can protect such biologicals in storage under high temperature and humid conditions. The present invention provides a solution to all of these challenges as is described below. The dehydration process of the present invention is very gentle and does not expose the active agent to boiling or foaming and is therefore advantageous over conventional freeze drying and foam drying techniques which would subject the sample to one or both of these stresses.

SUMMARY OF THE INVENTION

[0010] The present disclosure~~invention~~ includes compositions and drying methods for preserving sensitive bioactive materials, such as peptides, proteins, enzymes, hormones, vitamins, carotenoids, minerals, drugs, antibiotics, microbiocides, fungicides, herbicides, insecticides, spermicides, nucleic acids, antibodies, vaccines, bacteria (probiotic or otherwise), viruses and/or cell suspensions in storage. The drying methods provide a process for drying of a formulation comprising the bioactive materials, a matrix forming agent, and a glass forming agent. The formulation is prepared by dispersing all the solid components and the bioactive materials in a solution. The solution is snap-frozen by means known in the art such as liquid nitrogen or dry ice to form an amorphous composition in small beads, strings or droplets. The frozen particles can be stored in a deep-freezer (between -30°C and -80°C) until drying or immediately placed on trays in a frozen amorphous state for liquid drying in a conventional freeze dryer. The drying method is initiated by a short purging and structure stabilizing step of the frozen particles under a vacuum pressure of less than <2000 mTORR followed by a primary drying step under higher than >2000 mTORR of vacuum pressure and at a desired temperature. During the secondary and final drying step of the glassy amorphous material, a full vacuum pressure and elevated temperature are applied, to achieve a final desirable water activity of the dry material.

[0010a] In one aspect, there is provided a dry composition, comprising a bioactive material, at least one matrix forming agent and at least two glass forming agents, wherein the composition is prepared in accordance with a method for preparing the dry composition without boiling or foaming, said method comprising:

(a) combining said bioactive material, said matrix forming agent and two glass forming agents in an aqueous solvent to form a viscous slurry;

(b) snap-freezing the slurry in liquid nitrogen to form solid frozen particles in the form of beads, droplets or strings;

(c) primary drying the frozen particles by evaporation, under vacuum, at a temperature above the freezing temperature of the frozen particles, whereby a primarily dried formulation is formed; and

(d) secondary drying the primarily dried formulation at full vacuum and a temperature of 20°C or higher for a time sufficient to reduce the water activity of the formulation to Aw - 0.3 or lower.

[0010b] In another aspect, there is provided a method for preparing a dry composition without boiling or foaming, said method comprising:

(a) combining a bioactive material, a matrix forming agent and two glass forming agents in an aqueous solvent to form a viscous slurry;

(b) snap-freezing the slurry in liquid nitrogen to form solid frozen particles in the form of beads, droplets or strings;

(c) primary drying the frozen particles by evaporation, under vacuum, at a temperature above the freezing temperature of the frozen particles, whereby a primarily dried formulation is formed; and

(d) secondary drying the primarily dried formulation at full vacuum and a temperature of 20°C or higher for a time sufficient to reduce the water activity of the formulation to Aw - 0.3 or lower, whereby the composition is prepared.

[0011] In one embodiment, the formulation comprises sufficient amounts of matrix forming agents, in which the bioactive material is embedded. Examples of a suitable matrix agent include, but are not limited to, cellulose acetate phthalate (CAP), carboxy-methyl-cellulose, pectin, sodium alginate, salts of alginic acid, hydroxyl propyl methyl cellulose (HPMC), methyl cellulose, carrageenan, guar gum, gum

acacia, xanthan gum, locust bean gum, chitosan and chitosan derivatives, collagen, polyglycolic acid, starches and modified starches, cyclodextrins and oligosaccharides (inulin, maltodextrins, dextrans, etc.); and combinations thereof. In one particular embodiment, the preferred matrix forming agent is sodium alginate. Preferably, the formulation comprises, in percent by weight of total dry matter, 0.1-20% and more preferably 1-12%.

[0012] In an additional embodiment, the matrix forming agent comprises a mixture of sodium alginate and oligosaccharides in a weight ratio of 1:1-10, more preferably 1:1-5 of sodium alginate/oligosaccharides.

[0013] In yet another embodiment ~~of the present invention~~, the matrix forming agent is cross-linked with divalent metals ions to form a firm hydrogel. The cross-linked hydrogel formulation is formed by atomizing or extruding the slurry in a bath containing divalent metal ions solution or by adding divalent metal ions directly into the slurry and allowing the formulation to harden and form a hydrogel. The hydrogel formulation is then flash frozen and dried according to the drying methods of the invention.

[0014] In yet another embodiment, the formulation comprises significant amounts of glass forming agents, in which the microorganisms are embedded. Examples of a suitable agent include but are not limited to proteins such as egg albumen, egg white, gelatin, immunoglobulin, isolated soy protein, wheat protein, pea protein, cotton seed protein skim milk powder, caseinate, whey protein and any hydrolyzed protein; carbohydrates including monosaccharides (*e.g.,* galactose, D-mannose, sorbose, *etc.*), disaccharides (*e.g.,* lactose, trehalose, sucrose, etc.), an amino acid such as lysine, glutamate, glycine, alanine, arginine or histidine, as well as hydrophobic amino acids (tryptophan, tyrosine, leucine, phenylalanine, etc.); a methylamine such as betaine; an excipient salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, (*e.g.* glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol); propylene glycol; polyethylene glycol; pluronics; surfactants; and combinations thereof.

