(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2011 (24.11.2011)

PCT

(10) International Publication Number
WO 2011/146140 A1

(51) **International Patent Classification**:
A23L 1/20 (2006.01)

(21) **International Application Number**:
PCT/US2011/000914

(22) **International Filing Date**:
21 May 2011 (21.05.2011)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/347,339 21 May 2010 (21.05.2010) US
61/445,501 22 February 2011 (22.02.2011) US

(71) **Applicant** *(for all designated States except US)*: **CHI'S RESEARCH CORPORATION** [US/US]; 5354 Beachside Drive, Minnetonka, MN 55343 (US).

(72) **Inventor; and**

(75) **Inventor/Applicant** *(for US only)*: **CHUKWU, Uchenna** [US/US]; 5354 Beachside Drive, Minnetonka, MN 55343 (US).

(74) **Agent**: **CHUKWU, Uchenna, N.**; c/o Chi's Research Corporation, 5354 Beachside Drive, Minnetonka, MN 55343 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/146140 A1

(54) **Title**: NOVEL BIOACTIVE SOY COMPOSITIONS

(57) **Abstract**: The present invention includes bioactive composition containing bioactive soy peptides having a molecular weight of about 200 grams per mole to about 7000 grams per mole, and such that the bioactive soy peptides are effective to neutralize free radicals and reduce oxidative stress.

NOVEL BIOACTIVE SOY COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is an international application that claims priority from U.S. Serial Application Number 61/347,339, entitled "METHOD OF ENZYMATIC REDUCTION OF SOY ALLERGEN PROTEIN" by Uchenna Chukwu filed May 21, 2010; and U.S. Serial Application No. 61/445,501, entitled "NOVEL ANTIAGING SOYMILK COMPOSITIONS, by Uchenna Chukwu filed February 22, 2011; all of which are incorporated herein in their entirety.

BACKGROUND OF THE INVENTION

The present invention generally relates to soy compositions. More specifically, the present invention relates to novel bioactive soy compositions that include hydrolyzed soy protein fragments and/or soy peptides having a molecular weight of about 200 grams per mole to about 7,000 grams per mole that are effective in neutralizing free radicals, free radical damage and/or reducing oxidative stress.

Oxidative stress has been implicated in the development of many neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson; and is also a factor in aging, atherosclerosis (AT), carcinogenesis, cardiovascular disease (CVD), diabetes, hypertension, inflammation and obesity (OB). Oxidative stress is observed when an imbalance between the production of ROS (superoxide anion (SO), hydrogen peroxide, hydroxyl radical (OH), peroxynitrite (ONOO)) and a biological system's ability to readily detoxify the reactive intermediates using antioxidant enzymes occurs. This imbalance between ROS and antioxidant defense systems in tissues can induce intracellular lethal and sublethal alterations to DNA, proteins, carbohydrates and lipids, and induce apoptosis through the production of peroxides and free radicals that damage cellular components.

Overtime, unrepaired oxidative damage products induce oxidative stress on biological systems leading to the manifestation of adverse effects; and the initiation, development, and progression of diseases. For example, oxygen free radicals, such as superoxide anion, H2O2, and hydroxyl radicals have been implicated in the etiology of OB and AD. Hydroxyl radicals can also react with pyrimidines, purines and chromatin protein resulting in base modifications, genomic instability and alterations in gene expression. These reactions in connection with oncogenes or tumor suppressor genes may result in the initiation of cancer. Primary sources of ROS implicated in the development of CVD include the superoxide anion, hypochlorous acid, the hydroxyl radical and peroxynitrite. Therefore, diverse oxidants are responsible for mediating free radical damage and ultimately oxidative stress in diseases.

SUMMARY OF THE INVENTION

The present invention includes a soy composition containing hydrolyzed soy protein fragments having a molecular weight of about 200 grams per mole to about 7000 grams per mole,

such that the soy protein are effective to neutralize free radicals, free radical damage and/or reduce oxidative stress.

DETAILED DESCRIPTION

For the purposes of the invention disclosed and claimed herein, the following terms and abbreviations have the following meanings.

I. Definitions

The term "soybean" as used herein refers to an edible seed of the genus *Glycine*, a soy plant commonly cultivated for human and animal consumption and as a nitrogen-fixing ground cover. In addition, the term "soybean" is also meant to include any edible seed of the species *Plantarum*, *Phaseolus max L.* and/or *Glycine max (L.) Merr.* Furthermore, the term "soybean" as used herein is meant to encompass any edible seed from the genus *Glycine Willd.*, including any edible seed from the plant categorized into subgenera, *Glycine* or *Soja*. Suitable non-exhaustive examples soybeans include black, brown, blue, yellow, light brown, green, mottled, or any combinations thereof.

The term "soy composition" as used herein refers to any solid, liquid, foam, gaseous (vapor) composition, or any combination of solid, liquid, foam, or gaseous composition that contains soy. In addition, the term "soy composition" is meant to encompass raw soy including hulled soybean, immature soybean and the like. Furthermore, the term "soy composition" is meant to include processed soy, such as whole (hulled), dehulled, dehulled/defatted versions of the following: soybean, soy meal, soy grits, soy paste, soy nuts, soy flakes, soy flours, soy protein concentrates, soy protein isolates, soy beverages, soy powders, soy milk, soy milk concentrates, soy gels, soy foams, soy cheese, soy fiber, soy yogurt, and/or purified and/or isolated soy fractions like soy peptide fractions, soy protein fragments, hydrolyzed soy protein fractions, hydrolyzed soy protein fragments, and the like.

The term "soy-based" as used herein refers to any component, ingredient, and/or product that include one or more soy components, ingredients and/or product. In addition, the term "soy-based" can include edible and/or non-edible soy-based component, ingredient and/or product like soy gels, soy-based lotions, soy-based creams, and the like.

As used herein, the term "raw" refers to soybeans that are uncooked, un-boiled, dry, edible, in the naturally occurring form, as being in a natural condition, not processed or any combination of any of these. It is also to be understood that the term "soybean" is meant to encompass broken a raw soybean that (1) has a first outer layer (hull or seed coat) that is in adhesive contact with a second layer or inner portion (cotyledon), even though the inner portion is exposed.

The term "whole" as used herein refers to soybeans that have not been subjected to techniques like maceration, pulverization, comminution, grating, grinding or the like in a manner that removes the hull (seed coat), fat, protein or any other component of and/or in the vegetable.

In an example, a whole soybean is one in which the hull (seed coat) is still connected to the soybean.

The term "soy protein" as used herein refers to protein molecules held in discrete particles called "protein bodies" within a soybean. In addition, the term "soy protein" is also meant to encompass the 2S (Bowman-Birk Inhibitor), 7S (β-conglycinin), 11S (glycinin) and 15S (soy protein polymers) soy fractions; enzymes, trypsin inhibitors, hemagglutinins, and cysteine proteases. The term "soy protein" is also meant to include any protein that can be extracted by water, alkaline water having a pH of about 7 to about 9, and/or aqueous solutions of 0.5–2 Molar sodium chloride from soybean. In general, exemplary soy compositions for use in the present invention comprise protein concentrations ranging up to about 40% by weight on a dry weight basis; and a protein non-aggregated molecular weight ranging up to about 70 kDa.

The term "soy protein isolate" as used herein refers to a purified form of soy protein (hydrolyzed or intact) having a minimum protein content of 90% on a moisture-free basis. In addition, the term "soy protein isolate" is meant to also refer to the soy protein product remaining after removal of non-protein components, fats and/or carbohydrates from defatted and/or dehulled soy flour.

The term "soy protein concentrate" as used herein refers to a purified form of soy protein having a minimum soy protein content 70% by weight. In addition, the term "soy protein concentrate" is meant to include the soy protein product remaining after removal of carbohydrates from dehulled and/or defatted soybean.

The term "whole soy protein flour" as used herein refers to hulled soybean with a moisture content of 30% by weight or less that have been ground into a fine power and subjected to processing steps that concentrate/enrich the protein levels in the fine powder.

The term "whole soy grits" as used herein refers to soy flour derived from hulled soybean with a moisture content of 30% by weight or less that have been toasted and cracked into coarse pieces.

The term "whole soy flour" as used herein refers to hulled soybeans with a moisture content of 30% by weight or less that have been ground into powder form.

The term "soy protein flour" as used herein refers to dehulled and/or defatted soybean that have been ground into a fine power and subjected to processing steps that concentrate/enrich the protein levels in the flour.

The term "soy grits" as used herein refers to soy flour derived from de-hulled and/or defatted soybean with a moisture content of 30% by weight or less that have been toasted and cracked into coarse pieces.

The term "soy flour" as used herein refers to dehulled soybeans generally steam treated to inactivate anti-nutritional and to denature lipoxygenase that have been ground into powder form.

In general, bioactive compositions in the form of granular or powdered soy protein isolates, soy protein concentrates, whole soy protein flour, soy flour, whole soy protein isolate, whole soy protein flour of the present invention will have at least about 95% of the particles with a mean particle size of below about 70 microns, at least about 70% of the particles with a mean particle size of below about 30 microns, and/or at least about 50% of the particles with a mean particle size of below about 25 microns. In addition, in order to minimize the sensation of gritty mouth feel, the particle sizes of bioactive compositions in the form of soy protein isolates, soy protein concentrates, whole soy protein flour, soy flour, whole soy protein isolate, whole soy protein flour generally have a mean particle size of about 5 to about 20 microns. Furthermore, bioactive compositions in the form of whole soy grits and/or soy grits of the present invention will have at least about 99% of the particles with a mean particle size of below 4 millimeters, or below about 6 mesh (3.36 millimeters).

The term "treatment" as used herein is defined to include preventing, lowering, stopping, ameliorating and/or reversing the progression or severity of a condition or symptom being treated. As such, the present invention includes both medical therapeutic and/or prophylactic administration, as appropriate.

The term "effective amount" as used herein is an amount of a pharmaceutical or nutraceutical composition that is effective in treating the target condition or symptom.

The term "oral dosage form" as used herein is used in a general sense to refer to nutraceutical and/or pharmaceutical compositions administered via the mouth. Oral dosage forms are recognized by those skilled in the art to include such forms as liquid formulations, tablets, capsules, and gelcaps. Solid oral dosage forms are recognized by those skilled in the art to include such forms as tablets, capsules, gel caps and aerosols.

The term "pharmaceutically acceptable" as used herein means carriers, excipients, diluents, and other formulation ingredients that are compatible with all other ingredients of a composition, and are not deleterious to an individual treated with the composition.

The term 'anti-aging" as used herein is any composition, treatment, process, product and/or device with the ability to slow down and/or reverse the aging process. The term "anti-aging" is also meant to refer to the ability of a composition, treatment, product, process and/ or device to extend both the maximum and average lifespan of an organism. The term "anti-aging" is also meant to encompass the ability of a composition, product, treatment, process and/or device to combat or defend an organism against the aging process.

The term "activity profile" as used herein refers to being capable of modulating a specific target to result in a therapeutic effect. Furthermore, the term "activity profile", as used herein refers to exertion of biological efficacy or activity against one or more targets *in vitro* or *in vivo*.

As used herein, the term "therapeutically effective amount" refers to an amount of a biologically active component, extract and/or substance which is sufficient to alleviate, ameliorate,

prevent, and/or clear the symptoms and/or the pathology of a condition or disease. The methods in accordance with the disclosure contemplate administration of biologically active compositions comprising bioactive soy peptides, bioactive soy protein fragments, and/or bioactive soy protein extracts whether or not symptoms are manifest, i.e., prophylactic administration is contemplated. Because preferred dosages of agents for a variety of therapeutic purposes are well known in the art, appropriate dosages of purified and/or concentrated biologically active compositions comprising bioactive soy peptides, bioactive soy protein fragments and/or bioactive soy protein extracts for incorporation into nutraceutical or pharmaceutical compositions may be easily determined by standard methods.

As used herein, the term "drugability" refers to the feasibility of a target to be effectively modulated by a small molecule ligand that has the appropriate (a) potency, (b) selectivity, (c) specificity, (d) defined mechanism of action (MOA), (e) ADME-Tox properties, (f) relatively easy synthesis and/or production route, and (g) patentability to be developed into a drug candidate with appropriate properties for the desired therapeutic use.

As used herein, "blood-brain-barrier" refers to the dense layer of endothelial cells that create a barrier between the blood and brain parenchyma.

As used herein, the term "free radical" or "radical" refers to atoms, molecules, or ions with unpaired electrons on an open shell configuration that may have positive, negative or zero charge. Some non-exhaustive exemplary free radicals include superoxide anion, hydrogen peroxide, hydroxyl radical, organic hydroperoxide, alkoxy radical, peroxy radical, hypochlorous acid, and/or peroxynitrite.

The term "bioactive" as used herein means any substance that has biological and/or chemical efficacy against one or more biological and/or chemical targets.

The term "soymilk" as used herein refers to a liquid composition derived from the cooking and processing of whole soybeans and/or soy protein with water that contains at least about 2.5% to 6% by weight nonhdyrolyzed or unhydrolzyed soy protein, at least about 1.0% by weight soybean fat (or 0.5% soybean fat for low fat versions) and no less than 7.0% total solids. In addition, the term "soymilk' is meant to encompass a liquid food obtained as a result of combining (1) aqueous-extracted whole soybean solids and water, or (2) other edible-quality soy protein solids, soybean oil, and water; to provide at least about 2.5% to about 6% nonhydrolyzed or unhydrolyzed soy protein, at least about 1.0% by weight soybean fat (or 0.5% soybean fat for low fat versions) and no less than about 7% total solids.

The terms "soymilk drink" or "soymilk beverage" as used herein refer to a liquid soy composition derived from whole soybeans and/or soy protein that contains about 0.9% to about 3% nonhydrolyzed or unhydrolyzed soy protein, no less than about 0.5% soybean fat and no less than about 3.9% total solids.

The terms "soymilk concentrate" as used herein means a liquid soymilk composition obtained by modifying the level of water in soymilk to include a liquid composition containing no less than about 6.0% nonhydrolyzed or unhydrolyzed soy protein, no less than 2.0% soy fat, and no less than 14.0% total solids.

The term "bioactive soymilk" as used herein refers to a liquid soy composition that contains (1) at least about 2.5% to 6% by weight nonhydrolyzed or unhydrolyzed soy protein, (2) at least about 1.0% by weight soybean fat (or 0.5% soybean fat for low fat versions), (3) no less than 7.0% total solids, and (4) an effective amount of bioactive soy peptides having a molecular weight of about 200 grams per mole to about 7000 grams per mole. In addition, the term "bioactive soymilk' is meant to encompass a liquid food obtained as a result of combining (1) aqueous-extracted whole soybean solids and water, (2) other edible-quality soy protein solids, soybean oil, and water that provides (a) at least about 2.5% to 6% soy protein, (b) at least about 1.0% by weight soybean fat (or 0.5% soybean fat for low fat versions), (c) no less than about 7% total solids, (4) and an effective amount of bioactive soy peptides having a molecular weight of ranging from about 200 grams per mole to about 7000 grams per mole.

