

STABILIZED HEMEPROTEIN COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[001] The present application claims priority from the U.S. Provisional Application No. 63/271,423, filed on October 25, 2021, the entire contents of which are hereby incorporated by reference.

FIELD

[002] Compositions for use in non-animal food products comprising one or more purified hemeproteins and one or more antioxidants, methods of making, and methods of use thereof.

BACKGROUND

[003] Consumer demand for alternatives to animal-based foods, such as meat, eggs, and milk, continues to rise because of growing environmental, health, and ethical concerns associated with the rearing and slaughter of livestock animals. In response to these demands, the food and biotechnology sectors have developed a number of innovative approaches to designing animal-free food products; however, many of these non-animal based alternatives to animal proteins do not fully emulate the sensory and functional properties of animal proteins.

[004] Current difficulties with creating non-animal sourced food products include generating products having the appropriate color and taste profile. As an example, there has been great interest in finding non-animal alternatives to the hemeproteins normally found in meat, such as myoglobin, because these proteins provide a desirable red color and meaty flavor comparable to conventional animal-based foods. The characteristic red color associated with hemeproteins is determined by the protein's redox state, which depends on environmental conditions, such as pH, ionic strength, temperature, and oxygen levels. The problem in isolating hemeproteins from plant sources or producing them using cellular agriculture approaches (e.g., fermentation), is that the resulting isolated hemeproteins are highly susceptible to chemical degradation during storage and utilization and the desired red color and taste profile is lost soon after production. As such, there is a need to develop non-animal based hemeproteins with increased stability, thereby extending their quality and shelf-life while maintaining a color and taste profile that emulates animal-based foods such as muscle-based meat products.

SUMMARY OF THE INVENTION

[005] The present disclosure provides novel compositions suitable for use in food products wherein the compositions herein have improved color stability and reduced protein degradation (e.g., aggregation) over time.

[006] Embodiments of the present disclosure provide compositions for use in a food product comprising one or more purified hemeprotein compositions and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a non-animal source.

[007] In certain embodiments, one or more purified hemeprotein compositions herein may comprise a globin. In some embodiments, one or more purified hemeproteins compositions herein may comprise a leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrucorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytoglobin, or any combination thereof.

[008] In certain embodiments, one or more purified hemeproteins compositions herein may comprise a hemeprotein from a genetically modified non-animal source. In some embodiments, a genetically modified non-animal source herein may comprise a genetically modified plant, a genetically modified bacteria, a genetically modified yeast, or any combination thereof.

[009] In some embodiments the current disclosure comprises hemeprotein composition for use in a food product comprising one or more purified hemeprotein and one or more antioxidants. In some embodiment, the one or more purified hemeprotein comprises a globin. In some aspects, the one or more purified hemeprotein comprise leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrucorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytoglobin, or any combination thereof. In some embodiments, the hemeprotein compositions comprise a purified hemeprotein from a genetically modified source. In some aspects, the source comprises a genetically modified plant, a genetically modified bacteria, a genetically modified yeast, or any combination thereof. In some embodiments, the one or more purified hemeprotein compositions comprise a polypeptide expressed and/or secreted from a source, wherein the source comprises plants, fungi, bacteria, yeasts, algae, archaea, genetically modified plants, genetically modified fungi, genetically modified bacteria, genetically modified yeasts, genetically modified algae, genetically

modified archaea, or any combination thereof. In some aspects, the polynucleotide sequence encoding the hemeprotein is derived from animals, plants, fungi, bacteria, yeasts, algae, archaea, or any combination thereof.

[010] In certain embodiments, one or more antioxidants herein may comprise antioxidant vitamins, polyphenols, or any combination thereof. In some embodiments, one or more antioxidants herein may comprise vitamin C, vitamin E, any derivatives thereof, or any combination thereof. In some embodiments, one or more antioxidants herein may comprise flavonoids or any derivatives thereof. In some embodiments, one or more antioxidants herein may comprise isorhamnetin, kaempferol, myricetin, proanthocyanidins, quercetin, rutin, taxifolin, catechin, gallic catechin, gallic catechin gallate esters, epicatechin, epigallocatechin, epigallocatechin gallate esters, theaflavin, theaflavin gallate esters, thearubigins, or any combination thereof. In some embodiments, one or more antioxidants herein may have a reduction potential less than about 500 mV.

[011] In certain embodiments, compositions herein may comprise a ratio of the total amount by weight of one or more purified hemeproteins to the total amount of one or more antioxidants as about 1:1 to about 30:1, for example about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 21:1, about 22:1, about 23:1, about 24:1, about 25:1, about 26:1, about 27:1, about 28:1, about 29:1, or about 30:1. In certain embodiments, hemeprotein compositions herein may be stable for at least 7 days at about 4°C.

[012] In certain embodiments, hemeprotein compositions herein may comprise one or more hemeproteins herein that may comprise a heme group bound to oxygen, a heme group bound to carbon monoxide, or a combination thereof for at least 7 days. In certain embodiments, hemeprotein composition results in an increase in peak height of the UV-visible absorption spectrum for the heme protein compositions at one or more of about 550 nm and about 582 nm. In some embodiments, the increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm is detectable for at least about 7 days after addition of the one or more antioxidants.

[013] In certain embodiments, the hemeprotein compositions herein may comprise one or more purified hemeproteins from a genetically modified non-animal source and one or more

antioxidants herein that stabilizes the oxidation state of the one or more purified hemeproteins. In some embodiments, hemeprotein compositions herein may comprise one or more purified hemeproteins from a genetically modified non-animal source such as genetically modified plant, a genetically modified bacteria, or a genetically modified yeast, and one or more antioxidants herein that stabilizes the oxidation state of the one or more purified hemeproteins.

[014] In certain embodiments, one or more purified hemeproteins herein may be recombinant hemeproteins produced from a genetically modified non-animal source. In some embodiments, recombinant hemeproteins herein may be encoded from a polynucleotide, wherein the polynucleotide may comprise an nucleic acid sequence from a plant, animal, fungus, or bacteria, encoding a hemeprotein. In some embodiments, the nucleic acid sequence encoding a hemeprotein herein may be derived from a legume. In some embodiments, the nucleic acid sequence encoding a hemeprotein herein may be derived from a leopard, a bovine, or a whale. In some embodiments, recombinant hemeproteins herein may be recombinant globin. In some embodiments, recombinant hemeproteins herein may be recombinant leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytooglobin, or any combination thereof.

[015] In certain embodiments, one or more purified hemeproteins herein may be recombinant hemeproteins produced from a genetically modified yeast source, wherein the recombinant hemeproteins may be encoded from a polynucleotide, wherein the polynucleotide may comprise an endogenous nucleic acid sequence for a hemeprotein derived from a legume, an equine, a leopard, a bovine, a whale, or any combination thereof.

[016] Other embodiments of the present disclosure provide for methods of making any of the hemeprotein compositions disclosed herein. In certain embodiments, methods herein may stabilize an oxidative state of one or more purified hemeproteins from a non-animal source herein by combining the one or more purified hemeproteins with one or more antioxidants. In some embodiments, methods herein may stabilize the visual appearance of the purified hemeproteins for at least 7 days. In some embodiments, methods herein may stabilize the purified hemeproteins from aggregation at pH ranging from about 5 to about 9. In some embodiments, methods herein may stabilize the oxidation state of the purified hemeproteins for at least 7 days. In certain embodiments, methods of making a hemeprotein composition

herein for use in a food product may comprise obtaining one or more purified hemeproteins from a non-animal source and combining the one or more purified hemeproteins with at least one or more antioxidants herein. In some embodiments, methods herein may comprise recombinantly producing the one or more purified hemeproteins from a genetically modified non-animal source. In some embodiments, methods herein may comprise combining hemeproteins herein with about 0.01% to about 10% by weight of the composition of the one or more antioxidants. In some embodiments, methods herein may comprise combining antioxidants herein with about 1% to about 99% by weight of the composition of the one or more purified hemeproteins. In some embodiments, the method herein may comprise combining a comprise combining antioxidants herein with one or more purified hemeproteins wherein the hemeprotein composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more hemeprotein compositions at one or more of about 550 nm and about 582 nm, that is detectable for at least about 7 days after addition of the one or more antioxidants. In some embodiments, methods herein may further comprise combining hemeproteins and antioxidants herein with in a buffer solution, wherein the buffer solution may have a pH ranging from about 5 to about 9.

[017] In certain embodiments, the current disclosure also encompasses composition comprising one or more purified hemeprotein compositions and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a genetically modified non-animal source selected from the group consisting of a genetically modified plant, a genetically modified bacteria, or a genetically modified yeast; and wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm that is detectable for at least about 7 days after addition of the one or more antioxidants. In certain embodiments, the one or more antioxidants has a reduction potential ranging from about 280 mv to about 500 mV. In certain embodiments, the one or more antioxidants is selected from ascorbic acid, quercetin, taxifolin, Trolox, and combinations thereof. In certain embodiments, the composition comprises a weight ratio of the one or more purified hemeprotein to the one or more antioxidants of about 1:1 to about 30:1 (for example about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 21:1, about 22:1, about 23:1, about 24:1, about 25:1, about

26:1, about 27:1, about 28:1, about 29:1, or about 30:1).

[018] In certain embodiments, the hemeprotein of the compositions comprising the hemeprotein composition are encoded from a polynucleotide, wherein the polynucleotide comprises an endogenous nucleic acid sequence for a hemeprotein derived from a plant, animal, fungus, or bacteria source. In certain embodiments the endogenous nucleic acid sequence for the hemeprotein is derived from a legume. In certain embodiments, the endogenous nucleic acid sequence for the hemeprotein is derived from an animal source selected from a group consisting of equine, leopard, bovine, or whale. In certain embodiments, the hemeprotein is a globin selected from a group consisting of leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, and phytooglobin. In certain embodiments, wherein the one or more purified hemeproteins are recombinant hemeproteins produced from a genetically modified yeast source, wherein the recombinant hemeproteins are encoded from a polynucleotide, wherein the polynucleotide comprises an endogenous nucleic acid sequence for a hemeprotein derived from a legume, an equine, a leopard, a bovine, a whale, or any combination thereof. In certain embodiments, the hemeprotein composition comprises a myoglobin and wherein combining the one or more purified hemeprotein compositions with one or more antioxidants causes an increase in the relative amount of oxymyoglobin and carboxymyoglobin to metmyoglobin in the composition. In certain embodiments, the increase in relative amount of oxymyoglobin and carboxymyoglobin to metmyoglobin in the composition is detectable for at least about 7 days after addition of the antioxidant. In certain embodiments, the increase in the relative amount of oxymyoglobin and carboxymyoglobin to metmyoglobin in the composition ranges from about 1.1-fold to about 5-fold.

[019] In certain embodiments the current disclosure encompasses a hemeprotein composition comprising one or more purified hemeprotein and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a genetically modified source selected from the group consisting of a genetically modified plant, a genetically modified bacteria, a genetically modified fungi, or a genetically modified yeast; and wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm that is detectable for at least about 7 days after addition of the one or more

antioxidants.

[020] In some embodiments, the hemeprotein is encoded by a polynucleotide comprising a nucleic acid sequence from a plant, animal, fungus, or bacteria. In some embodiments, the polynucleotide comprises a nucleic acid sequence derived from a legume, wherein the nucleic acid sequence encodes a hemeprotein. In some embodiments, the polynucleotide sequence comprises a nucleic acid sequence derived from a group consisting of an equine, a feline, a bovine, or a whale. In some embodiments, the hemeprotein is a globin selected from a group consisting of leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, and phytooglobin. In some embodiments, the one or more purified hemeproteins are recombinant hemeproteins produced from a genetically modified yeast source, wherein the recombinant hemeproteins are encoded from a polynucleotide, comprising a nucleic acid sequence derived from a legume, an equine, a leopard, a bovine, a whale and encoding a hemeprotein.

