comprises measuring a cell density of outflow from the adherent bioreactors 410 to infer an adherent cell density.

[0063] The cells grow into adherent tissue within the adherent bioreactors 410 whilst the cell culture media is circulated through the bioreactors 410 to promote and stimulate cell growth. Once they have grown to a target density, either according to a learned timing or according to a measured fluctuation in cell metabolism of components such as glucose and oxygen, then the adherent tissue is ready for removal. The removal process of the disclosed method uses a high-pressure flow to shear the adherent tissue off the substrate surfaces. In one example, wash buffer from a wash tank 408 is flowed across the substrates in the adherent bioreactors 410. The wash buffer and cell mixture are flowed through a filter 412 where the cells are collected into one or more cell masses 414.

[0064] In one or more implementations, the cell masses 414 and the circulated cell culture media are together removed from the bioreactor system during harvesting, then separated by centrifugation or other separation procedures. For example, FIG. 5 illustrates an example apparatus for separating cultivated cells, such as cell masses 414 and other cells remaining in the expended cell culture media, into a heavy phase 506 (e.g., cell masses 414) and a light phase 504 (e.g., a substantially liquid component of the expended cell culture media). In particular, FIG. 5 illustrates a centrifuge apparatus 502 (e.g., a bacto-fuge or other centrifuge system) that utilizes centrifugal forces to separate the heavy phase 506 from the light phase 504. While FIG. 5 illustrates a mechanical method for separating the cultivated cells and/or cell masses 414 from cell culture media and other process liquids (e.g., the wash buffer mentioned above), other methods may be used, such as filtering, sedimentation methods, and so forth. In some implementations, such as suspension cultivation, cells are continually expanded in one or more large vessels (e.g., vessel 316) and are harvested directly from such vessels into the centrifuge apparatus 502 or other apparatus for separation.

[0065] In various implementations, the light phase 504 separated from the heavy phase 506 (from which a comestible food base may be produced) may contain various components of the expended cell culture media and/or portions of the cultivated animal cells (e.g., from the cell masses 414). In some implementations, for example, the light phase 504 retains at least some of the cells, nutrients, and/or additives remaining in the expended cell culture media after cultivation (e.g., cells, nutrients, or additives not exhausted during cultivation of animal cells). Also, in some implementations, the light phase 504 contains a portion of the animal cells grown during the cultivation (e.g., animal cells separated from the adherent cells of the heavy phase during cultivation, harvesting, and/or separation of the light phase 504 and the heavy phase 506). In particular, the light phase 504 can contain broken, damaged, or partial animal cells grown in the bioreactor. Furthermore, the light phase 504 can include animal cells that are not damaged (e.g., entire or whole animal cells) that have broken off or otherwise separated from the cell sheet.

[0066] As also shown in FIG. 5, the cell masses 414 may be further processed to adjust moisture content. For instance, FIG. 5 illustrates an example apparatus for reducing moisture content in the cells. In particular, FIG. 5 illustrates a pressure apparatus 510 that compresses the cell masses 508a and 508b. While FIG. 5 illustrates a mechanical method for adjusting moisture content of the cell masses 508a and 508b, other methods may be used to adjust moisture content. For example, the cell masses 508a and 508b may be mixed with a drying agent, vacuum dried, centrifuged, or otherwise dried. A moisture-adjusted-cell mass may be transferred to a container 512 for additional processing. For example, the cell mass 508a or 508b may be removed from the container 512 to be formed into a cell-based-meat product.

[0067] In some implementations, the light phase 504 comprised of extracted cell culture media may be further processed to produce a comestible food base. For example, FIG. 6 illustrates an example method for producing a comestible food base 610 utilizing expended cell culture media previously utilized during cultivation of one or more cell masses of a cell-based animal food product. As shown, in some implementations, expended cell culture media 602 can be further filtered (e.g., utilizing a filter 604 or a more complex filtering apparatus) to remove sediment remaining after separation of the expended cell culture media 602 and the cell-based animal food product (e.g., as described in relation to FIG. 5).

