IN THE UNITED STATES DISTRICT COURT
FOR DISTRICT OF DELAWARE

IMPOSSIBLE FOODS INC.,	)	CASE NO.:
	)	
Plaintiff,	)	**COMPLAINT FOR DAMAGES AND INJUNCTIVE RELIEF**
	)	
v.	)	
	)	
MOTIF FOODWORKS, INC.,	)	**JURY TRIAL DEMANDED**
	)	
Defendant.	)	
	)	

Plaintiff Impossible Foods Inc. ("Impossible Foods") brings this Complaint against defendant Motif FoodWorks, Inc. ("Motif" or "Defendant") and alleges, on personal knowledge as to its own actions and on information and belief as to the actions of others, as follows:

THE PARTIES

1. Plaintiff Impossible Foods is a Delaware corporation with its principal place of business at 400 Saginaw Drive, Redwood City, California. Impossible Foods develops and distributes plant-based meat products, including the well-known IMPOSSIBLE BURGER, IMPOSSIBLE SAUSAGE and IMPOSSIBLE MEATBALLS ("IMPOSSIBLE Products").

2. Defendant Motif is a Delaware corporation with its principal place of business at 27 Drydock Avenue, Boston, Massachusetts. Defendant advertises itself as a provider of plant-based food ingredients, ingredient systems, and finished formulations of plant-based food.

3. Founded in 2011, Impossible Foods seeks to restore biodiversity and reduce the impact of climate change by transforming the global food system. To do this, it makes delicious, nutritious, affordable, and sustainable meat from plants. Impossible Foods' innovative approach to food science, powered by proprietary research and patent-protected

technology, has allowed it to develop plant-based foods, including the award-winning IMPOSSIBLE BURGER, that recreates the entire sensory experience of eating meat despite being made from plants, without any actual meat or meat-derived ingredients. Impossible Foods has invested hundreds of millions of dollars in the research and development of these market-leading meat alternatives and has secured patents covering its innovative ingredients, food products and manufacturing processes.

4. Defendant has sought to compete with Impossible Foods with ingredients and products that allegedly taste, smell and feel like meat.

5. Impossible Foods brings this action for damages and injunctive relief to protect its innovative technology and products against Defendant's patent infringement.

NATURE OF THE ACTION

6. This is an action for patent infringement under title 35 of the United States Code.

7. As set forth in more detail below, Defendant has been infringing Impossible Foods' patents, including at least United States Patent No. 10,863,761 ("the '761 Patent"), and continues to do so through the present date.

8. Impossible Foods thus seeks injunctive relief against Defendant's infringement of its patent, as well as damages for Defendant's past and ongoing patent infringement.

JURISDICTION AND VENUE

9. This Court has subject matter jurisdiction of this suit for patent infringement pursuant to 28 U.S.C. §§ 1331 and 1338(a).

10. This Court has personal jurisdiction over Defendant because Defendant is incorporated in the State of Delaware.

11. Venue is proper in this District under 28 U.S.C. § 1400(b), because Defendant is incorporated in, and thus resides in, this District.

BACKGROUND

A. Impossible Foods' Innovative Technology and Patents

12. Founded in 2011 by Dr. Patrick O. Brown, Impossible Foods is a food innovator and seeks to develop and sell delicious, nutritious, affordable, and sustainable meat made from plants.

13. Early in its history, Impossible Foods assembled a team of scientists for an ambitious research investigation: determining which biological molecules make meat look, cook, and taste the way it does. The company discovered that heme, a biological molecule involved in oxygen transport, is a central component of meat's appeal, leading to meat's savory flavor and aroma and influencing how meat cooks. Specifically, heme is "the molecule that gives meat its bloody taste when raw and creates the intense, meaty flavors and aromas when it's cooked."[1] Impossible Foods set out to make plant-based foods that incorporate heme to replicate the taste, aroma, and overall sensory experience of meat. The IMPOSSIBLE Products include heme.

14. The heme in the IMPOSSIBLE Products is part of a hemoprotein molecule called soy leghemoglobin, or LegH. LegH occurs naturally in the root nodules of soy plants and, on information and belief, is not naturally produced in the body of any animal species. Impossible Foods discovered that the inclusion of leghemoglobin "transformed what would

[1] https://impossiblefoods.com/blog/how-gmos-can-save-civilization-and-probably-already-have

otherwise have been a dull tasting veggie burger into [] meat! And the meat cooked, smelled and tasted like meat from a cow."[2]

15. LegH can be produced by growing soy plants, harvesting the root nodules, and isolating the hemoprotein—but Impossible Foods discovered that this process was too inefficient for commercial production. Impossible Foods thus developed a proprietary strain of genetically modified *Pichia* yeast that produces LegH through a fermentation process.

16. Impossible Foods released IMPOSSIBLE BURGER in 2016 and reformulated it in 2019. IMPOSSIBLE BURGER is a meat replica product that replicates the flavor and texture of ground beef and can be used as a hamburger meat replacement for multiple applications. IMPOSSIBLE BURGER has won numerous awards, including "Best of the Best" at the 2019 Consumer Electronics Show, and is available in thousands of restaurants and grocery stores nationwide.

17. Impossible Foods has applied for, and has been awarded, patents regarding many elements and aspects of the manufacturing and composition of heme-containing meat replica products.

18. On December 15, 2020, the United States Patent and Trademark Office issued U.S. Patent No. 10,863,761 to Impossible Foods, entitled "Methods and Compositions for Consumables." A true and correct copy of the '761 Patent is attached as Exhibit 1.

B. Defendant's Product Development

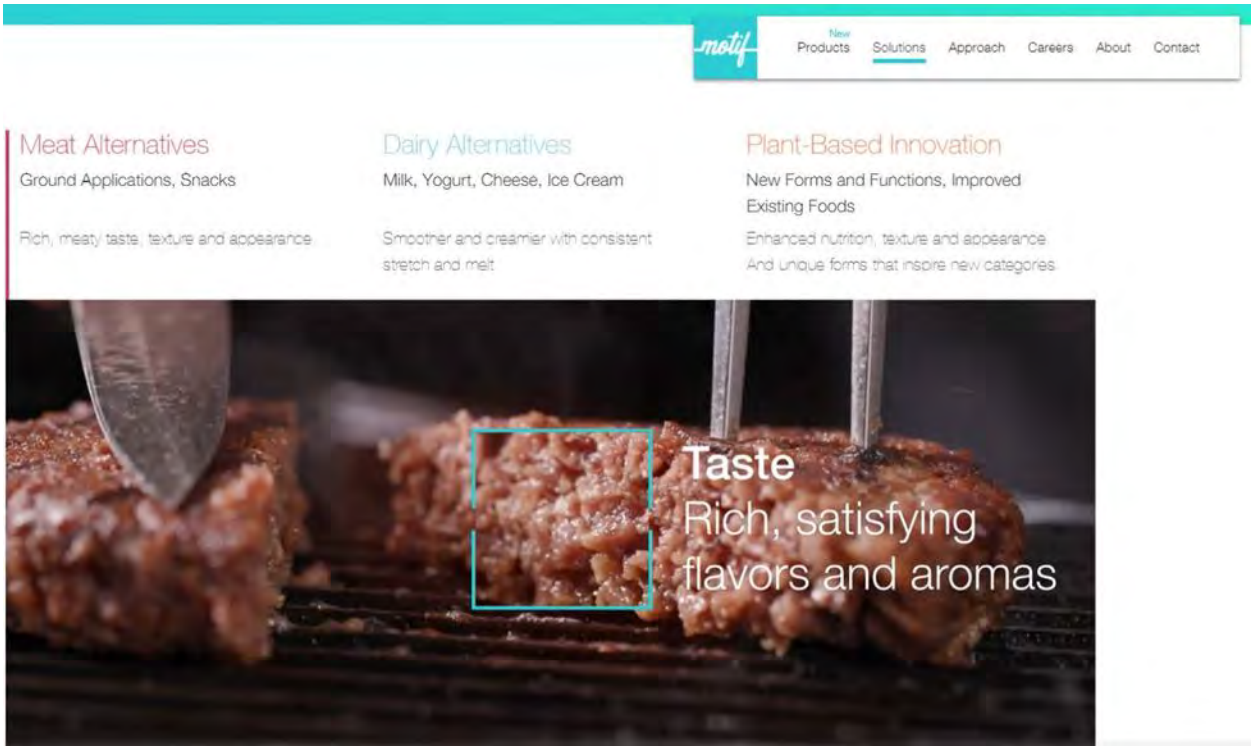
19. Defendant spun out of Ginkgo Bioworks Inc. ("Ginkgo Bioworks"), a genetic engineering company, in early 2019. Ginkgo Bioworks is still a major investor in Defendant.