[020] In certain embodiments, the current disclosure also encompasses a composition comprising one or more purified hemeprotein compositions and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytooglobin, or any combination thereof; wherein the one or more antioxidants is selected from the group consisting of ascorbic acid, quercetin, taxifolin, Trolox, and combinations thereof; wherein the composition comprises a weight ratio of the one or more purified hemeprotein to the one or more antioxidants of about 1:1 to about 30:1 (for example: about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 21:1, about 22:1, about 23:1, about 24:1, about 25:1, about 26:1, about 27:1, about 28:1, about 29:1, or about 30:1), and wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more hemeprotein compositions at one or more of about 550 nm and about 582 nm, that is detectable for at least about 7 days after addition of the one or more antioxidants.

[021] Other embodiments of the present disclosure include meat substitute (e.g., meat replica) compositions and methods of making thereof. In some embodiments, methods of preparing a meat substitute comprise combining any of the compositions herein with a meat replica matrix.

In some embodiments, methods that comprise combining any of the compositions herein to a meat replica matrix may result in the composition imparting a meat-like (e.g., a beef-like) appearance to the meat substitutes herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[022] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. The following drawings form part of the present specification and are included to further demonstrate certain embodiments of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein. Embodiments of the present inventive concept are illustrated by way of example in which like reference numerals indicate similar elements and in which as follows.

[023] **Fig. 1** depicts a representative graph of zeta-potential versus pH profile of equine heart myoglobin (1 mg/mL) measured by electrophoresis.

[024] **Fig. 2** depicts a representative graph of protein content of equine heart myoglobin (1 mg/mL) at pH 2.5 to 8.5.

[025] **Fig. 3A** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for 0 days.

[026] **Fig. 3B** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for 1 days.

[027] **Fig. 3C** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for 2 days.

[028] **Fig. 3D** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for 3 days.

[029] **Fig. 3E** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for

4 days.

[030] **Fig. 3F** depicts a representative graph of absorption spectra and representative image of visual appearance of equine heart myoglobin solutions (~1 mg/mL) stored at 4°C for 5 days.

[031] **Fig. 4A** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions samples stored at 4°C in combination with (1 mM) Ascorbic Acid.

[032] **Fig. 4B** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution samples stored at 4°C in combination with (1 mM) Ascorbic Acid.

[033] **Fig. 4C** are a series of representative images of visual appearance over time (from day 0 to day 27 as indicated) of equine heart myoglobin solutions samples stored at 4°C in combination with (1 mM) Ascorbic Acid.

[034] **Fig. 4D** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions samples stored at 4°C in combination with (1 mM) Quercetin.

[035] **Fig. 4E** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution samples stored at 4°C in combination with (1 mM) Quercetin.

[036] **Fig. 4F** are a series of representative images of visual appearance over time (from day 0 to day 27 as indicated) of equine heart myoglobin solutionssamples stored at 4°C in combination with (1 mM) Quercetin.

[037] **Fig. 4G** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with (1 mM) Epigallocatechin gallate (EGCG).

[038] **Fig. 4H** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution samples stored at 4°C in combination with (1 mM) EGCG.

[039] **Fig. 4I** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions samples stored at 4°C in combination with (1 mM) Taxifolin.

[040] **Fig. 4J** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution samples stored at 4°C in combination with (1 mM) Taxifolin.

[041] **Fig. 4K** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions samples stored at 4°C in combination with (1 mM) 4-Methylcatechol.

[042] **Fig. 4L** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution samples stored at 4°C in combination with (1 mM) 4-Methylcatechol.

[043] **Fig. 4M** are a series of representative images of visual appearance over time (from day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with antioxidants EGCG, Taxifolin and Methylcatechol respectively.

[044] **Fig. 4N** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with Trolox.

[045] **Fig. 4O** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution (1 mM) samples stored at 4°C in combination with Trolox.

[046] **Fig. 4P** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with Caffeic Acid.

[047] **Fig. 4Q** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution (1 mM) samples stored at 4°C in combination with Caffeic Acid.

[048] **Fig. 4R** depicts a representative graph of absorption spectra (from day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with Gallic Acid.

[049] **Fig. 4S** depicts a representative graph of absorption spectra intensity (at 544 nm and 582 nm) over time (from day 0 to day 27 as indicated) of equine heart myoglobin solution (1 mM) samples stored at 4°C in combination with Gallic Acid.

[050] **Fig. 4T** are a series of representative images of visual appearance over time (from

day 0 to day 27 as indicated) of equine heart myoglobin solutions (1 mM) samples stored at 4°C in combination with antioxidants Trolox, Caffeic Acid and Gallic Acid respectively.

[051] **Fig. 5A** depict representative graphs of zeta potential of recombinant leopard, bovine, whale, and soy hemeprotein samples in solutions having a pH of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5.

[052] **Fig. 5B** are a series of representative images of visual appearance of recombinant leopard, bovine, whale, and soy hemeprotein samples in solutions having a pH of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5.

[053] **Fig. 6** depicts a representative graph showing the impact of pH on the solubility of myoglobin solutions (0.5 mg/mL) produced by cellular agriculture.

[054] **Fig. 7A** depicts representative graphs of absorption spectra of a 1 mg/mL solution of recombinant hemeprotein from *Panthera pardus* (leopard) stored at 4°C for 27 days.

[055] **Fig. 7B** depicts representative graphs of absorption spectra of a 5mg/mL solution of recombinant hemeprotein from *Panthera pardus* (leopard) stored at 4°C for 27 days.

[056] **Fig. 7C** are a series of representative images of visual appearance of 1 mg/mL and 5mg/mL solutions of recombinant hemeprotein from *Panthera pardus* (leopard) stored at 4°C for 27 days.

[057] **Fig. 7D** depicts representative graphs of absorption spectra of a 1 mg/mL solution of recombinant hemeprotein from *Bos taurus* (bovine) stored at 4°C for 27 days.

[058] **Fig. 7E** depicts representative graphs of absorption spectra of a 5mg/mL solution of recombinant hemeprotein from *Bos taurus* (bovine) stored at 4°C for 27 days.

[059] **Fig. 7F** are a series of representative images of visual appearance of 1 mg/mL and 5mg/mL solutions of recombinant hemeprotein from *Bos taurus* (bovine) stored at 4°C for 27 days.

[060] **Fig. 7G** depicts representative graphs of absorption spectra of a 1 mg/mL solution of recombinant hemeprotein from *Physeter macrocephalus* (sperm whale) stored at 4°C for 27 days.

[061] **Fig. 7H** depicts representative graphs of absorption spectra of a 5mg/mL solution of recombinant hemeprotein from *Physeter macrocephalus* (sperm whale) stored at 4°C for 27 days.

[062] **Fig. 7I** are a series of representative images of visual appearance of 1 mg/mL and

5mg/mL solutions of recombinant hemeprotein from *Physeter macrocephalus* (sperm whale) stored at 4°C for 27 days.

[063] **Fig. 7J** depicts representative graphs of absorption spectra of a 1 mg/mL solution of recombinant hemeprotein from soy leghemoglobin stored at 4°C for 27 days.

[064] **Fig. 7K** depicts representative graphs of absorption spectra of a 5mg/mL solution of recombinant hemeprotein from soy leghemoglobin stored at 4°C for 27 days.

[065] **Fig. 7L** are a series of representative images of visual appearance of 1 mg/mL and 5mg/mL solutions of recombinant hemeprotein from soy leghemoglobin stored at 4°C for 27 days.

DETAILED DESCRIPTION

[066] The following detailed description references the accompanying drawings that illustrate various embodiments of the present inventive concept. The drawings and description are intended to describe embodiments and embodiments of the present inventive concept in sufficient detail to enable those skilled in the art to practice the present inventive concept. Other components can be utilized and changes can be made without departing from the scope of the present inventive concept. The following description is, therefore, not to be taken in a limiting sense. The scope of the present inventive concept is defined only by the appended claims, along with the full scope of equivalents to which such claims are entitled.

I. Terminology

[067] The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. For example, the use of a singular term, such as, “a” is not intended as limiting of the number of items. Also, the use of relational terms such as, but not limited to, “top,” “bottom,” “left,” “right,” “upper,” “lower,” “down,” “up,” and “side,” are used in the description for clarity in specific reference to the figures and are not intended to limit the scope of the present inventive concept or the appended claims.

[068] Further, as the present inventive concept is susceptible to embodiments of many different forms, it is intended that the present disclosure be considered as an example of the principles of the present inventive concept and not intended to limit the present inventive concept to the specific embodiments shown and described. Any one of the features of the present inventive concept may be used separately or in combination with any other feature. References to the terms “embodiment,” “embodiments,” and/or the like in the description mean

that the feature and/or features being referred to are included in, at least, one aspect of the description. Separate references to the terms “embodiment,” “embodiments,” and/or the like in the description do not necessarily refer to the same embodiment and are also not mutually exclusive unless so stated and/or except as will be readily apparent to those skilled in the art from the description. For example, a feature, structure, process, step, action, or the like described in one embodiment may also be included in other embodiments, but is not necessarily included. Thus, the present inventive concept may include a variety of combinations and/or integrations of the embodiments described herein. Additionally, all embodiments of the present disclosure, as described herein, are not essential for its practice. Likewise, other systems, methods, features, and advantages of the present inventive concept will be, or become, apparent to one with skill in the art upon examination of the figures and the description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present inventive concept, and be encompassed by the claims.

[069] As used herein, the term “about,” can mean relative to the recited value, *e.g.*, amount, dose, temperature, time, percentage, etc., $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$.

[070] The terms “comprising,” “including,” “encompassing” and “having” are used interchangeably in this disclosure. The terms “comprising,” “including,” “encompassing” and “having” mean to include, but not necessarily be limited to the things so described.

[071] The terms “or” and “and/or,” as used herein, are to be interpreted as inclusive or meaning any one or any combination. Therefore, “A, B or C” or “A, B and/or C” mean any of the following: “A,” “B” or “C”; “A and B”; “A and C”; “B and C”; “A, B and C.” An exception to this definition will occur only when a combination of elements, functions, steps or acts are in some way inherently mutually exclusive.

[072] The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles,

orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)).

[073] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

[074] As used herein, “recombinant” refers to a cell, nucleic acid, protein, or vector, which has been modified due to the introduction of an exogenous nucleic acid or the alteration of a native nucleic acid. The nucleic acid can be of genomic, cDNA, semisynthetic, and/or synthetic origin, which, by virtue of its origin or manipulation, is not associated with all or a portion of the polynucleotide with which it is associated in nature.

[075] “Transformation” refers to the transfer of a nucleic acid fragment into a host organism or the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “recombinant”, “transgenic” or “transformed” organisms. Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. Typically, expression vectors include, for example, one or more cloned genes under the transcriptional control of 5' and 3' regulatory sequences and a selectable marker. Such vectors also can contain a promoter regulatory region

(e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or location-specific expression), a transcription initiation start site, a ribosome binding site, a transcription termination site, and/or a polyadenylation signal.

[076] “Stabilization” or “stabilized” in the context of hemeprotein compositions provided herein, refers to a composition wherein the combination of hemeprotein with an antioxidant causes a lasting increase in the levels of oxygenated hemeprotein or carboxy hemeprotein, as indicated by a change in visual appearance to a more red composition, a change in the oxidation state (from Met state to oxygen or Carbon monoxide bound state), or a change in UV-Visible spectra such that the peak height at 550 nm (corresponding to CarboxyMb), and/or at 582nm (corresponding to OxyMb) increases in comparison to a composition without antioxidant. In some aspects, the increase in the peak height can be detected within 1-48 hours of combining the antioxidant with the hemeprotein. This period between adding the antioxidant and a detectible increase in the peak height is referred to as the lag-time or lag-phase. In some aspects, depending on the antioxidant used and the storage conditions, the increase in peak height can last or is “stably” maintained for about 0.5 days to about 90 days, though in some embodiments, the absolute peak height may decrease through this stable period.