[0068] As further shown in FIG. 6, the expended cell culture media is subjected to a heating process 606, such as boiling, simmering, and/or an ultra-high-temperature (UHT) process. By heating the expended cell culture media 602, for instance, unwanted aromas may be reduced and the comestible food base 610 can undergo sterilization. In some implementations, as shown in FIG. 6, one or more supplementary ingredients 608 are mixed with the expended cell culture media 602 and, in some cases, heated together to integrate the supplementary ingredients with the expended cell culture media 602. For example, the supplementary ingredients 608 can include, but are not limited to, flavoring ingredients, ingredients configured to alter a texture, appearance, or smell of the expended cell culture media 602, and/or nutritional supplements (e.g., vitamins, minerals, protein).

[0069] Accordingly, in various implementations, the comestible food base 610 can be produced to include a variety of food base products comprising various comestible ingredients. Indeed, the comestible food base 610 can contain the various components of the extracted cell culture media (e.g., the light phase 504), such as suspension cells, cultivated animal cells, proteins, nutritional additives, and so forth. Further, additional ingredients can be integrated with the extracted cell culture media (e.g., the light phase 504) to further enhance or improve the flavor, texture, aroma, and/or nutritional content of the resulting comestible food base 610. Moreover, in various embodiments, the comestible food base 610 can include a liquid stock or broth, can be

dehydrated to comprise a bouillon, seasoning, or nutritional supplement, or can be utilized in preparing completed food products such as soups, sauces, and any manner of culinary preparation.

[0070] FIGS. 1-6, the corresponding text, and the examples provide several different systems, methods, techniques, components, and/or devices relating to producing a comestible food base in accordance with one or more implementations. In addition to the above description, one or more implementations can also be described in terms of flowcharts including acts for accomplishing a particular result. FIG. 7, for example, illustrates such a flowchart of acts. The acts described herein may be repeated or performed in parallel with one another or in parallel with different instances of the same or similar acts.

[0071] FIG. 7 illustrates a series of acts 700 comprising an act 702 of growing animal cells by circulating cell culture media through a bioreactor system, an act 704 of extracting circulated cell culture media from the bioreactor system, and an act 706 of producing a comestible food base with the extracted cell culture media.

[0072] The series of acts 700 comprises the act 702 of growing animal cells by circulating cell culture media through a bioreactor system. In some implementations, the act 702 includes circulating the cell culture media through the bioreactor system to grow adherent cell masses of the cell-based animal food product within the bioreactor system. Alternatively, act 702 may comprise growing animal cells in a suspension format, where cell culture media is added in discrete batches, circulated between a bioreactor and media tank, continuously added and removed, or some combination thereof.

[0073] The series of acts 700 includes the act 704 of extracting circulated cell culture media from the bioreactor system. In some implementations, the act 704 also includes separating the animal cells from the extracted cell culture media. Also, in some implementations, separating the animal cells from the extracted cell culture media comprises utilizing a centrifuge to remove a heavy phase from a light phase from contents of the bioreactor system. Further, in some implementations, remnants of the animal cells (e.g., material excreted from cells, materials from lysed cells, etc.) are retained within the extracted cell culture media and form part of the comestible food base.

[0074] The series of acts 700 also includes the act 706 of producing a comestible food base with the extracted cell culture media. In particular, the act 706 comprises producing the comestible food base by integrating one or more flavoring ingredients with the extracted cell culture media. In some implementations, integrating the one or more flavoring ingredients with the extracted cell culture media comprises integrating one or more of fresh vegetables, processed vegetables, fresh herbs, dried herbs, spices, or natural flavors. Also, in some implementations, the act 706 further

includes mixing one or more of an emulsifier or a thickening agent to the extracted cell culture media.

[0075] In some implementations, the series of acts 700 further comprises heating the extracted cell culture media. Moreover, in some implementations, the series of acts 700 further comprises sterilizing the comestible food base with an ultra-high-temperature process. Further, in some implementations, producing the comestible food base comprises producing an animal stock or producing a broth. Also, in some implementations, the series of acts 700 further comprises dehydrating the comestible food base to produce a powdered comestible food base.