---

[2] https://impossiblefoods.com/blog/heme-health-the-essentials

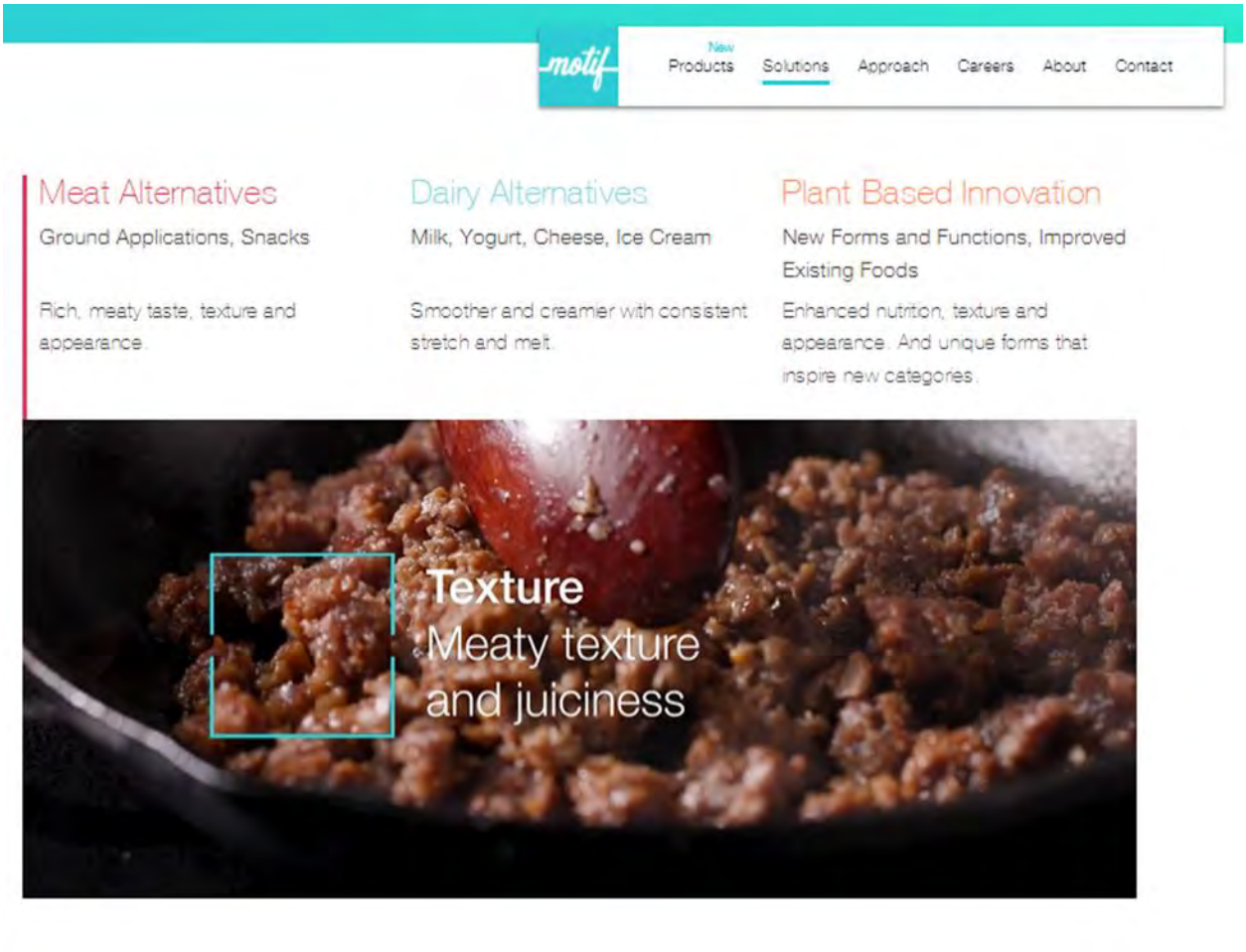
20. Defendant describes its products as "Meat alternative options that consumers crave."

21. On its website, Defendant advertises that it sells "individual ingredients," "ingredient systems," and "finished formulations" of "plant-based food." Defendant includes pictures of various foods that purportedly replicate the "taste, texture and appearance" of meat. For instance, Defendant's website depicts the following with respect to taste:



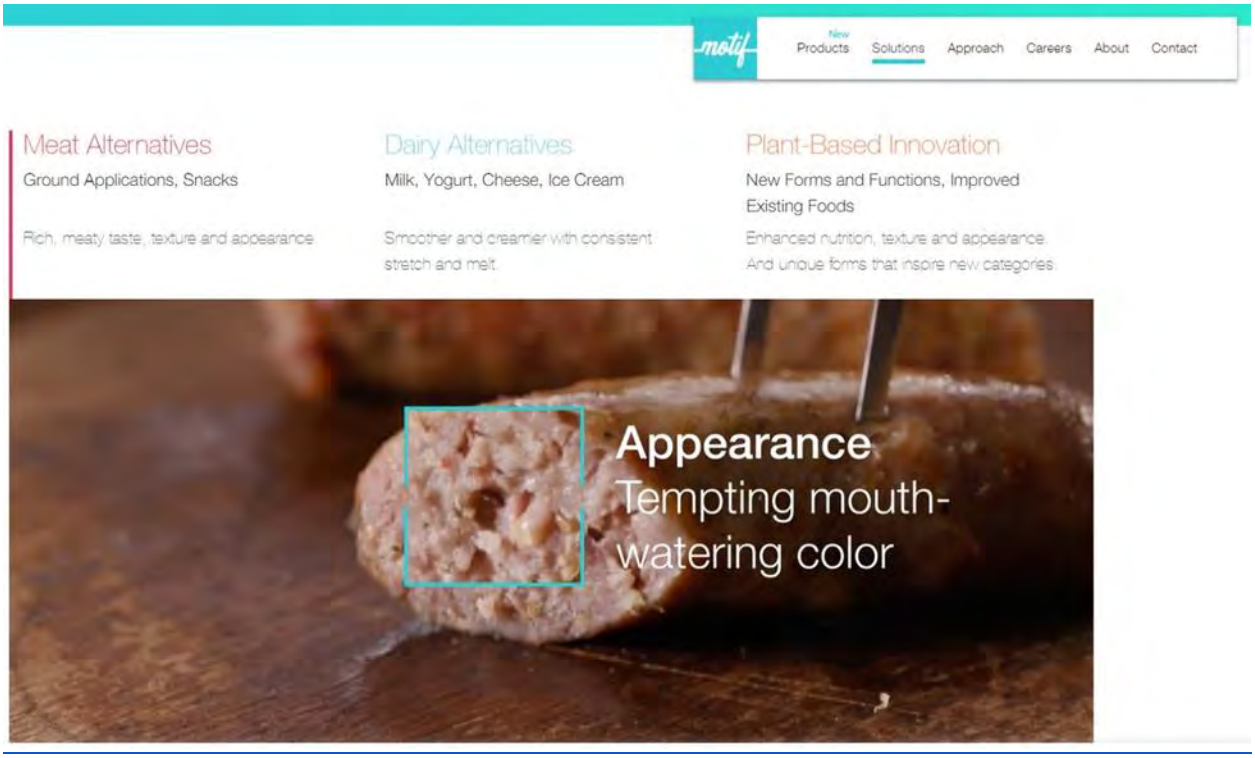
https://madewithmotif.com/solutions/

22. Defendant's website depicts the following with respect to texture:



https://madewithmotif.com/solutions/

23. Defendant's website depicts the following with respect to appearance:



https://madewithmotif.com/solutions/

24. Defendant markets meat alternatives that include the ingredient HEMAMI, which purportedly provides "meat alternatives" with "[t]he real umami flavors, appearance and aromas of meat."[3] Its website notes that HEMAMI "tastes and smells like meat because it uses the same naturally occurring heme protein" and bestows "[m]outh-watering aromas that engage the senses—while cooked and right before you take your first bite."[4]

25. As Defendant's website notes, HEMAMI contains heme.

26. In an April 2021 submission to the Food and Drug Administration (FDA) Office of Food Additive Safety (hereinafter "the Motif GRAS Notice"), Defendant stated that "Motif

[3] https://madewithmotif.com/solutions/

[4] https://madewithmotif.com/solutions/

FoodWorks' myoglobin ingredient is a liquid flavoring preparation (herein referred to as Myoglobin Preparation) containing myoglobin produced by fermentation from a modified strain of *Pichia pastoris* expressing the myoglobin gene from *Bos taurus*." A true and correct copy of the Motif GRAS Notice is attached as Exhibit 2.

27. On information and belief, HEMAMI contains the bovine myoglobin preparation that is the subject of the Motif GRAS Notice.

C. Defendant's Infringing Products and Activities

28. Defendant has made and demonstrated a replica burger at trade shows, including the Plant-Based World Expo in New York in December 2021. Defendant also announced plans to demonstrate the replica burger at Natural Foods Expo West and at Future Food-Tech SF in California. The replica burger that Defendant demonstrated infringes the '761 Patent.

29. Defendant's replica burger includes its HEMAMI ingredient.

30. In the summer of 2021, Defendant partnered with Coolgreens, a restaurant chain with a location in Dallas, TX, to produce and sell replica burger products containing HEMAMI. Defendant and Coolgreens sold infringing products from Coolgreens' Dallas restaurant.

31. On information and belief, Defendant has had opportunities to obtain non-public information regarding Impossible Foods' proprietary yeast and methods of making its proprietary heme-containing protein.

32. Defendant recently launched HEMAMI for sale to customers with intent that customers integrate HEMAMI into their own plant-based meat alternatives.

33. Defendant is currently constructing a research, development, and production facility in Massachusetts that it intends to use for fermentation, ingredient production, and finished product production, including, on information and belief, manufacturing the heme-

containing bovine myoglobin included in HEMAMI, production of HEMAMI, and production of finished products integrating HEMAMI. Defendant plans for the facility to be fully online this year.

34. Solar Biotech, which has a facility in Virginia that has been used to manufacture HEMAMI, also announced, on January 28, 2022, that it would continue to manufacture HEMAMI for Defendant.

35. On information and belief, the finished meat replica products that include HEMAMI and which Defendant has sold, offered for sale, and/or demonstrated for marketing purposes directly or indirectly infringe the '761 Patent.

36. Defendant is and has been aware that the inclusion of HEMAMI in meat replica products is a violation of Impossible Foods' patent rights and has touted HEMAMI as a substitute for Impossible Foods' patented technology in its marketing communications.

37. For example, Defendant's official Twitter account, @motiffoodworks, retweeted a link to an article in The Spoon, a food technology trade publication, from February 7, 2022. That article stated that the launch of HEMAMI "is good news if you're a maker of alt-meat products who wants to replicate Impossible's proprietary plant-based heme, because now instead of spending tens of millions trying to build it yourself, now you can buy a similar technology from Motif."[5]

38. As another example, Defendant's CEO, Jonathan McIntyre, provided a quote pertaining to Defendant's new Massachusetts facility that was included in a December 8, 2021 article in Vegconomist, a vegan industry trade publication. That article noted that "heme

---

5 https://twitter.com/RethinkFoodVC/status/1490690290147078145?s=20&t=DD9WjVymcPhHCw3z0SpqUA

represented Impossible Foods' most closely-guarded flavor secret, and the $2 billion company held exclusive patent rights and knowledge on producing and commercializing it."[6]

39. In addition, Ginkgo Bioworks' CEO, in an article announcing a round of fundraising completed by Defendant, described Defendant's strategy as follows: "We'll brew up the next 100 hemes so that we can see many more Impossible Burgers in the next few years."[7]

COUNT I: INFRINGEMENT OF THE '761 PATENT

40. Impossible Foods incorporates and re-alleges ¶¶ 1-39 as though fully stated herein.

41. Impossible Foods is the owner of all right, title, and interest in and to the '761 Patent. Impossible Foods has the exclusive right to make, use, sell, and offer to sell any product embodying the '761 Patent throughout the United States, and to import any product embodying the '761 Patent into the United States.