[077] “Cellular Agriculture” is a method of producing animal products from cell culture, rather than animals using a combination of biotechnology, tissue engineering, molecular biology, and synthetic biology to create and design new methods of producing proteins, fats, and tissues that would otherwise come from traditional agriculture. In the context of the current application, in some aspects, hemeproteins can be sourced and purified from cellular agriculture.

[078] As used herein, a polynucleotide encoding a hemeprotein “derived from” or which is a “derivative of” an endogenous polynucleotide refers to a polynucleotide related to the endogenous polynucleotide by sequence. In some aspects the polynucleotide may be a variant or comprise a fragment of the endogenous polynucleotide and may comprise mutations, insertions, deletions, truncations, modifications, or combinations thereof compared to an endogenous polynucleotide. In some aspects, the polynucleotide may comprise a nucleic acid sequence at least about 60% identical to an endogenous polynucleotide or a fragment thereof, encoding a hemeprotein.

[079] As used herein, the term “source” refers to an organism that comprises a polynucleotide sequence encoding an endogenous or a recombinant hemeprotein which can be

purified for use in the compositions and methods disclosed herein. In some aspects, a source can comprise a polynucleotide sequence that encodes an endogenous hemeprotein, for example *Glycine max* (soybean) comprises a polynucleotide sequence encoding soy leghemoglobin which can be purified and used in the compositions and methods disclosed herein. In some aspects, the source may be a genetically modified source. As used herein, the term “genetically modified source” refers to a recombinant organism for example a genetically modified plant, genetically modified fungi, genetically modified bacteria, genetically modified yeast, genetically modified algae, genetically modified archaea comprising a polynucleotide sequence encoding a hemeprotein and from which the hemeprotein can be purified for use in the compositions and methods of the current disclosure. In some aspects, the recombinant organism comprises a polynucleotide sequence that is at least about 60% identical to an endogenous polynucleotide or a fragment thereof from a plant or at least about 60% identical to an endogenous polynucleotide or a fragment thereof from a bovine, equine, feline or whale.

[080] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

II. Compositions

[081] The present disclosure provides for hemeprotein compositions suitable for use in food products having improved color stability and reduced protein degradation over time. In some embodiments, the current disclosure results from the surprising result that addition of certain antioxidants to these compositions greatly increases the desirable characteristics of the hemeproteins. In some embodiments, compositions herein may have one or more purified hemeproteins and one or more antioxidants.

A. Hemeproteins

[082] In certain embodiments, hemeprotein compositions suitable for use in food products herein may have one or more purified hemeproteins. As used herein, the term “hemeprotein” includes any polypeptide that can covalently or noncovalently bind to a heme moiety. In some embodiments, hemeproteins herein may be a monomer (i.e., a single polypeptide chain), a dimer, a trimer, tetramer, a higher order oligomer, or any combination thereof.

[083] In some embodiments, hemeproteins herein may be a globin. Non-limiting

examples of globins that can covalently or noncovalently bind to a heme moiety for use herein can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, an erythrocrurin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a histoglobin, a neuroglobin, a chlorocruorin, a truncated hemoglobin (e.g., HbN, HbO, a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3)), a cytochrome, or a peroxidase. In accordance with certain embodiments herein, globins may have a globin fold having a series of about seven to about nine alpha helices. In accordance with certain embodiments herein, globins may be of any class (e.g., class I, class II, or class III). In accordance with certain embodiments herein, globins may transport and/or store oxygen.

[084] In some embodiments, hemeproteins herein may have an oxygenated Fe^+ state similar to that of globin (e.g., myoglobin). In some embodiments, hemeproteins herein may have an oxygenated Fe (iron)⁺ state higher than globin (e.g., myoglobin). In some embodiments, hemeproteins herein may have an oxygenated Fe^+ state about 10%, 20%, 30%, 40%, 50%, 100% or higher than globin (e.g., myoglobin). In some embodiments, hemeproteins herein may be similar to oxymyoglobin. As used herein “oxymyoglobin” refers to the oxygenated form of myoglobin which is a single chain globular protein.

[085] In some embodiments, hemeproteins herein may be a non-symbiotic hemoglobin, a leghemoglobin, a chlorocruorin, an erythrocrurin, a protoglobin, a cytochrome, a cyanoglobin, a flavohemoglobin, a myoglobin, a phytoglobin, or any combination thereof.

[086] In some embodiments, hemeproteins herein may be derived from non-animal sources. Non-limiting examples of non-animal sources include plants, fungi, bacteria, yeasts, algae, archaea, genetically modified organisms such as genetically modified bacteria, plants, or yeast, chemical or in vitro synthesis. In some embodiments, hemeproteins herein may be a polypeptide derived from non-animal sources. In some embodiments, hemeproteins herein may be a polypeptide expressed and/or secreted from a non-animal source. In some embodiments, hemeproteins herein may be a polypeptide expressed and/or secreted from a non-animal source wherein the polypeptide may be encoded from a polynucleotide derived from animals, plants, fungi, bacteria, yeasts, algae, archaea, or any combination thereof.

[087] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a “wild-type” source. A wild-type source of hemeproteins herein may be mammals, fish, birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, bacteria, or any combination thereof.

[088] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a mammal belonging to any of the 27 orders of mammalian species, the orders including: Afrosoricida; Carnivora; Cetartiodactyla; Chiroptera; Cingulata; Dasyuromorphia; Dermoptera; Didelphimorphia; Diprotodontia; Eulipotyphla; Hyracoidea; Lagomorpha; Macroscelidea; Microbiotheria; Monotremata; Notoryctemorphia; Paucituberculata; Peramelemorphia; Perissodactyla; Pholidota; Pilosa; Primates; Proboscidea; Rodentia; Scandentia; Sirenia; and Tubulidentata.

[089] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from human, non-human primate (e.g., gibbon, rhesus macaque, bonobo, chimpanzee, gorilla, orangutan, lemur, loris, tarsier), bovine (e.g., cow, zebu, bison, water buffalo, African buffalo, antelopes), ovine, caprine, camelid, canine (e.g., domestic dog, wolves, coyotes, jackals, foxes), cetacean (e.g., whales, dolphins, porpoises), feline (e.g., domestic cat, tiger, lion, cheetah, leopard, jaguar, bobcat, caracal, margay, oncilla, cougar, serval, ocelot, lynx, puma), equine (e.g., horses, donkeys, mules, zebras), marsupial, or from any other mammal of interest. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a mammal such as a cow, goat, sheep, horse, pig, ox, mule, rabbit, yak, llama, camel, deer, cat, dog, bear, or any combination thereof.

[090] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a bird. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from Anseriformes (e.g., ducks, swans, geese), Falconiformes (e.g., falcons, eagles, hawks), Galliformes (e.g., chickens, turkeys, pheasants), Struthioniformes (e.g., emus, ostriches, kiwis), Passeriformes (e.g., perching birds and songbirds such as sparrows, larks, crows, swallows, and the like), Sphenisciformes (e.g., penguins), Pelecaniformes (e.g., Ibis, herons, pelicans), Strigiformes (e.g., owls), Gaviiformes (e.g., loons), Gruiformes (e.g., terrestrial, marsh birds), or any combination thereof.

[091] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a fish. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a Scombridae (e.g., tuna), Salmonidae (e.g., salmon), Gadidae (e.g., cod, haddock), Clupeidae (e.g., herrings, shads, sardines, hilsa, menhadens), Engraulidae (e.g., anchovies), and the like. In some

embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from shrimp, oysters, clams, mussels, and the like.

[092] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from a plant. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from *Nicotiana tabacum* or *Nicotiana glauca* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* sps. (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* sps. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium* sp. (quinoa); *Sesamum* sp. (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley).

[093] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from fungi. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, or *Fusarium oxysporum*.

[094] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from bacteria. In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from *Escherichia coli*, *Bacillus subtilis*, *Synechocystis* sp., *Aquifex aeolicus*, *Methylobacterium extorquens*, or thermophilic bacteria such as *Thermophilus*.

[095] In some embodiments, hemeproteins herein may be isolated from or may be encoded from a polynucleotide derived from non-symbiotic hemoglobin. In some embodiments, hemeproteins herein may be non-symbiotic hemoglobins isolated from or encoded from a polynucleotide derived from soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, rice, or any combination thereof.

[096] In some embodiments, hemeproteins described herein may have an amino acid sequence corresponding to a wild-type hemeprotein, fragments, truncations, variants or fusions thereof that contain a heme-binding motif. One of skill in the art will appreciate that the amino acid sequences of any of the wild-type hemeproteins contemplated herein can be found in sequence databases such as, but not limited to, the UniProtKB/Swiss-Prot database and the Heme Protein Database. In some embodiments, hemeproteins described herein may have an amino acid sequence with at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence corresponding to a wild-type hemeprotein, fragments, truncations, variants or fusions thereof that contain a heme-binding motif. One of skill in the art will appreciate how to determine the percent identity between two amino acid sequences using methods known in the art. Such methods include, but are not limited to, use of a BLAST 2 Sequences (BL2seq) program provided by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the like.

[097] In certain embodiments, hemeproteins herein may be from a genetically modified non-animal source. In accordance with certain embodiments herein, a genetically modified non-animal source may be a genetically modified plant, a genetically modified bacteria, a genetically modified yeast, or any combination thereof. In some embodiments, hemeproteins herein may be recombinant hemeproteins. As used herein “recombinant hemeproteins” refers to hemeproteins recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast, plant cells such as tobacco, soybean, or *Arabidopsis*, or mammalian cells). In some embodiments, recombinant hemeproteins herein may be a polypeptide encoded from a polynucleotide, wherein the polynucleotide may have an endogenous (i.e., wild-type) nucleic acid sequence for a hemeprotein derived from a plant, animal, fish, bird, fungus, or bacteria source as described herein. In accordance with certain embodiments herein, an endogenous nucleic acid sequence for the hemeprotein may be derived from a plant. In accordance with certain embodiments herein, an endogenous nucleic acid sequence for the hemeprotein may be derived from a legume. In accordance with certain embodiments herein, an endogenous nucleic acid sequence for the hemeprotein may be derived from a mammal. In accordance with certain embodiments herein, an endogenous nucleic acid sequence for the hemeprotein may be derived from an equine, feline (e.g., leopard), bovine, or cetacean (e.g., whale).

[098] In some embodiments, standard polypeptide synthesis techniques (e.g., liquid-phase

polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) may be used to produce any of the recombinant hemeproteins herein. In some embodiments, in vitro transcription-translation techniques may be used to produce any of the recombinant hemeproteins herein

[0099] In some embodiments, recombinant hemeproteins may be expressed by a microbial expression system. In some embodiments, microbial expression systems for use herein may comprise at least one expression vector. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of recombinant proteins are well known to those skilled in the art, any of which may be used to produce the any one of the gene products (e.g., recombinant hemeproteins) of the polynucleotides disclosed herein. Vectors or cassettes useful for the transformation of suitable host cells are well known in the art. "Expression vector" or "expression construct" or "plasmid" or "recombinant DNA construct" refers to a vehicle for introducing a nucleic acid into a host cell. A nucleic acid for use herein can be one that has been generated via human intervention, including by recombinant means or direct chemical synthesis, with a series of specified nucleic acid elements that permit transcription and/or translation of a particular nucleic acid. In some embodiments, expression vectors for use herein can be part of a plasmid, virus, or nucleic acid fragment, or other suitable vehicle. In some embodiments, expression vectors for use herein may further include a nucleic acid to be transcribed operably linked to a promoter.

