CLAIMS

1. A method for producing a culture medium for the cultivation of non-human metazoan cells, comprising:

mixing a source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, with at least one proteolytic enzyme to cleave peptide bonds to provide a protein hydrolysate;

further mixing the protein hydrolysate with at least one enzyme having phytase activity to cleave phosphate ester bonds of inositol hexaphosphate, a derivative thereof, or their combination thereof, to provide a modified protein hydrolysate, wherein the modified protein hydrolysate comprises phosphate ions, wherein the phosphate ions originate from the cleavage of the phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof;

removing solid residues from the modified protein hydrolysate to provide a purified protein hydrolysate; and adding at least one nutritional additive to the purified protein hydrolysate to produce the culture medium for the cultivation of non-human metazoan cells.

2. The method according to claim 1, wherein the source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, is at least one source of protein selected from soy, pea, rice, wheat, corn, fava beans, alfalfa, hemp, chickpea, potato, pumpkin, rapeseed, red lentil, Spirulina, Chlorella, sunflower, water lentil, mung beans, flax, brewer spent grain, distillers spent grain (DDGS), duckweed, tomato pomace, or a combination thereof.

3. The method according to claim 1, wherein the source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, is pretreated with at least one treatment selected from steeping in water to induce germination, soaking, blanching, and milling.

4. The method according to claim 3, wherein the pretreatment of the source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, is performed to improve solubility and susceptibility of hydrolysis by blanching at a temperature in a range of 75 ºC to 95 ºC for a time period in a range of 5 minutes to 120 minutes.

5. The method according to claim 1, wherein the cleavage of the source of protein comprising inositol hexaphosphate, a derivvative thereof, or a combination thereof with proteolytic enzyme takes place at a pH in a range of 2 to 12, at a temperature in a range of 25 °C to 80 °C, and for at least 2 hours and wherein the resulting protein hydrolysate comprises 20% to 100% free amino acids.

6. The method according to claim 1, wherein the proteolytic enzyme is protease, peptidase, or their combination, and wherein the enzyme having phytase activity is phytase, phosphatase, or their combination.

7. The method according to claim 1, wherein the cleavage of phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof by the enzyme having phytase activity, takes place at a pH in a range of 2 to 10, at temperature in a range of 20 °C to 70 °C, and for a time period of 5 minutes to 320 minutes.

8. The method according to claim 1, wherein the purified protein hydrolysate comprises:
    (a) peptides having molecular weight higher than 17 kDa in a range of 0 % to 10 % of the purified purified protein hydrolysate;
    (b) peptides having a molecular weight ranging from 6.7 kDa t0 17 kDa in a range of 2 % to 35 % of the purified protein hydrolysate;
    (c) peptides having a molecular weight ranging from 1.7 kDa to 6.7 kDa in a range of 5 % to 40 % of the purified protein hydrolysate;
    (d) peptides having a molecular weight ranging from 1 kDa to 1.7 kDa in a range of 5 % to 40 % of the purified protein hydrolysate;
    (e) peptides having a molecular weight less than 1 kDa in a range of 20 % to 70 % of the purified protein hydrolysate; and
    (f) free amino acids in a range of 30 % to 50 % of the purified protein hydrolysate.

9. The method according to claim 1, wherein the purified protein hydrolysate comprises:
    (a) peptides having a molecular weight ranging from 1000 Da to 6500 Da in a range of 5 % to 50% of the purified protein hydrolysate;
    (b) peptides having a molecular weight ranging from 600 Da to 1000 Da in a range of 10 % to 70 % of purified protein hydrolysate;
    (c) peptides having a molecular weight ranging from 300 Da to 600 Da in a range of 10 % to 65 % of purified protein hydrolysate;
    (d) amino acids or peptides having a molecular weight less than 300 Da in a range of 10 % to 90 % of purified protein hydrolysate.