42. The '761 Patent claims and describes an invention comprising a beef replica product, which comprises a) a muscle replica comprising 0.1%-5% of a heme-containing protein, at least one sugar compound and at least one sulfur compound; and b) a fat tissue replica comprising at least one plant oil and a denatured plant protein, wherein said muscle replica and fat tissue replica are assembled in a manner that approximates the physical organization of meat.

43. On information and belief, Defendant has been and is now infringing at least claim 1 of the '761 Patent in the United States by, among other things, directly or through intermediaries, making, using, selling, and/or offering for sale an imitation burger including

[6] https://vegconomist.com/products-launches/motif-foodworks-heme/
[7] https://www.inc.com/jeff-bercovici/motif-food-biotech.html

HEMAMI (the "Infringing Burger"), which is covered by one or more claims of the '761 Patent, to the injury of Impossible Foods. In particular, the Infringing Burger is a beef replica product comprising a) a muscle replica comprising 0.1%-5% of a heme-containing protein, at least one sugar compound and at least one sulfur compound; and b) a fat tissue replica comprising at least one plant oil and a denatured plant protein, wherein said muscle replica and fat tissue replica are assembled in a manner that approximates the physical organization of meat.

44. Defendant is directly infringing, literally and/or under the doctrine of equivalents, the '761 Patent. Defendant is thus liable for infringement of the '761 Patent pursuant to 35 U.S.C. § 271(a).

45. Defendant infringes the '761 Patent because it makes, uses, sells and/or offers for sale the invention of the '761 Patent. In particular, Defendant infringes at least claim 1 of the '761 Patent by making, using, selling and/or offering for sale the Infringing Burger.

46. On information and belief, Defendant will continue to infringe the '761 Patent unless enjoined.

47. Defendant contributes to infringement of the '761 Patent under 35 U.S.C. § 271(c) inasmuch as it provides a component of the Infringing Burger, *e.g.*, HEMAMI, which constitutes a material part of Impossible Foods' invention, to another, knowing the same to be especially made or especially adapted for use in infringement of the '761 Patent.

48. On information and belief, Defendant will continue to contribute to infringement of the '761 Patent unless enjoined.

49. Defendant actively encourages its business partners to make, use, sell, and/or offer for sale the Infringing Burger. Defendant is aware of Impossible Foods' proprietary

IMPOSSIBLE BURGER. Moreover, Defendant is aware of the '761 Patent. Despite such knowledge, Defendant has actively induced its business partners to make, use, sell, and/or offer for sale the Infringing Burger in a way that constitutes infringement. Defendant has encouraged this infringement with a specific intent to cause its business partners and customers to infringe. Defendant's acts thus constitute active inducement of patent infringement in violation of 35 U.S.C. § 271(b).

50. On information and belief, Defendant will continue to induce infringement of the '761 Patent unless enjoined.

51. Defendant's direct infringement, contributory infringement, and inducement of infringement have irreparably harmed Impossible Foods.

52. Pursuant to 35 U.S.C. § 284, Impossible Foods is entitled to damages adequate to compensate for Defendant's infringement.

53. Defendant's infringement has been and is willful and, pursuant to 35 U.S.C. § 284, Impossible Foods is entitled to treble damages. Defendant's willful infringement is based at least on Defendant's knowledge of Impossible Foods, its manufacturing techniques, its products, and its patents (*see, e.g.,* ¶¶ 26, 36-39, *supra*). Defendant's conduct is egregious as it has continued offering, selling, making and/or using the Infringing Burger despite knowledge of the infringement. Defendant has either willfully and wantonly infringed the '761 Patent or has recklessly avoided knowledge of its own infringement, even when faced with knowledge of Impossible Foods' own products and patents.

54. This case is "exceptional" within the meaning of 35 U.S.C. § 285, and Impossible Foods is entitled to an award of attorneys' fees.

DEMAND FOR A JURY TRIAL

Impossible Foods demands a jury trial on all issues so triable.

PRAYER FOR RELIEF

WHEREFORE, Impossible Foods demands judgment as follows:

a. Judgment that Defendant has infringed and is infringing the '761 Patent;

b. That Impossible Foods be granted injunctive relief against Defendant and its officers, employees, agents, servants, attorneys, instrumentalities, and/or those in privity with them, to prevent the recurrence of the infringing activities complained of herein, including ceasing manufacture, use, sale, offering for sale, and importation of the Infringing Burger, ceasing contribution to and/or inducement of others to do the same, and for all further proper injunctive relief pursuant to 35 U.S.C. § 283;

c. Judgment that Defendant account for and pay to Impossible Foods all damages and costs incurred by Impossible Foods, caused by Defendant's infringing activities complained of herein;

d. Judgment that Defendant has willfully infringed the '761 Patent and an increase in the damages award to Impossible Foods up to three times the amount assessed, pursuant to 35 U.S.C. § 284;

e. That Impossible Foods be granted pre- and post-judgment interest on the damages;

f. That this Court declare this case exceptional and award Impossible Foods reasonable attorneys' fees and costs in accordance with 35 U.S.C. § 285; and

g. That Impossible Foods be granted such other and further relief as the Court may deem just and proper under the circumstances.

WILSON SONSINI GOODRICH & ROSATI, P.C.

/s/ Ian R. Liston
Ian R. Liston (#5507)
Jennifer A. Ward (#6476)
222 Delaware Avenue, Suite 800
Wilmington, DE 19801
(302) 304-7600
iliston@wsgr.com
jward@wsgr.com

Counsel for Plaintiff Impossible Foods Inc.

OF COUNSEL:

Matthew R. Reed
WILSON SONSINI GOODRICH & ROSATI, P.C.
650 Page Mill Road
Palo Alto, CA 94304
(650) 493-9300
mreed@wsgr.com

Wendy L. Devine
WILSON SONSINI GOODRICH & ROSATI, P.C.
One Market Plaza
Spear Tower, Suite 3300
San Francisco, CA 94105
(415) 947-2000
wdevine@wsgr.com

Lori P. Westin
WILSON SONSINI GOODRICH & ROSATI, P.C.
12235 El Camino Real
San Diego, CA 92130
(858) 350-2300
lwestin@wsgr.com

G. Edward Powell III
WILSON SONSINI GOODRICH & ROSATI, P.C.
1700 K Street NW, Fifth Floor
Washington, DC 20006
(202) 973-8800
epowell@wsgr.com

Dated: March 9, 2022

EXHIBIT 1

US010863761B2

(12) **United States Patent**
Brown et al.

(10) **Patent No.: US 10,863,761 B2**
(45) **Date of Patent: Dec. 15, 2020**

(54) **METHODS AND COMPOSITIONS FOR CONSUMABLES**

(71) Applicant: **Impossible Foods Inc.**, Redwood City, CA (US)

(72) Inventors: **Patrick O'Reilly Brown**, Stanford, CA (US); **Marija Vrljic**, San Mateo, CA (US); **Ranjani Varadan**, Fremont, CA (US); **Michael Eisen**, Berkeley, CA (US); **Sergey Solomatin**, Mountain View, CA (US)

(73) Assignee: **Impossible Foods Inc.**, Redwood City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 74 days.

(21) Appl. No.: **15/985,697**

(22) Filed: **May 21, 2018**

(65) **Prior Publication Data**

US 2018/0368453 A1 Dec. 27, 2018

Related U.S. Application Data

(63) Continuation of application No. 13/941,211, filed on Jul. 12, 2013, now abandoned, which is a (Continued)

(51) **Int. Cl.**
A23L 27/26 (2016.01)
A23L 13/40 (2016.01)
(Continued)

(52) **U.S. Cl.**
CPC ***A23L 27/26*** (2016.08); ***A23J 1/12*** (2013.01); ***A23J 1/14*** (2013.01); ***A23J 3/14*** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC A23J 1/12; A23J 1/14; A23J 3/227; A23L 27/00; A23L 27/20; A23L 33/185; A23L 27/26; A23L 13/424
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,934,435 A 4/1960 May
2,934,436 A 4/1960 May
(Continued)

FOREIGN PATENT DOCUMENTS

CN 1252231 A 5/2000
CN 1301811 A 7/2001
(Continued)

OTHER PUBLICATIONS

Proulx et al., "Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model". J. Agric. Food Chem. 54, pp. 1518-1522. (Year: 2006).*
(Continued)

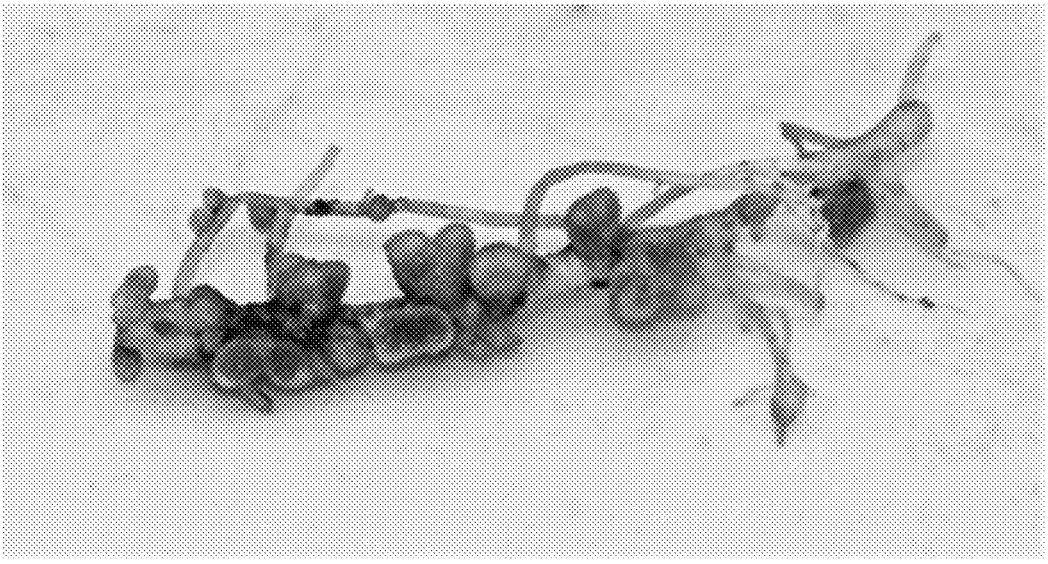
Primary Examiner — Erik Kashnikow
Assistant Examiner — Assaf Zilbering
(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(57) **ABSTRACT**

Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

17 Claims, 11 Drawing Sheets

Specification includes a Sequence Listing.