[0100] In accordance with certain embodiments herein, vectors comprising polynucleotides disclosed herein can be introduced into appropriate microorganisms (i.e., host cells) via transformation techniques to provide high-level expression of the recombinant hemeproteins for use herein. Expression of a polypeptide (e.g., recombinant hemeprotein) of the disclosure may include transient expression and/or constitutive expression (e.g., developing of a stable cell line) in a suitable host cell. Host cells herein may be transformed by any suitable technique including, e.g., biolistics, electroporation, glass bead transformation and silicon carbide whisker transformation. Any convenient technique for introducing a transgene into a microorganism can be employed in the present invention. Transformation can be achieved by, for example, the method of D. M. Morrison (Methods in Enzymology 68, 326 (1979)), the method by increasing permeability of recipient cells for DNA with calcium chloride (Mandel, M. and Higa, A., J. Mol. Biol., 53, 159 (1970)), or the like.

[0101] In some embodiments, a suitable host cell for production of recombinant

hemeproteins herein may be from a genetically modified organism. In certain embodiments, a genetically modified organism herein may be a bacterium, a yeast, a fungus, an algae, a mammalian cell, an insect cell, or any combination thereof. In some embodiments, a suitable host cell for production of recombinant hemeproteins herein may be from a genetically modified plant, a genetically modified bacteria, and/or a genetically modified yeast. In certain embodiments, a genetically engineered organism suitable for production of recombinant hemeproteins herein may be *Acetobacter*, *Acinetobacter calcoaceticus*, *Alcaligenes eutropha*, *Arxula adeninivorans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aurantiochytrium* spp., *Bacillus licheniformis*, *Bacillus methanolicus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Candida utilis*, *Chlamydomonas reinhardtii*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Corynebacterium glutamicum*, *Escherichia coli*, *Hansenula polymorpha*, *Isochrysis* spp., *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Lactococcus lactis*, *Micrococcus lysodeikticus*, *Nannochloropsis* spp., *Ogataea*, *Paracoccus denitrificans*, *Pavlova* spp., *Penicillium chrysogenum*, *Pichia guilliermondii*, *Pichia pastoris*, *Pichia stipitis*, *Pseudomonas putida*, *Rhizopus* spp., *Rhodopiridium* spp., *Rhodotorula* spp., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Streptococcus lactis*, *Streptomyces*, *Synechococcus elongatus*, *Tetraselmis* spp., *Thermoanaerobacter* spp., *Thermoanaerobacterium* spp., *Trichoderma reesei*, *Xanthaomonas campestris*, and/or *Yarrowia lipolytica*.

[0102] In some embodiments, following introduction of a polynucleotide comprising the coding sequence for a hemeprotein of the disclosure, a host cell may be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, and/or amplifying expression of a polypeptide-encoding polynucleotide. In some embodiments, following the introduction of a polynucleotide comprising the coding sequence for a hemeprotein of the disclosure, a host cell may be cultured by fermentation. Culturing may be accomplished in a growth medium having one or more supplements to aid in culture growth including, but not limited to, aqueous mineral salts medium, organic growth factors, carbon and/or energy source material, molecular oxygen, and the like. In some embodiments, polypeptides (e.g., hemeproteins) may be recovered from the culture (e.g., by centrifugation, purification, etc.), and purified as described herein.

[0103] In certain embodiments, hemeproteins for use herein may be purified hemeproteins. As used herein, the term “purified” refers to a polypeptide or protein (e.g., a hemeprotein) that

has been separated from other components of the source material (e.g., other animal, fish, plant, fungal, algal, bacterial, genetically modified plant, genetically modified bacteria, or genetically modified yeast proteins). In some embodiments, purified hemeproteins herein may be free of least about 2% to about 100% (e.g., about 5%, 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%) of the other components of the source material. Hemeproteins herein can be purified using methods of protein separation known in the art, including but not limited to, size exclusion chromatography, affinity chromatography, anion exchange chromatography, cation exchange chromatography, ultrafiltration through membranes, or density centrifugation, isoelectric precipitation, ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents, and solvent extraction.

B. Antioxidants

[0104] In certain embodiments, compositions suitable for use in food products herein may have one or more purified hemeproteins and one or more antioxidants. As used herein, “antioxidants” are agents that inhibit oxidation and thus can be used to prevent the deterioration of preparations by the oxidative process. In some embodiments, antioxidants suitable for use herein may be an antioxidant vitamin, a polyphenol, or any combination thereof.

[0105] In some embodiments, an antioxidant herein may be a naturally occurring or a synthetic form of a vitamin having antioxidant properties. In some embodiments, an antioxidant vitamin may be vitamin C, a derivative thereof, and/or an analogue thereof. In accordance with certain embodiments herein, an antioxidant vitamin may be ascorbic acid, L-ascorbic acid, ethylated L-ascorbic acid, vitamin C, or of the erythorbic acid isomer thereof, or salts or esters thereof. In some embodiments, an antioxidant vitamin may be vitamin E, a derivative thereof, and/or an analogue thereof. Vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols. In accordance with certain embodiments herein, an antioxidant vitamin may be alpha-Tocopherol, beta-Tocopherol, gamma-Tocopherol, delta-Tocopherol, Tocopheryl acetate, RRR-alpha-tocopherol, SSR-alpha-tocopherol, alpha-tocotrienol, vitamin E, or salts or esters thereof.

[0106] In some embodiments, an antioxidant herein may be a naturally occurring or a synthetic form of a polyphenol having antioxidant properties. Polyphenols are common constituents of foods of plant origin and contribute the major antioxidants found in diets. The main dietary sources of polyphenols include, but are not limited to, fruits, vegetables, and

beverages (e.g., coffee). Non-limiting examples of antioxidants are polyphenolic compounds, chlorogenic acids, flavonoids, tocopherols, di- or tri-carboxylic acids (such as citric acid), EDTA (ethylenediaminetetraacetic acid), ascorbic acid (vitamin C), anthocyanins, catechins, quercetin, resveratrol, rosmarinic acid, carnosol, Maillard reaction products, enzymes such as superoxide dismutase, certain proteins, amino acids, and protein hydrolyzates, etc. Several thousand different polyphenols have been identified in foods. In some embodiments, antioxidant polyphenols herein may be a naturally occurring or a synthetic form of a flavonoid having antioxidant properties. Non-limiting examples flavonoids include quercetin (found in onion, tea, apple), catechin (tea, fruit), hesperidin (citrus fruits), and cyanidin (red fruits). In some embodiments, antioxidant flavonoids herein may be isorhamnetin, kaempferol, myricetin, proanthocyanidins, quercetin, rutin, taxifolin, catechin, gallic acid, gallic acid gallate esters, epicatechin, epigallocatechin, epigallocatechin gallate esters, theaflavin, theaflavin gallate esters, thearubigins, or any combination thereof. In some embodiments, antioxidant polyphenols herein may be a naturally occurring or a synthetic form of a phenolic acids having antioxidant properties. A non-limiting example of a phenolic acid includes caffeic acid which is present in many fruits and vegetables. Caffeic acid, most often esterified with quinic acid as in chlorogenic acid, is the major phenolic compound in coffee.

[0107] In some embodiments, an antioxidant herein may be ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate and sodium metabisulfite and other materials known to one of ordinary skill in the art.

[0108] In some preferred embodiments, an antioxidant herein may be ascorbic acid, quercetin, epigallocatechin gallate, EGCG, trolox, taxifolin, 4-methycatechol, caffeic acid, gallic acid, or any combination thereof.

[0109] In some embodiments, antioxidants for use herein may have one or more characteristics that impart a desirable property to the compositions described herein. In some embodiments, antioxidants for use herein may have a strong reducing potential. Standard reduction potential describes the ability of a compound to accept electrons. In some embodiments, antioxidants for use herein may have reduction potential less than about 500 mV (e.g., about 0.5, 1, 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 mV). In some embodiments, antioxidants for use herein may have

reduction potential less than about 200, or 250, or 300, or 350, or 400, or 450, or 500 mV.

C. Hemeprotein Compositions

[0110] In some embodiments, hemeprotein compositions as described herein may include one or more purified hemeproteins. In some embodiments, the compositions described herein may comprise about 1% to about 99% (e.g., about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%) by weight of the composition of one or more purified hemeprotein compositions, as disclosed herein. As used herein, a “hemeprotein composition” includes the hemeprotein hydrated or in solution. In certain embodiments, the hemeprotein content can be calculated on a dry basis, meaning the hemeprotein content and concentration is determined with the liquid from the hemeprotein composition removed. In such embodiments, the compositions described herein may comprise about 1% to about 99% (e.g., about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%) by weight of the composition of one or more purified hemeproteins on a dry weight basis.

[0111] In some embodiments, the compositions described herein may comprise about 0.01% to about 10% (e.g., about 0.01%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%) by weight of the composition of one or more antioxidants disclosed herein. In some embodiments, compositions herein may comprise about 1% to about 99% (e.g., about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%) by weight of the composition of one or more purified hemeproteins disclosed herein and about 0.01% to about 10% (e.g., about 0.01%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%) by weight of the composition of one or more antioxidants disclosed herein. In some embodiments, compositions herein may comprise a ratio of total hemeprotein amount to total antioxidant amount. In some embodiments, compositions herein may have a weight ratio of total hemeprotein composition content to antioxidant content ranging from about 1:1 to about 30:1. In certain embodiments, the weight ratio of total hemeprotein content to total antioxidant content is about 1:1 to about 30:1, for example about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 21:1, about 22:1, about 23:1, about 24:1, about 25:1, about 26:1, about 27:1, about 28:1, about 29:1, or about 30:1..

[0112] In certain embodiments, the composition comprises a leghemoglobin and one or more antioxidants. In some embodiments, the composition comprises a soy leghemoglobin and one or more antioxidant selected from any one of Quercetin, Ascorbic acid, EGCG, Trolox or Taxifolin. In some embodiments, the compositions herein may have a weight ratio of total soy leghemoglobin content to total antioxidant content of about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, or about 20:1. In some additional embodiments, the composition comprises a myoglobin and one or more antioxidants. In some embodiments, the composition comprises one or more myoglobin selected from an equine, bovine, feline (for example leopard) or whale (for example, sperm whale) and one or more antioxidant selected from any one of Quercetin, Ascorbic acid, EGCG, Trolox or Taxifolin. In some embodiments, the compositions herein may have a weight ratio of total soy leghemoglobin content to total antioxidant content of about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, or about 20:1.

[0113] In some embodiments, hemeprotein compositions herein may have one or more purified hemeproteins as disclosed herein and one or more antioxidants as disclosed herein in a buffer solution. A buffer solution for use herein may be any solution suitable for use in a food product. In some embodiments, hemeprotein compositions herein may also include a buffer solution having one or more buffering agents wherein “buffering agents” are compounds used to resist change in pH upon dilution or addition of acid or alkali. Buffering agents for use herein can include, by way of example and without limitation, potassium metaphosphate, potassium phosphate, monobasic sodium acetate and sodium citrate anhydrous and dihydrate and other materials known to one of ordinary skill in the art. In some embodiments, any food-grade organic or inorganic buffer can be used. In some embodiments, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more buffering agents by total weight of the composition. In some embodiments, the amount of one or more buffering agents may depend on the desired pH level of compositions herein. In some embodiments, buffer solutions herein may have a pH ranging from about 5 to about 9 (e.g., about 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.4, 8.5, 9). In some embodiments, compositions disclosed herein may have a pH

ranging from about 4 to about 9 (e.g., about 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9).