Example 1

[0076] Example 1 describes one example implementation of forming a chicken broth; however, it should be appreciated that the following is merely one working example, and no aspect thereof should be considered limiting. As a first step, food grade cell culture media components, comprising one or more dried powders, are mixed and hydrated to form a fresh cell culture media that is edible. The fresh cell culture media is flooded into a bioreactor and inoculated with chicken cells. After a growth period (e.g., 1-7 days), the chicken cells have proliferated extensively and are ready for harvesting. Harvesting of the cells includes centrifuging the contents of the bioreactor into a heavy phase and a light phase. The heavy phase includes a majority of the cells and is processed into a cell-based food product. The light phase includes a majority of the expended cell culture media, which is collected for further processing.

[0077] The light phase of Example 1 is filtered and mixed with typical cooking ingredients found in chicken broth. Specifically, 1 liter of expended cell culture media is combined with 25 grams of carrots, 25 grams of celery, 5 grams of yeast extract and natural flavors, 0.5 grams of onion powder, and 0.5 grams of garlic powder. The combined ingredients are simmered for 5 to 10 minutes on a burner. After simmering, the combined ingredients are thermally processed for sterility and packaged for storage as ready-to-use chicken broth. Alternatively, the combined and sterilized ingredients are dehydrated and packaged for storage as a chicken bouillon or similar food base product.

[0078] As shown by Fig. 8, a comestible food base (e.g., chicken broth) formulated from expended media exhibits liking and preference scores similar to conventional chicken broth across 54 testers. Specifically, chicken broth formulated from expended media ranked within 0.2 points (across a 9 point scale) of conventional chicken broth scores for overall liking, color, overall flavor, chicken flavor, overall mouthfeel, and aftertaste. Only one metric deviated more than 0.2 points, and that was overall appearance, which was 1 point lower for chicken broth formulated from expended media. This data supports that there is no significant organoleptic difference between chicken broth formulated from expended media and conventional chicken broth. Furthermore, the

chicken broth formulated from expended media was tested in soup, risotto, and potsticker recipes and performed on par with conventional chicken broth, thereby showing its capacity for similar versatility. Indeed, as illustrated by the results shown in FIG. 8, comestible food bases formulated from expended cell culture media according to one or more implementations of the present disclosure can be implemented as a satisfactory substitute for conventional broths, such as commercially-produced chicken broth, in terms of both nutritional composition, taste, and marketability.

[0079] In accordance with common practice, the various features illustrated in the drawings may not be drawn to scale. The illustrations presented in the present disclosure are not meant to be actual views of any particular apparatus (e.g., device, system, etc.) or method, but are merely idealized representations that are employed to describe various implementations of the disclosure. Accordingly, the dimensions of the various features may be arbitrarily expanded or reduced for clarity. In addition, some of the drawings may be simplified for clarity. Thus, the drawings may not depict all of the components of a given apparatus (e.g., device) or all operations of a particular method.

[0080] Terms used herein and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as “open” terms (e.g., the term “including” should be interpreted as “including, but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as “includes, but is not limited to,” etc.).

[0081] Additionally, if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases “at least one” and “one or more” to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to implementations containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or “at least one” and indefinite articles such as “a” or “an” (e.g., “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations.

[0082] In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of “two recitations,” without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to “at least one of A, B, and C, etc.” or “one or more of A, B, and C, etc.” is used, in general such a construction is intended to include A alone, B alone, C alone, A and B

together, A and C together, B and C together, or A, B, and C together, etc. For example, the use of the term “and/or” is intended to be construed in this manner.

[0083] Further, any disjunctive word or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase “A or B” should be understood to include the possibilities of “A” or “B” or “A and B.”

[0084] However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to implementations containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or “at least one” and indefinite articles such as “a” or “an” (e.g., “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations.

[0085] Additionally, the use of the terms “first,” “second,” “third,” etc., are not necessarily used herein to connote a specific order or number of elements. Generally, the terms “first,” “second,” “third,” etc., are used to distinguish between different elements as generic identifiers. Absent a showing that the terms “first,” “second,” “third,” etc., connote a specific order, these terms should not be understood to connote a specific order. Furthermore, absent a showing that the terms “first,” “second,” “third,” etc., connote a specific number of elements, these terms should not be understood to connote a specific number of elements. For example, a first widget may be described as having a first side and a second widget may be described as having a second side. The use of the term “second side” with respect to the second widget may be to distinguish such side of the second widget from the “first side” of the first widget and not to connote that the second widget has two sides.