Related U.S. Application Data

continuation-in-part of application No. PCT/US2012/046560, filed on Jul. 12, 2012.

(60) Provisional application No. 61/671,069, filed on Jul. 12, 2012, provisional application No. 61/572,205, filed on Jul. 12, 2011.

(51) **Int. Cl.**

A23J 3/22	(2006.01)
A23J 1/12	(2006.01)
A23J 1/14	(2006.01)
A23J 3/14	(2006.01)
A23L 27/00	(2016.01)
A23L 27/10	(2016.01)
A23L 27/20	(2016.01)
A23L 33/185	(2016.01)

(52) **U.S. Cl.**

CPC ***A23J 3/227*** (2013.01); ***A23L 13/424*** (2016.08); ***A23L 13/426*** (2016.08); ***A23L 27/00*** (2016.08); ***A23L 27/10*** (2016.08); ***A23L 27/20*** (2016.08); ***A23L 33/185*** (2016.08)

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,934,437	A	4/1960	Morton et al.
2,955,041	A	10/1960	Broderick
3,157,516	A	11/1964	Huber
3,271,167	A	9/1966	Perret
3,316,099	A	4/1967	Hoersch
3,365,306	A	1/1968	Perret
3,394,015	A	7/1968	Giacino et al.
3,394,016	A	7/1968	Bidmead
3,394,017	A	7/1968	Giacino et al.
3,480,447	A	11/1969	Hack
3,493,395	A	2/1970	Soeters
3,519,437	A	7/1970	Giacino et al.
3,524,747	A	8/1970	O'Hara
3,532,514	A	10/1970	May
3,532,515	A	10/1970	Broderick
3,578,465	A	5/1971	van der Zijden
3,615,600	A	10/1971	Zevenaar
3,620,772	A	11/1971	Nagayoshi
3,642,497	A	2/1972	Gunther
3,645,753	A	2/1972	Gasser
3,645,754	A	2/1972	Wiener
3,658,550	A	4/1972	Hawley
3,660,114	A	5/1972	Thomas
3,689,289	A	9/1972	Perret
3,693,533	A	9/1972	Liepa
3,697,295	A	10/1972	van der Ouweland
3,716,379	A	2/1973	de la Potterie
3,716,380	A	2/1973	de la Potterie
3,719,499	A	3/1973	Hai et al.
3,741,775	A	6/1973	Lee
3,761,287	A	9/1973	Jaeggi et al.
3,804,953	A	4/1974	Bentz et al.
3,829,582	A	8/1974	Guadagni et al.
3,840,674	A	10/1974	Joseph et al.
3,857,970	A	12/1974	Tsumura
3,870,801	A	3/1975	Tombs
3,879,561	A	4/1975	Smith et al.
3,881,022	A	4/1975	Gasser
3,928,643	A	12/1975	Ishiguro et al.
3,930,046	A	12/1975	Baugher
3,966,985	A	6/1976	Jonas
3,973,043	A	8/1976	Lynn
4,045,587	A	8/1977	Katz et al.
4,066,793	A	1/1978	Eguchi
4,076,852	A	2/1978	Van Delft et al.
4,094,997	A	6/1978	Aishima et al.
4,098,913	A	7/1978	Baugher
4,132,809	A	1/1979	Desrosier
4,161,550	A	7/1979	Bernhardt et al.
4,165,391	A	8/1979	Corbett nee Rolison
4,197,324	A	4/1980	Ziminski et al.
4,218,487	A	8/1980	Jaeggi
4,324,807	A	4/1982	Kim et al.
4,411,915	A	10/1983	Eriksson
4,435,438	A	3/1984	Lehnhardt et al.
4,604,290	A	8/1986	Lee et al.
4,678,676	A	7/1987	Ishizuka et al.
4,994,285	A	2/1991	Hisano et al.
5,039,543	A	8/1991	Lee et al.
5,055,310	A	10/1991	Nonaka et al.
5,264,239	A	11/1993	Cornet et al.
5,443,852	A	8/1995	Shahidi et al.
5,597,594	A	1/1997	Matsuura et al.
5,650,554	A	7/1997	Moloney et al.
5,753,295	A	5/1998	Goldman
5,807,601	A	9/1998	Carpenter et al.
5,856,452	A	1/1999	Moloney et al.
5,922,392	A	7/1999	Kelly et al.
6,093,424	A	7/2000	Han et al.
6,146,645	A	11/2000	Deckers et al.
6,183,762	B1	2/2001	Deckers et al.
6,210,742	B1	4/2001	Deckers et al.
6,228,418	B1	5/2001	Gluck
6,242,036	B1	6/2001	Han et al.
6,287,620	B1	9/2001	Van Den Ouweland et al.
6,372,234	B1	4/2002	Deckers et al.
6,372,961	B1	4/2002	Tarczynski
6,379,738	B1	4/2002	Dingman et al.
6,383,531	B1	5/2002	Gottemoller
6,399,135	B2	6/2002	Gottemoller
6,413,569	B1	7/2002	Borders et al.
6,416,797	B1	7/2002	Han et al.
6,420,148	B2	7/2002	Yamaguchi
6,495,184	B1	12/2002	Zheng et al.
6,495,187	B1	12/2002	Borders et al.
6,509,453	B1	1/2003	Moloney
6,582,710	B2	6/2003	Deckers et al.
6,596,287	B2	7/2003	Deckers et al.
6,599,513	B2	7/2003	Deckers et al.
6,692,788	B1	2/2004	Mottram et al.
6,761,914	B2	7/2004	Deckers et al.
6,908,634	B2	6/2005	Hwang
6,936,749	B1	8/2005	Guy et al.
7,052,879	B2	5/2006	Shaw et al.
7,332,587	B2	2/2008	Moloney
7,407,786	B2	8/2008	Giver et al.
7,479,472	B1	1/2009	Harbury et al.
7,585,645	B2	9/2009	Deckers et al.
7,622,290	B2	11/2009	Brunstedt et al.
7,666,618	B2	2/2010	Miasnikov et al.
7,666,628	B2	2/2010	Moloney
7,674,953	B2	3/2010	Mulet Salort et al.
7,709,044	B2	5/2010	Ishimoto
7,807,870	B2	10/2010	Geigenberger et al.
7,931,925	B2	4/2011	Nielsen
8,012,732	B2	9/2011	Brunstedt et al.
8,021,695	B2	9/2011	Gruber et al.
8,188,415	B2	5/2012	Kats et al.
8,304,522	B2	11/2012	Kungitani
8,597,694	B2	12/2013	Guth et al.
9,011,949	B2	4/2015	Brown et al.
9,700,067	B2	7/2017	Fraser
9,808,029	B2	11/2017	Fraser et al.
9,826,772	B2	11/2017	Fraser et al.
9,943,096	B2	4/2018	Fraser et al.
10,039,306	B2	8/2018	Vrljic et al.
10,172,380	B2	1/2019	Varadan et al.
10,172,381	B2	1/2019	Vrljic et al.
2001/0024677	A1	9/2001	Bringe
2001/0049132	A1	12/2001	Kringelum et al.
2002/0034570	A1	3/2002	Krammer et al.
2003/0198700	A1	10/2003	Gruber
2003/0212281	A1	11/2003	Sinha et al.
2003/0224476	A1	12/2003	Chou
2004/0151778	A1	8/2004	Richard et al.
2004/0161513	A1	8/2004	Akashe et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2005/0037111	A1	2/2005	Berry	
2005/0112271	A1*	5/2005	Pickarski	A23J 3/227 426/656
2006/0035003	A1	2/2006	McMindes et al.	
2006/0035006	A1	2/2006	McMindes et al.	
2006/0204644	A1	9/2006	Cavallini et al.	
2006/0233721	A1	10/2006	Tamarkin et al.	
2007/0269567	A1	11/2007	McMindes et al.	
2007/0269571	A1	11/2007	Akita et al.	
2007/0269583	A1	11/2007	McMindes et al.	
2008/0026128	A1	1/2008	Yamaguchi	
2008/0226810	A1	9/2008	Passe et al.	
2008/0254168	A1	10/2008	Mueller et al.	
2008/0254199	A1	10/2008	Orcutt et al.	
2008/0268112	A1	10/2008	Rolan et al.	
2008/0292749	A1	11/2008	Goodwins et al.	
2008/0299254	A1	12/2008	Kim et al.	
2009/0264520	A1	10/2009	Bhagat et al.	
2009/0274817	A1	11/2009	Yamaguchi et al.	
2010/0074998	A1	3/2010	Vega et al.	
2010/0136201	A1	6/2010	Bigeard et al.	
2010/0196575	A1	8/2010	Sanchez	
2010/0233347	A1	9/2010	Uhrhan	
2010/0249560	A1	9/2010	Levinson et al.	
2010/0281765	A1	11/2010	Schwartz	
2010/0310738	A1	12/2010	Ludwig	
2010/0311950	A1	12/2010	Kugitani	
2011/0008502	A1	1/2011	Hosomi et al.	
2011/0064862	A1	3/2011	McCready et al.	
2011/0081386	A1	4/2011	Guth et al.	
2011/0081435	A1	4/2011	Guth et al.	
2011/0117180	A1	5/2011	Yan et al.	
2011/0286992	A1	11/2011	Gruber et al.	
2011/0287467	A1	11/2011	Crane et al.	
2011/0288389	A9	11/2011	Levinson et al.	
2012/0059150	A1	3/2012	Moloney et al.	
2012/0093994	A1	4/2012	Hsieh et al.	
2013/0004617	A1	1/2013	Zhang et al.	
2014/0011347	A1	1/2014	Yerushalmi et al.	
2014/0220217	A1	8/2014	Brown et al.	
2015/0289541	A1	10/2015	Brown et al.	
2015/0296834	A1	10/2015	Geistlinger et al.	
2015/0296835	A1	10/2015	Anderson et al.	
2015/0366233	A1	12/2015	Brown et al.	
2016/0305361	A1	10/2016	Akita	
2017/0105438	A1	4/2017	Ajami et al.	
2017/0172169	A1	6/2017	Grzanich	
2017/0188612	A1	7/2017	Varadan et al.	
2017/0321204	A1	11/2017	Kale et al.	
2018/0027851	A1	2/2018	Vrlijic et al.	
2018/0168209	A1	6/2018	Fraser et al.	
2018/0192680	A1	7/2018	Fraser et al.	
2018/0199605	A1	7/2018	Fraser et al.	
2018/0199606	A1	7/2018	Fraser et al.	
2019/0008192	A1	1/2019	Brown et al.	
2019/0116855	A1	4/2019	Vrljic et al.	
2019/0133162	A1	5/2019	Varadan et al.	
2019/0133163	A1	5/2019	Varadan et al.	
2019/0200658	A1	7/2019	Vrljic et al.	