[0114] In some embodiments, the hemeprotein compositions herein may comprise additional components for example, binding agents, flavor enhancers, oligosaccharides, stabilizing agents, pH regulators, preservatives, non-heme proteins, dietary fibers, gelling agents, surfactants, water, fats, oils emulsifiers, starches, coloring agents and combinations thereof. In some embodiments, the additional components may include for example, one or more of, glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, sunflower oil, canola oil, olive oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, vitamins, dietary fibers like vegetable fibers from carrots, bamboo, peas, broccoli, potatoes, sweet potatoes, corn, whole grains, alfalfa, collard greens, celery, celery root, parsley, cabbage, squash, green beans, common beans, black beans, red beans, white beans, beets, cauliflower, nuts, apple peels, oats, wheat or plantain, or mixtures thereof, onion flavor, garlic flavor, or herb flavors, basil, celery leaves, chervil, chives, cilantro, parsley, oregano, tarragon, thyme, spice extracts, spice oils, natural smoke solutions, natural smoke extracts, yeast extract, and shiitake extract.

[0115] In some embodiments, the hemeprotein compositions herein comprising one or more of the antioxidants described herein and one or more of the hemeproteins described herein can be formulated into for example, liquids, gels, pastes, sauces, powder or cubes, ingredients of flavor packets, seasoning packets or shakers. In some embodiments, the compositions herein may be formulated into or added to, for example, soup or stew bases, bouillon or broths.

D. Characteristics of Hemeprotein Compositions

[0116] The present disclosure provides for hemeprotein compositions suitable for use in food products having improved color stability and reduced protein degradation over time.

[0117] In certain embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins.

The iron atom in the heme group of a hemeprotein can be in the ferrous (Fe^{2+}) oxidation state to support oxygen and other gases' binding and transport. Hemoglobin in normal red blood cells is protected by a reduction system to stabilize these states. Initial oxidation to the ferric (Fe^{3+}) state without oxygen converts hemoglobin into "methemoglobin" which cannot bind oxygen. For example, the hemeprotein myoglobin can exist in an oxygen bound ferrous (Fe^{2+}) state referred herein as Oxy-myoglobin (OxyMb), in a deoxygenated state referred herein as Deoxy-myoglobin (DeoxyMb), a state bound to carbon monoxide referred herein as Carboxy-myoglobin (CarboxyMb) or in the ferric state referred to as Met-myoglobin (MetMb). Changes in the oxidative states of hemeprotein can be determined from the UV-visible absorption spectra, for example of a solution comprising the composition disclosed herein. For example, the absorption spectra may comprise peaks consistent with CarboxyMb (550 nm), OxyMb (582 nm), and MetMb (503 nm and 632 nm).

[0118] In some embodiments, change in the oxidative states can also result in change in the appearance of the solution comprising the composition disclosed herein. For example, a solution comprising higher amounts of CarboxyMb or OxyMb is characterized by a brighter red color, while MetMb results in a more undesirable brown coloration. In some embodiments as provided herein, presence of antioxidant results in change and/or stabilization of the oxidative state of the hemeprotein.

[0119] In some embodiments, hemeprotein compositions herein may comprise antioxidants to act as a reduction system for changing and/or stabilization of the oxidation state of the one or more purified hemeproteins. "Stabilization" or "stabilized" in context of hemeprotein compositions provided herein, refers to a composition wherein the combination of hemeprotein with an antioxidant causes a lasting increase in the levels of oxygenated hemeprotein or carboxy hemeprotein, as indicated by a change in appearance to a more red composition, or a change in UV-Visible spectra such that the peak height at 550 nm (corresponding to CarboxyMb), and/or at 582nm (corresponding to OxyMb) increases in comparison to a composition without antioxidant. In some aspects, the increase in the peak height can be detected within 1-48 hours of combining the antioxidant with the hemeprotein. This period between adding the antioxidant and a detectible increase in the peak height is referred to as the lag-time or lag-phase. In some aspects, depending on the antioxidant used and the storage conditions, the increase in peak height can last or is "stably" maintained for about 0.5 days to about 90 days, though in some embodiments, the absolute peak height may decrease

through this stable period. A guidance for some exemplary antioxidants and their impact on peak heights is provided in the Examples included herein.

[0120] In some embodiments, the current disclosure encompasses hemeprotein compositions comprising a hemeprotein and antioxidant, wherein the antioxidant causes an increase in relative amount of oxygenated or carboxygenated hemeprotein compared to the oxidized Met state in the composition. In certain embodiments of the compositions disclosed herein, the hemeprotein is a myoglobin and the antioxidant causes an increase in relative amount of oxymyoglobin to metmyoglobin in the composition as measured by the change in the UV-visible spectrum of a solution of the composition. In some embodiments, the increase in relative amount of oxymyoglobin to metmyoglobin in the composition is detectable at about 0.5 to about 90 days, or at about 0.5 to 1 day, or about 1-10 days or about 10-20 days, or about 20-30 days, or about 30-40 days, or about 40-50 days after addition of the antioxidant and stored at refrigeration temperatures (e.g., about 2°C to about 8°C). In some embodiments, the increase in the relative amount of oxymyoglobin to metmyoglobin in the composition is at least about 1.1 to about 5-fold, or about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 3, about 4 or about 5-fold. In some embodiments the antioxidant causes a change in the UV-visible absorption spectrum with an increase in peak height at about 550 nm and about 582 nm, at about 4 hours to about 50 days, or about 0.5 to about 1 day, or about 1-10 days or about 10-20 days, or about 20-30 days, or about 30-40 days, or about 40-50 days after addition of the antioxidant and stored in refrigeration (e.g., about 2°C to about 8°C).

[0121] In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days. In some embodiments, compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for at least about 7 days (e.g., about 0.5, 1, 2, 3, 4, 5, 6, 7 days).

[0122] In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at temperatures below freezing (e.g., below 0°C). In some embodiments, hemeprotein compositions herein may

comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored in refrigeration (e.g., about 2°C to about 8°C). In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at room temperature (e.g., about 22°C to about 27°C). In some embodiments, compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at about -30°C to about 30°C (e.g., about -30°C, about -20°C, about -10°C, about 0°C, about 10°C, about 20°C, about 30°C, about 40°C). In some embodiments, compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at about 4°C. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 7 days when the hemeprotein composition is stored at about 4°C. In some embodiments, compositions herein may comprise antioxidants for stabilization of the oxidation state of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored sequentially at two or more temperatures ranging from about -30°C, to about 40°C.

[0123] In some embodiments, hemeprotein compositions herein may comprise antioxidants and hemeproteins wherein the heme group of the hemeprotein is bound to oxygen, carbon monoxide, or a combination thereof. In some embodiments, hemeprotein compositions herein may comprise antioxidants and hemeproteins wherein the heme group of the hemeprotein is bound to oxygen, carbon monoxide, or a combination thereof for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days. In some embodiments, hemeprotein compositions herein may comprise antioxidants and hemeproteins wherein the heme group of a higher fraction of

hemeprotein is bound to oxygen, carbon monoxide, or a combination thereof for at least about 0.5 days, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, or at least about 7 days or at least about 8, or at least about 9, or at least about 10, or at least about 11, at least about 12, or at least about 13, or at least about 14, or at least about 15, or at least about 16, or at least about 17, or at least about 18, or at least about 19, or at least about 20 or more days. In some embodiments, compositions herein may comprise antioxidants and hemeproteins wherein the heme group of a higher fraction of the hemeprotein is bound to oxygen, carbon monoxide, or a combination thereof for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at about -30°C to about 40°C (e.g., about -30°C, -20°C, -10°C, 0°C, 10°C, 20°C, 30°C, 40°C).

[0124] In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins. As used herein, a stabilized visual appearance of a hemeprotein herein refers to the appearance of a red composition in visible light. As used herein, a destabilized visual appearance of a hemeprotein herein refers to the appearance of a brown composition in visible light. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins for about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins for at least about 0.5 days, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, or at least about 7 days, or at least about 8, or at least about 9, or at least about 10, or at least about 11, at least about 12, or at least about 13, or at least about 14, or at least about 15, or at least about 16, or at least about 17, or at least about 18, or at least about 19, or at least about 20, or at least about 21, or at least about 22, or at least about 23, or at least about 24, or at least about 25, or at least about 26, or at least about 27, or at least about 28, or at least about 29, or at least about 30 or more days. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins for at least about 0.5 days, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, or at least about 7 days, or at least about 8, or at

least about 9, or at least about 10, or at least about 11, at least about 12, or at least about 13, or at least about 14, or at least about 15, or at least about 16, or at least about 17, or at least about 18, or at least about 19, or at least about 20, or at least about 21, or at least about 22, or at least about 23, or at least about 24, or at least about 25, or at least about 26, or at least about 27, or at least about 28, or at least about 29, or at least about 30 or more days, when the hemeprotein composition is stored at about -30°C to about 40°C (e.g., about -30°C, about -20°C, about -10°C, about 0°C, about 10°C, about 20°C, about 30°C, about 40°C).

[0125] In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins, wherein intensity of the red color of the composition decreases slowly over time compared to compositions only comprising hemeproteins. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins, wherein intensity of the red color of the composition decreases by about 0.5% to about 70% after about 30 days (e.g., about 0.5, about 1, about 5, about 10, about 15, about 20, about 25, about 30 days). In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins, wherein intensity of the red color of the composition decreases in a linear manner per day after making the compositions according to the methods herein. In some embodiments, hemeprotein compositions herein may comprise antioxidants for stabilization of the visual appearance of the one or more purified hemeproteins, wherein intensity of the red color of the composition decreases by about 0.001% to about 0.5% per day after making the compositions according to the methods herein.

[0126] In some embodiments, hemeprotein compositions herein comprising one or more antioxidants herein and one or more hemeproteins herein may have almost no protein degradation of the hemeprotein. In some embodiments, hemeprotein compositions herein comprising one or more antioxidants herein and one or more hemeproteins herein may have about 0.01% to about 15% (e.g., about 0.01%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%) protein degradation of the hemeprotein. In some embodiments, hemeprotein compositions herein comprising one or more antioxidants herein and one or more hemeproteins herein may have about 0.01% to about 15% (e.g., about 0.01%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about

6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%) protein degradation of the hemeprotein after about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days. In some embodiments, hemeprotein compositions herein comprising one or more antioxidants herein and one or more hemeproteins herein may have about 0.01% to about 15% (e.g., (e.g., about 0.01%, about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%)) protein degradation of the hemeprotein after about 1 to about 120 days, about 2 to about 100 days, about 3 to about 80 days, about 4 to about 60 days, or about 7 to about 30 days when the composition is stored at about -30°C to about 40°C (e.g., about -30°C, about -20°C, about -10°C, about 0°C, about 10°C, about 20°C, about 30°C, about 40°C).

III. Methods of Use

[0127] The present disclosure provides for food products and methods of making such food products using the hemeprotein compositions herein. In certain embodiments, hemeprotein compositions herein can be used for producing meat substitute food products (“meat replicas” or “meat analogs”). As used herein, the term “meat replica” or “meat analog” has the same meaning as commonly understood by one of ordinary skill in the art and includes but is not restricted to plant-derived meat, vegan meat, meat substitute, mock meat, meat alternative, imitation meat, vegetarian meat, fake meat or faux meat. The terms “meat replica” or “meat analog” refer to a food product aiming to have a realistic meat-like appearance without containing an animal-based component. In some embodiments, compositions herein can be used as a materials in and in methods of making meat replicas, including, but not limited to ground meat replicas (e.g., ground beef, ground chicken, ground turkey, ground lamb, or ground pork), as well as replicas of cuts of meat and fish.

