boiling, Bronshtein (U.S. Patent Nos.: 6,884,866, 6,306,345) has proposed special chambers and a controlled temperature/pressure application protocol that reduces overheating to an acceptable level. Another approach to contain the eruption and excessive foaming is described in US. Pat. App. No.: 2008/0229609, in which the bioactive solution is enclosed in a container or a bag covered with breathable membranes. Once again, these protocols are difficult to implement in industrial level and they are difficult to reliably replicate with different formulations.

[0011] A need remains for a suitable protective formulation that can be dried in a liquid state and an industrially scaleable method to dry bioactive microorganisms such as live or dead viruses, bacteria and cells, particularly at temperatures above freezing. There is a need particularly for a cost effective scaleable drying process that is also suitable for applications outside the pharmaceutical industry such as food and agriculture industries. Protective formulations and mild drying processes are required to provide adequate drying without exposure to high temperatures. A composition is needed that can protect such biologicals in storage under high temperature and humid conditions. The present invention, as described below, provides a solution to all of these challenges.

SUMMARY ~~OF THE INVENTION~~

[0012] The present disclosure~~invention~~ includes compositions and methods for preserving bioactive materials, such as peptides, proteins, hormones, vitamins, minerals, drugs, microbiocides, fungicides, herbicides, insecticides, spermicides, nucleic acids, antibodies, vaccines, and/or bioactive microorganism such as bacteria (probiotic or otherwise), viruses and/or cell suspensions, in storage. The drying methods provide a process of controllable expansion of a formulation comprising the bioactive microorganism or material, a formulation stabilizer agent, and a protective agent. The formulation is prepared by dispersing all the solid components in a solution, with or without a vacuum. The solution is cooled to a temperature above its freezing temperature and dried under vacuum into a dry composition, which exhibits an unexpectedly high stability. The methods include a primary drying step of the formulation at a desired temperature and time period, and an accelerated secondary

xylitol, sorbitol, and mannitol); propylene glycol; polyethylene glycol; pluronics; surfactants; and combinations thereof.

[0016] In one preferred embodiment, the protecting agent comprises a mixture of a disaccharide, a protein, and a protein hydrolysate. In a particular embodiment, the preferred protecting agent is a mixture of trehalose, soy protein isolate or whey protein and their hydrolysates. Preferably, the formulation comprises, in percent by weight of total dry matter, 10-90%, of trehalose, 0.1-30% soy protein isolate or whey proteins and 0.1-30% soy or whey protein hydrolysate. Preferably 20-80% of trehalose, 0.1-20% soy protein isolate or whey proteins and 1-20% soy or whey protein hydrolysate, more preferably 40-80% of trehalose, 0.1-20% soy protein isolate or whey proteins and 1-20% soy or whey protein hydrolysate.

[0017[The method of the disclosure~~invention~~ typically includes blending with or without a vacuum, concentrated solution or dry powder of bioactive microorganism (*e.g.,* live or dead vaccines, bacteria, algae, viruses and/or cell suspensions) or a bioactive material (*e.g.,* peptides, proteins, hormones, vitamins, minerals, drugs, microbiocides, fungicides, herbicides, insecticides, spermicides, nucleic acids, antibodies, vaccines), a stabilizer agent, and a protective agent into a homogeneous formulation, cooling the formulation to a temperature above its freezing temperature, and drying under vacuum at a shelf temperature above 20°C. According to the disclosed method~~invention~~, the drying process can involve a primary vacuum drying at a shelf temperature of 20°C or above, followed by an accelerating secondary drying of the formulation under maximum vacuum and elevated temperature for a time sufficient to reduce the water activity of the dried formulation to 0.3 Aw or less.