FOREIGN PATENT DOCUMENTS

CN	1407108	A	4/2003
CN	1466903		1/2004
CN	1557188		12/2004
CN	1593223	A	3/2005
CN	1634524	A	7/2005
CN	101138405		3/2008
CN	101156632		4/2008
CN	101489422		7/2009
CN	101541187	A	9/2009
CN	101606574	A	12/2009
CN	101861895		10/2010
CN	101897418		12/2010
CN	102440302		5/2012
CN	102578544		7/2012
CN	102835460		12/2012
DE	102007061256		6/2009
DE	202011002097		3/2011
EP	0136428		4/1985
EP	0500132		8/1992
EP	0815736		1/1998
EP	1166653		1/2002
EP	1254601		11/2002
EP	0680751		11/2004
EP	1529444		5/2005
EP	1759593		3/2007
EP	1759593	A1 *	3/2007 A23J 3/10
EP	1361264		4/2007
EP	1952695		8/2008
EP	2138052		12/2009
EP	2943072		11/2015
GB	836694		6/1960
GB	858333		1/1961
GB	858660		1/1961
GB	1032334		6/1966
GB	1069104		5/1967
GB	1076948		7/1967
GB	1082504		9/1967
GB	1084619		9/1967
GB	1099711		1/1968
GB	1115610		5/1968
GB	1126889		9/1968
GB	1130631		10/1968
GB	1135123		11/1968
GB	1146337		3/1969
GB	1148449		4/1969
GB	1182976		3/1970
GB	1198398		7/1970
GB	1205882		9/1970
GB	1206265		9/1970
GB	1221482		2/1971
GB	1224989		3/1971
GB	1232719		5/1971
GB	1234927		6/1971
GB	1256462		12/1971
GB	1283913		8/1972
GB	1284357		8/1972
GB	1302525		1/1973
GB	1311638		3/1973
GB	1313830		4/1973
GB	1318460		5/1973
GB	1325335		8/1973
GB	1357091		6/1974
GB	1364747		8/1974
GB	1382335		1/1975
GB	1384332		2/1975
GB	1447730		8/1976
GB	1471907		4/1977
GB	1515961		6/1978
GB	1515962		6/1978
GB	1525541		9/1978
GB	2016255		9/1979
JP	S42-22194		10/1942
JP	S49-39824		10/1974
JP	S51-63971		10/1975
JP	52156962		12/1977
JP	S53115846		10/1978
JP	S54122766		9/1979
JP	S5959151		4/1984
JP	S6283842		4/1987
JP	H08140627		6/1996
JP	H08173024		7/1996
JP	H08289761		11/1996
JP	H0970272		3/1997
JP	H11-508448		7/1999
JP	2001/037434		2/2001
JP	2001/061415		3/2001
JP	2001/346533		12/2001
JP	2002/101835		4/2002
JP	2005/021163		1/2005
JP	2005/530483		10/2005
JP	2009-516522		4/2009

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

JP	2009/171877	8/2009
JP	2009/284893	12/2009
JP	2010/512788	4/2010
JP	2010-523125	7/2010
JP	2011/000073	1/2011
JP	2012/016336	1/2012
JP	2013-192528	9/2013
JP	2014/113112	6/2014
KR	10-2009-0009990	1/2009
RU	2144293	1/2000
RU	2010137628	3/2012
SU	291395	6/1971
SU	301014	1/1974
WO	WO 1993/025697	12/1993
WO	WO 1994/017673	8/1994
WO	WO 1996/017981	6/1996
WO	WO 1997/01961	1/1997
WO	WO 1998/012913	4/1998
WO	WO 1998/053698	12/1998
WO	WO 2001/022829	4/2001
WO	WO 2001/022830	4/2001
WO	WO 2003/070172	8/2003
WO	WO 2004/113543	12/2004
WO	WO 2005/013713	2/2005
WO	WO 2005/046354	5/2005
WO	WO 2005/097059	10/2005
WO	WO 2006/042608	4/2006
WO	WO 2007/060288	5/2007
WO	WO 2007/115899	10/2007
WO	WO 2007/137125	11/2007
WO	WO 2007/137128	11/2007
WO	WO 2007/118751	12/2007
WO	WO 2008/017499	2/2008
WO	WO 2008/030089	3/2008
WO	WO 2008/083117	7/2008
WO	WO 2009/060678	5/2009
WO	WO 2010/101625	9/2010
WO	WO 2012/106751	8/2012
WO	WO 2012/110797	8/2012
WO	WO 2012/116703	9/2012
WO	WO 2010/110493	10/2012
WO	WO 2012/157544	11/2012
WO	WO 2013/010037	1/2013
WO	WO 2013/010042	1/2013
WO	WO 2013/013292	1/2013
WO	WO 2013/138793	9/2013
WO	WO 2014/110532	7/2014
WO	WO 2014/110540	7/2014
WO	WO 2015/127388	8/2015

OTHER PUBLICATIONS

Brewer, "The Chemistry of Beef Flavor—Executive Summary". Available online at http://beefresearch.org/CMDocs/BeefResearch/The%20Chemistry%20of%20Beef%20Flavor.pdf (Year: 2006).*

Asgar et al., "Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs". Comprehensive Reviews in Food Science and Food Safety vol. 9. (Year: 2010).*

Analysis of soy hydrolysate, Avomen Analytical Services Laboratory Test Results, Jul. 25, 2019.

Biede, et al., "Swiss cheese flavor: I. chemical analysis," Journal of Dairy Science, 1979, 62:227-237.

Declaration of Dr. Jorge Ruiz-Carrascal, dated Jul. 29, 2019, 72 pages.

EP Opposition Letter from Opponent in EP Appln. No. 14737766.7 dated Aug. 6, 2019, 55 pages.

Estevez, et al., "Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS," Journal of Agricultural and Food Chemistry, 2003, 51:3429-3435.

Friedman and Brandon, "Nutritional and health benefits of soy proteins," J. Agric. Food Chem., 2001, 49(3):1069-1086.

Ibrahim, et al., "Mechanism of the CO-sensing heme protein CooA: new insights from the truncated heme domain and UVRR spectroscopy, Journal of Inorganic Biochemistry," 2007, 101:1776-1785.

Kendrick and Watts, "Acceleration of inhibition of lipid oxidation by heme compounds," Lipids, 1969, 4:454-458.

Mahajan, et al., "Aroma compounds in sweet whey powder," Journal of Dairy Science, 2004, 87:4057-4063.

Meisinger, et al., Flavor relationships among muscles from the beef chuck and round, Journal of Animal Science, 2006, 84:2826-2833.

Singh, et al., "Functional and edible uses of soy protein products," Comp. Rev. Food Sci. Food Safety, 2008, 7:14-28.

Vaghefi, et al., "Influence of the extent of hemoglobin hydrolysis on the digestive absorption of heme iron. An in vitro study," Journal of Agricultural and Food Chemistry, 2002, 50:4969-4973.

Yeast Metabolom Database, "Heme (YMDB0041)," (http://www.ymdb.ca/compounds/YMDB00041).

Young and Pellett, "Plant proteins in relation to human protein and amino acid nutrition," Am. J. Clin. Nutr., 1994, 59(Suppl):1203S-1212S.

"Heterologous," Merriam-Webster Dictionary, retrieved on Sep. 10, 2015, http://www.merriam-webster.com/dictionary/heterologous, 1 page.

"Rethink Meat," Presented at the 6th Annual Sustainable Innovation Forum, Paris, France, Dec. 7-8, 2015, retrieved on Feb. 1, 2016, https://amp.twimg.com/v/7c7f7084-b173-42cb-bc12-723f35994dff, 1 page (Video Submission).

"Silicon Valley gets a taste for food," The Economist Technology Quarterly, Mar. 7, 2015, http://cdn.static-economist.com/sites/default/files/sponsorships/accenture_tq_march2015/20150307_tq_mailout.pdf, pp. 11-13.

"Veggie burgers that look, taste, and bleed like real meat," CBS News, Aug. 9, 2016, retrieved Aug. 25, 2016 <http://www.cbsnews.com/news/food-trend-veggie-burgers-that-look-bleed-taste-like-real-meat/>, 4 pages.

"Watch Momofuku Cook Impossible Foods' Plant-Based Burger that 'Bleeds'," Vice, Jul. 27, 2016, retrieved Aug. 25, 2016, <https://munchies.vice.com/en/videos/watch-momofuku-cook-impossible-foods-plant-based-burger-that-bleeds>, 3 pages.

[No Author Listed] Impossible Foods Inc. "GRAS Notification for Soybean Leghemoglobin Protein Derived from Pichia Pastoris." GRAS notice 000737, Retrieved from internet <<URL:https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htmf>, 1063 pages (Oct. 2, 2017).