[0128] In some embodiments, methods of making food products (e.g., meat replicas) may include combining the hemeprotein compositions herein with non-animal-based fat, non-animal-based matrixes, non-animal-based edible fibrous components, or any combination thereof. In some embodiments, methods of making food products (e.g., meat replicas) may include combining the compositions herein with non-animal-based fat, non-animal-based matrixes, non-animal-based edible fibrous components, or any combination thereof wherein

the addition of the composition herein provides a meat-like appearance to the meat substitute. In some embodiments, methods of making food products (e.g., meat replicas) may include combining the compositions herein with non-animal-based fat, non-animal-based matrices, non-animal-based edible fibrous components, or any combination thereof wherein the addition of the composition herein provides a meat-like taste to the meat substitute. In some embodiments, methods of making food products (e.g., meat replicas) may include combining the compositions herein with non-animal-based fat, non-animal-based matrixes, non-animal-based edible fibrous components, or any combination thereof wherein the addition of the composition herein provides a meat-like smell to the meat substitute. In accordance with some embodiments herein, a meat-like appearance, taste, or smell may be a beef-like appearance, taste, or smell, a poultry-like appearance, taste, or smell, a seafood-like appearance, taste, or smell, a game-like appearance, taste, or smell, a pork-like appearance, taste, or smell, a lamb-like appearance, taste, or smell, or any combination thereof.

[0129] In some embodiments, the methods of making food-products may include combining the compositions herein with non-animal-based meat-like, poultry-like, or seafood-like base dough, that can be used in meat replicas sold in a form such as “ground meat”, burgers/patties, or other forms, for example comparable to Impossible® Burger (from Impossible™ Foods), Beyond Burger® (from Beyond Meat®), Veggie Chik Patty® (from Morningstar Farms®), and Plant-Based Patties from Good & Gather™. Other examples of poultry, meat and seafood analog products that may include compositions provided herein include products like Veggie Meal Starters® from Morningstar Farms®, such as Veggie CHIK’N Nugget, Veggie Popcorn CHIK’N, Veggie CHIK’N Strips, Veggie Grillers®, Veggie Buffalo, beef analogue products made by Beyond Meat® products such as Beyond Beef® Crumbles, Beyond Beef® Ground Beef, and Beyond Beef® Sausage, or fish analog products made by Good Catch like salmon burgers, fish sticks, fish fillets, crab cakes, fish burgers and fish cakes.

[0130] In some embodiments, the methods of making food-products may include combining the liquids, gels, pastes, sauces, powder or cubes, ingredients of flavor packets, seasoning packets or shakers, soup or stew bases, bouillon or broths into a food product before, during, or after cooking of the consumable food product. In some embodiments, the compositions herein can be used to modulate the flavor and/or aroma profile for a variety of consumable food products.

[0131] In some embodiments, food products herein (e.g., meat replicas) may comprise about 1% to about 99% (e.g., about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%) of any of the hemeprotein compositions herein by weight. In some embodiments, food products herein (e.g., meat replicas) may comprise about 0.01% to about 99% (e.g., about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%) of any of the antioxidants herein by weight. In some embodiments, food products herein (e.g., meat replicas) may comprise about 1% to about 99% (e.g., about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%) of any of the hemeproteins herein by weight.

EXAMPLES

[0132] The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the present disclosure, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present disclosure.

[0133] The Examples 1-4 below were conducted using the exemplary techniques provided herein for guidance, though equivalent techniques known to those skilled in the art can be used.

Exemplary Techniques

[0134] In exemplary methods as disclosed in Examples 1-4 below, the following techniques were performed as detailed below.

[0135] ***Preparation of equine heart myoglobin solution.*** 50 mg/ml of equine heart myoglobin solution was prepared by dispersing a weighed mass of the protein powder into a buffer solution (2.5 mM sodium phosphate/2.5 mM histidine, pH 9.0). The resulting myoglobin solution was stirred continually for at least 1 hour to ensure the protein was fully dissolved. The conversion of myoglobin to metmyoglobin was carried out according to the method described by Tang et al., *Journal of Food Science*, 69(9) (2004) with some slight modifications. In brief, potassium ferricyanide (5 mg/mL) was added to the myoglobin solution and then the mixture was stirred for 1 hour in an ice bath (4°C). Any residual ferricyanide was then removed from the mixture using a desalting column (Sephadex G-25 PD-10).

[0136] The metmyoglobin (MetMb) was then reduced to oxymyoglobin (OxyMb) by

adding either sodium hydrosulfite or antioxidants: ascorbic acid, caffeic acid, EGCG, gallic acid, quercetin, taxifolin, Trolox, and 4-methylcatechol. The desalted MetMb solution was diluted to 2 mg Mb/mL with buffer solution (2.5 mM sodium phosphate/2.5 mM histidine, pH 9.0) and then reduced by adding either sodium hydrosulfite (0.5 mg/mL) or antioxidant (1 mM) solutions. This was achieved by adding a weighed mass of the reducing agent to the OxyMb solution and mixing well. Any excess sodium hydrosulfite was subsequently removed using a desalting column (Sephadex G-25 PD-10). For addition of the antioxidants to the myoglobin solution (~ 2.0 mg Mb/mL – desalted), non-water-soluble antioxidants (caffeic acid, quercetin, or taxifolin) were first added to ethanol (5 to 10% total volume) prior to adding to the MetMb solutions. The reduction potential and solubility of antioxidants used are listed in **Table 1**. For all systems, the pH of the myoglobin solutions was measured after incubation for at least 2 hours. All samples were stored at 4°C in screw-capped glass vials or 1.5 mL plastic cuvettes with a lid for up to 52 days.

[0137] *Preparation of cellular agriculture hemeprotein solutions.* Powdered hemeprotein samples obtained using a cellular agriculture approach (e.g., fermentation) were used to prepare the protein solutions: bovine, leopard, and sperm whale myoglobin and soy leghemoglobin. Weighed masses of each myoglobin powder were dissolved in aqueous buffer solutions (2.5 mM sodium phosphate/2.5 mM histidine, pH 9.0) to obtain a range of protein concentrations (0.5, 1.0, and 5 mg Mb/mL). The resulting mixtures were then stirred continuously for at least 1 hour to fully dissolve the protein. All solutions were prepared fresh daily or stored at 4°C for a maximum of 1 day, prior to being analyzed.

[0138] *Appearance.* The appearance of the myoglobin samples was captured using a digital camera.

[0139] *Electrical characteristics.* The zeta-potential (ζ -potential) of the protein solutions (1.0 or 5.0 mg/mL) was measured using a particle electrophoresis instrument (Zetasizer Pro, Malvern Instruments Ltd., Worcestershire, UK). The ζ -potential was measured from pH 8.5 to 2.5 by titrating the initial solutions with either acid (0.1 to 0.25 M HCl) or alkaline (0.25 M NaOH) solutions with continuous stirring.

[0140] *Solubility.* The pH-dependence of the solubility of the proteins in the myoglobin solutions was determined by measuring the soluble and total protein concentrations over a range of pH values. The myoglobin solutions (0.5 mg/mL) were stored overnight (4°C) and then the protein concentrations were measured using a Bradford assay kit. The total protein

concentration (c_{total}) was determined by directly analyzing the myoglobin solutions, whereas the soluble protein concentration (c_{soluble}) was determined by centrifuging them at 13,000 rpm for 15 minutes and then analyzing the supernatant. The protein concentration measurements were performed according to the instructions provided with the Bradford assay kit. In brief, 5 μL protein samples (centrifuged or non-centrifuged) were pipetted into the wells of a 96-well micro plate (Costar, Corning Inc., Corning, NY, USA) and then 250 μL of Bradford reagent was added to each well, and the solutions were mixed well. The mixtures were then incubated at room temperature for 5 minutes before being analyzed. The absorbance of the samples was then measured at 595 nm using a UV-visible spectrophotometer (SpectraMax M2e, Sunnyvale, CA, USA). A standard curve was prepared by measuring the absorbance of a series of bovine serum albumin (BSA) solutions with different protein concentrations (0.125 mg/mL to 1 mg/mL), which led to a linear calibration curve with $R^2 = 0.995$. The myoglobin solubility was calculated using the following expression: $\% \text{Solubility} = 100 \times c_{\text{soluble}} / c_{\text{total}}$.

[0141] Absorption spectra measurement. The absorption spectra of myoglobin solutions (1 and 5 mg/mL) were measured from 450 to 750 nm using a UV-visible spectrophotometer (Cary 100, Agilent Technologies, Santa Clara, CA, USA) (Tang et al., 2004). All samples were contained in cuvettes (volume = 1.5 mL; path length = 1 cm) that were stored at 4°C throughout the storage study. The myoglobin redox state proportions (deoxygenated myoglobin (DeoMb), oxygenated myoglobin (OxyMb), and metmyoglobin (MetMb)) in the myoglobin systems containing antioxidant were calculated according to the modified Krzywicki equations by similar to that described in Tang et al., *Journal of Food Science*, 69(9) (2004).

[0142] Computational Modeling. Some insights into the nature of the molecular interactions involved between the antioxidants and the proteins were obtained by carrying out molecular docking analysis for equine heart myoglobin and selected antioxidants. Molecular docking was carried out using Glide software (Version 2019-3, Schrodinger, LLC, New York) to obtain some additional insights into the nature of the binding interactions between the antioxidants and myoglobin. The myoglobin crystal structure (PDB ID 1MNH) was used as a starting configuration and was processed and optimized using the Protein Preparation Wizard, setting PROPKA at pH = 8.0. The 3D structures of four ligands (ascorbic acid, quercetin, gallic acid, , and 4-methylcatechol) were built using Maestro 3D Builder. All the ligand structures were then prepared with LigPrep with OPLS3 force field. Ligand chirality was maintained, and possible states at the target pH 8.0 ± 1.0 were generated. A docking grid was created and

centered on the heme group, which enabled the entire myoglobin to be covered by adjusting the size of ligand diameter midpoint box, and no other constraint was applied. Docking experiments were then performed using ligand docking with extra precision (XP) mode. The number of poses to be reported was not limited, but post-docking minimization was applied with 20 poses per ligand included with strain correction terms. Then, all the reported results were ranked by docking scores, and poses with a docking score above zero were excluded. Only the specific configurations (binding site and binding pose) with the highest Glide docking scores (most negative) in docking experiments are discussed and illustrated in the Results section.

[0143] **Statistical analysis.** For each myoglobin system, replicate analyses were performed and at least triplicate measurements were carried out for each analytical test. The mean and standard deviations of the combined data were calculated from the 6 or more experimental data points using Microsoft Excel 2016.

Example 1. Effect of pH on properties of equine heart myoglobin

[0144] In an exemplary method, an initial characterization of the physical properties, including electrical charge, solubility and stability of an exemplary myoglobin (equine heart myoglobin) was conducted at different pH.

Electrical charge:

[0145] The electrical characteristics of equine heart myoglobin were determined by measuring the pH-dependence of their ζ -potential values. In particular, the isoelectric point (pI) of each protein was determined from the point where their net charge was zero. The isoelectric point of the equine heart myoglobin (1 mg/mL) was around pH 5.5 (**Fig. 1**). Visual observation indicated that a thin sediment layer formed at the bottom of the samples around and below pH 5.5 (data not shown), which can be attributed to aggregation of the protein.

Protein solubility

[0146] The effect of pH on solubility of the equine heart myoglobin was determined by measuring the soluble and total protein contents of the solutions using the Bradford assay. The protein was highly soluble across the entire pH range studied, with the solubility always being greater than 90% (**Fig. 2**).

Example 2. Impact of antioxidant addition on oxymyoglobin stability

[0147] In another exemplary method, the impact of various natural and synthetic antioxidants to convert metmyoglobin to oxymyoglobin in the form of solutions instead of meat

muscle, and thereby improve the color characteristics of myoglobin solutions for application in food product development was examined. The oxygenated form of myoglobin (oxymyoglobin; OxyMb) plays a critical role in determining the desirable bright red color of many meat products. However, it is highly susceptible to oxidation to metmyoglobin (MetMb), which causes a change in color from red to brown.