The terms "bioactive soymilk beverage" or "bioactive soymilk drink" as used herein refers to a liquid soy composition derived from whole soybeans and/or soy protein that contains (1) about 0.9% to about 3% nonhydrolzyed or unhydrolyzed soy protein, (2) no less than about 0.5% soybean fat, (3) no less than about 3.9% total solids, and (4) an effective amount of bioactive soy peptides having a molecular weight of about 200 grams per mole to about 7000 grams per mole.

The terms "bioactive soymilk concentrate" as used herein means a liquid soymilk composition obtained by modifying the level of water in soymilk to include a liquid composition containing (1) no less than about 6.0% nonhydrolzyed or unhydrolyzed soy protein, (2) no less than 2.0% soy fat, (3) no less than 14.0% total solids and (4) an effective amount of bioactive soy peptides having a molecular weight of about 200 grams per mole to about 7000 grams per mole.

The term "soy peptide" as used herein refers to hydrolyzed soy protein, one or more soy peptides, soy protein fraction, and/or soy protein fragments having a molecular weight of less than about 10kDa, or about 200 grams per mole to about 7000 grams per mole or 7 kiloDaltons (kDa). In addition, the terms "soy peptide" or "hydrolyzed soy protein" or "hydrolyzed soy protein fragment" or "soy protein fragment" or "hydrolyzed soy protein fraction" as used herein is meant to include soy peptides, hydrolyzed soy protein, hydrolyzed soy protein fragments, or soy protein fragments that are derived from hydrolysis of native soy protein or protein fragments, denatured soy protein or soy protein fragments, and any combination of native and denatured soy protein or protein fragments.

The term "bioactive soy peptides" as used herein refers to soy peptides having a molecular weight of less than about 10kDa, or about 200 grams per mole to about 7kDa that are effective to alleviate, ameliorate, prevent, reduce, lower and/or clear the symptoms and/or the pathology of a

condition or disease. Furthermore, it is to be understood the term "bioactive soy peptide," as used herein, refers to soy peptides or soy protein fragments or hydrolyzed soy protein or hydrolyzed soy protein fragment or hydrolyzed soy protein fractions that are released from native protein, denatured protein and/or any combination of native and denatured after hydrolysis of soy protein with acid, base, enzymes, or any combination thereof. In addition, the terms "bioactive soy peptide" or "bioactive soy protein fragments" or "hydrolyzed soy protein fragment" or "soy protein fragment" or "hydrolyzed soy protein fraction" are meant to encompass peptide or protein fragments having a molecular weight of about 200 grams per mole to about 7kDa that are released from non-hydrolyzed (or unhydrolzyed) soy protein during protein hydrolysis. The term "bioactive soy peptides" as used herein is also meant to encompass the biological activity or efficacy of soy peptides having a molecular weight of about 200 grams per mole to about 7kDa in preventing, treating, reducing, and/or minimizing the aging process.

The terms "bioactive soy protein fragment" or "bioactive soy fragment" as used herein refer to soy protein fragments or soy protein fractions (obtained via protein hydrolysis) having a molecular weight of less than about 10kDa, or about 200 grams per mole to about 7kDa that are to alleviate, ameliorate, prevent, reduce, lower and/or clear the symptoms and/or the pathology of a condition or disease. The terms "bioactive soy protein fragment" or "bioactive soy fragment" as used herein is also meant to encompass the biological activity or efficacy of soy protein fragments or soy protein fraction (obtained via protein hydrolysis) having a molecular weight of about 200 grams per mole to about 10,000 grams per mole in preventing, treating, reducing, and/or minimizing the aging process.

The term "hydrolyzed soy protein" as used herein refers to any soy protein molecule that has been broken down into smaller sizes, fragments or molecular weight pieces. Furthermore, the term "hydrolyzed soy protein" may encompass any soy peptide, soy protein fragment, soy protein fraction, or any combination thereof that is derived from acid, base and/or enzymatic hydrolysis of native, denatured, and any combination of native and/or denatured soy protein. In addition, hydrolyzed soy protein, hydrolyzed soy protein fraction and/or hydrolyzed soy protein fragment may be characterized as "bioactive" so long as the hydrolyzed soy protein and/or hydrolyzed soy protein fragment is effective to (1) neutralize free radicals, (2) inhibit, reduce and/or minimize free radical damage, (3) enhance antioxidant activity, (4) promote detoxification of free radicals, reactive intermediates and reactive oxygen species, (5) enhance the antioxidant status of an organism, (6) inhibit, reduce and/or minimize oxidative damage to proteins, DNA, carbohydrates, lipids and cellular tissue, (7) enhance antioxidant enzyme activity like glutathione (GTH) peroxidase (GTH-PX), superoxide dismutase (SOD), glutathione reductase (GTH-R), glutathione-S-transferases (GSTs), thiol-disulfide oxido-reductases, heme oxygenase (HO-1), glucose-6-phosphate dehydrogenase (G6PD) and catalase (CAT), and/or (8) prevent, treat, reduce, and/or minimize the aging process.

The term "native protein" as used herein refers to a protein that has not lost any quaternary, tertiary, and/or secondary structures present in the original unaltered protein. In addition, the term "native protein" is meant to encompass the naturally occurring form of a protein. Furthermore, the term "native protein" is meant to include the protein's operative or functional form that contains the highly specific three-dimensional shape known as the tertiary structure or the folded (conformational) protein shape that possesses a minimum of free energy and renders the protein capable of performing its intended biological function. It is also to be understood that the "protein" portion of the term "native protein" is also meant to encompass peptides, protein fractions, protein fragments, or any combination of any of these.

The term "undenatured protein" as used herein may also refer to a protein that has not lost any quaternary, tertiary, and/or secondary structures that are present in the original protein. Furthermore, the term "undenatured protein" is meant to include a protein's operative or functional form that contains the highly specific three-dimensional shape known as the tertiary structure or folded (conformational) protein shape that possesses a minimum of free energy state, condition or value. It is also to be understood that the "protein" portion of the term "undenatured protein" is also meant to encompass peptides, protein fractions, protein fragments, or any combination of any of these.

The term "undenatured" as used herein refers to a substance that has not lost any quaternary, tertiary, and/or secondary structures that are present in the original substance. The term "undenatured" is also meant to include any substance that retains the original quaternary, tertiary and/or secondary structures originally present in the substance. In addition, the term "undenatured" is also meant to refer to a substance that remains folded in the conformational protein shape having the least free energy state, condition or value.

The term "denatured protein" as used herein refers to a protein that has partially or completely lost any quaternary, tertiary, and/or secondary structures that are originally present in the native or undenatured protein. The term "denatured protein" is also meant to include protein that has undergone partial and/or complete unfolding into a conformational protein shape having a higher free energy state than the free energy state condition or value of the undenatured protein. It is also to be understood that the "protein" portion of the term "denatured protein" is also meant to encompass peptides, protein fractions, protein fragments, or any combination of any of these.

The term "denatured" as used herein refers to a substance that has undergone partial and/or complete loss of any quaternary, tertiary, and/or secondary structures that are originally present in the native or undenatured protein.

As used herein, the term "nonhydrolyzed" or "unhydrolyzed" (i.e. intact) soy protein refers to soy protein that retains its original molecular weight and that has not been subjected to protein degradation and/or hydrolysis. Furthermore, the term "nonhydrolzyed" or "unhydrolyzed" soy protein is meant to include the following without limitation nonhydrolzyed or unhydrolyzed:

2S (Bowman-Birk Inhibitor), 7S (β-conglycinin), 11S (glycinin) and 15S (soy protein polymers) soy fractions; enzymes, trypsin inhibitors, hemagglutinins, and cysteine proteases; and any combination of any of these unhydrolyzed or nonhydrolzyed soy protein fractions, soy peptides or soy protein fragments that have not been subjected to protein degradation and/or hydrolysis and/or are present in its original molecular weight.

The term "oxygen radical absorbance capacity" or "ORAC" as used herein refers to the antioxidant capacity of a substance. In addition, the term "ORAC" is generally reported in microMolar Trolox per gram sample or microMolar Trolox per 100 grams of sample in the present specification.

As used herein, the term "oxygen radical absorbance capacity assay" or "ORAC assay" refers to a method of measuring antioxidant capacities in a biological sample *in vitro*. The term "ORAC assay" as used herein is also meant to refer an assay that measures the oxidative degradation of a fluorescent molecule (either beta-phycoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds that produce the peroxyl radical upon heating, which damages the fluorescent molecule, and results in the loss of fluorescence that can be quantified using a fluorometer. In addition, the term "ORAC" assay is meant to include the oxidative degradation of a fluorescent molecule (normally fluorescein) after being mixed with free radical generators that produce the hydroxyl radical, the singlet oxygen radical, the peroxynitrite radical, the superoxide anion, or any other free radical, that can damage fluorescein and result in the loss of fluorescence. The loss of fluorescence can be quantified using a fluorometer. Furthermore, the ORAC assay as used herein is meant to encompass an assay that measures the free radical damage to a fluorescent probe through the change in its fluorescence intensity, such that the change of fluorescence intensity is an index of the degree of free radical damage, and such that in the presence of antioxidant which inhibits the free radical, the change in fluorescent intensity is correlated to the antioxidant capacity of the antioxidant against the free radical.

The term "degree of hydrolysis" as used herein means the degree to which protein has been hydrolyzed.

The term "protein hydrolysis" as used herein means a process by which protein is hydrolyzed or broken down into smaller peptides or protein fragments typically having a reduced molecular weight than the protein in its original (unhydrolyzed) state. In general, protein can be hydrolyzed using an acid, a base, one or more enzymes, or any combination of any of these.

As used herein, the term "oxidative damage" refers to intracellular lethal and sublethal alterations to DNA, protein, carbohydrates, lipids and/or apoptosis through the production of peroxides and free radicals that damage cellular components. In addition, the term "oxidative damage" can be quantified or measured by biomarkers of oxidative damage, such as malondialdehyde (MDA), 4-hydroxy-2-trans-nonenal (4-HNE), thiobarbituric acid reactive substances (TBARS), F2-isoprostanes (PGF2) like 8-isoPGF2alpha (8-iso-PGF2α) that indicate

oxidative damage to lipids; protein carbonyl groups (PCO) and lipofuscin that suggest oxidative damage to protein; 8-hydroxydeoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8OHG) that represent oxidative damage to DNA and RNA respectively; and antioxidant enzymes levels along with plasma antioxidant levels (reduced glutathione, alpha-tocopherol, beta-carotene, and vitamin C).

As used herein, the term "oxidative stress" refers to a biological condition or state observed when there is an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates that results in induction and/or propagation of intracellular lethal and sublethal alterations to DNA, proteins, carbohydrates and lipids, and cell death. Furthermore, the term "oxidative stress" is meant to encompass the expression and/or accumulation of unrepaired oxidative damage products overtime that leads to the manifestation of adverse effects; and the condition that leads to initiation, development, and progression of one or more diseases.

The term "isoflavone" as used herein is meant to refer to an organic class of plant hormones or phytoestrogens often occurring as glycosides, their respective malonates, or acetyl conjugates of isoflavone: genistein (5-OH, 7-OH, 4'-OH), daidzein (7-OH, 4'-OH) produced almost exclusively by the members of the *Fabaceae/Leguminosae* (bean) family that resembles human estrogen in chemical structure and have the ability to trap singlet oxygen. In addition, the term "isoflavone" is meant to include genistin, daidzin and/or glycitin (glucones) in which a glucose molecule is attached; and genistein, daidzein and/or glycitein (aglucones), which do not have a glucose molecule attached; formononetin, coumestrol and biochanin A.

II. Modes for Carrying Out the Invention

The present invention discloses novel bioactive soy compositions and/or soy-based compositions comprising hydrolyzed soy peptides, hydrolyzed soy protein fractions, and/or hydrolyzed soy protein fragments having a molecular of about 200 grams per mole to about 10kDa. The hydrolyzed soy peptides, hydrolyzed soy protein fractions, and/or hydrolyzed soy protein fragments are effective to (1) neutralize free radicals, (2) inhibit, reduce and/or minimize free radical damage, (3) enhance antioxidant activity, (4) promote detoxification of free radicals, reactive intermediates and reactive oxygen species, (5) enhance the antioxidant status of an organism, (6) inhibit, reduce and/or minimize oxidative damage to proteins, DNA, carbohydrates, lipids and cellular tissue, and/or (7) enhance antioxidant enzyme activity like glutathione peroxidase (GTH-PX), superoxide dismutase (SOD), glutathione reductase (GTH-R), glutathiones (GSTs), thiol-disulfide oxido-reductases, heme oxygenase-1 (HO-1), glucose-6 phosphate dehydrogenase (G6PD) and catalase (CAT). In addition, the term "Dalton" and "grams per mole" is used interchangeably throughout the specification. Although the term "soy composition" and "soy-based composition" are defined separately, the terms will be used interchangeably throughout the specification.

The bioactive soy compositions disclosed herein further include multifunctional antioxidants (AOXs) in the form of soy peptide, soy protein fractions and/or soy protein fragments (also referred collectively as "AOX-BPs") having a molecular weight of about 200 grams per moles to about 10kDa, or about 200 grams per moles to about 7kDa that may be effective to (1) target the site of reactive oxygen species (ROS) generation, (2) neutralize multiple oxidants (3) inhibit, reduce and/or minimize free radical damage, (4) enhance antioxidant activity, (5) promote detoxification of free radicals, reactive intermediates and reactive oxygen species, (6) enhance the antioxidant status of an organism, (7) inhibit, reduce and/or minimize oxidative damage to proteins, DNA, carbohydrates, lipids and cellular tissue, and/or (8) enhance antioxidant enzyme activity (i.e. GTH-PX, SOD, HO-1, CAT, etc.) and/or (9) target enzymes (like NADPH oxidase and xanthine oxidase) responsible for generating ROS by inhibiting enzyme activity.

In addition, soy protein, soy protein fraction, soy peptide and/or soy protein fragments may be present in their native protein form, denatured protein form or in any combination of native and denatured protein form when practicing the present invention. As used herein, bioactive soy peptides, bioactive soy protein fragments, bioactive soy protein fractions, bioactive hydrolyzed soy protein, bioactive hydrolyzed soy protein fragments, bioactive hydrolyzed soy protein fractions, and/or any combination of any of these, comprising a molecular weight range of about 200 Daltons to about 7,000 Daltons are collectively referred to as "AOX-BSPs."