"Acidified Milk Products and Protein Stabilisation," Herbstreith & Fox, retrieved on Mar. 3, 2014, http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html, 15 pages.

"Ice Cream and Ice Cream Desserts," Herbstreith & Fox, retrieved on Mar. 3, 2014, http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html, 5 pages.

"Innovation at Its Best: 5 Years of Food Valley Awards," Food Valley, retrieved on Mar. 7, 2014, http://www.foodvalley.nl/English/Afbeeldingen/FVAjubileumuitgave/Innovation%20at%20Its%20Best%20-%205%20Years%20of%20Food%20Valley%20Awards.pdf, 51 pages.

"Low Methylester Amidated Pectins," Herbstreith & Fox, retrieved on Mar. 3, 2014, http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html, 13 pages.

"Stabilisation of Whey and Whey Mix Products with Pectin," Herbstreith & Fox, retrieved on Mar. 3, 2014, http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html, 6 Pages.

"Texturising of Fermented Milk Products," Herbstreith & Fox, retrieved on Mar. 3, 2014, http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html, 6 pages.

Alemán et al., "Oxidative stability of a heme iron-fortified bakery product: Effectiveness of ascorbyl palmitate and co-spray-drying of heme iron with calcium caseinate," Food Chemistry, 2016, 196:567-576.

(56) **References Cited**

OTHER PUBLICATIONS

Asgar et al., "Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs," Comprehensive Reviews in Food Science and Food Safety, 2010, 9:513-529.
Aubrey, "Food For Thought: Saving The Planet, One Burger At A Time: This Juicy Patty Is Meat-Free," The Salt, Feb. 11, 2017, retrieved on Feb. 14, 2017, retrieved from <http://www.npr.org/sections/thesalt/2017/02/11/514544431/saving-the-planet-one-burger-at-a-time-this-juicy-patty-is-meat-free>, 14 pages.
Australian Patent Examination Report No. 1 in Australian Application No. 2012281064, dated Jan. 25, 2016, 5 pages.
Australian Patent Examination Report No. 1 in Australian Application No. 2012281069, dated Sep. 25, 2015, 5 pages.
Baek, "Process Flavors," Handbook of Meat, Poultry and Seafood Quality, Second Edition, 2012, Chapter 7, 91-104.
Baohua, "Animal products processing,", China agricultural science and technology press, 2008, pp. 224-222, English Translation.
Bastide et al., "Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved.", Cancer Prev Res; 4(2); 177-84, 2011.
Bastide et al., "Heme Iron from Meat and Risk of Colorectal Cancer: A Meta-analysis and a Review of the Mechanisms Involved," Cancer Prevention Research, 2011, vol. 4, pp. 177-184.
Battaglia et al., "The Enigmatic LEA Proteins and Other HydroPhilins1[W]," Plant Physiology, Sep. 2008, 148:6-24.
Belitz et al., "Aroma Compounds," Food Chemistry, Springer 2009, pp. 340-402.
Belitz et al., Food Chemistry, 3rd revised edition. Springer-Verlag, Berlin (2006), p. 368.
Beuchat et al., "Fermentation of Peanut Milk with Lactobacillus bulgaricus and L. acidophilus," J. Food Sci, 1978, 43:1109-1112.
Beyond Better Order page and Nutritional Facts, retrieved on Feb. 6, 2014, http://www.beyond-better.com/order.html, 8 pages.
Beyond Meat, posted on or before Feb. 24, 2001, accessed Jan. 7, 2014, http://beyondmeat.com/, 2 pages.
Boca Bruschetta Tomato Basil Parmesan Veggie Patties Package Ingredients, posted on or before Jul. 22, 2008, accessed on Jan. 7, 2014, http://www.bocaburger.com/products/nutrition-info.aspx?product=5928360103, 1 page.
Boca Flame Grilled Meatless Burgers Package Ingredients, posted on or before Jul. 14, 2008, accessed on Jan. 7, 2014, http://www.bocaburger.com/products/nutrition-info.aspx?product=5928367321, 1 page.
Boca Original Meatless Chik'n Nuggets Package Ingredients, posted on or beforeJul. 22, 2008, accessed Jan. 7, 2014, http://www.bocaburger.com/products/nutrition-info.aspx?product=5928360012, 1 page.
Boca Original Vegan Meatless Burgers Package Ingredients, posted on or before Jul. 14, 2008, accessed Jan. 7, 2014, http://www.bocaburger.com/products/nutrition-info.aspx?product=5928333445, 1 page.
Boral and Bohidar , "Effect Of Ionic Strength On Surface-Selective Patch Binding-Induced Phase Separation And Coacervation In Similarly Charged Gelatin-Agar Molecular Systems," Journal of Physical Chemistry B, 2010, 114(37): 12027-35.
Bradshaw, "Food 2.0: the future of what we eat," FT Magazine, Oct. 31, 2014, retrieved on Nov. 11, 2014, http://www.ft.com/cms/s/2/bfa6fca0-5fbb-11e4-8c27-00144feabdc0.html#axzz3InGaCIdL, 6 pages.
Brewer, "The Chemistry of Beef Flavor," Dec. 2006, retrieved on Aug. 30, 2016, <http://beefresearch.org/CMDocs/BeefResearch/The%20Chemistry%20of%20Beef%20Flavor.pdf>, 16 pages.
Brooks et al., "Prediction of beef flavor by precursor and volatile compounds Principal Investigators: Funded by The Beef Checkoff," Texas Tech University, May 31, 2012, retrieved Aug. 30, 2016, <http://www.beefresearch.org/CMDocs/BeefResearch/PE_Project_Summaries_FY11Prediction_of_beef_flavor.pdf>.
Brown et al., "The structure and function of mammalian and plant globins," International Review of Scientific Synthesis, Sep. 2013, 2014, 21 pages.
Bunge et al., "Quest Heats up for Alternatives to Beef," The Wall Street Journal, Business News, Nov. 4, 2016, p. B5.
Burdock, "Fenaroli's handbook of flavor ingredients," CRC press, 17 pages (2016).
Bute Island Foods, "Sheese," posted on or before Dec. 5, 2006, retrieved on Feb. 6, 2014, http://www.buteisland.com/a_sheese_home.htm, 2 pages.
Cadwallader and Macleod, "16 Instrumental methods for analyzing the flavor of muscle foods," Flavor of Meat, Meat Products and Seafoods, 18 pages (1998).
Calkins et al., "A fresh look at meat flavor," Meat Science, 77(1):63-80 (2007).
Carlsen et al., "Heme-iron in lipid oxidation," Coordination Chemistry Review, 2005, 249:485-498.
Cerny et al., "Formation of Aroma Compounds from Ribose and Cysteine during the Maillard Reaction," J. Agric. Food Chem., 2003, 51, pp. 2714-2721.
Chamlee, "Why Do People Want Veggie Burgers That Bleed?," Eater, Jul. 25, 2016, retrieved Aug. 25, 2016, <http://www.eater.com/2016/7/25/12270698/lab-grown-meat-beyond-burger-impossible-foods>, 11 pages.
Chau, "Uncanny Patty," The Ringer, Feb. 27, 2017, retrieved on Feb. 28, 2017, retrieved from <https://theringer.com/impossible-burger-last-meal-on-earth-week-food-f9f14acdb99d#.vocb2hi6e>, 19 pages.
Chaudhari et al., "The cell biology of taste," 190(3):285-296 (Aug. 2010).
Chen et al., "Effect of Urea on Volatile Generation from Maillard Reaction of Cysteine and Ribose," J. Agric. Food Chem., 48:3512-3516 (2000).
Chen et al., "Influence of DNA on Volatile Generation from Maillard Reaction of Cysteine and Ribose," Nutraceutical Beverages, American Chemical Society, pp. 427-442 (Dec. 2003).
Chicago Vegan Foods, accessed on Jan. 7, 2014, http://chicagoveganfoods.com/products/teese-vegan-cheese/, 8 pages.
Christlbauer et al., "Characterization of the Key Aroma Compounds in Beef and Pork Vegetable Gravies á la Chef by Application of the Aroma Extract Dilution Analysis," J. Agric. Food Chem., 2009, 57:9114-9122.
Clare et al., "Effects of Transglutaminase Catalysis on the Functional and Immunoglobulin Binding Properties of Peanut Flour Dispersions Containing Casein," J. Agric. Food Chem., 2008, 56(22):10913-10921.
Connelly and Piper, "Person of the Year: Tal Ronnen," VegNews, Nov./Dec. 2013, 29-32.
Cott et al., "The 'Impossible' Veggie Burger: A Tech Industry Answer to the Big Mac," Business Day, Jan. 13, 2017, retrieved on Jan. 17, 2017, <https://mobile.nytimes.com/2017/01/13/business/veggie-burger-impossible-burger.html?referer=http://www.drudgereport.com/>, 7 pages.
Cross et al., "Developing a heme iron database for meats according to meat type, cooking method and doneness level", Food Nutr Sci., 3(7): 905-913, 2012.
Dai, "David Chang Adds Plant Based 'Impossible Burger' to Nishi Menu," Jul. 26, 2016, retrieved Jul. 27, 2016 <http://ny.eater.com/2016/7/26/12277310/david-chang-impossible-burger-nishi>, 6 pages.
Daiya, Deliciously Dairy Free, "Say Cheese, Dairy-Free cheesy deliciousness," posted on or before Jan. 26, 2010, accessed Jan. 7, 2014, http://www.daiyafoods.com, 6 pages.
Datar, I. et al., "Possibilities for an in vitro meat production system" Innovative Food Science and Emerging Technologies, vol. 11, 13-22, 2010.
Davis et al., "Some Rheological Properties of Aqueous Peanut Flour Dispersions," J. Texture Studies, 2007, 38:253-272.
Deliciously Healthy Nacheez, Products and Nutrition Facts, posted on or before Jan. 23, 2011, retrieved on Feb. 7, 2014, http://nacheez.com/, 9 pages.
Dixie Diner's Club, Cheese (Not!) Sauce Nutrition Facts, posted on or before Sep. 3, 2009, retrieved on Feb. 7, 2014, http://www.dixiediner.com/cheese-notÂ™-sauce-regular-cheese-p-69.html, 2 pages.
D'Onfro, "I tried the plant-based meat that Google wanted to buy and I never want to eat a 'real' hamburger again" Business Insider,