[0018] In one embodiment of the mixing method the bioactive microorganism or material is in a dry stabilized form and is further dry blended with the dry stabilizer agents and protective agents. This dry blend is then added to water and mixed under the appropriate vacuum and agitation to give a homogeneous slurry of the desired density.

delbrueckii ss. bulgaricus, L farciminis, L. fermentum, L. gasseri, L. helveticus, L. lactis, L. plantarum, L. johnsonii, L. reuteri, L. rhamnosus, L. sakei, L. salivarius, Leuconostoc mesenteroides, P. cereviseae (damnosus), Pediococcus acidilactici, P. pentosaceus, Propionibacterium freudenreichii, Prop. shermanii, Saccharomyces cereviseae, Staphylococcus carnosus, Staph. xylosus, Streptococcus infantarius, Strep. salivarius ss. thermophilus, Strep. Thermophilus and *Strep. lactis.*

[0023] In preferred methods, the formulation is mixed under vacuum at room temperature (*e.g.,* from 20°C to 30°C). After mixing to homogeneity, the formulation is then cooled to a temperature above the freezing temperature of the formulation. Typically, the formulation is cooled to between -10°C to +10°C, more preferably the formulation is cooled to between -5°C and +5°C. In a preferred embodiment, the cooled formulation is then transferred to a drying chamber where heating is applied (20°C or more) while controlling an initial vacuum pressure at a level to maintain the original pre-cooling temperature. Typically, the desirable vacuum pressure is below 7 Torr but no less than 3 Torr. Under these preferred conditions a controlled expansion of the formulation and subsequent faster primary drying of the formulation is achieved. To accelerate the secondary drying, a maximum vacuum pressure is applied and heat supply temperature may be further 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 water at a pressure of less than 1 Torr, and in an especially preferred embodiment to less than 0.2 Torr.

[0024] The wet formulation can be in the form of viscous slurry or hydrogel particles ranging from 0.05 to 10 mm. The dried formulation can be used directly as a flake, or ground into a powder with an average particle size from about 10 μm to about 1000 μm. The formulation can be administrated directly to an animal, including human, 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.

[0024a] In one aspect, there is provided a composition comprising (i) a bioactive microorganism or material as fresh, frozen or dry powder, (ii) at least two stabilizer agents, and (iii) at least two protective agents, wherein the composition is suitable for a liquid state drying and stabilizing the bioactive microorganism or material.

[0024b] In another aspect, there is provided a method for preparing a stable dry powder composition as defined above, such method comprising: (i) combining under vacuum a bioactive microorganism or material in the form of fresh, frozen or dry powder, dry stabilizer agents and dry protective agents in an aqueous solvent; (ii) cooling the mixture of step (i) to a temperature above its freezing temperature; (iii) primary drying of the cooled mixture by evaporation, under vacuum, at a temperature above its freezing temperature; (iv) secondary drying of the mixture at a temperature of 20°C or more for a time sufficient to reduce the water activity of the mixture to Aw – 0.3 or less.

[0024c] In yet another aspect, there is provided a composition comprising (i) a bioactive microorganism or material as fresh, frozen or dry powder, (ii) at least two stabilizer agents, and (iii) at least two protective agents in an aqueous solution, wherein the protecting agents are dissolved in the solution and wherein the composition has a viscosity from about 10,000 cP to about 450,000 cP and is suitable for a liquid state drying and stabilizing the bioactive microorganism or material.

[0024d] In still another aspect, there is provided a method for preparing a stable dry powder composition, the method comprising: (i) preparing an aqueous solution comprising dry stabilizer agents, dry protective agents and a bioactive microorganism or material in the form of fresh, frozen or dry powder, wherein the protecting agents are dissolved in the aqueous solution and the viscosity of the solution is from about 10,000 cP to about 450,000 cP to form a homogeneous mixture; (ii) cooling the mixture of step (i) to a temperature above its freezing temperature; (iii) primary drying of the cooled mixture by evaporation, under vacuum in a range from about 1 Torr to 5 Torr and at a temperature above its freezing temperature; (iv) secondary drying of the mixture at a temperature of 20°C or more and at a vacuum pressure of less than 0.2 Torr for a time sufficient to reduce the water activity of the mixture to Aw – 0.3 or less to form a dry mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

24. The composition of claim 22, wherein the bioactive microorganism or material is selected from a cell, a microbe, a virus, a culture, a probiotic, a yeast, an algae, a protein, a recombinant protein, an enzyme, a peptide, a hormone, a vaccine, a drug, a vitamin, a mineral, a microbiocide, a fungicide, a herbicide, an insecticide or a spermicide.