[0148] The UV-visible absorption spectra of equine heart myoglobin (OxyMb and MetMb) solutions without antioxidant are shown in **Fig. 3A**. The oxymyoglobin spectrum had two distinct absorption peaks around 544 and 582 nm, which are responsible for the bright red color of these solutions. Conversely, the metmyoglobin only had relatively small absorption peaks around 503 and 632 nm, which are responsible for its brown color. The oxymyoglobin solutions only remained bright red for about 1 day before turning brown and exhibiting a UV-visible spectrum similar to that of metmyoglobin. **Figs. 3B-3F** shows the concentration of oxygenated myoglobin decreased gradually over time and metmyoglobin concentration increased to about 100% on day 5.

[0149] Antioxidants with different reduction potential values (E^0) were examined for their potential to convert metmyoglobin to oxymyoglobin, thereby creating a desirable red color in the system. The exemplary antioxidants used herein are provided in **Table 1**.

TABLE 1

Type	Reduction Potential (E^0 , mV)*	LogP / xLogP ³	Solubility in Water (mg/mL or mM)
Ascorbic Acid	282	-1.6 to -2.15	100 mg/mL
Quercetin	330	0.9 to 1.5	Soluble in Ethanol; Water: < 1 mg/mL
Epigallocatechin gallate; EGCG	430	1.2 to 2.984	Soluble in Ethanol; Water: < 10 mM
Trolox	480	1.272	Soluble in Ethanol Water: 0.38 mg/mL
Taxifolin	500	0.803	Soluble in Ethanol; Water: < 1 mg/mL
4-methycatechol	520	1.37	38 mg/mL
Caffeic Acid	534	0.89 to 1.2	Soluble in Ethanol, DMSO Water: <1 mg/mL
Gallic Acid	560	0.7 to 0.96	11.5 mg/mL or 70 mM

[0150] It was found that addition of 1 mM ascorbic acid to the metmyoglobin solutions (~2 mg/mL) converted the MetMb to OxyMb, as seen in the UV-visible absorption and color measurements (**Figs. 4A-4C**). After forming OxyMb and reaching peak concentration (99.9%) at Day 1, the OxyMb portion decreased gradually over 13 days in the presence of the ascorbic acid, compared to only 2 to 3 days in its absence (**Figs. 4A-4C**). Interestingly, there was a “lag time” that ranged from about 4 to 24 hours for the OxyMb concentration to reach maximum and the color of the solution became bright red (**Figs. 4A-4C**). The origin of this effect may have been due to the delay in the ability of the ascorbic acid to reach the heme group. Consequently, the observed lag-time may have been due to the time taken for the ascorbic acid molecules to move from the aqueous solution to the active site where they could reduce the heme group. Without wishing to be bound by theory, the longer lag-time observed in the samples herein may have been because they were stored at 4°C, which could have slowed down the reaction kinetics. Moreover, the lag-time time observed the myoglobin solutions herein may be related to the “bloom” time observed in freshly cut meat, *i.e.*, the time for the interior of the meat to turn from brown to red due to oxygenation of the myoglobin. **Fig. 4A** also shows that deoxygenated myoglobin (DeoMb) concentration was increasing at day 10 and onward. This occurrence could be due to depletion of oxygen around the myoglobin molecules and formation of a low partial oxygen pressure environment, as ascorbic acid is an oxygen scavenger, and hence the DeoMb conversion from MetMb.

[0151] Quercetin, a natural hydrophobic antioxidant, was also studied for its potential in stabilizing the oxymyoglobin. 1 mM quercetin (originally dissolved in ethanol) reduced the metmyoglobin to oxymyoglobin, with 70% oxymyoglobin formed at day 1 and then decreased gradually over 21 days (**Figs. 4D-4F**). The lag-time for the red color to stabilize was around 2 to 10 hours (data not shown). The quercetin was observed to have a shorter lag-time.

[0152] Epigallocatechin gallate (EGCG), which is soluble in both water and organic solvents, was also examined. The addition of EGCG (1 mM) was also effective at reducing the metmyoglobin to oxymyoglobin (around 40%) and turning the protein solution to slightly red color (**Figs. 4G, 4H and 4M**). The 40% oxymyoglobin formed was not very stable and reverted back to metmyoglobin by 10% after 24 hours, causing the solution to turn brown quickly.

[0153] The addition of taxifolin (1 mM), a hydrophobic antioxidant, to the solution converted the MetMb to OxyMb and the red color remained relatively stable over 27 days (**Figs. 4I, 4J and 4M**). There was around a 24-hour lag-time for the oxymyoglobin to fully

stabilize and reach a peak concentration of about 65% (**Fig. 4I**) and form a bright red color in the system. The characteristic absorption peaks and red color associated with the OxyMb slowly decreased in intensity over 27 days, with OxyMb concentration decreasing gradually to 22% and the MetMb state reaching 72% concentration.

[0154] The addition of 1 mM 4-methylcatechol to myoglobin did not yield the typical characteristic double-peak spectra of oxymyoglobin, with OxyMb state reaching 10 to 20% only (**Figs. 4K-4M**). This suggested that 4-methylcatechol at 1 mM was not an effective reducing agent or antioxidant. Instead, there was a large increase in the measured absorbance at the lower wavelengths (**Fig. 4K**), which suggested that some pigmented molecules or colloidal particles were formed that absorbed or scattered light, respectively. A “blood red” color was observed in these samples, which may have applications in some food systems.

[0155] The addition of 1 mM Trolox, a water-soluble analog of vitamin E, to the protein solution was also able to reduce the MetMb to OxyMb (52%), which then remained stable for up to 26 days (**Figs. 4N, 4O and 4T**). This system had a 24-hour lag-time before the characteristic absorption spectra, OxyMb maximum concentration and red color associated with OxyMb was first observed (**Fig. 4T**). The OxyMb concentration decreased gradually from 52% to 30% over 26 days with the MetMb portion reaching 61%. The deoxygenated myoglobin concentration increased almost linearly on day 7 onward, which could be due to oxygen being depleted (**Fig. 4O**).

[0156] The addition of 1 mM caffeic acid, a hydrophobic antioxidant, was able to reduce the metmyoglobin solution to only about 25% oxymyoglobin after 24 hours. Only slight red color was observed in the solution on day 1 and it then turned brown (**Figs. 4P, 4Q and 4T**). The 24 hour lag-time with only slight red color forming may have been because of the relatively low reducing potential power of caffeic acid. Interestingly, the metmyoglobin concentration also decreased over time after reaching a peak at 78% on day 4 while the DeoMb portion increased linearly from day 0 over 15 days (**Fig. 4P**). Again, the DeoMb conversion from MetMb could be due to a low oxygen partial pressure formed in the system as a result of the radical scavenging properties of caffeic acid.

[0157] The addition of 1 mM gallic acid (water-soluble) was able to reduce metmyoglobin and form about 35% oxymyoglobin (**Figs. 4R, 4S and 4T**). The slightly reddish myoglobin solution observed on Day 0 turned dark brown after 1 day of storage and the OxyMb concentration decreased to 14% only after 24 hours (Day 1). The low conversion of MetMb to

OxyMb may have been due to the relatively high reduction potential of gallic acid among the other antioxidants used (**Table 1**). Around 20% DeoMb was measured in the system throughout the storage span (**Fig. 4R**).

[0158] Overall, the results of these exemplary methods showed that different natural and synthetic antioxidants had different abilities to convert MetMb to OxyMb and then stabilize the oxymyoglobin form. In particular, ascorbic acid and quercetin, which both have relatively low reduction potentials (**Table 1**), were effective at stabilizing the color of the oxymyoglobin. These insights are important for the application of myoglobin in food products that are expected to have a long shelf life so that the color is maintained for prolonged periods.

Example 3. Molecular docking experiments

[0159] In another exemplary method, molecular docking experiments were performed for selected antioxidants using Glide (Schrodinger Suite) to obtain some insight into key molecular interactions involved when antioxidants bind to myoglobin. The docking results showed that there was a similar ligand binding site close to the heme group for ascorbic acid, quercetin, and gallic acid and the distance of these antioxidant ligands to the heme group were around 10 to 12 angstroms.

[0160] *Ascorbic acid*: For ascorbic acid, three lysines (K42, K47, and K98) and an aspartic acid (D44) on the myoglobin were involved in the binding interaction. In terms of the nature of the bonds, a salt-bridge and five hydrogen bonds were formed between hydroxyl/carbonyl groups on the ascorbic acid and these amino acids on the protein.

[0161] *Quercetin*: For quercetin, two lysines (K96 and K98), an aspartic acid (D44), and a glutamic acid (E41) participated in the binding interactions. In this case, several hydrogen bonds were formed between the hydroxyl/carbonyl groups on the quercetin and the amino acids on the protein.

[0162] *Gallic acid*: For gallic acid, three lysines (K42, K96, and K98) and an aspartic acid (D44) participated in the binding interaction. In this case, two salt-bridges were formed between the carboxylate on the gallic acid and the K42/K98 side chains. In addition, three hydrogen bonds were formed between the hydroxyl groups on the gallic acid and the K47/D44 side chains and K96 carbonyl on the protein.

[0163] *4-methylcatechol*: Also performed was a molecular docking experiment for 4-methylcatechol, since this compound did not yield a typical oxymyoglobin spectra when added to the myoglobin solution (**Figs. 4K and 4L**). Indeed, this compound was bound to a different

site on the protein molecule, near to the K34 and E52 residues, which is far away from the heme group. There were only two hydrogen bonds formed between the hydroxyl groups on the 4-methylcatechol and the K34/E52 side chains on the protein. The different binding site for the 4-methylcatechol may be the reason for the different absorption spectra measured in the mixed system.

Example 4. Characteristics of myoglobin produced by cellular agriculture

[0164] In another exemplary method, four different myoglobin samples (leopard, bovine, sperm whale, and soy legume) generated using a cellular agriculture (fermentation) approach were characterized. In particular, the electrical charge, color, solubility, and redox stability of the proteins was measured using similar methods as for the equine heart myoglobin discussed in Examples 1 and 2 and described in detail herein.

[0165] **Electrical charge.** The ζ -potential versus pH profiles of the four different myoglobin samples was measured to determine their isoelectric points (pI) and stability to aggregation under different solution conditions (**Fig. 5A**). The leopard myoglobin, bovine myoglobin, and soy leghemoglobin all had similar isoelectric points (pH 4.5), while the sperm whale myoglobin had a much higher isoelectric point (pH 6.5) and the equine myoglobin had isoelectric point in between these four samples, *i.e.*, at pH 5.5. When the pH was reduced from 8.5 to 2.5, the ζ -potential changed from about -18 to +14 mV for leopard myoglobin, -30 to +17 mV for bovine myoglobin and soy leghemoglobin, and -18 to +28 mV for sperm whale myoglobin.

[0166] **Appearance.** All four myoglobin solutions turned from red to brown when the pH was reduced from 8.5 to 2.5, with this effect being most pronounced for the animal-origin myoglobin samples (**Fig. 5B**), including the equine heart myoglobin. This change in color can be attributed to protein unfolding and exposure of the heme group at low pH values, which leads to the loss of oxygen molecules bound to the iron heme. Visually, the soy and leopard myoglobin solutions appeared to maintain their strong red color longer than the other samples, which may be an advantage for food applications.