[0086] All examples and conditional language recited herein are intended for pedagogical objects to aid the reader in understanding the invention and the concepts contributed by the inventor to furthering the art and are to be construed as being without limitation to such specifically recited examples and conditions. Although implementations of the present disclosure have been described in detail, it should be understood that the various changes, substitutions, and alterations could be made hereto without departing from the spirit and scope of the present disclosure.

[0087] The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. Indeed, the described implementations are to be considered in all respects only as illustrative and not restrictive. For example, the methods described herein may be performed with less or more steps/acts or the steps/acts may be performed in differing orders. Additionally, the steps/acts described herein may be repeated or performed in

parallel to one another or in parallel to different instances of the same or similar steps/acts. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS

What is claimed is:

1. A method for producing a comestible food base, the method comprising:
growing animal cells within a bioreactor system by circulating cell culture media through the bioreactor system;
extracting circulated cell culture media from the bioreactor system; and
producing the comestible food base by integrating one or more flavoring ingredients with the extracted cell culture media.
2. The method of claim 1, wherein growing the animal cells further comprises circulating the cell culture media through the bioreactor system to grow adherent cell masses of a cell-based animal food product within the bioreactor system.
3. The method of claim 1, further comprising separating the animal cells from the extracted cell culture media.
4. The method of claim 3, wherein remnants of the animal cells are retained within the extracted cell culture media and form part of the comestible food base.
5. The method of claim 3, wherein separating the animal cells from the extracted cell culture media comprises utilizing a centrifuge to remove a heavy phase from a light phase from contents of the bioreactor system.
6. The method of claim 1, further comprising heating the extracted cell culture media.
7. The method of claim 1, wherein integrating the one or more flavoring ingredients with the extracted cell culture media comprises integrating one or more of fresh vegetables, processed vegetables, fresh herbs, dried herbs, spices, or natural flavors.
8. The method of claim 1, further comprising sterilizing the comestible food base with an ultra-high-temperature process.
9. The method of claim 1, wherein producing the comestible food base comprises producing an animal stock.

10. The method of claim 1, wherein producing the comestible food base comprises producing a broth.

11. The method of claim 1, further comprising mixing one or more of an emulsifier or a thickening agent to the extracted cell culture media.

12. The method of claim 1, further comprising dehydrating the comestible food base to produce a powdered comestible food base.

13. A comestible food base comprising:
expended cell culture media previously utilized during cultivation of one or more cell masses of a cell-based animal food product;
proteins released from the one or more cell masses of the cell-based animal food product during the cultivation; and
one or more supplementary ingredients configured to enhance a flavor, a texture, or a nutritional content of the expended cell culture media.

14. The comestible food base of claim 13, wherein the expended cell culture media comprises a plurality of edible ingredients with concentrations at or below an acceptable daily intake (ADI) value.

15. The comestible food base of claim 14, wherein the plurality of edible ingredients includes a growth factor configured to stimulate cell growth within a bioreactor system.

16. The comestible food base of claim 14, wherein the plurality of edible ingredients comprises additives configured for cultivation of the cell-based animal food product, the additives including one or more of an adhesion protein, amino acids, carbohydrates, vitamins, inorganic salts, trace metals, lipids, or food oils.

17. The comestible food base of claim 14, wherein the plurality of edible ingredients comprises one or more glycolysis intermediates, iron carriers, ferric maltol, ferrous gluconate, sheer protectants, polyethylene glycol, methylcellulose, simethicone, food-grade plant oils, or hydrolysates.

18. The comestible food base of claim 13, wherein the one or more supplementary ingredients comprise one or more of fresh vegetables, processed vegetables, fresh herbs, dried herbs, spices, natural flavors, an emulsifier, or a thickening agent.

19. The comestible food base of claim 13, wherein the comestible food base comprises a dehydrated food additive.

20. The comestible food base of claim 13, wherein the comestible food base comprises a liquid stock or broth.