Furthermore, it is to be understood that protein hydrolysis of raw (hulled) whole soybeans in accordance with the present invention generally includes hydrolysis of native protein or protein in its naturally occurring form that has not been denatured. This is because raw whole soybeans generally comprise undenatured and/or native protein; and conventional processing of raw whole soybeans typically results in the denaturation of soy protein. Therefore, AOX-BSPs can be generated in their native protein form.

In another example, the AOX-BPs can be denatured particularly if denatured soy protein is the staring material. In a third example, the AOX-BSPs are in both native and denatured form, such as is observed during partial protein hydrolysis of raw and/or whole (hulled) soybeans using the techniques disclosed herein followed by application of conventional soy processing techniques like extraction, or the like that deanatures any unhydrolyzed soy protein.

Furthermore, the bioactive compositions disclosed herein have substantially less anti-nutritional factors, such as isoflavones, trypsin inhibitors, other protease inhibitors, lectins, goitrogens, phytic acid, and/or saponins when compared to (untreated) raw (non-hydrolyzed), processed, and/or whole (hulled) soybeans or soy materials. In one example, bioactive compositions of the present invention have less than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, or about 10% of the isoflavones,

lectins, trypsin inhibitors, other protease inhibitors, phytic acid, goitrogens and/or saponins originally present in raw whole (untreated) soybeans.

In addition, bioactive compositions derived from enzymatic degradation of raw whole soybeans will have substantially less concentrations of anti-nutritional factors in the form of enzyme inhibitors (trypsin inhibitor as measured using the techniques described in Marzo et al, 2002, J. Anim. Sci. 2002. 80:875–879, which is incorporated herein by reference. In one example, bioactive compositions in the form of raw whole (hulled) soybeans comprising AOX-BSPs has less than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, or about 1% of the trypsin inhibitor levels of untreated raw whole soybeans comprising non-hydrolyzed soy protein (as measured by the American Oil Chemists Society (AOCS) method Ba 12-75; or in Emiola et al, 2007). In another example, the trypsin inhibitor levels in raw soybeans ranges from about 4 trypsin inhibitor units (TIU) per gram raw soy to about 200 TIU per gram raw soy; and from less than or equal to about 2 TIU/g in bioactive raw hulled soy compositions comprising AOX-BSPs. In a third example, the trypsin inhibitor levels comprises about 4 to about 20 TIU per gram raw soy; and less than about 0.5, about 0.4, about 0.3, about 0.2, about 0.1 TIU per gram in a bioactive soy composition.

Lectins levels in bioactive compositions generally have less than or equal to about 75%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% of the lectin levels originally present in raw soybeans. It is also noted that bitter soy peptides levels are reduced and the reduction is believed due to hydrolysis of native soy protein.

The novel bioactive soy compositions are also substantially free of isoflavones. By "substantially free of isoflavones" is meant the novel bioactive soy compositions have less than about 150 mg per gram soybean, less than about 140, less than about 130, less than about 120, less than about 110, less than about 100, less than about 90, less than about 80, less than about 70, less than about 60, less than about 50, less than about 50, less than about 40, less than about 30, less than about 20, or less than about 10 mg isoflavones per gram soybean, soy composition, soy-based product and/or soyfood.

In one example, when the bioactive soy composition is bioactive soymilk, the isoflavone content is generally less than about 5, about 4, about 3, about 2, about 1, about 0.5, about 0.4, about 0.3, about 0.2, or less than about 0.1 mg isoflavones per gram bioactive soymilk. In another example, when the bioactive soy composition is bioactive soy flour containing at least about 50% by weight soy protein, the isoflavone content is generally less than about 50, about 40, about 30, about 20, about 10, about 5, about 4, about 3, about 2, or less than about 1 mg isoflavone per gram bioactive soy flour. In a third example, the bioactive soy composition is bioactive whole soybean and the isoflavone content is less than about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, or less than about 0.5 mg isoflavones per gram whole soybean.

The AOX-BSPs in the novel soy compositions generally comprise a molecular weight of about 200 grams per moles (Daltons, Da) to about 7,000 Daltons. In one example, the AOX-BSPs comprise a molecular weight of less than about 500 Daltons. In a second example, the AOX-BSPs comprise a molecular weight of less than about 1,000 Daltons. In a third example, the AOX-BSPs comprise a molecular weight of about 200 Daltons to about 1,000 Daltons. In a fourth example, the AOX-BSPs comprise a molecular weight of up to about 1,500 Daltons. In a fifth example, the AOX-BSPs comprise a molecular weight of up to about 2kDa. In a sixth example, the AOX-BSPs comprise a molecular weight of about 2,500 Daltons. In a seventh example, the AOX-BSPs comprise a molecular weight of up to about 3kDa. In an eighth example, the AOX-BSPs comprise a molecular weight of up to about 1,800 Daltons. In a ninth example, the AOX-BSPs have a molecular weight of up to about 5,000 Daltons. In a tenth example, the AOX-BSPs have a molecular weight of up to about 7,000 grams per mole.

The methods and compositions in accordance with the disclosure contemplate consumption and/or administration of biologically active compositions comprising AOX-BSPs for prevention, reduction, lowering, and/or treatment of free radical damage, oxidative damage, oxidative stress and/or those diseases that are exacerbated by free radical damage, oxidative damage, and/or oxidative stress.

In addition, the methods and compositions in accordance with the disclosure contemplate consumption and/or administration of biologically active compositions comprising AOX-BSPs for prevention, reduction, lowering, and/or treatment of the aging process in an organism. In one example, biologically active compositions that include AOX-BSPs with a molecular weight of about 300 grams per moles to about 7kDa may be effective to (1) neutralize free radicals, (2) inhibit, reduce and/or minimize free radical damage, (3) enhance antioxidant activity, (4) promote detoxification of free radicals, reactive intermediates and reactive oxygen species, (5) enhance the antioxidant levels (beta-carotene, alpha-tocopherol, and vitamin C) of an organism, (6) inhibit, reduce and/or minimize oxidative damage to proteins, DNA, carbohydrates, lipids and cellular tissue, and/or (7) enhance antioxidant enzymes like GTH-PX, SOD, GTH-R, GST, thiol-disulfide oxido-reductases, HO-1, G6PD, and CAT; and may be characterized as "anti-aging" when practicing the present invention.

The "anti-aging" capacity of a substance, as used herein, can be characterized in terms of the (1) oxygen radical absorbance capacity of the substance, (2) the degree to which any antioxidant enzyme activity is increased by the substance when compared to a control (or before treatment and/or exposure to the substance), (3) the degree to which internal or endogenous antioxidant levels, such as beta-carotene, alpha-tocopherol and vitamin C levels are increased following exposure, administration and/or treatment of the substance, (4) the degree to which biomarkers of oxidative damage, such as MDA, HNE, TBARS, PGF2alpha, PCO, lipofuscin, 8-

OHdG, 8OHG, are reduced after exposure, treatment and/or administration to the anti-aging substance; and (5) any combination of any of these.

Furthermore, the compositions and methods disclosed herein contemplate consumption and/or administration of biologically active compositions comprising AOX-BSPs, whether or not symptoms are manifest, i.e., prophylactic administration is contemplated.

In general, the AOX-BSPs concentration in a bioactive composition can range from about 1 ppm to about 100% (w/w), based on the total weight of the bioactive composition. In one example, the concentration of AOX-BSPs is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 5%, or less than about 1% by weight of the total weight of the bioactive composition.

In another example, the bioactive compositions are raw whole (hulled) bioactive soybeans derived from raw whole soybeans, and the concentration of AOX-BSPs is generally less than about 40%, less than about 35%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, or less than about 0.05% by weight, based on the total weight of the raw bioactive soybeans.

In a third example, the bioactive soy compositions are raw whole (hulled) bioactive soybeans and the concentration of AOX-BSPs is generally less than about 20%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.4%, less than about 0.3%, or less than about 0.2%, or less than about 0.1%, or less than about 0.05% by weight, based on the total weight of the raw whole bioactive soybeans.

In a fourth example, the bioactive soybean compositions are raw whole bioactive soybeans and the concentration of AOX-BSPs is generally less than about 4%, less than about 3.5%, less than about 3%, less than about 2.5%, less than about 2%, less than about 1.5%, less than about 1%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, about 0.1%, about 0.05%, about 0.025%, or about 0.010% by weight, based on the total weight of the raw whole (hulled) bioactive soybeans.

In a fifth example, the bioactive composition is enclosed in a capsule and the AOX-BSPs in the capsule comprise about 100%, about 80%, about 60%, about 40%, about 20%, about 10%, about 5%, or about 1% by weight, based on the total weight of the bioactive composition in the capsule.

In another example, at least about 100%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% of the non-hydrolyzed soy protein in raw whole (hulled) soybeans has been hydrolyzed. In as second example, at least about

50%, about 40% about 30%, about 20%, about 10%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.5% of the non-hydrolyzed soy protein in raw whole (hulled) soybeans has been hydrolyzed. In a third example, at least about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, about 0.25%, or about 0.01% of the non-hydrolyzed soy protein in raw whole (hulled) soybeans has been hydrolyzed. In a fourth example, at least about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, about 0.25%, or about 0.01% of the non-hydrolyzed soy protein in raw whole soy has been hydrolyzed. Soy protein levels are determined using techniques disclosed in the Examples section.

When the bioactive composition is in granular (powder) form, the bioactive composition generally comprises about 100%, about 90%, about 80%, about 70%, about 60%, or about 50% by weight hydrolyzed protein, based on the total weight of the bioactive composition in powder form; and about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% non-hydrolyzed (intact) protein. In another example, the bioactive soy composition is in powder form and the AOX-BSPs comprise about 100%, about 90%, about 80%, about 70%, about 60%, or about 50% by weight, based on the total weight of the bioactive soy composition in powder form.

In another example, the bioactive composition is bioactive whole soy concentrate and the AOX-BSPs comprise about 100%, about 90%, about 80%, about 70%, about 60%, or about 50% by weight, based on the total weight of the whole soy concentrate. In a second example, the bioactive composition is whole soy concentrate and the AOX-BSPs comprise about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% by weight, based on the total weight of the whole soy concentrate. In a third example, the bioactive composition is a whole soy concentrate and the concentration of AOX-BSPs ranges up to about 90% by weight, based on the total weight of the whole soy concentrate. In a fourth example, the bioactive composition is soy protein concentrate that includes non-hydrolyzed soy protein at a concentration of up to about 50% by weight, based on the total weight of the soy protein concentrate while the AOX-BSPs comprise up to about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% by weight, based on the total weight of the soy protein concentrate. In a fifth example, the bioactive composition is soy protein concentrate that includes non-hydrolyzed soy protein at a concentration of up to about 50% by weight, based on the total weight of the soy protein concentrate; the AOX-BSPs comprise less than about 50%, about 40%, about 30%, about 20%, about 10%, or about 5% by weight, based on the total weight of the soy protein concentrate.

In another example, the bioactive composition is soy protein isolate having a non-hydrolyzed soy protein concentration of at least about 90% by weight on a moisture-free basis, based on the total weight of the soy protein concentrate while the AOX-BSPs generally comprise less than about 10%, about 9%, about 8%, about 7%, about 6%, or about 5% by weight on a moisture-free basis, based on the total weight of the soy protein isolate. In a second example, the soy protein isolate comprises up to about 90% by weight AOX-BSPs, on a moisture-free basis,

based on the total weight of the soy protein isolate; and up to about 10% by weight non-hydrolyzed soy protein, based on the total weight of the soy protein isolate.

Liquid bioactive soymilk compositions can generally comprise from about 0.0001% to about 20% by weight non-hydrolyzed soy protein, based on the total weight (or volume) of the liquid soymilk composition, while the AOX-BSPs generally comprise less than about 20%, about 19%, about 18%, about 17%, about 16%, or about 15%, about 14%, about 13%, about 12%, about 11%, or about 10%, about 9.9%, about 9.8%, about 9.7%, about 9.6%, about 9.5%, about 9.0%, about 8.5%, about 8.0%, about 7.5%, about 7.0%, about 6.5%, about 6.0%, about 5.5%, about 5.0%, about 4.5%, about 4.0%, about 3.5%, about 3.0%, about 2.6%, about 2.5%, about 2.0%, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, about 0.1%, about 0.05%, about 0.025%, or about 0.010% by weight, based on the total weight (or volume) of the bioactive soymilk composition.

Bioactive liquid compositions can generally comprise from about 0.0001% to about 20% by weight AOX-BSPs, based on the total weight (or volume) of the bioactive liquid composition. As used herein, bioactive liquid compositions can include soymilk, soymilk drinks, soymilk concentrates, liquid concentrates and/or soymilk beverages. In one example, the AOX-BSPs comprise about 10%, about 9.9%, about 9.8%, about 9.7%, about 9.6%, about 9.5%, about 9.0%, about 8.5%, about 8.0%, about 7.5%, about 7.0%, about 6.5%, about 6.0%, about 5.5%, about 5.0%, about 4.5%, about 4.0%, about 3.5%, about 3.0%, about 2.6%, about 2.5%, about 2.0%, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, about 0.1%, about 0.05%, about 0.025%, or about 0.010% by weight, based on the total weight of the bioactive soymilk composition.

In another example, the AOX-BSPs comprise about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, about 0.1%, about 0.05%, about 0.025%, or about 0.010% by weight, based on the total weight of the bioactive soymilk composition.

When liquid soymilk compositions contain about 0.1% to about 20% unhydrolyzed soy protein, the AOX-BSPs concentration can range from 0.0001% to about 20% having a molecular weight of about 300 grams per mole to about 7000 grams per mole, based on the total liquid soymilk composition. In one example, the AOX-BSPs concentration ranges from about 0.0001% to about 10% by weight having a molecular weight of about 200 grams per mole to about 7000 grams per mole, based on the total liquid soymilk composition. In a third example, the AOX-BSPs concentration ranges from about 0.0001% to about 10% by weight having a molecular weight of

about 300 grams per mole to about 7000 grams per mole, based on the total liquid soymilk composition.

The bioactive soy compositions of the present invention can be characterized in terms of the oxygen radical absorbance capacity. The ORAC value is typically reported in microMolar Trolox per gram of product or microMolar Trolox per 100 grams of product. In general, bioactive soy compositions disclosed herein generally increases the ORAC value from about 1% to about 1000%, when compared to a soy composition containing non-hydrolyzed (intact) soy protein at the same moisture content of the bioactive composition. Furthermore, bioactive soy compositions of the present invention containing AOX-BSPs generally have higher ORAC values than soy compositions that do not contain hydrolyzed soy protein even though the latter generally include appreciable levels of isoflavones that are known to have antioxidant capacity.