10. The method according to claim 1, wherein the at least one nutritional additive comprises saccharides, mineral compounds, vitamins, amino acids, peptides, organic amines, signaling compounds, oligonucleotides, fatty acids, phospholipids, organic micronutrients, or a combination thereof.

11. The method according to claim 1, wherein at least 50 % of the phosphate ions in the purified protein hydrolysate are provided by cleavage of the phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof.

12. The method according to claim 1, wherein the purified protein hydrolysate is combined with at least one shear protectants, anti-foaming agent, or their combination, wherein the shear protectant comprises at lease one of polyethylene glycol (PEG), methyl cellulose (MC), (hydroxypropyl)methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC), and dextran sulfate, and wherein the anti-foaming agent comprises at least one of polyethylene glycol (PEG), poly vinyl alcohol (PVA), polydimethylsiloxane, polysorbate 80, vegetable oils, or their combination.

13. The method according to claim 1, wherein the non-human metazoan cells are bovine, avian, porcine, equine, cervine, or cricetine cell lines, and have characteristics or properties of: hepatocytes, myocytes, myoblasts,

osteoblasts, fibroblasts, lipoblasts, odontoblasts, keratinocytes, mesenchymal stem cells, multipotent progenitor cells, embryonic stem cells, myofibroblasts, myosatellite cells, or a combination thereof.

14. The method according to claim 1, wherein the non-human metazoan cells cultivated using the culture medium prepared by the method are used for preparation of food products for human or animal consumption.

15. The method according to claim 1, wherein the culture medium is prepared in a cultivation system, wherein the cultivation system comprises at least one hydrolysis tank, and wherein the hydrolysis tank is used for mixing a source of protein with at least one protealytic enzyme to cleave peptide bonds to provide a protein hydrolysate.

16. The method according to claim 15, wherein the cultivation system comprises at least one filtration unit or at least one centrifuge unit for the removal of solid residues, or both.

17. A method for producing a culture medium for the cultivation Of non-human metazoan cells, comprising:

mixing a source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, with an enzyme having phytase activity to cleave phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof;

further mixing with the source of protein treated with the enzyme having phytase activity with a proteolytic enzyme, wherein the proteplytic enzyme cleaves peptides bonds of the source of protein to provide a modified protein hydrolysate, wherein the modified protein hydrolysate comprises phosphate ions, and wherein the phosphate ions originate from the cleavage of the inositol hexaphosphate, a derivative thereof, or a combination thereof;

removing solid residues from the modified protein hydrolysate to provide a purified protein hydrolysate; and

adding at least one nutritional additive to the purified protein hydrolysate to produce the culture medium for the cultivation of non-human metazoan cells.

18. The method according to claim 17, wherein the proteolytic enzymes and enzymes having phytase activity are loaded from a loading tank, wherein the loading tank is equipped with cooling system to maintain a consistent temperature essential for enzyme stability.

19. The method according to claim 17, wherein the cleavage of phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof by the enzyme having phytase activity takes place at a pH in a range of 2 to 20, at a temperature in a range of 20 ºC to 70 ºC, and for a time period of 5 minutes to 320 minutes.

20. The method according to claim 17, wherein the at least one nutritional additive is selected from the group consisting of saccharides, mineral compounds, vitamins, amino acids, peptides, organic amines, signaling compounds, oligonucleotides, fatty acids, phospholipids, and organic micronutrients, wherein the purified protein hydrolysate provides phosphate ions.

21. The method according to claim 17, wherein the nutritional additive comprises saccharides, mineral compounds or their combination, wherein saccharides are selected from the group consisting of glucose, fructose, galactose, sucrose, lactose, and maltose, and wherein the mineral compound is selected from at least one ion from the group consisting of Ca^{2+}, Cl^{-}, Cu^{2+} , $S0_4^{2-}$, Fe^{3+}, NO_3^{-},Fe^{2+} , Mg^{2+} , K^{+}, Na^{+} , $C0_3^{2-}$, HCO_3^{-}, $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}, $Zn^{2+}$and SeO_3^{2-}.