(56) **References Cited**

OTHER PUBLICATIONS

Jun. 12, 2016, retrieved Jun. 14, 2016, <http://www.businessinsider.com/impossible-burgers-taste-test-2016-6>, 14 pages.
Donnelly, "Meet the Impossible Burger: It Looks and Tastes Like the Real Thing But Is Totally Meat-Free," Vogue, Aug. 1, 2016, retrieved Aug. 25, 2016 <http://www.vogue.com/13462891/impossible-burger-meat-free-vegan-david-chang/>, 6 pages.
Door 86 Vegan Cheese, Discover a New World of Vegan Cheese and Menu, posted on or before Dec. 5, 2013, retrieved Feb. 7, 2014, http://door86vegancheese.wix.com/door-86-vegan-cheese#, 14 pages.
Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Brazil Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-cashew-brazil.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Crystal Algae Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-cashew-crystal.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Dulse Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-cashew-dulse.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Hemp Seeds Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-cashew-hemp.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Cashew Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-cashew-nut-cheese.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Macadamia & Hemp Seeds Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-macadam-hemp.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Aged Macadamia Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, http://www.dr-cow.com/products/aged-macadam-nut-cheese.html, 1 page.
Dr. Cow, Natural Living & Organic Foods, "Cashew Nut Cream Cheese," posted on or before Sep. 22, 2008, accessed Jan. 7, 2014, http://www.dr-cow.com/products/cashew-nut-cream-cheese.html, 1 page.
Duane, "Engineering the Future of Artisanal Vegan Cheese," Food & Wine, Nov. 2013, http://www.foodandwine.com/articles/engineering-the-future-of-artisanal-vegan-cheese, 5 pages.
DuFosse et al., "Importance of lactones in food flavours," Sciences Des Aliments, 14:17-25 (1994).
Dwivedi, Basant K. et al., "Meat flavor" Critical Reviews in Food Science & Nutrition, vol. 5, 487-535, 1975.
Edris et al., "Application of headspace-solid-phase microextraction and HPLC for the analysis of the aroma volatile components of treacle and determination of its content of 5-hydroxymethylfurfural (HMF)", Food Chemistry vol. 104, Issue 3, pp. 1310-1314, 2007.
Elise, "Classic Meatloaf Recipe," Simply Recipes, 2009, https://www.simplyrecipes.com/recipes/classic meatloaf/, downloaded Nov. 22, 2017, 11 pages.
Ellfolk, Nils, "Crystalline Leghemoglobin" ACTA Chemica Scandinavica, vol. 15, 545-554, 1961.
Ellis et al., "Structure of ferric soybean leghemoglobin a nicotinate at 2.3 A resolution," Acta Crystallographica, May 1997, Section D, 53(3):302-310.
Elmore et al., "Effect of the Polyunsaturated Fatty Acid Composition of Beef Muscle on the Profile of Aroma Volatiles," J. Agric. Food Chem. 47:1619-1625 (1999).
Elzerman et al., Exploring meat substitutes: consumer 2013 experiences and contextual factors: British Food Journal, vol. 115 Issue: 5, pp. 700-710, 705, 2013.
EPO Opposition, "Proprietor's observations and amendments under Rule 79(1) EPC," Feb. 27, 2019, 50 pages.
Etienne, "Eating the plant-derived Impossible Burger cooked by Momofuku's David Chang," Tech Crunch, Jul. 26, 2016, retrieved Aug. 25, 2016, <https://techcrunch.com/2016/07/26/eating-the-plant-derived-impossible-burger-cooked-by-momofukus-david-chang/>, 9 pages.
European Search Report (Supplementary) in European Application No. 12810661.4, dated Mar. 12, 2015, 14 pages.
European Search Report (Supplementary) in European Application No. 12811683.7, dated Mar. 12, 2015, 9 pages.
European Search Report for International Application No. EP 14737766, dated Jul. 15, 2016, 11 pages.
European Search Report for International Application No. EP 17210528, dated May 14, 2018, 10 pages.
Fang et al., "Food Nutrition health theory and technology," China light industry press, p. 448, Jan. 31, 1997 (English Translation).
Fantastic World Foods, "Fantastic Foods Nature's Burger (Meatless Burger Mix)," posted on or before Jan. 6, 2009, accessed on Jan. 7, 2014, http://fantasticfoods.elsstore.com/view/product/?id=8715&cid=1967, 2 pages.
Felt, "Raw Vegan Almond Ricotta Cheese," FeedYourSkull, Mar. 12, 2012, https://feedyourskull.com/2012/03/12/raw-vegan-almond-ricotta-cheese/, 15 pages.
Fengyi et al., "Soybean protein production and application,", China light industry press, 2004, pp. 275-277, English Translation.
Field et al., "Heme Pigment Content of Bovine Hemopoietic Marrow and Muscle," J. Food Sci., 45:1109-1112, 1980.
Follow Your Heart Homepage, posted on or before Nov. 28, 1999, accessed Jan. 7, 2014, http://www.followyourheart.com, 3 pages.
Follow Your Heart, Products and Nutrition Facts, posted on or before Nov. 28, 1999, accessed Feb. 7, 2014, http://www.followyourheart.com/products/, 26 pages.
Foo, "Beef and Scallop Stir-Fry," Food & Wine, Jul. 2001, retrieved on Sep. 10, 2015, http://www.foodandwine.com/recipes/beef-and-scallop-stir-fry/print, 3 pages.
Food for Lovers, Vegan Queso Original & Vegan Queso Mild, posted on or before Oct. 27, 2011, retrieved Feb. 7, 2014, http://www.foodforlovers.com/products, 3 pages.
Fountoulakis and Lahm, "Hydrolysis and amino acid composition analysis of proteins", J. Chromatogr A, 826: 109-134, 1998.
Fourth Chinese Office Action in Chinese Application No. 201280041713.1, dated Nov. 11, 2016, 18 pages (with translation).
Free & Easy Dairy Free Cheese Flavour Sauce Mix, Holland & Barrett, posted on or before Jun. 22, 2013, retrieved Feb. 7, 2014, http://www.hollandandbarrett.com/pages/product_detail.asp?pid=2686, 2 pages.
Fromson, "The Race to Build a Fake-Meat Burger That Just Might Save the World, Free the cows!" New York Magazine, Jun. 1-7, 2015, 46-48.
Galaxy Foods Vegan Soy Grated Parmesan, ShopRite, retrieved Feb. 7, 2014, http://www.shoprite.com/pd/Galaxy-Nutritional-Foods/Vegan-Grated-Soy-Topping-Parmesan-Flavor/4-oz/077172640006/, 6 pages.
Gardein The Ultimate Beefless Burger Package Ingredients, posted on or before 2013, accessed Jan. 7, 2014, http://gardein.com/products/beefless-burger/, 12 pages.
Gardenburger The Original Veggie Burger Package Ingredients, posted on or before Oct. 5, 2008, accessed Jan. 7, 2014, http://www.gardenburger.com/product.aspx?id=11630, 1 page.
GenBank Accession No. AAA02168.1, May 21, 1993, 1 page.
GenBank Accession No. AFK42304.1, unknown [Medicago truncatula], May 25, 2012, 1 page.
Gharst, "Biochemical and Rheological Characteristics of Peanut Proteins Crosslinked with Microbial Transglutaminase," A dissertation submitted to the Graduate Faculty of North Carolina State University, Raleigh NC, 2007, 149 pages.
Gharst, "Effects of Transglutaminase Catalysis on the Functional and Immunoglobulin Binding Properties of Peanut Flour Dispersions Containing Casein," J. Agric. Food Chem., 2008, 56:10913-10921.
Gharst, "The Effect of Transglutaminase Crosslinking on the Rheological Characteristics of Heated Peanut Flour Dispersions," J. Food Sci., 2007, 72(7):C369-C375.
Gilbert et al., "The revolutionary meatless burger from Impossible Foods is perfect for vegetarians and carnivores alike," Tech Insider,