In an example, when the bioactive compositions are hulled bioactive soybeans having a concentration of AOX-BSPs of about 40% by weight or less, based on the total weight of the raw whole bioactive soybeans the ORAC value is generally increased from about 1000%, about 900%, about 800%, about 700%, about 600%, about 500%, about 400%, about 300%, about 200%, about 100%, about 90%, or about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 25%, about 20%, about 15%, or about 10%, about 5%, or about 1% more than the ORAC value of raw soybeans having a concentration of about 40% by weight, or less non-hydrolyzed soy protein at the same moisture content. In a second example, when the bioactive compositions are raw whole (hulled) bioactive soybeans having a concentration of AOX-BSPs of about 20% by weight or less, based on the total weight of the raw whole bioactive soybeans the ORAC value is generally increased from about 25%, about 20%, about 15%, or about 10%, about 5%, or about 1% more than the ORAC value of raw soybeans having a concentration of about 20% by weight, or less non-hydrolyzed soy protein at the same moisture content.

In a third example, the bioactive compositions are hulled whole soybeans containing up to about 40% by weight AOX-BSPs, based on the total weight of the hulled whole bioactive soybeans, and the ORAC value is at least about 65 microMolar Trolox per gram raw whole bioactive soybean. In a four example, In a third example, the bioactive compositions are raw whole soybeans containing up to about 40% by weight AOX-BSPs, based on the total weight of the raw whole bioactive soybeans, and the ORAC value is at least about 70 microMolar Trolox per gram raw whole bioactive legume. In a fifth example, the bioactive compositions are raw whole soybeans containing up to about 40% by weight AOX-BSPs, based on the total weight of the raw whole bioactive soybeans, and the ORAC value is at least about 75 microMolar Trolox per gram raw whole bioactive soybean. In a sixth example, the bioactive compositions are raw whole soybeans containing up to about 40% by weight AOX-BSPs, based on the total weight of the raw whole bioactive soybeans, and the ORAC value is at least about 80 microMolar Trolox per gram raw whole bioactive soybeans. In a sixth example, when the bioactive soy compositions are raw

whole bioactive soybeans having a concentration of AOX-BSPs of more than about 20% by weight, based on the total weight of raw whole bioactive soybeans, the ORAC value is at least about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, about 110, about 115, about 120, or about 125 microMolar Trolox per gram of raw whole bioactive soybeans.

In a seventh example, the ORAC value of a bioactive liquid soymilk composition containing 0.001% to about 20% AOX-BSPs having molecular weights ranging from about 200 grams per mole to about 7000 grams per mole, is increased more than about 100%, about 100%, about 99%, about 98%, about 97%, about 96%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 26%, about 25%, about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1%, when compared to the ORAC value of the liquid soymilk composition that contains 0.001% to about 20% non-hydrolyzed soy protein.

In an eighth example, the ORAC value of a bioactive soymilk composition containing 0.9% to about 10% AOX-BSPs, having molecular weights ranging from about 200 grams per mole to about 7000 grams per mole, is generally increased by more than 100%, about 100%, about 99%, about 98%, about 97%, about 96%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 26%, about 25%, about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1%, when compared to the ORAC value of the liquid soymilk composition that contains 0.9% to about 10% non-hydrolyzed soy protein.

Similarly, the ORAC value of bioactive soymilk composition is increased from about 100%, about 99%, about 98%, about 97%, about 96%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 26%, about 25%, about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1% more, when compared to the ORAC value of liquid soymilk composition in which about 100% of the soy protein present is in the form of non-hydrolyzed soy protein.

In another example, the ORAC value of bioactive soymilk composition is about 10 times (x), 9.5x, about 9x, about 8.5x, about 8x, about 7.5x, about 7x, about 6.5x, about 6x, about 5.5x, about 5x, about 4.5x, about 4x, about 3.5x, about 3x, about 2.5x, about 2x, about 1.5x, about 1.4x, about 1.3x, about 1.2x, or about 1.1x more than the ORAC value of a control soymilk composition

in which approximately 100% of the soy protein is in the form of unhydrolyzed soy protein. Likewise, the ORAC value of the novel bioactive soymilk compositions of the present invention generally ranges from about 10x, 9.5x, about 9x, about 8.5x, about 8x, about 7.5x, about 7x, about 6.5x, about 6x, about 5.5x, about 5x, about 4.5x, about 4x, about 3.5x, about 3x, about 2.5x, about 2x, about 1.5x, about 1.4x, about 1.3x, about 1.2x, or about 1.1x more than the ORAC value of a liquid soymilk composition in which approximately 100% of the soy protein is in the form of (1) unhydrolyzed soy protein and/or (2) unhydrolyzed soy protein that do not contain hydrolyzed soy peptides having a molecular weight of about 300 Daltons to about 7 kDa.

In another example, when the bioactive soy compositions are soymilks compositions containing more than 0.9% non-hydrolyzed soy protein and 0.9% bioactive soy peptides, the ORAC value generally ranges from about 1microMolar (μM) Trolox per gram soymilk composition to more than 10μM Trolox per gram soymilk composition. In a third example, the ORAC value of AOX-BSPs with a molecular weight of less than 7000 Daltons is typically more than about 1 microMolar Trolox per gram AOX-BSPs. In a fourth example, AOX-BSPs with a molecular weight of less than about 1000 Daltons have ORAC values of more than about 2 microMolar Trolox per gram AOX-BSPs.

III. Methods of Preparing Bioactive Soy Compositions

Some non-exhaustive techniques that can be used to obtain bioactive soy compositions of the present invention include (1) *in situ* protein hydrolysis of raw whole soybeans containing non-hydrolyzed (native, intact) soy protein into soy peptides with a molecular weight of about 200 grams per mole to about 7,000 grams per mole using acid, base, enzymes, or any combination thereof; followed by protein/peptide extraction, purification and/or further processing steps, (2) protein hydrolysis of dehulled and/or defatted soybean, soy meal, soy protein concentrate, soy protein isolate, soy flour or any fractionated, processed and/or unfractionated soy composition comprising non-hydrolyzed soy protein using acid, base, enzymes, or any combination thereof, (3) hydrolyzing liquid soy compositions comprising non-hydrolyzed soy protein using acid, base, enzymes, or any combination thereof, (4) adding hydrolyzed soy protein peptides or soy protein fragments having molecular weights that range from about 200 grams per mole to about 7,000 grams per mole to soy or soy slurry compositions followed by application of one or more conventional processing steps, (5) adding non-hydrolyzed soy protein to a soy composition also comprising non-hydrolyzed protein followed by protein hydrolysis into soy peptides with a molecular weight of about 200 grams per mole to about 7,000 grams per mole, (6) preparing soy-based compositions staring with soybeans or soy materials containing AOX-BSPs having a molecular weight of about 200 grams per mole to about 7,000 grams per moles, (7) adding AOX-BSPs having molecular weights that range from about 200 grams per mole to about 7000 grams per mole to soy compositions, soymilk compositions, or other compositions.

***In situ* hydrolysis of native soy protein present in raw whole legumes** - In general, acids, bases, enzymes, or any combination thereof, is applied to raw whole soybeans (hulled soybeans) for a time that is effective to degrade the hull, allow penetration and subsequent *in situ* hydrolysis within the interior of the cotyledon to form AOX-BSPs having molecular weights ranging from 200 grams per mole to 7000 grams per mole. In this method, soybeans are typically immersed or brought into contact with an aqueous composition comprising acid, base, enzyme, or any combination thereof, for a time that is effective to hydrolyze native soy protein into AOX-BSPs.

It is to be understood that the term "raw" refers to the state or condition of the first outer layer (hull), the second inner layer (cotyledon) or both the first and second layers of the soybean. The term "raw" is also meant to encompass the connection of the first outer layer to the second inner layer of the soybean having a moisture content of less than about 30%.

As used herein, the term "enzyme" means any complex protein produced by a living cell that is capable of at least catalyzing a specific biochemical reaction on one or more target substrates. The term "enzyme" is also meant to encompass any complex protein capable of catalyzing a specific biochemical reaction that is substantially free of any microorganism. All references to enzyme is also understood as encompassing any synthetically- or genetically-produced identical copy of the enzyme that is identical in molecular structure to the enzyme that originated in a living organism.

The present invention uses enzymes that are substantially free of microorganisms to hydrolyze, tenderize, and/or degrade the soy materials. By "substantially free" is meant an enzyme composition that has less than 1000 microbes per gram. For example, suitable enzyme compositions for use in the present invention generally comprise less than 1000, less than 900, less than 800, less than 700, less than 600, less than 500, less than 400, less than 300, less than 200, less than 100, less than 50, less than 30, and less than 10 microbes, coliforms, fungi per gram of enzyme composition. Furthermore, the term "substantially free" is also meant to encompass has substantially zero microorganisms per gram enzyme composition, or reads "negative" for pathogenic microbes, such as *Salmonella* and *E. coli*, for example.

It is further noted that enzymatic degradation, and not fermentation, is one desired outcome of the present invention. Indeed, avoidance of fermentation can be optionally pursued by the incorporation of a sufficient amount of one or more microbial and/or fungal inhibitors (like sulfites, lysozyme chloride) in combination with the acid, base, enzymes, or any combinations thereof being used to hydrolyze protein in a manner that prevents growth, and therefore subsequent fermentation (or spoilage) of soy. In another example, oxygenation during protein hydrolysis may also be undertaken to prevent undesirable fermentation by spoilage bacteria, for example.

Generally, the temperature during protein hydrolysis may range from about 75°F (23.8°C) to about 150°F (65.5°C) before adding the soy material. In another example, the temperature of the aqueous enzyme composition ranges from about 80°F (26°C) to about 140°F (60°C) when

practicing the present invention. In one example, an enzyme composition comprising water, cellulase, pectinase, hemicellulase, amylase, alpha-galactosidase, and several endo- and exo-proteases like papain, bromelain, fungal proteases and neutral proteases at an initial pH ranging from about 3 to about 6, and temperature of about 120°F (48°C) to about 160°F (72°C) is applied to a raw (hulled) soybean composition for a time that is effective to hydrolyze the native soy protein into one or more peptides having a molecular weight of less than about 7000 Daltons. In this example, more than about 50%, about 60%, about 70%, about 80%, or more than about 90% by weight reduction in alpha-glycinin and/or beta-conglycinin levels, when compared to alpha-glycinin and/or beta-conglycinin levels in raw non-hydrolyzed (hulled) whole soybeans, is attained. Furthermore, the soy peptides and/or soy protein fragments that are generated have antioxidant activity, and therefore, are considered bioactive, and able to neutralize free radical damage, neutralize free radicals and/or reduce oxidative stress.

While not wanting to be bound to theory, it is believed that the inclusion of multiple and/or broad-spectrum endo- and exo-proteases (and broad-spectrum carbohydrases) into the enzyme composition are effective to significantly and randomly hydrolyze soy protein into soy peptides and protein fragments with molecular weights of less than 7000 Daltons, in a manner that is also effective to reduce allergenic soy protein content while generating AOX-BSPs.

Soy proteins generally include the 2S (Bowman-Birk Inhibitor), 7S (β-conglycinin), 11S (glycinin) and 15S (soy protein polymers) fractions. There are currently 38 soy proteins that have been identified as allergens (FAARP Allergen Protein Database 2010) with molecular weights ranging from 7kDa to 71kDa. Small regions in the allergenic proteins, called epitopes, provoke the immunoglobulin E (IgE)-mediated allergenic response. The 11S and 7S globulin fractions account for the source of most allergenic soy proteins.

In general, conventional enzymatic hydrolysis of soy protein typically starts with processed soy compositions, such as soybean meal, soy protein concentrate, soy flour and/or soy protein isolate in which the soy protein has already been denatured. However, reduction of allergenic soy protein, and in particular the epitope region is not observed using conventional enzyme hydrolysis. In contrast, the present invention uses a combination of broad-spectrum proteases (and carbohydrases) to enable efficient random fragmentation of native soy protein in a manner that significantly reduces the allergenic soy protein levels and elicits a reduced allergenic response after consumption of soymilks prepared therefrom. While not wanting to be bound to theory, it is believed that the combination of broad-spectrum proteases and carbohydrases along with hydrolysis of soy protein in native form is effective to enable conversion of soy protein into smaller fragments as well as destruction (hydrolysis) of the epitopes of allergenic soy protein. The activity of the carbohydrases allows for removal of any carbohydrate portions that surround (protect) the protein bodies. Hence, reduction of carbohydrates from within the cotyledon helps

the proteases access and degrade protein, and in particular, destroys the epitopes of the allergenic soy protein.

Furthermore, the combination of enzymes is believed effective to reduce the molecular weight of soy protein to less than about 7000 daltons since the scientific literature reports that soy peptides having a molecular weight of about 7kDa are able to elicit an allergenic response; and soy peptides having a molecular weight of less than about 10kDa are lower in allergenicity than soy peptides having a molecular weight of more than 10KDa.

In another example, the enzyme composition comprises water, cellulase, pectinase, hemicellulase, and several endo- and exo-proteases like papain, bromelain, fungal proteases and neutral proteases at an initial pH ranging from about 3 to about 6. In this example, amylases are not included since the starch levels in soybeans are low. In addition, alpha-galactosidase may also be an optional component of the enzyme composition since the oligosaccharide levels in soybeans is also less than about 10%.

In addition, the potency (concentration of enzymes) and/or application time can be varied to maximize protein hydrolysis when using enzyme compositions. Typically, a higher level of enzymes are used in order to attain AOX-BSPs having a molecular weight of less than about 7000 grams per mole and/or facilitate epitope destruction.

In another example, enzymatic degradation using an aqueous enzyme composition comprising cellulase, proteases, alpha-galactosidase, and pectinase applied to a raw whole hulled soybeans, is effective to reduce the allergenic soy protein, as measured by 7S and 11S fractions, by about 99%, about 98%, about 97%, about 96%, about 95%, about 94%, about 93%, about 92%, about 91%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, or about 10% by weight, relative to raw hulled soybeans that have not been enzymatically hydrolyzed.

Alternatively, raw whole (hulled) soybeans can be hydrolyzed by an aqueous acidic composition at an initial pH of about 3 to about 5 and temperature of about 120°F (48°C) to about 160°F (72°C) for a time that is effective in randomly hydrolyzing soy protein *in situ* to generate antioxidant soy peptides and/or soy protein fragments having molecular weights of less than 7000 Daltons. Similarly, raw whole soybeans can be hydrolyzed by an aqueous basic composition at an initial pH of about 8 to about 11 and temperature of about 120°F (48°C) to about 160°F (72°C) for a time that is effective in randomly hydrolyzing soy protein *in situ* to generate antioxidant soy peptides and/or soy protein fragments having molecular weights of less than 7000 Daltons.