[0015] In one preferred embodiment, the glass forming agent comprises a mixture of a disaccharide and a hydrolyzed protein. In a particular embodiment, the preferred glass forming agent is a mixture of trehalose and hydrolyzed protein. Preferably, the formulation comprises, in percent by weight of total dry matter, 10-90%, of trehalose and 0.1-30% hydrolyzed protein, more preferably 20-80% of trehalose and 0.1-20% hydrolyzed protein, and most preferably 40-80% of trehalose and 0.1-20% hydrolyzed protein.

[0016] The disclosed method ~~of the invention~~ typically includes mixing in a solution bioactive materials (*e.g.*, peptides, proteins, enzymes, hormones, vitamins, carotenoids, minerals, drugs, antibiotics, microbiocides, fungicides, herbicides, insecticides, spermicides, nucleic acids, antibodies, vaccines, bacteria, viruses and/or cell suspensions), at least one matrix forming agent, and at least two glass forming agents into a homogeneous slurry, snap-freezing the slurry by atomizing, dropping or extruding into liquid nitrogen bath. Collecting the beads, microbeads, strings or droplets from the liquid nitrogen bath and drying in a liquid state in a freeze drier, or alternatively storing them in a deep freezer (between -30°C and -80°C) until drying.

[0017] In one embodiment ~~a variation of the present invention~~, the amount of the matrix forming agent in the formulation is adjusted to achieve a desired formulation viscosity and density that allowed an efficient primary drying while avoiding boiling and excessive foaming that typically occur during the primary liquid drying step. A desired density of the liquid formulation can be achieved by any means known in the art, for example, whipping or injecting gas such as air, nitrogen, carbon dioxide, argon etc. Preferably, nitrogen is injected into the viscous slurry formulation under mixing to form stable porous or creamy slurry before the snap-freezing step.

[0018] According to an embodiment ~~the invention~~, the drying process involves three major steps; 1. Short purging and structure stabilizing step of the frozen particles under a vacuum pressure of less than <2000 mTORR, 2. Primary liquid drying step under vacuum pressure of more than >2000 mTORR and at a desired temperature, 3. Secondary and final drying step of the glassy material under full vacuum pressure and elevated temperature for a time sufficient to reduce the water activity of the dried formulation to 0.3 Aw or less.

is snap-frozen to between -30°C to -180°C, more preferably the formulation is snap-frozen in liquid nitrogen.

[0021] In a preferred embodiment, the snap-frozen particles are immediately dried or otherwise stored in a deep freezer, preferably at -80°C, until drying. The frozen particles are then loaded on trays and immediately transferred to a vacuum drying chamber where they are dried according to the present disclosure~~invention~~. Preferably, the drying is initiated by subjecting the frozen particles under vacuum pressure between 0 and 2000 mTORR. The frozen particles are degassed and their structure and volume allowed to develop and stabilize for a short period of time. Typically, the desirable time period for subjecting the frozen particle to high vacuum pressure is no more than 30 minutes, more preferably the time period is between 1 and 20 min. After the short initial degassing and structure stabilizing of the frozen particles the vacuum is adjusted to between 2000 and 10,000 mTORR and heat applied to thaw the particles at a temperature above their freezing point. Typically, the vacuum is adjusted to between 2000 and 4000 mTORR and the particle temperature is increased to between -5°C and +5°C. Under these preferred primary drying conditions the frozen particles are rapidly thawed and loosely maintain their original shape while an accelerated dehydration begins. The subsequent secondary drying is established after removing about 60-90% of the free water, a maximum vacuum pressure is applied and heat supplying temperature to the formulation is elevated to from 30°C to 60°C. To maximize the stability of the final product, the formulation is preferably dried for a time sufficient to reduce the water activity of the formulation to Aw=0.3 or less. In a preferred embodiment of the invention, the secondary drying comprises removal of bound water at a pressure of less than 1000 mTORR.

[0022] The dried formulation can be used directly as a flake, or ground into a powder and sieved to an average particle size from about 10 μm to about 1000 μm. The formulation can be administrated directly to an animal, including man, as a concentrated powder, as a reconstituted liquid, (e.g., beverage), or it can be incorporated either in flake or powder form into an existing food or feed product.

BRIEF DESCRIPTION OF THE FIGURES

11. The method of claim 10, wherein the primary drying step is carried out at a temperature greater than -10°C.

12. The method of claim 10, wherein the primary drying step is carried out at a temperature between -5°C and +5°C .

13. The method of claim 10, wherein the primary drying is primary drying step is carried out under a vacuum pressure greater than 2,000 mTORR.

14. The method of claim 10, wherein the primary drying is carried out under a vacuum pressure between 2,000 and 10,000 mTORR.

15. The method of claim 10, wherein the temperature or vacuum pressure is increased during the secondary drying step.

16. The method of claim 10, wherein the dry composition is cut, crushed, milled or respectively pulverized into a free flowing powder.

17. The method of claim 16, wherein particle size of the powder is less than about 1000 μm.

18. The composition of claim 1 for administration to animals or plants as a reconstituted liquid or as a ground powder incorporated into a food or feed product.

19. The method of claim 10, for further comprising mixing the dry composition with a component selected from the group consisting of infant formula, functional beverages, and pet food.

beverages, and pet food.