[0167] **Protein solubility.** Visually, some protein aggregation was observed around and below the isoelectric point of the myoglobin solutions, which can be attributed to the reduction in electrostatic repulsion and protein unfolding effects. In general, however, the four myoglobin samples had a relatively high solubility (> 78%) across the entire pH range studied (Figs. 7A-7D). This suggested that there was either a strong repulsion (electrostatic or steric) or weak

attraction (van der Waals or hydrophobic) between the myoglobin molecules. For the sake of comparison, the average solubilities of the proteins across the whole pH range was calculated: soy (101%) > leopard (97%) > sperm whale (94%) > bovine (91%). Overall, these results suggested that all the hemeproteins had a high water-solubility across the whole pH range but that there were some differences between them. These differences may have arisen due to differences in the primary structure (amino acid sequence) of the proteins, which led to differences in their conformations, surface charges, and hydrophobicity.

[0168] *Oxidative stability.* The absorption spectra of the freshly prepared myoglobin solutions (1 and 5 mg/mL) were measured to determine their initial redox state. The leopard myoglobin solutions appeared red with the intensity of the color increasing with protein concentration. These solutions had distinct absorption peaks at 550 and 582 nm (**Fig. 6**); however, the spectrum differed somewhat from that expected for oxymyoglobin (**Fig. 4A**). In particular, the peak at 550 nm was strongest for the leopard myoglobin, while the peak at 582 nm was strongest for the reduced equine heart myoglobin. When myoglobin binds carbon monoxide it produces a double-peak spectrum that closely resembles that of OxyMb but with a more prominent peak around 541 nm. Both OxyMb and CarboxyMb also have a fairly similar bright cherry red color. It was therefore possible that mixtures of OxyMb and CarboxyMb, as well as some MetMb (small peak around 635 nm) were formed in the initial leopard myoglobin system. This may have occurred because some carbon monoxide attached to the myoglobin during the production, isolation, and dehydration of the protein. Over time, the OxyMb and CarboxyMb were gradually oxidized to MetMb, as shown by the change in absorption spectra, *i.e.*, the formation of peaks around 503 and 632 nm (**Figs. 7A-7C**). A higher absorbance intensity and a longer redox stability were observed for the more concentrated myoglobin solution (5 mg/mL), which would be expected because there is more active compound present.

[0169] The absorption spectra of the bovine myoglobin solution also suggested that it contained a mixture of CarboxyMb, OxyMb, and MetMb, with peaks being observed at 554 and 630 nm (**Fig. 7D-7F**). The bovine myoglobin had slightly better oxidative stability than the leopard myoglobin, with the characteristic double-peak spectra still being observed at day 21. The bovine myoglobin solutions did not, however, display a strong red color even on Day 0, and over time the solutions turned brown. This change in color was consistent with the gradual change of the absorption spectra from CarboxyMb/OxyMb to MetMb, with a prominent peak gradually forming at 632 nm over time (**Figs. 7D-7F**).

[0170] **Fig. 7G** show that the initial sperm whale myoglobin had a slightly different redox state than the other proteins examined herein. The absorption spectrum contained peaks consistent with CarboxyMb (550 nm), OxyMb (582 nm), and Metmyoglobin (503 nm and 632 nm). Over about 5 days storage, however, the absorption spectrum gradually became more similar to that of pure MetMb, with the solution turning from red to brown (**Figs. 7G-7I**). This study suggested that the sperm whale sample used in this exemplary study may have undergone some autoxidation.

[0171] Soy leghemoglobin produced solutions herein with a much stronger bright cherry red color than the three animal myoglobin solutions. Initially, the absorption spectrum of this sample had peaks that were consistent with the presence of CarboxyMb and OxyMb (**Fig. 7J-JL**). A double-peak absorption spectrum was still observed in the soy leghemoglobin at Day 52, suggesting that this hemeprotein was highly resistant to oxidation. Consequently, the soy leghemoglobin used in this study may have applications in food products that have a long shelf life.

[0172] In comparison to the equine heart myoglobin without antioxidant addition, the cellular agriculture-produced myoglobin had longer oxidative stability (compare **Fig. 3** and **Figs. 7A-7L**). This may be contributed to the method used to yield these myoglobins. Nonetheless, all these systems had typical oxymyoglobin/carboxymyoglobin absorption spectra.

[0173] The foregoing discussion of the disclosure has been presented for purposes of illustration and description. The foregoing is not intended to limit the disclosure to the form or forms disclosed herein. Although the description of the disclosure has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the disclosure, *e.g.*, as can be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

CLAIMS

What is claimed is:

1. A composition for use in a food product comprising one or more purified hemeprotein compositions and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a non-animal source.
2. The composition according to claim 1, wherein the one or more purified hemeprotein compositions comprises a globin.
3. The composition according to claim 1, wherein the one or more purified hemeprotein compositions comprise leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrucorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytohemoglobin, or any combination thereof.
4. The composition according to claim 1, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a genetically modified non-animal source.
5. The composition according to claim 4, wherein the genetically modified non-animal source comprises a genetically modified plant, a genetically modified bacteria, a genetically modified yeast, or any combination thereof.
6. The composition according to claim 1, wherein the one or more purified hemeprotein compositions comprise a polypeptide expressed and/or secreted from a non-animal source, wherein the non-animal source comprises plants, fungi, bacteria, yeasts, algae, archaea, genetically modified plants, genetically modified fungi, genetically modified bacteria, genetically modified yeasts, genetically modified algae, genetically modified archaea, or any combination thereof.
7. The composition according to claim 6, wherein the polypeptide expressed and/or secreted from a non-animal source, is encoded from a polynucleotide derived from animals, plants, fungi, bacteria, yeasts, algae, archaea, or any combination thereof.

8. The composition according to claim 1, wherein the one or more antioxidants comprise vitamins, polyphenols, and any combination thereof.
9. The composition according to claim 1, wherein the one or more antioxidants comprise vitamin C and any derivatives thereof, vitamin E and any derivatives thereof, and any combination thereof.
10. The composition according to claim 1, wherein the one or more antioxidants comprises flavonoids or any derivatives thereof.
11. The composition according to claim 10, wherein the one or more antioxidants comprise isorhamnetin, kaempferol, myricetin, proanthocyanidins, quercetin, rutin, taxifolin, catechin, gallocatechin, gallocatechin gallate esters, epicatechin, epigallocatechin, epigallocatechin gallate esters, theaflavin, theaflavin gallate esters, thearubigins, or any combination thereof.
12. The composition according to claim 1, wherein the one or more antioxidants has a reduction potential ranging from about 150 mV to about 500 mV.
13. The composition according to claim 1, comprising a weight ratio of the one or more purified hemeprotein compositions to the one or more antioxidants ranging from about 1:1 to about 30:1.
14. The composition according to claim 13, comprising a weight ratio of the one or more hemeprotein compositions to the one or more antioxidants ranging from about 2:1 to about 10:1.
15. The composition according to claim 1, wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm.

16. The composition of claim 15, wherein the increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm is detectable for at least about 7 days after addition of the one or more antioxidants.

17. A composition for use in a food product comprising one or more purified hemeprotein compositions and one or more antioxidants, wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a non-animal source, wherein,

the non-animal source comprises plants, fungi, bacteria, yeasts, algae, archaea, genetically modified plants, genetically modified fungi, genetically modified bacteria, genetically modified yeasts, genetically modified algae, genetically modified archaea, or any combination thereof;

the hemeprotein is expressed and/or secreted from the non-animal source, wherein the polypeptides are encoded from a polynucleotide comprising a nucleic acid derived from animals, plants, fungi, bacteria, yeasts, algae, archaea, or any combination thereof;

the polypeptides are selected from a group consisting of leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, and phytooglobin; and

wherein the one or more antioxidants has a reduction potential ranging from about 280 mv to about 500 mV,

wherein the composition comprises a weight ratio of the one or more purified hemeprotein compositions to the one or more antioxidants of about 1:1 to about 30:1.

18. The composition of claim 17, wherein the polypeptide is selected from leghemoglobin and myoglobin.

19. The composition of claim 17, wherein the one or more antioxidants is selected from the group consisting of ascorbic acid, quercetin, taxifolin, and Trolox.

20. The composition of claim 17, wherein the composition comprises a weight ratio of the one or more purified hemeprotein compositions to the one or more antioxidants of about 2:1 to about 10:1.

21. The composition of claim 17, wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm.

22. The composition of claim 21, wherein the increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm is detectable for at least about 7 days after addition of the one or more antioxidants.

23. A composition comprising one or more purified hemeprotein compositions and one or more antioxidants,

wherein the one or more purified hemeprotein compositions comprise a hemeprotein from a genetically modified non-animal source selected from the group consisting of a genetically modified plant, a genetically modified bacteria, a genetically modified fungi, or a genetically modified yeast; and wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more heme protein compositions at one or more of about 550 nm and about 582 nm that is detectable for at least about 7 days after addition of the one or more antioxidants.

24. The composition according to claim 23, wherein the one or more antioxidants has a reduction potential ranging from about 150 mV to about 500 mV.

25. The composition according to claim 23, wherein the one or more antioxidants is selected from ascorbic acid, quercetin, taxifolin, Trolox, and combinations thereof.

26. The composition according to claim 24, wherein the composition comprises a weight ratio of the one or more purified hemeprotein compositions to the one or more antioxidants of about 2:1 to about 10:1.

27. The composition according to claim 23, wherein the purified hemeprotein is encoded by a polynucleotide comprising a nucleic acid sequence from a plant, animal, fungus, or bacteria.

28. The composition according to claim 27, wherein the polynucleotide comprises a nucleic acid sequence derived from a legume encoding a hemeprotein.
29. The composition according to claim 28, wherein the polynucleotide comprises a nucleic acid sequence derived from any one of an equine, a leopard, a bovine, or a whale.
30. The composition according to claim 27, wherein the one or more purified hemeprotein is a globin selected from a group consisting of leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, and phytooglobin.
31. The composition according to claim 27, wherein the one or more purified hemeproteins are recombinant hemeproteins produced from a genetically modified yeast source, wherein the recombinant hemeproteins are encoded from a polynucleotide, wherein the polynucleotide comprises a nucleic acid sequence derived from a legume, an equine, a leopard, a bovine, a whale, or any combination thereof and encoding a hemeprotein.
32. The composition of any one of claims 1, 17 or 23, wherein the hemeprotein composition comprises a myoglobin and wherein combining the one or more purified hemeprotein compositions with one or more antioxidants causes an increase in the relative amount of oxymyoglobin and conversion of carboxymyoglobin to metmyoglobin in the composition.
33. The composition of claim 32, wherein the increase in relative amount of oxymyoglobin and conversion of carboxymyoglobin to metmyoglobin in the composition is detectable for at least about 7 days after addition of the antioxidant.
34. The composition of claim 33, wherein the increase in the relative amount of oxymyoglobin and conversion of carboxymyoglobin to metmyoglobin in the composition ranges from about 1.1-fold to about 5-fold.
35. A composition comprising one or more purified hemeprotein compositions and one or more antioxidants,

wherein the one or more purified hemeprotein compositions comprise leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, protoglobin, cytochrome, cyanoglobin, flavohemoglobin, myoglobin, phytooglobin, or any combination thereof;

wherein the one or more antioxidants is selected from the group consisting of ascorbic acid, quercetin, taxifolin, Trolox, and combinations thereof;

wherein the composition comprises a weight ratio of the one or more purified hemeprotein compositions to the one or more antioxidants of about 2:1 to about 10:1, and

wherein the composition results in an increase in peak height of the UV-visible absorption spectrum for the one or more hemeprotein compositions at one or more of about 550 nm and about 582 nm, that is detectable for at least about 7 days after addition of the one or more antioxidants.

36. A method of preparing a meat substitute, the method comprising combining the composition according to any one of claims 1, 17, 23, or 35 to a meat replica matrix, wherein the composition according to any one of 1, 17, 23, or 35 results in an increase in peak height of the UV-visible absorption spectrum for the one or more hemeprotein compositions at one or more of about 550 nm and about 582 nm, that is detectable for at least about 7 days after addition of the one or more antioxidants.