As noted, broad-spectrum carbohydrases, besides cellulase is suitable for use in practicing the present invention. Besides cellulase, it is believed that other carbohydrases, such as hemicellulase, alpha-galactosidase, invertase, mannanase, beta-gluconase, beta-glucanase, arabanase, polygalacturonase, ferulic acid esterase, xylanase, beta-galactosidase, beta-fructofuranosidase, alpha-amylase, beta-amylase, pectinase, pectin depolymerase, pectin methyl

esterase, pectin lyase, glucoamylase, oligo-1,6 glucosidase, lactase, beta-*d*-glucosidase, or any combination of any of these are suitable additional non-exhaustive examples of carbohydrases that may be used separately or in combination with cellulase in accordance with the present invention.

Some non-exhaustive examples of cellulases or carbohydrases that can be used in the present invention include Cellulase AP, AP10 and/or Cellulase T (Amano Enzymes USA, Chicago, IL); Enzeco cellulase CEP and/or Enzeco cellulase CE-2 (Enzyme Development Corporation (EDC), New York, NY); Cellulase 4000 or Crystalzyme Cran (Valley Research Inc., South Bend, IN); Viscozyme L, or Cellubrix, Peelzym, Gamanase 1.0L (Novozymes, Franklinton, NC); Multifect cellulases (Danisco, CA); or Rapidase tropical cloud, Cytolase PCl5, Cytolase CL (Gist Brocades, NJ).

Some non-exhaustive examples of suitable pectinases include pectinase 500,000 AJDU/GM or pectinase 3,500 ENDO-PG/GM (Bio-cat), pectinase p-II (Amano Enzymes USA); or Multifect pectinase FE (Danisco). Suitable amylases for the present invention include alpha-amylases, beta-amylases, glucoamylases, Enzeco fungal amylase (EDC), amylase DS, Amylase S Amano, Amylase THS Amano, and Amylase AY Amano (Amano Enzymes USA).

Some non-exhaustive examples of proteases include Alcalase®, Neutrase® Esperase®, Protamex, Novozym® FM, Flavourzyme®, and Kojizyme®, all available from Novo Nordisk Biochem North America of Franklinton, North Carolina, and Enzeco® exoprotease, bromelain, Enzeco fungal protease 180 and neutral bacterial protease 160K all available from Enzyme Development Corporation of New York, New York.

Suitable alpha-galactosidases include α-d-galactosidase or α-d-galactosidase DS (Amano Enzymes USA), Enzeco alpha-galactosidase concentrate (EDC); and Validase AGS (Valley Research, Inc). Suitable proteases that can be used in the present invention include Enzeco purified papain concentrate, Panol purified papain, Enzeco fungal acid protease, and Enzeco fungal protease 100 (EDC). Suitable hemicellulases that can be used in the present invention include Enzeco hemicellulase 20M (EDC); Hemicellulase Amano 90 (Amano Enzymes USA); and Multifect XL (Danisco).

The aqueous enzyme composition includes a pH-modifying component to obtain a pH of about 2 to about 7 of the soaking system. Preferably, the pH is initially at about 4 to 6 to obtain an aqueous enzyme composition that has sufficient ability to hydrolyze protein *in situ*. In general, a pH-modifying component can be added to the aqueous enzyme composition in order to maintain a pH range of about 3 to about 7, about 3 to about 6, or about 4 to 6. The pH-modifying component can be in the form of an acidulant, a basic agent, a buffering agent, a salt, or any combinations thereof that are effective to modify the pH of an aqueous composition and activate the enzyme component. The pH component can be optional or included depending on the water source and quality that is used during enzymatic degradation.

Some non-exhaustive examples of ingredients that can be used to form the pH-modifying component include organic acids, such as acetic acid, gluconic acid, tartaric acid, malic acid, ascorbic acid, fumaric acid, succinic acid, citric acid, or the like; phosphoric acid; buffering agents of such organic acids, such as calcium citrate, ferrous gluconate, ferrous citrate, calcium acetate, magnesium acetate, zinc citrate, zinc gluconate, calcium maleate, calcium succinate, sodium acetate, sodium maleate, sodium succinate, iron fumarate, sodium citrate, or the like; and/or any combinations thereof. Basic compounds like sodium hydroxide or the like may also be included as part of the pH-modifying component in the present invention. In one example, a combination of citric acid and calcium citrate is used to maintain a pH of about 4 to about 6 during enzymatic degradation. In a second example, calcium citrate is used drop the pH to about 4 prior to enzymatic degradation. When calcium citrate is used to modify the pH, the calcium citrate serves as a source of calcium ions, which are absorbed by the raw whole vegetable composition during enzymatic modification. Similarly, calcium citrate, ferrous citrate, ferrous gluconate, zinc citrate or any combination thereof can be used to modify the pH of the soak water before, during or after enzymatic degradation has been started. Furthermore, absorption of calcium, ferrous, and/or zinc occurs during enzymatic modification and serves to increase the levels of ions present.

There is typically a sufficient amount of water is present in the aqueous enzyme composition to affect an increase in moisture content of the legumes during protein hydrolysis. More specifically, the weight ratio of aqueous enzyme composition to legume is sufficient to rehydrate the legumes to attain the moisture levels described herein. Preferably, the weight ratio of aqueous enzyme composition to raw legumes or even suitable legume compositions undergoing biotransformation to bioactive legume compositions range from about 1:1 to about 10:1, and more preferably from about 1:1 to about 8:1 and most preferably from about 2:1 to about 4:1. Water sources known to the skilled artisan may be utilized in the present invention. By "water source", it is meant the water used to soak the legumes or any water subsequently added to the soak water. The term "water source" refers to any source of water or moisture, including steam. Preferably, the water source is tap water, deionized water, distilled water or combinations thereof.

Hydrolyzing dehulled and/or defatted soybean, soymeal, soy flour, soy protein concentrate/isolate comprising non-hydrolyzed soy protein - In this example, dehulled and/or defatted soybean, soybean meal or dehulled and/or defatted soybean flour are hydrolyzed using acid, base, enzymes, or any combination thereof to obtain AOX-BSPs. The starting soy material is typically transformed into liquid or slurry form prior to protein hydrolysis. The dehulled and/or defatted soybean, soybean meal or dehulled and/or defatted soybean flour can also be hydrolyzed using an aqueous enzyme composition that includes water, cellulase, hemicellulase, pectinase, alpha-galactosidase, amylases and/or several endo- and exo-proteases like papain, bromelain, fungal proteases and neutral proteases at an initial pH ranging from about 3 to about 6 into AOX-BSPs. However, care must be taken to avoid premature deactivation of the enzymes due to the

exposure of the enzymes to other components present in the soybean meal and/or flour that are known to inactivate enzymes, such as protease inhibitors.

Similarly, an aqueous acidic (pH 3 to 5) or basic (pH 7 to 11) composition at temperatures of about 100°F (37°C) to about 150°F (66°C) may also be used to hydrolyze protein instead of using enzymes. Other soy compositions, such as other fractionated, processed and/or un-fractionated soy compositions comprising non-hydrolyzed soy protein may be hydrolyzed using acid, base, enzymes, or any combination thereof, to obtain bioactive soy compositions and/or AOX-BSPs of the present invention. After protein hydrolysis, any non-hydrolyzed protein and/or hydrolyzed protein (mainly AOX-BSPs) can be extracted, purified, and/or further processed to yield bioactive soy compositions for use in foods, beverages, supplements, non-food (edible) compositions or the like.

In addition, care is required to avoid protein denaturation during protein hydrolysis, particularly since the hulls may be removed in this example, and/or size reduction of the starting soy material may have been undertaken. In this example, the temperature of the aqueous enzyme composition (or acidic or basic composition) can be maintained below the temperature at which soy protein is denatured or less than 133°F (56°C), less than 158°F (70°C), and/or less than 194°F (90°C).

Hydrolyzing liquid soy compositions comprising non-hydrolyzed soy protein - Liquid soy compositions, such as soymilks, soymilk beverages, soymilk concentrates, and the like, that contain up to about 20% by weight non-hydrolyzed soy protein, based on the total weight of the starting soy composition, can also be hydrolyzed using acid, base, enzymes, or any combinations thereof. In one example, soymilks comprising at least about 0.9% intact soy protein are prepared conventionally by extracting soy protein from dehulled soybeans that have been comminuted. After extraction, non-protein solids are removed via a decanting centrifuge and the liquid portion is hydrolyzed using acidic conditions and/or an aqueous enzyme composition comprising the enzymes disclosed above for a time that is sufficient to form AOX-BSPs having a molecular weight of less than about 7000 Daltons. In a second example, soymilk concentrates comprising up to about 20% non-hydrolyzed soy protein is hydrolyzed using an aqueous enzyme composition that includes water, cellulase, hemicellulase, pectinase, papain, bromelain, fungal and/or neutral proteases for a time that is effective to form AOX-BSPs.

As noted, hydrolyzing the non-hydrolyzed soy protein present in conventionally-prepared soymilks, soymilk concentrates, or soymilk beverages using acid and/or enzymes can be used to obtain bioactive soymilks, bioactive soymilk concentrates, or bioactive soymilks beverages. Alternatively, bioactive liquid soy compositions can be prepared by (1) adding AOX-BSPs having molecular weights that range from about 200 grams per mole to about 7000 grams per mole to conventionally-prepared soymilk compositions and/or soymilk beverages, (2) adding unhydrolyzed soy protein to a conventionally-prepared soymilk composition also comprising

unhydrolyzed protein followed by protein hydrolysis using acid, base, enzymes, or any combination thereof, into AOX-BSPs with a molecular weight of about 200 grams per mole to about 7000 grams per mole, and/or (3) preparing soymilk compositions and/or soymilk beverages using whole soybeans containing AOX-BSPs having a molecular weight of about 200 grams per mole to about 7000 grams per moles. The bioactive liquid soy compositions/concentrates can be used as is or enriched further with more AOX-BSPs to attain a desired bioactive functionality. In another example, AOX-BSPs having molecular weights that range from about 200 grams per mole to about 7,000 grams per mole can be added to edible and/or non-edible compositions followed by application of one or more conventional processing steps.

Bioactive soy protein concentrates in powder form can be obtained from bioactive soymilk compositions by simply pre-concentrating the soymilk composition followed by spraying drying to form a powder. Bioactive soy protein concentrate can also be prepared by washing raw whole (hulled) soybeans to remove surface dirt and then subjecting the clean soybeans to protein hydrolysis using acid, base, enzymes, or any combination thereof. Thereafter, the beans are drained of the soak water, transferred into a wet-mill that grinds the soybeans now having a moisture content of about 50% to about 70% by weight into a slurry that is subsequently extracted using warm (below the temperature at which soy protein denatures) or hot (150°F-170°F) water at a bean:water ratio of about 1:5 to 1:10 and an initial pH of about 8 or more. The temperature and pH renders soy protein soluble for maximum extraction. Next, optional precipitation of extracted soy protein is accomplished by dropping the pH to about 4.2 to 4.5 using an acid, such as citric acid or hydrochloric acid. Thereafter, the precipitated protein is transferred into any equipment, such as a decanting centrifuge that separates the protein from the whey. The separated protein is transferred into a spray drier that spray dries the protein into a powder. The hydrolysis water and the whey expelled during acidic precipitation is expected to contain appreciable amounts of hydrolyzed soy protein, therefore, both liquids can be filtered using an ultrafiltration (UF) or reverse osmosis (RO) membrane that enables selective retention of the hydrolyzed soy protein which can also be spray dried into a powder. Alternatively, the hydrolysis water can be used to wet-mill the raw beans instead being removed. Thereafter, the same processing steps are performed after wet-milling as described above.

In another example, the hydrolyzed soybeans are dried down to a moisture content (by weight) of less than about 20%, or less than about 10%, and then processed according to the conventional steps used to produce soy protein concentrates. These processing steps include dehulling, fat extraction, dry-milling, acidic precipitation, and spray drying. The remaining whey can be processed to remove any bioactive soy peptides and/or protein fragments and added back to soy protein concentrate before, during or after spray drying. Alternatively, the processing steps after drying include dehulling, cracking, and optionally packaging the cracked soybeans or continuing on with defatting, and grinding to less than 150 micron in size.

Method of producing AOX-BSPs from hulled soybeans - In general, AOX-BSPs can be isolated by transferring hydrolyzed soybeans into a wet-mill, grinding to a size of less than about 200 microns, solubulizing the protein by bringing to a pH of about 8, separating the non-protein (oligosaccharides and/or fibrous) fraction, using a decanting centrifuge for example; and transferring the soluble protein fraction into a spray drier that dries the protein fraction into a granular powder. Alternatively, the soluble proteins are precipitated by bringing the pH to a range of about 4.2 to about 4.5 after removing the non-protein fraction, followed by recovery of the AOX-BSPs via decanting centrifuge for example, before spraying drying. In another example, the soluble protein may be fractionated using HPLC preparative and/or ultrafiltration (UF) membranes to isolate AOX-BSPs into the following molecular weight ranges of less than about 500 Daltons; less than about 1000 Daltons; less than about 1500 Daltons; less than about 2000 Daltons; less than about 2500 Daltons; less than about 3000 Daltons; less than bout 5000 Daltons; and/or less than about 7000 Daltons. In a third example, the soluble protein after removal of the non-protein fraction is concentrated using a reverse osmosis (RO) membrane system. Any whey expelled during optional acidic precipitation is expected to contain appreciable amounts of hydrolyzed soy protein, therefore, the expelled whey can also be filtered using an ultrafiltration (UF) or reverse osmosis (RO) membrane that enables selective retention of the hydrolyzed soy protein which can also be spray dried into a powder.

Use of multiple enzyme hydrolysis steps to produce AOX-BSPs - AOX-BPS can be produced by subjected raw whole (hulled) and/or dehulled soybeans to protein hydrolysis preferably using a combination of enzymes that include cellulase, hemicellulase, pectinase, alpha-galactosidase, and/or amylases; and/or one or more proteases, such as papain, bromelain, neutral proteases and fungal proteases in water at a pH of about 4.5 to 5.5, at an initial temperature of about 120°F (48°C) for up to about 12 hours. Thereafter, the soak water is drained and processed to recover and any AOX-BPs using an RO and/or UF membrane system prior to drying. After removal of soak water, hydrolyzed soybeans are wet-milled into a slurry having a size of less than about 200 microns, and subjected to a second enzyme hydrolysis step in order to attain greater levels of AOX-BPs having a molecular weight of less than 7kDa. After the 2nd enzymatic hydrolysis step, the slurry is brought to a pH of about 8 to solubilize the protein fraction. The non-protein fraction is separated from soluble AOX-BPs/protein and transferred into a spray drier that dries the protein fraction into a granular powder. Alternatively, the soluble protein fraction is precipitated prior to recovery, before spraying drying. Similarly, additional processing and/or separation can be performed as well to obtain desired molecular weight fractions.