25. The composition according to claim 22, wherein the stabilizer agents comprise a polysaccharide and an oligosaccharide.

26. The composition of claim 25, wherein the polysaccharide is selected from 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 combinations thereof.

27. The composition of claim 25, wherein the oligosaccharide is selected from inulin, maltodextrins, dextrans and combinations thereof.

28. The composition according to claim 22, wherein the protective agents do not thicken or polymerize upon contact with water.

29. The composition according to claim 22, wherein the protective agent is selected from a group consisting of human serum, bovine serum albumin, egg albumen, gelatin, immunoglobulins, isolated soya protein, wheat protein, skim milk powder, caseinate, whey protein, pea protein, a protein hydrolysate, galactose, D-mannose, sorbose, lactose, trehalose, sucrose, cyclodextrins, lysine, monosodium glutamate, glycine, alanine, arginine, histidine, tryptophan, tyrosine, leucine, phenylalanine, betaine, magnesium sulfate, glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, mannitol; propylene glycol; polyethylene glycol; pluronics; surfactants; and combinations thereof.

30. A method for preparing a stable dry powder composition, the method comprising: (i) preparing an aqueous solution comprising dry stabilizer agents, dry protective agents and a bioactive microorganism or material in the form of fresh, frozen or dry powder,

wherein the protecting agents are dissolved in the aqueous solution and the viscosity of the solution is from about 10,000 cP to about 450,000 cP to form a homogenous mixture; (ii) cooling the mixture of step (i) to a temperature above its freezing temperature; (iii) primary drying of the cooled mixture by evaporation, under vacuum in a range from about 1 Torr to 5 Torr and at a temperature above its freezing temperature; (iv) secondary drying of the mixture at a temperature of 20°C or more and at a vacuum pressure of less than 0.2 Torr for a time sufficient to reduce the water activity of the mixture to Aw – 0.3 or less to form a dry mixture.

31. The method according to claim 30, wherein the dried mixture is cut, crushed, milled or respectively pulverized into a free flowing powder.

32. The method according to claim 30, wherein particle size of the dried mixture is less than about 1000 μm.

33. The method according to claim 30, wherein the bioactive microorganism or material is selected from a cell, a microbe, a virus, a culture, a probiotic, a yeast, an algae, a protein, a recombinant protein, an enzyme, a peptide, a hormone, a vaccine, a drug, a vitamin, a mineral, a microbiocide, a fungicide, a herbicide, an insecticide or a spermicide.

34. The method according to claim 30, wherein the dry stabilizer agents comprise a polysaccharide and an oligosaccharide.

35. The method according to claim 34, wherein the polysaccharide is selected from 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 combinations thereof.

36. The method according to claim 34, wherein the oligosaccharide is selected from inulin, maltodextrins, dextrans and combinations thereof.

37. The method according to claim 30, wherein the protective agents do not thicken or polymerize upon contact with water.

38. The method according to claim 30, wherein the protective agent is selected from a group consisting of human serum, bovine serum albumin, egg albumen, gelatin, immunoglobulins, isolated soya protein, wheat protein, skim milk powder, caseinate, whey protein, pea protein, a protein hydrolysate, galactose, D-mannose, sorbose, lactose, trehalose, sucrose, cyclodextrins, lysine, monosodium glutamate, glycine, alanine, arginine, histidine, tryptophan, tyrosine, leucine, phenylalanine, betaine, magnesium sulfate, glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, mannitol; propylene glycol; polyethylene glycol; pluronics; surfactants; and combinations thereof.

39. The method of claim 30, wherein the bioactive microorganism or material is added to the aqueous solution after the protecting agents are dissolved.

40. ~~A method of feeding an animal a bioactive microorganism or material, the method comprising:~~

~~administering a~~The composition according to any of claims 22 to 29~~.~~, for use as an animal feed.