22. The method according to claim 17, wherein the purified protein hydrolysate is combined with at least one nutritional additive, wherein nutritional additive comprises a vitamin selected from the group consisting of alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), vitamin B 12, biotin, choline, pantothenic acid, folic acid, niacinamide, pyridoxine, riboflavin, thiamine, and i-inositol, and wherein the nutritional additives in the culture medium are in a range of 0.1 mg/l to 1000 mg/l.

23. The method according to claim 17, wherein the nutritional additive comprises at least one amino acid or an amino acid derivative selected from the group comprising glycine, L-alanine, L-arginine, L-asparagine L- aspartic acid, L-cystine I— glutamic acid, L-glutamine, L-histidine, L-hydroxyproline, L- ornithine, Is-citrulline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L- proline, L-serine, LA-threonine, L-pyroglutamic acid, L-phosphoserine, L-tryptophan, IA-tyrosine, and L-valine.

24. The method according to claim 17, wherein nutritional additive comprises at least one organic amine, signaling compounds or their combination, wherein organic amine is selected from putrescine and ethanolamine, and wherein the signaling compound for stimulation and proliferation is selected from the group consisting of FGF family ligands, insulin, insulin and IGF family ligands, TGF family ligands, and transferrin, and wherein a concentration of organic amine in the culture medium is in a range of 0.01 mg/l to 1000 mg/l.

25. The method according to claim 17, wherein the purified protein hydrolysate is combined with at least one stabilizing agent, wherein the stabilizing agent comprises at least one shear protectant, anti-foaming agent, or their combination, wherein the shear protectant comprises at least one of polyethylene glycol (PEG), methyl cellulose (MC), (hydroxypropyl)methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC), and dextran sulfate, and wherein the anti-foaming agent comprises at least one of polyethylene glycol (PEG), polyvinylalcohol (PVA), polydimethylsiloxane, polysorbate 80, and vegetable oil, and wherein a concentration of shear protectant in the culture medium is in a range of 0% to 5% by weight and a concentration of anti- foaming agent in the culture medium is in a range of 0.01% to 5%.

26. A method for generating phosphate ions in a culture medium for the cultivation of non-human metazoan cells, comprising: providing a hydrolyzed source of protein comprising inositol hexaphosphate, a derivative thereof, or a combination thereof, mixing the hydrolyzed source of protein with at least one enzyme having phytase activity to provide more phosphate ions by cleaving phosphate ester bonds of the inositol hexaphosphate, derivative thereof, or combination thereof to provide modified protein hydrolysate; adding at least one nutritional additive; and cultivating non-human metazoan cells in the culture medium.

27. The method according to claim 26, wherein the enzyme having phytase activity is derived from an animal, plant, or microorganism source, and wherein the concentration of enzymes having phytase activity is in a range of 0.00001% to 5% expressed as a ratio of the concentration of enzymes having phytase activity to the concentration of inositol hexaphosphate in the reaction mixture.

28. The method according to claim 26, wherein the cleavage of phosphate ester bonds of the inositol hexaphosphate, a derivative thereof, or a combination thereof, by an enzyme having phytase activity results in the generation of free inorganic

phosphate ions and at least one of inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate, inositol diphosphate, inositol monophosphate intermediates, or inositol, wherein the free phosphate ions generated by the cleavage of phosphate ester bonds of the inositol hexaphosphate, or the derivatives thereof, or a combination thereof, in the culture medium are in a range of 50% to 100% of total phosphate ions.

29. The culture medium according to claim 17, wherein the protein hydrolysate comprises derivatives of inositol hexaphosphate, including one or more lower phosphoric esters from the group of inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate, inositol diphosphate, inositol monophosphate, inositol, or combinations thereof.

30. The method according to claim 26, wherein the culture medium is produced without addition of an inorganic phosphate salt.