Production of AOX-BSPs from soybean slurry - AOX-BSPs can be produced by dehulling raw soybeans (or dehulled and/or defatted raw beans and/or soy meal, dehulled and/or defatted soy flour), comminution to a size of less than about 120 microns, forming a 20% by weight slurry, and subjecting the slurry enzymatic hydrolysis using cellulase, hemicellulase, pectinase, alpha-

galactosidase, and/or amylases; and/or one or more proteases, such as papain, bromelain, neutral proteases and fungal proteases at a pH of about 4.5 to 5.5. Since the starting soy material is dehulled, lesser concentrations of cellulase, hemicellulase and/or pectinase may be used. Enzymatic degradation typically continues until a maximum of 8 hours is attained at an initial temperature of about 120°F (48°C). Next, the slurry is brought to a pH of about 8 to solubilize the protein prior to removal of non-protein material (carbohydrates) by a decanting centrifuge, for example. The soluble protein fraction is optionally precipitated by bringing the pH to a range of 4.2 to 4.5 prior to spray drying. Alternatively, the soluble protein fraction can be directly transferred into a spray drier and dried into a powder. In another example, the AOX-BSPs can be fractionated into smaller molecular weight fractions to isolate specific bioactive fractions and/or enhance potency.

The optional step of precipitating the liquid protein stream by pH adjustment may be unnecessary since maximum retention and recovery of AOX-BSPs is desired from the present invention. Therefore, any processing steps including the optional protein precipitation steps is implemented only after careful consideration of any factors that would result in undesired elimination of AOX-BSPs.

Enzyme hydrolysis of isolated soy protein - AOX-BSPs can be produced by soaking raw hulled soybeans in water for up to about 12 hours followed by wet-milling to a particle size of less than about 200 microns. Next, the wet-milled soybeans are subjected to filtration or a decanting centrifuge to remove the non-protein fraction. Thereafter, the pH of the filtrate is brought to a range of about 8 or more to solubilize the protein. After protein solubulization, additional carbohydrates and insoluble matter may be removed via decanting centrifuge or a membrane filter. The liquid protein filtrate is then subjected to enzymatic hydrolysis for up to 8 hours at a pH of about 5 to about 6 using enzymes that include multiple broad spectrum proteases, such as papain, bromelain, fungal and/or neutral proteases. The hydrolyzed protein can be spray dried into a powder or may be optionally concentrated using an RO system or subjected to additional fractionation using a UF membrane system (not shown). Alternatively, soy protein concentrate and/or isolate can be directly hydrolyzed to form AOX-BSPs.

In addition, the discarded non-protein fraction, bioactive okara derived from using bioactive hulled soybeans to prepare bioactive soymilks, in combination with the filtered soak water obtained protein hydrolysis can be transformed into biofuels as each soy fraction disclosed above contains appreciable levels of soy lipids.

IV. Nutraceutical compositions

Nutraceutical compositions disclosed herein include any edible food product, a beverage, a supplement, tablet, capsule, concentrate or gel. Suitable examples of beverages include ready-to-drink (RTD) beverages or dry-blended beverages (DBB). The beverage may be a substantially cloudy beverage or a substantially clear beverage. Non-limiting examples of suitable beverages

containing AOX-BSPs include milk-based beverages, milk analog beverages (e.g., soymilk, rice milk, etc), weight management beverages, protein shakes, meal replacement drinks, coffee-based beverages, nutritional drinks, energy drinks, infant formulas, fruit juice-based drinks, fruit drinks, fruit-flavored drinks, vegetable-based drinks, sports drinks, and the like. The pH of the beverage will generally range from about pH 2.8 to about pH 7.5, or from about pH 4.5 to about pH 7.5, or about pH 7.0.

In another example, the food product may be a beverage comprising AOX-BSPs. Non-limiting examples of suitable edible materials that can be used to formulate the beverage include skim milk, skim soymilk, reduced fat milk, reduced fat soymilk, 2% milk, whole milk, cream, evaporated milk, evaporated soymilk, yoghurt, buttermilk, dry milk powder, dry soymilk powder, non-fat dry milk powder, nonfat dry soymilk powder, milk proteins, acid casein, caseinate (e.g., sodium caseinate, calcium caseinate, etc.), whey protein concetrate, whey protein isolate, soy protein isolate, soy protein hydrolysate, soy flour, whey hydrolysate, chocolate, cocoa powder, coffee, tea, fruit juices, vegetable juices, and so forth. The beverage food product may further comprise sweetening agents (such as glucose, sucrose, fructose, maltodextrin, sucralose, corn syrup, honey, maple syrup, etc.), flavoring agents (e.g., chocolate, cocoa, chocolate flavor, vanilla extract, vanilla flavor, fruit flavors, etc), emulsifying or thickening agents (e.g., lecithin, carrageenan, cellulose gum, cellulose gel, starch, gum, arabic, xanthan gum, and the like); stabilizing agents, lipid materials (e.g., canola oil, sunflower oil, high oleic sunflower oil, fat powder, etc.), preservatives (e.g., potassium sorbate, sorbic acid, and so forth), antioxidants (e.g., ascorbic acid, sodium ascorbate, etc.), coloring agents, vitamins, minerals, and combinations thereof.

In another example, the food product including AOX-BSPs may be a food bar, such as a granola bar, a cereal bar, a nutrition bar, or an energy bar. In a second example, the food product may be a cereal-based product. Non-limiting examples of cereal-based food products include breakfast cereals, pasta, breads, baked products (i.e., cakes, pies, rolls, cookies, crackers), and snack products (e.g., chips, pretzels, etc.). The edible material of a cereal-based food product may be derived from wheat (e.g., bleached flour, whole wheat flour, wheat germ, wheat bran, etc.), corn (e.g., corn flour, cornmeal, cornstarch, etc.), oats (e.g., puffed oats, oatmeal, oat flour, etc), rice (e.g., puffed rice, rice flour, rice starch), and so forth. In a third example, the food product may be a "solid" dairy-based product. Non-limiting examples of suitable "solid" dairy-based food products include hard cheese products, soft cheese products, ice cream products, yoghurt products, frozen yoghurt products, whipped dairy-like products, sherbets, and the like. Alternatively, the food product may be a nutritional supplement. The nutritional supplement may be liquid, foam, or solid. In another example, the food product may be a meat product or a meat analog product. Examples of meat food products include, but are not limited to, processed meats, comminuted meats, and whole muscle meat products. The meat material may be animal meat or seafood meat. The meat

analog may also be a textured vegetable or dairy protein that mimics animal or seafood meat in texture. The meat analog may be part or all of the meat material in a meat food product.

V. Methods of administration of AOX-BSPs

Nutraceutical, pharmaceutical compositions and/or pharmaceutically acceptable salts of AOX-BSPs described herein may be prepared or synthesized according to methods known to those skilled in this art, see, for example Pharmaceutical Salts: Properties, Selection, and Use, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor) June 2002. Generally, such salts are prepared by reacting the free base forms of these compounds with a stoichiometric amount of the appropriate acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of some appropriate salts are found, for example, in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985.

The AOX-BSPs described herein may be administered as complex mixtures comprising bioactive soy protein fractions of a specific molecular weight and/or molecular weight range. The AOX-BSPs may be administered in oral dosage forms that include tablets, capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Further, the compounds may be administered in intravenous (bolus or infusion), intraperitoneal, intrathecal, directly into the brain or spinal fluid, subcutaneous, or intramuscular form.

The AOX-BSPs described herein are typically to be administered in admixture with suitable pharmaceutical diluents, excipients, extenders, or carriers (termed herein as a pharmaceutically acceptable carrier, or a carrier) suitably selected with respect to the intended form of administration and as consistent with conventional pharmaceutical practices. The deliverable compound will be in a form suitable for oral, rectal, topical, intravenous injection or parenteral administration. Carriers include solids or liquids, and the type of carrier is chosen based on the type of administration being used. The compounds may be administered as a dosage that has a known quantity of the compound.

Techniques and compositions for making dosage forms useful for materials and methods described herein are described, for example, in the following references: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Pharmaceutical Dosage Forms: Tablets (Lieberman et al., 1981); Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976); Remington's Pharmaceutical Sciences, 17th ed. (Mack Publishing Company, Easton, Pa., 1985); Advances in Pharmaceutical Sciences (David Ganderton, Trevor Jones, Eds., 1992); Advances in Pharmaceutical Sciences Vol 7. (David Ganderton, Trevor Jones, James McGinity, Eds., 1995); Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms (Drugs and the Pharmaceutical Sciences, Series 36 (James McGinity, Ed., 1989); Pharmaceutical Particulate Carriers: Therapeutic Applications: Drugs and the Pharmaceutical Sciences, Vol 61 (Alain Rolland, Ed., 1993); Drug Delivery to the Gastrointestinal Tract (Ellis Horwood Books in the Biological Sciences. Series in

Pharmaceutical Technology; J. G. Hardy, S. S. Davis, Clive G. Wilson, Eds.); Modern Pharmaceutics Drugs and the Pharmaceutical Sciences, Vol 40 (Gilbert S. Banker, Christopher T. Rhodes, Eds.).

Suitable binders, lubricants, disintegrating compounds, coloring compounds, flavoring compounds, flow-inducing compounds, and melting compounds may be included as carriers, e.g., for pills. For instance, an active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, gelatin, agar, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like.

Suitable binders include, for example, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, for example, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

The compounds may also be used with liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The compounds may also be coupled to polymers as targetable drug carriers or as a prodrug. Suitable biodegradable polymers useful in achieving controlled release of a drug include, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, caprolactones, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacylates, and hydrogels, preferably covalently crosslinked hydrogels.

The AOX-BSPs can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active compounds can also be administered parenterally, in sterile liquid dosage forms.

Capsules may contain the AOX-BSPs and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similarly, such diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as immediate release products or as sustained release products to provide for continuous or long-term release of the active compounds. The deliverable form of the AOX-BSPs can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

For oral administration as a liquid, the AOX-BSPs may be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Examples liquid forms include solutions or suspensions in water, pharmaceutically acceptable fats

and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying compounds, suspending compounds, diluents, sweeteners, thickeners, and melting compounds.

Liquid dosage forms for oral administration can contain coloring and flavoring, as needed. In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water-soluble salt of the active ingredient, suitable stabilizing compounds, and if necessary, buffer substances. Antioxidizing compounds such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing compounds. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field.

The compounds described herein may also be administered in intranasal form via use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches known to those skilled in these arts. To be administered in the form of a transdermal delivery system, the dosage administration will generally be continuous rather than intermittent throughout the dosage regimen. Parenteral and intravenous forms may also include minerals and other materials to make them compatible with the type of injection or delivery system chosen.

The AOX-BSPs set forth herein may also be used in pharmaceutical kits for the treatment of diseases, or other purposes, which comprise one or more containers containing a pharmaceutical composition comprising a therapeutically effective amount of any of the AOX-BSPs. Such kits may further include, if desired, one or more of various components, such as, for example, containers with the bioactive compound, containers with one or more pharmaceutically acceptable carriers, additional containers, and instructions. The instructions may be in printed or electronic form provided, for example, as inserts or labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the bioactive component.

Dosage levels include from about 0.01 mg to about 2000 mg of the AOX-BSPs, per kilogram of body weight per day are preferable dosages. As used herein, the term "active compound" refers complex mixtures of the bioactive soy peptides, bioactive hydrolyzed soy protein, bioactive soy protein fragments and/or bioactive hydrolyzed soy protein fragments having the desired molecular weight range or fraction of about 200 Daltons to about 7000 Daltons.

Persons of ordinary skill in these arts will recognize that all doses and ranges between these explicit values are contemplated, e.g., 0.01 to 100, and 0.1 to 50 mg/kg per day. The amount

of active compound that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 0.01 mg to about 10,000 mg of an active compound; persons of ordinary skill in these arts will recognize that all doses and ranges between these explicit values are contemplated. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration and rate of excretion, drug combination and the severity of the particular disease undergoing therapy. For example, a suitable dosage adopted for oral or intravenous administration of any of the bioactive soy peptides, bioactive hydrolyzed soy protein, bioactive soy protein fragments and/or bioactive hydrolyzed soy protein fragments may range from about 0.01 to about 1000 mg per dose, from once per week to 5 times daily and may be easily calculated from IC_{50} values.

Nevertheless, in practice, it is believed that a physician and/or individual will determine the actual dosing regimen which is most suitable for an individual patient when administering pharmaceutical compositions that include any combination of the active compounds, and the dosage is expected to vary with the age, weight, and response of the particular patient. Furthermore, the above dosages are exemplary of an average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope of this invention.

VI. Method of treating free radical damage and/or oxidative stress

In one example, a nutraceutical composition comprising up to about 25 grams AOX-BSPs is administered to an individual experiencing oxidative damage, stress, has elevated levels of 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, and/or PCO; and the nutraceutical composition is effective to (1) reduce plasma levels of 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, and/or PCO by at least about 70%, about 60%, about 50%, about 40%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 1%, (2) increase urinary 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, and/or PCO by at least about 0.1%, about 0.25%, about 0.5%, about 1%, about 2%, about 3%, about 4%, or about 5%, when compared to an individual experiencing oxidative damage and stress that did not consume the nutraceutical composition.

In another example, a nutraceutical composition comprising up to about 7 grams of AOX-BSPs with a molecular weight of less than about 7000 Daltons is administered to an individual experiencing oxidative damage and stress, has elevated levels of 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, or PCO; and the nutraceutical composition is effective to (1) reduce plasma levels of 8-OHdG, 8OHG, 8-iso-prostaglandin-2α and/or PCO by at least about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 1%, and/or (2) increase urinary 8-OHdG, 8OHG, 8-iso-prostaglandin-2α and/or PCO by at least about 0.1%, about 0.25%, about 0.5%, about 1%, about

2%, about 3%, about 4%, or about 5%, when compared to an individual with elevated levels of biomarkers of oxidative stress that did not consume the nutraceutical composition.

In another example, a pharmaceutical composition comprising up to about 1000 mg of AOX-BSPs having a molecular weight of up to about 7000 Daltons is administered to a patient or sample having elevated levels 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, 8-iso-prostaglandin-2α and/or PCO; and the pharmaceutical composition is effective to reduce 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, 8-iso-prostaglandin-2α and/or PCO by at least about 50%, about 40%, about 30%, about 20%, or about 10%, when compared to the original levels in an untreated patient or control sample.

VII. Examples

All enzyme concentrations are in weight percent of soybean unless otherwise noted. Enzeco papain concentrate, fungal amylase concentrate, Enzeco neutral bacterial protease 160K, Pectinase P-10, alpha-galactosidase, Enzeco fungal protease 180, Enzeco hemicellulase 20M, Enzeco hemicellulase (2.5x) concentrate, bromelain concentrate, cellulase CEP were supplied by Enzyme Development Corporation. Alpha-galactosidase DS, alpha-galactosidase "Amano 10", and Pectinase P-II were supplied by Amano Enzymes USA. Alpha-gal™ and Viscozyme L were supplied by Novo Nordisk. Tate and Lyle supplied citric acid, sodium citrate and calcium citrate. Bio-Cat supplied pectinase 3500-ENDO-PGM. Temperatures are initial readings unless otherwise noted.

Protein hydrolysis – Approximately 300 grams (gr) of raw whole soybeans (from Whole Foods, Minneapolis, MN) were washed to remove surface dirt and placed in approximately 900 grams of water at the temperature and pH conditions listed in Tables 1, 2 and 3. Thereafter, enzyme concentrations based on the component concentrations of Tables 1, 2 and 3 were added to the soybean water mixture. The individual weights are also listed in Tables 1, 2 and 3. For acidic or neutral/basic protein hydrolysis, no enzymes were added, only acid, salt and/or buffers to obtain and maintain the desired pH range. The mixture was stirred for about 2 minutes and enzymatic hydrolysis (or soaking) was allowed to proceed for a total of eight hours. For the second enzymatic degradation step, soybeans (or soy starting materials) were soaked for up to 4 hours using the enzyme concentrations and amounts listed in Table 2 and 3.

Bioactive soy compositions - After protein hydrolyses, processing into AOX-BPs, and/or bioactive compositions in the form of whole soy, whole soybean protein flour, soybean protein flour, soybean flour, whole soybean flour, soybean protein isolate, soybean protein concentrate, soybean grits, soybean bars, soybean powders, protein blends, soy beverages, beverages, and/or beverage mixes was undertaken. As noted, AOX-BPs can be included into any edible or non-edible compositions in order to confer bioactive properties to the compositions. Thereafter, samples were taken for analysis.

Fractionation of AOX-BSPs – Liquid compositions containing the AOX-BSPs were further separated using a series of HPLC, preparative columns and/or UF membranes in the following MWCO ranges - <500Da; <1000Da; <1.5kDa; <2KDa; <2.5kDa; 2-4KDa; <5kDa; 4-6KDa; and/or <7kDa and/or 10kDa; molecular weight fractions were confirmed by SDS-PAGE, and were tested for bioactivity (antioxidant activity).

Food, beverage and soymilk sample preparation – Approximately 200 grams of soybeans were added to the amount of regular tap water (1200 or 1250 grams) and placed in a SoyQuick Automatic Soymilk maker purchased from the Internet. Bean weights, moisture content and added water details are listed in Table 2. Soymilk preparation was operated as specified by the manufacturer's instruction manual. The machine was turned on and beans were extracted and thermally processed into soymilk. Samples were taken for analysis. For the 2nd enzyme hydrolysis step, the amount of soymilk hydrolyzed is 900 grams and listed in Table 3. Soy okara from soymilks preparation was used to fortify a variety of food products including baked goods (cakes, cookies, pies), meat products (meat loaf), beverages, soups, chilis, and the like at concentrations of 10% to 50% by weight and samples were taken for analysis.

Protein concentration – in micrograms per mL (ug/mL) was determined by first agitating liquid soy composition samples before placing 5 grams of the liquid soy composition (or soymilk) sample and 10 ml of extracting buffer (30mM of Tris-HCl buffer with 10 mM β-mercaptoethanol at pH 8.0). Samples were shaken for 1 hour at room temperature and then concentrated using a rotovap to remove the β-mercaptoethanol. After vacuum drying, liquid soy:buffer (or soymilk:buffer) samples were re-suspended in 10 ml of extracting buffer and tested for total protein with the MicroBCA kit from Thermo Scientific. For solid whole soybeans were dried, ground to a particle size of less than 200 microns, and placed in the extracting buffer. 1 gram of whole soybeans was extracted using 10 ml of the extracting buffer (30mM of Tris-HCl buffer with 10 mM β-mercaptoethanol at pH 8.0). Samples were shaken for 1 hour at room temperature and then concentrated using a rotovap to remove the β-mercaptoethanol. Protein concentrations were tested using the MicroBCA kit from Thermo Scientific.

Using this, tests indicated the raw non-hydrolyzed control soybeans had 64.98 ± 3.68 mg/g soy protein with 23.27 ± 1.08 mg/g 7S and 14.21 ± 0.34 mg/g 11S fractions present on a dry basis. Enzymatically degraded soybeans had 27.77 ± 4.65 mg/g total protein with 1.7 ± 0.02 mg/g 7S (beta-conglycinin) and 0.04 ± 0.01 mg/g 11S (glycinin) fractions present. This represents a 93% to 99% reduction in allergenic soy protein as measured by alpha-glycinin (glycinin) and beta-conglycinin. Furthermore, preparation of soymilks using the enzymatically degraded soybeans elicited, little, if any soy sensitivity in the form of 24-72 hours of itching and scratching, after consumption of 1 cup of soymilk by a soy sensitive individual. The soy sensitive individual typically suffers from scratching and itching for 24 to 72 hours after consuming soymilk products.

ORAC analysis – To obtain the ORAC value, solid bean samples were ground to a particle size of less than 200 microns and dried. 1.00±0.01 g ground beans were weighed out and the mass accurately recorded. Five ml of methanol:1.2 N hydrochloric acid was added, and mixing was performed for 2 hours (±10 minutes). Samples were spun to sediment the solids and the aqueous phase was collected. An additional 5 ml of solvent was added and samples were mixed for an additional 2 hours. Samples were spun again and supernatents were combined with previously collected samples. The extract was mixed, and then diluted 50 fold (20 µl in 980 µl) with the phosphate buffered saline (PBS) listed in the ORAC procedure.

Approximately1.00±0.01 g of soymilk sample was weighed out and the mass accurately recorded. Next, five ml of solvent (methanol:1.2 N hydrochloric acid) was added, and mixing was performed for 2 hours (±10 minutes) at room temperature. Next, if samples contained precipitated protein fractions, samples were centrifuged to separate the solids from the aqueous phase. Thereafter, the aqueous phase was withdrawn, an additional five ml of methanol:HCl solvent was added, and samples were mixed for an additional 2 hours. Centrifugation was performed to remove the solids from the aqueous phase, which was subsequently withdrawn. The aqueous phases (supernatants) were combined, and diluted 50 fold (20 µl in 980 µl) with 74 mM phosphate buffered saline (PBS), pH 7.4.

10 µl of Trolox was added to the sample wells followed by 25 µl of each soymilk sample. Samples were tested in triplicate. Fluoroscein working solutions made by diluting 0.6 ml of the fluoroscein stock solution in 15 ml of PBS was prepared, as was 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH) solution by dissolving 0.1656 g AAPH in 2 ml PBS and mixied vigorously until dissolved. Next, approximately 150 µl of the fluorescein working reagent was also added to each well containing the soymilk (or control) samples followed by addition of adding 25 µl of the AAPH solution to each well after 7 minutes of incubation. The fluorescence emitted over time was measured using an automated fluorometer. ORAC values were calculated as the fluorescence emitted over time (area under the curve) with respect to a control sample, and reported as microMolar Trolox per gram sample.

SDS-PAGE Analysis – whole soybean, soy compositions, soymilk and/or AOX-BSPs samples were prepared as described above. Aliquots of each were resolved by SDS-PAGE using standard procedures to permit comparison of molecular sizes with those of the starting soy materials and proteins. An image of a Coomassie stained gel will show molecular weights range from about 500 Daltons to about 100 kDa. Furthermore, 7S and 11S subunit bands will not be present.

Measurement of biomarkers of oxidative stress - 8-OHdG, 8OHG, MDA, 4-HNE, TBARS, or PCO levels were performed using standard methods (ELISA kits and/or HPLC detection and quantification) after extraction from primary culture, tissue samples, and/or tissue culture.

The results are presented in Tables 1-5 below. Table Key: "**NA**" refers to not applicable. "**ND**" refers to not determined. **"ENZ HYD"** refers protein hydrolysis using enzymes. **"ACID"** refers to protein hydrolysis under acidic conditions. **"BASIC"** refers to protein hydrolysis under basic conditions. **"WF"** refers to Whole Foods. **"SILK"** refers to Silk® Soymilk Beverages. **"EDC"** refers to Enzyme Development Corporation.

Soymilk compositions containing varying concentrations of nonhydrolyzed and hydrolyzed soy protein (AOX-BSPs) were also prepared (Table 4). Samples were tested for antioxidant activity using the ORAC assay. A brief description of each soymilk sample is provided below:

Control A – is a soymilk composition (Silk® unflavored soymilk beverage).

Control B – is a soymilk composition (Whole Foods unflavored and unsweetened soymilk beverage).

Samples D1, D2 and D3 are soymilk compositions prepared from whole soybeans that were enriched with bioactive native soy peptides and/or soy protein fragments derived from enzymatic hydrolysis of native soy protein.

Samples E1, E2 and E3 are soymilk compositions prepared with bioactive soy peptides and/or soy protein fragments derived from acidic pH hydrolysis of native soy protein.

Samples F1, F2 and F3 are soymilk compositions prepared with bioactive soy protein fragments and/or soy peptides derived from hydrolysis of native soy protein under near neutral and/or basic pH conditions.

Samples G1, G2 and G3 are soymilk compositions that were enriched with bioactive soy protein fragments and/or soy peptides derived from enzymatic hydrolysis of native and denatured soy protein.

Samples H1 and H2 are soymilk compositions containing bioactive soy peptides and/or soy protein fragments derived from hydrolysis of native soy protein under near neutral and/or slightly basic pH conditions.

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to a composition containing "a compound" includes a mixture of two or more compounds. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

TABLE 1 – ENZYMATIC DEGRADATION OF RAW WHOLE SOYBEANS

Composition	Control beans	VGZO FORMULA	ENZ HYD 1	ENZ HYD 2	ENZ HYD 3	ACID 1	ACID 2	ACID 3	BASIC 1	BASIC 2	BASIC 3
Cellulase AP 10	NA	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pectinase P-10	NA	0.000	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pectinase P-II	NA	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 20M	NA	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 2.5X	NA	0.000	NA	NA	NA	NA	NA	NA	NA	NA	NA
Papain	NA	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA
Citric Acid	NA	0.200	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alpha-galactosidase (EDC)	NA	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA
alpha-amylase (EDC)	NA	0.125	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total (enzyme conc %) or (enzyme wgt g)	NA	0.645	1.941	1.937	1.936	NA	NA	NA	NA	NA	NA
Bromelain (EDC)	NA	0.020	0.060	0.060	0.061	NA	NA	NA	NA	NA	NA
Fungal protease 180 (EDC)	NA	0.020	0.062	0.062	0.063	NA	NA	NA	NA	NA	NA
neutral bacterial protease 160K (EDC)	NA	0.020	0.058	0.063	0.058	NA	NA	NA	NA	NA	NA
Hemicellulase 20M	NA	0.045	0.136	0.134	0.134	NA	NA	NA	NA	NA	NA
Total (conc %) or (wgt g)	NA	0.750	2.257	2.256	2.252	NA	NA	NA	NA	NA	NA
Calcium citrate (g)	NA	NA	~3 g	~3 g	NA	~3 g	~3 g	NA	NA	NA	NA
Sodium Citrate (g)	NA	NA	NA	NA	~3g	NA	NA	~3 g	NA	NA	NA
bean:water ratio	NA	NA	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3
Initial pH	NA	NA	4.210	4.130	4.52	4.220	4.220	4.740	ND	4.540	4.520
Final pH	NA	NA	5.510	5.580	4.99	5.230	5.660	5.200	ND	5.720	5.720
Initial temperature	NA	NA	121.1°F/49.5°C	122.5°F/50.3°C	119.0°F/48.3°C	121.1°F/49.5°C	117.2°F/47.3°C	120.0°F/48.9°C	69.1°F/20.6C	69.2°F/20.7°C	68.0°F/28°C
Final temperature	NA	NA	75.7°F/24.3°C	74.5°F/23.6°C	77.0°F/25°C	75.6°F/24.2°C	76.8°F/24.9°C	78.3°F/25.7C	73.7°F/23.2C	73.1°F/22.8°C	74.5°F/24.6°C
Initial Bean wgt (g)	NA	NA	300.000	300.000	300.000	300.000	300.000	300.000	300.000	300.000	300.000
Final bean wgt (g)	NA	NA	652.000	655.000	623.000	644.000	645.000	615.000	569.000	577.000	579.000
Absorbed water (g)	NA	NA	352.000	355.000	323.000	344.000	345.000	315.000	269.000	277.000	279.000
% moisture	NA	NA	53.988	54.198	51.846	53.416	53.488	51.220	47.276	48.007	48.187
Enzymatic degradation time	NA	NA	8 hours	8 hours	8 hours	8 hours	8 hours	8 hours	8 hours	8 hours	8 hours
mg 7S soy protein fraction per gram bean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
% reduction 7S soy protein	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
% reduction 11S soy protein	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mg 11S soy protein fraction per gram bean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Percent protein hydrolysis	NA	NA	53.460	40.880	64.260	18.000	24.620	22.410	7.810	30.290	15.960
ORAC (uM Trolox per g bean)	62±6.28	NA	81.42±10.34	85.90±9.67	80.68±5.19	81.41±15.07	79.81±5.00	75.91±9.85	75.10±10.56	65.93±13.25	68.24±10.90

TABLE 2 – BIOACTIVE SOYMILK COMPOSITIONS

Composition	VGZO FORMULA	BASIC 1	BASIC 2	BASIC 3	BASIC 2ND ENZ HYD 1	BASIC 2ND ENZ HYD 2	BASIC 2ND ENZ HYD 3	WF SOYMILKS 1	WF SOYMILKS 2	SILK SOYMILK 1	SILK SOYMILK 2
Initial pH	NA	ND	ND	ND	NA	NA	NA	NA	NA	NA	NA
Final pH	NA	ND	ND	ND	NA	NA	NA	NA	NA	NA	NA
Initial temperature	NA	69.1°F/20.6°C	69.2°F/20.7°C	68.0°F/20°C	116.1°F/46.7°C	121.3°F/49.6°C	121.2°F/49.6°C	NA	NA	NA	NA
Final temperature	NA	73.7°F/23.2C	73.1°F/22.8C	74.5°F/23.6°C	79.4°F/26.3°C	81.0°F/27.2°C	79.9°F/26.6°C	NA	NA	NA	NA
Initial Bean wgt (g)	NA	300.000	300.000	300.000	NA	NA	NA	NA	NA	NA	NA
Final bean wgt (g)	NA	569.000	577.000	579.000	NA	NA	NA	NA	NA	NA	NA
Added water	NA	269.000	277.000	279.000	NA	NA	NA	NA	NA	NA	NA
% moisture	NA	47.276	48.007	48.187	NA	NA	NA	NA	NA	NA	NA
Enzymatic degradation or soak time	NA	8 hours	8 hours	8 hours	4 hours	4 hours	4 hours	NA	NA	NA	NA
Beans	NA	200.000	200.000	200.000	NA	NA	NA	NA	NA	NA	NA
Water	NA	1241.000	1260.000	1250.000	NA	NA	NA	NA	NA	NA	NA
Soymilk wgt	NA	NA	NA	NA	1000.000	1000.000	1000.000	NA	NA	NA	NA
Cellulase AP 10	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pectinase P-II	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 20M	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 2.5X	0.000	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Papain	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Citric Acid	0.200	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alpha-galactosidase (EDC)	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
alpha-amylase (EDC)	0.125	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total (enzyme conc %) or (enzyme wgt g)	0.645	NA	NA	NA	0.651	0.645	0.652	NA	NA	NA	NA
Bromelain (EDC)	0.020	NA	NA	NA	0.066	0.021	0.023	NA	NA	NA	NA
Fungal protease 180 (EDC)	0.020	NA	NA	NA	0.045	0.016	0.019	NA	NA	NA	NA
neutral bacterial protease 160K (EDC)	0.020	NA	NA	NA	0.022	0.016	0.022	NA	NA	NA	NA
Hemicellulase 20M	0.045	NA	NA	NA	0.053	0.046	0.046	NA	NA	NA	NA
Total (conc %) or (wgt g)	0.750	NA	NA	NA	0.837	0.744	0.762	NA	NA	NA	NA
Calcium citrate (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sodium Citrate (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Initial soymilk pH	NA	NA	NA	NA	6.44	6.420	4.680	NA	NA	NA	NA
Final soymilk pH	NA	NA	NA	NA	6.37	6.340	4.790	NA	NA	NA	NA
mg 7S soy protein fraction per gram bean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
% reduction 7S soy protein	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
% reduction 11S soy protein	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mg 11S soy protein fraction per gram bean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Percent protein hydrolysis	NA	7.810	30.290	15.960	ND	ND	ND	NA	NA	NA	NA
ORAC (uM Trolox per g extract)	NA	2.74 ± 0.76	ND	3.42 ± 0.22	6.50 ± 1.07	6.38 ± 0.47	6.70 ± 0.43	0.75 ± 0.18	4.40 ± 0.98	1.04 ± 0.03	3.34 ± 0.38

TABLE 3 – BIOACTIVE SOY COMPOSITIONS

Composition	VGZO FORMULA	2ND ENZ HYD 1	2ND ENZ SHYD 2	2ND ENZ HYD 3	ACID 2ND ENZ HYD 1	ACID 2ND ENZ HYD 2	ACID 2ND ENZ HYD 3	WF SOYMILKS 1	WF SOYMILKS 2	SILK SOYMILK 1	SILK SOYMILK 2
Initial temperature	NA	132°F/56.4°C	135°F/57.2°C	130.5°F/54.6°C	121.3°F/49.5°C	121.7°F/50.8°C	130°F/54.2°C	NA	NA	NA	NA
Final temperature	NA	84.9°F/29°C	85.1°F/29.1°C	93.4°F/34.1°C	78.9°F/26.1°C	86°F/30°C	76.1°F/24.6°C	NA	NA	NA	NA
Enzymatic degradation or soak time	NA	4 hours	4 hours	4 hours	4 hours	3.5 hours	2 hours	NA	NA	NA	NA
Beans	NA	200.000	200.000	200.000	200.000	200.000	200.000	NA	NA	NA	NA
Water	NA	1250.000	1250.000	1254.000	1254.000	1254.000	1250.000	NA	NA	NA	NA
Soymilk wgt	NA	900.000	900.000	900.000	900.000	900.000	900.000	NA	NA	NA	NA
Cellulase AP 10	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pectinase P-II	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 20M	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 2.5X	0.000	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Papain	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Citric Acid	0.200	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alpha-galactosidase (EDC)	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
alpha-amylase (EDC)	0.125	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total (enzyme conc %) or (enzyme wgt g)	0.645	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bromelain (EDC)	0.020	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
neutral bacterial protease 160K (EDC)	0.020	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fungal protease 180 (EDC)	0.020	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hemicellulase 20M	0.045	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total (conc %) or (wgt g)	0.750	0.675	0.689	0.675	0.675	0.675	0.652	NA	NA	NA	NA
Calcium citrate (g)	NA	0.900	1.040	0.900	NA	NA	NA	NA	NA	NA	NA
Sodium Citrate (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Initial soymilk pH	NA	6.10	6.07	6.14	6.41	6.36	6.35	NA	NA	NA	NA
Final soymilk pH	NA	6.36	6.29	6.34	6.46	6.37	6.37	NA	NA	NA	NA
mg 7S soy protein fraction per gram bean	NA	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
% reduction 7S soy protein	NA	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
% reduction 11S soy protein	NA	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
mg 11S soy protein fraction per gram bean	NA	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
Percent protein hydrolysis	NA	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
ORAC (uM Trolox per g extract)	NA	6.32 ± 0.37	6.99 ± 0.64	9.00 ± 0.65	7.90 ± 1.00	6.87 ± 0.58	7.07 ± 0.52	0.75 ± 0.18	4.40 ± 0.98	1.04 ± 0.03	3.34 ± 0.38

TABLE 4 – ORAC VALUES OF BIOACTIVE SOY COMPOSITIONS

Composition	ENZ HYD 1	ENZ HYD 2	ENZ HYD 3	ACID 1	ACID 2	ACID 3	BASIC 1	BASIC 2	BASIC 3
Bean weight (grams)	200.000	200.000	200.000	200.000	200.000	200.000	200.000	200.000	200.000
Water weight (grams)	1200.000	1200.000	1200.000	1200.000	1200.000	1206.000	1200.000	1200.000	1200.000
percent moisture	53.988	54.198	51.846	53.416	53.488	51.220	47.276	48.007	48.187
Percent protein hydrolysis	53.460	40.880	64.260	18.000	24.620	22.410	7.810	30.290	15.960
ORAC (uM Trolox per g sample)	1.01 ± 0.11	1.96 ± 0.18	3.29 ± 0.05	2.55 ± 0.37	2.80 ± 0.25	3.44 ± 0.23	4.07 ± 0.62	4.41 ± 0.99	2.35 ± 0.36

TABLE 5 – ORAC VALUES FOR SOYMILK COMPOSITIONS

Sample	Protein (ug/ml) average of three samples	hydrolyzed protein bioactive soy peptide %	unhydro-lyzed protein %	ORAC (uM Trolox/g sample)
Control A	2792.60303	0.00	100.00	1.05
Control B	3012.28828	0.00	100.00	0.75
Sample D1	871.941915	53.46	46.54	1.10
Sample D2	1422.63183	40.88	59.12	1.90
Sample D3	5888.15964	64.26	35.74	3.29
Sample E1	1524.4566	17.99	82.01	2.55
Sample E2	1595.18392	24.62	75.38	2.80
Sample E3	1120.02872	22.41	77.59	3.44
Sample F1	3595.37189	7.81	92.19	4.07
Sample F2	3431.86263	30.29	69.71	4.41
Sample F3	5285.69847	15.96	84.04	2.35
Sample G1	11087.5559	not determined	not determined	6.50
Sample G2	4043.53778	not determined	not determined	6.38
Sample G3	7253.28654	not determined	not determined	6.70
Sample H1	5782.41174	not determined	not determined	2.74
Sample H2	6910.34733	not determined	not determined	3.42

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications might be made while remaining within the spirit and scope of the invention.

WHAT IS CAIMED IS:

1. A bioactive composition comprising:

up to about 40% by weight hydrolyzed soy protein fragments having a molecular weight of about 200 Daltons to about 7000 Daltons, based on a total weight of the bioactive composition;

up to 40% by weight non-hydrolyzed soy protein, based on the total weight of the bioactive composition; and

wherein the bioactive composition has an oxygen radical absorbance capacity of at least about 65 microMolar Trolox per gram bioactive composition.

2. A method of producing a bioactive soy composition comprising:

providing a raw hulled soybeans comprising unhydrolzyed native soy protein;

immersing the raw hulled soybeans in an aqueous enzyme composition for a time that is effective to degrade the hulls, wherein the aqueous enzyme composition is effective to degrade an inner portion of the raw hulled soybeans, wherein the aqueous enzyme composition comprises water, cellulase, at least one protease, and at least one carbohydrase, wherein the aqueous enzyme composition has an initial pH of about 3 to 6, and wherein the aqueous enzyme composition is effective to hydrolyze the raw hulled soybeans and form the bioactive soy composition; and

deactivating the aqueous enzyme composition.

3. The method of claim 2 wherein the aqueous enzyme composition is effective to degrade up to about 40% by weight native protein, based on the total weight of the raw hulled soybean.

4. The method of claim 3 wherein the native soy protein is hydrolyzed into bioactive peptides, bioactive protein fragments, bioactive protein fractions, or any combination thereof.

5. The method of claim 3 wherein the bioactive composition has less isoflavone content than the raw hulled soybean comprising native soy protein.

6. The method of claim 2 and further comprising drying the bioactive composition to a moisture content of less than about 10% by weight, grinding the dried bioactive composition, defatting the ground bioactive composition, concentration of a protein fraction of the defatted bioactive composition via acid precipitation, and spray drying to form a bioactive soy protein concentrate.

7. The method of claim 2 and further comprising extracting the bioactive composition to form a liquid bioactive composition comprising hydrolyzed and unhydrolyzed soy protein.

8. The method of claim 7 and wherein the bioactive legume composition has about 92% by weight, or less, of the polyphenol content of the raw whole legume.

9. The method of claim 2 and further comprising wet-milling the bioactive composition, solubulizing the protein fraction, and separating non-protein solids to form a liquid bioactive composition.

10. The method of claim 9 and further comprising spray drying to form a granular bioactive composition.

11. The method of claim 9 and further comprising filtering the bioactive liquid composition using an ultrafiltration membrane comprising a molecular weight cutoff value of about 7,000 Daltons.

12. A food product, the food product comprising:

an edible material; and

a bioactive composition comprising a mixture of bioactive peptides, bioactive protein fragment, or bioactive protein fractions having a molecular weight of about 200 daltons to about 7000 daltons, wherein the bioactive composition has an oxygen radical absorbance capacity of at least about 0.9 microMolar Trolox per gram bioactive composition.

13. The food product of claim 12, wherein the food product further comprises an ingredient selected from the group consisting of a sweetening agent, an emulsifying agent, a thickening agent, a stabilizer, a lipid material, a preservative, an antioxidant, a flavoring agent, a coloring agent, a vitamin, a mineral, and combinations thereof.

14. The food product of claim 12, wherein the food product is selected from the group consisting of a food bar, a food beverage, a liquid concentrate, a nutritional supplement, a cereal-based product, a meat or meat analog product, and a dairy or dairy analog product.

15. A nutraceutical composition comprising:

a therapeutically effective amount of a bioactive composition comprising bioactive legume peptides, bioactive legume protein fragment, bioactive legume protein fractions, bioactive hydrolyzed legume fractions, hydrolyzed legume protein fragments, or any combination thereof;

wherein the bioactive composition has a molecular weight of up to about 1000 Daltons;

wherein the bioactive composition has an oxygen radical absorbance capacity of at least about 2 microM Trolox per gram bioactive composition; and

wherein the nutraceutical composition is effective to reduce 8-OHdG, 8OHG, MDA, 4-HNE, 3-NT, TBARS, 8-iso-prostaglandin-2α or PCO.

16. The nutraceutical composition of claim 15 wherein the nutraceutical composition is effective to reduce SOD, CAT, or HO-1.

17. The nutraceutical composition of claim 15 wherein the nutraceutical composition is effective to increase plasma glutathione, alpha-tocopherol, or beta-carotene levels by at least about 5%.

18. The food product of claim 12, wherein the edible material comprises non hydrolyzed whole soybean, non hydrolyzed dehulled soybeans, comminuted non hydrolyzed whole soybeans, non hydrolyzed comminuted dehulled soybeans, non hydrolyzed soybean protein flour, non hydrolyzed soybean protein concentrate, non hydrolyzed soybean flour, non hydrolyzed soy grits, non hydrolyzed soy protein isolate, or any combination of any of these.

19. The food product of claim 12, wherein the food product is selected from the group consisting of soy protein flour, soy protein concentrate, soy flour, soy grits, non hydrolyzed soy protein isolate, or any combination of any of these.

20. A liquid soymilk composition comprising:

up to about 20% by weight bioactive soy peptides, based on the total volume of the liquid soymilk composition, wherein the bioactive soy peptides has a molecular weight of about 200 grams per mole to about 7000 grams per mole; and wherein the liquid soymilk composition has an oxygen radical absorbance capacity of at least about 1.1 microM Trolox per gram liquid soymilk composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/00914

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23L 1/20 (2001.01)
USPC - 426/634
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC -- 426/634, 424/757

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 348, 35, 65, 155; USPTO Web Page; Google Scholar; Search terms -- hydrolyzed soy protein, small peptides, unhydrolyzed soy protein, oxygen radical absorbance, Trolox, raw soybean hulls, grinding, extraction, protease, cellulase, acid pH, aqueous enzyme immersion, nutraceuti

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2005/0053705 A1 (GAO et al.) 10 March 2005 (10.03.2005) para [0004], [0006], [0009]-[0012], [0015], [0032], [0033], [0036], [0037], [0060], [0070], [0071], [0082], [0083], abstract	12-14, 19 ---------- 1-11, 15-18, 20
Y	US 2001/0024677 A1 (BRINGE et al.) 27 September 2001 (27.09.2001) para [0009], [0017], [0018], [0022], [0029], [0035], [0055], [0063], [0077], [0089], [0127], [0132]-[0134], [0136], [0137], [0164]	1-11, 15-18, 20
Y	US 2007/0237735 A1 (DENOMMEE) 11 October 2007 (11.10.2007) para [0017], [0029], [0047]	16
Y	US 2005/0175723 A1 (NAKAMURA et al.) 11 August 2008 (11.08.2008) para [0001], [0006], [0021]	17

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2011 (24.08.2011)	15 SEP 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)