(56) **References Cited**

OTHER PUBLICATIONS

Aug. 4, 2016, retrieved on Aug. 25, 2016, <http://www.techinsider.io/the-impossible-foods-burger-review-vegetarian-2016-8>, 9 pages.
Go Veggie!, "O% Dairy. 100% Yum.," posted on or before 2013, accessed Jan. 7, 2014, http://goveggiefoods.com/our-products/dairy-free-cheese-alternative-products/, 1 page.
Go Veggie!, Dairy Free Products and Nutrition Facts, posted on or before 2013, accessed Feb. 7, 2014, http://goveggiefoods.com/our-products/dairy-free-cheese-alternative-products/, 13 pages.
Gordinier, "Masters of Disguise Among Meatless Burgers," The New York Times, Mar. 22, 2011, accessed Jan. 7, 2014, http://www.nytimes.com/2011/03/23/dining/23meatless.html?pagewanted=all&_r=0, 5 pages.
Griffths, "XCIII. The Action of Gastric Juice on Beef Muscle-Globulin, With References to Anaemia," Biochemistry Journal, 28:671-675 (1934).
Grigorakis et al., "Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*): sensory differences and possible chemical basis," Aquaculture 225:109-119 (2003).
Grobart, "Making a Steak Without a Cow," Bloomberg Technology, Jun. 21, 2016, retrieved Jun. 23, 2016 <http://www.bloomberg.com/news/articles/2016-06-21/making-a-steak-without-the-cow>, 2 pages.
Grosch, "Evaluation of the Key Odorants of Food by Dilution Experiments, Aroma Models and Omission," Chem. Senses 26:533-545 (Jun. 2001).
Grounds for Opposition Against European Patent No. EP 2 943 072, dated Sep. 27, 2018, 44 pages.
Grunwald et al., "Mechanisms of Heme Protein-Mediated Lipid Oxidation Using Hemoglobin and Myoglobin Variants in Raw and Heated Washed Muscle," J. Agric. Food Chem., 2006, 54:8271-8280.
Hanlon, "Fake Meat: is science fiction on the verge of becoming fact?," The Guardian, Jun. 22, 2012, http://www.theguardian.com/science/2012/jun/22/fake-meat-scientific-breakthroughs-research, 7 pages.
Hannah, "A fermented feast," Bittersweet, retrieved on Nov. 3, 2016, retrieved from <https://bittersweetblog.com/2010/06/09/a-fermented-feast/>, 2 pages.
Heller, "Barbecued Soybeans," Vegetarian Soybean Recipes, Mother Earth News, Jan./Feb. 1985, http://motherearthnews.com/real-food/vegetarian-soybean-recipes-zmaz85asie.aspx.
Heme Protein Database, "Welcome to the Heme Protein Database," posted on or before Apr. 14, 2013, accessed Dec. 18, 2013, http://hemeprotein.info/heme.php, 1 page.
Heritage Health Food Creamy Veeta Cheese Sauce Mix, Vegan Essentials, posted on or before Aug. 13, 2013, retrieved Feb. 7, 2014, http://store.veganessentials.com/creamy-veeta-cheeze-sauce-mix-by-heritage-health-food-p3945.aspx, 1 page.
Herper, "Mission Impossible Burger: Tasting The Fake Meat That Wants To Save The World," Forbes, Jul. 28, 2016, retrieved on Aug. 25, 2016, <http://www.forbes.com/sites/matthewherper/2016/07/28/mission-impossible-burger-tasting-the-fake-meat-that-wants-to-save-the-world/#57781d823c43>, 6 pages.
Herper, "Drop that Burger," Forbes Online, Nov. 12, 2009, http://www.forbes.com/forbes/2009/1130/thought-leaders-mcdonalds-global-warming-drop-that-burger.html, 4 pages.
Homma et al. "Cheese-like food production from various nuts," Food Preservation Science, Japan 2009, Abstract.
Hoshaw, "Silicon Valley's Bloody Plant Burger Smells, Tastes and Sizzles Like Meat" the salt, Jun. 21, 2016, retrieved Jun. 21, 2016 <http://www.npr.org/sections/thesalt/2016/06/21/482322571/silicon-valley-s-bloody-plant-burger-smells-tastes-and-sizzles-like-meat>, 8 pages.
Hui et al., "Handbook of meat and meat processing," CRC Press, 2012, retrieved on Dec. 5, 2016, retrieved from <https://www.crcpress.com/Handbook-of-Meat-and-Meat-Processing-Second-Edition/Hui/p/book/9781439836835>, 3 pages.
International Preliminary Report on Patentability in International Application No. PCT/US2014/011361, dated Jul. 14, 2015, 13 pages.
International Preliminary Report on Patentability in International Application No. PCT/US2012/046560, dated Jan. 23, 2014, 12 pages.
International Preliminary Report on Patentability in International Application No. PCT/US2012/46552, dated Jan. 23, 2014, 9 pages.
International Preliminary Report on Patentability in International Application No. PCT/US14/11347, dated Jul. 14, 2015, 10 pages.
International Preliminary Report on Patentability in International Application No. PCT/US14/11362, dated Jul. 23, 2015, 10 pages.
International Search Report and Written Opinion in International Application No. PCT/US2012/046560, dated Dec. 14, 2012, 11 pages.
International Search Report and Written Opinion in International Application No. PCT/US2012/46552, dated Nov. 19, 2012, 12 pages.
International Search Report and Written Opinion in International Application No. PCT/US14/11347, dated Jul. 3, 2014, 20 pages.
International Search Report and Written Opinion in International Application No. PCT/US14/11361, dated Jun. 16, 2014, 26 pages.
International Search Report and Written Opinion in International Application No. PCT/US14/11362, dated Jun. 13, 2014, 19 pages.
International Search Report and Written Opinion in International Application No. PCT/US2015/017147, dated May 1, 2015, 15 pages.
International Search Report and Written Opinion in International Application No. PCT/US2015/023679, dated Aug. 28, 2015, 26 pages.
Invitation to Pay Fees in International Application No. PCT/US14/11361, dated Apr. 10, 2014, 4 pages.
Jamieson, "Iroquois Stew with Beef, Chicken and Pork," Epicurious, Nov. 1995, retrieved on Sep. 10, 2015, http://www.epicurious.com/recipes/food/printerfriendly/iroquois-stew-with-beef-chicken-and-pork-865, 2 pages.
Jensen, "Comparative Analysis of Autoxidation of Haemoglobin," J. Experimental Biology, 2001, 204:2029-2033.
Ju and Kilara, "Textural Properties of Cold-set Gels Induced from Heat-denatured Whey Protein Isolates," J. Food Science, 1998, 63(2): 288-292.
Jublot et al., "Quantitation of sulphur aroma compounds in maillard model reaction systems of different composition," Expression of Multidisciplinary Flavour Science, 4 pages (2010).
Kanani, "The Future of Meat is Meatless, Just as Tasty, And About to Change the World," Forbes, Mar. 6, 2014, retrieved on Sep. 11, 2015, http://www.forbes.com/sites/rahimkanani/2014/03/06/the-future-of-meat-is-meatless-just-as-tasty-and-about-to-change-the-world/, 8 pages.
Karahadian et al., "Action of Tocopherol-Type Compounds in Directing Reactions Forming Flavor Compounds in Autoxidizing Fish Oils," J. Amer. Oil Chem. Soc., 66:1302-8 (1989).
Kerscher et al., "Quantification of 2-Methyl-3-furnathiol, 2-Furfurylthiol, 3-Mercapto-2-pentanone in Heated Meat," J. Agric. Food Chem. 46:1954-1958 (1996).
Kerth and Miller, "Beef flavor: a review from chemistry to consumer," White Paper: Product Quality, Texas A&M University Dept of Animal Sciences, 25 pages (2013).
Khan et al., "Meat flavor precursors and factors influencing flavor precursors—A systematic review," Meat Science, 110:278-284 (Dec. 2010).
Konermann et al., "Acid-induced denaturation of myoglobin studied by time resolved electrospray ionization mass spectrometry", Biochemistry, 1997, vol. 36, pp. 6448-6454.
Koutsidis et al., "Water-soluble precursors of beef flavor: I. Effect of diet and breed," Meat Science, 79:124-130, 2008.
Kraft American Singles Package Ingredients, posted on or before Jun. 27, 2012, accessed on Jan. 7, 2014, http://www.kraftrecipes.com/Products/ProductInfoDisplay.aspx?SiteId=1&Product=2100060473, 1 page.
Kummer, "The Problem with Fake Meat," MIT Technology Review, Mar. 31, 2015, retrieved Apr. 20, 2016, <https://www.technologyreview.com/s/536296/the-problem-with-fake-meat/>, 11 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Kung et al., "Tobacco as a Potential Food Source and Smoke Material: Nutritional Evaluation of Tobacco Leaf Protein," J. Food Sci., 1980, 45(2):320-322, 327.
Lane et al., "The Variety of Odors Produced in Maillard Model Systems and How They are Influenced by Reaction Conditions," The Maillard Reaction in Foods and Nutrition, American Chemical Society, pp. 141-158 (Apr. 1983).
Leahy Gardens Vegan & Delicious, Macaroni & Cheese and Cheese Flavored Sauce Mix Product and Nutrition Facts, posted on or before Feb. 8, 2010, retrieved Feb. 7, 2014, http://www.leaheyfoods.com/products/MacCheese.aspx, 3 pages.
Leduc et al., "Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS," J. Sci. Food Agric., 92:2560-80 (2012).
Leghemoglobin, NCBI's database accession 004939, Mar. 1, 2002.
Lisanatti Foods, Vegan Cheeze Products and Nutrition Facts, posted on or before Mar. 26, 2013, retrieved Feb. 7, 2014, http://www.lisanatti.com/index.php?option=com_zoo&view=category&layout=category&Itemid=22, 5 pages.
Liu et al., "Intermolecular Interactions During Complex Coacervation Of Pea Protein Isolate And Gum Arabic," Journal of Agricultural and Food Chemistry, 2010, 58:552-556.
Lombardi et al., "Total Heme and Non-heme Iron in Raw and Cooked Meats," Journal of Food Science, 67(5):1738-1741 (2002).
Lopez, "We just tried the 'Impossible Burger'—the meatless burger NYC has been waiting for," Business Insider, Jul. 27, 2016, retrieved on Aug. 25, 2016, <http://www.businessinsider.com/what-the-impossible-burger-tastes-like-2016-7>, 5 pages.
Lugay and Kim, "Freeze alignment: A novel method for protein texturization," Utilization of Protein Resources, 1981, p. 177-187.
Luteness, "The Richest Source of Protein," Mosaic, May/Jun. 1979, 39-45.
Macleod, Glesni et al., "Natural and simulated meat flavors (with particular reference to beef)" Critical Reviews in Food Science & Nutrition, vol. 14, 309-437, 1981.
MacMunn, "An address on some of the applications of the spectroscope to medicine", The British Medical Journal, Jan. 3, 1891, pp. 3-9.
Maltais et al., "Formation of Soy Protein Isolate Cold-Set Gels: Proteins and Salt Effects," J. Food Science, 2005, 70 (1): C67-C73.
Maqsood et al., "Haemoglobin-mediated lipid oxidation in the fish muscle: A review," Trends in Food Science & Technology, 2012, 28:33-43.
Marshall et al., "We Tried the "Bleeding" Vegetarian Burger And It Was Actually Good," Jul. 27, 2016, retrieved Jul. 28, 2016 <https://www.buzzfeed.com/chelseamarshall/bleeding-vegetable-burger?utm_term=.jaa03Kyo7#.ogV0m7MAW>, 10 pages.
McGorrin, "Advances in Dairy Flavor Chemistry," Food Flavors and Chemistry: Advances of the New Millennium, Spanier, A. M.; Shahidi,F.; Parliment, T. H.; Mussinan, C. J.; Ho, C.-T.; Contis, E. T., Eds., RoyalSociety of Chemistry, Cambridge, pp. 67-84 (2001).
McGorrin, "Character-impact flavor and off-flavor compounds in foods," Flavor, Fragrance, and Odor Analysis, 2nd, 207-262 (2012).
McGorrin, "The significance of volatile sulfur compounds in food flavors," Volatile sulfur compounds in food 1068, 29 pages (2011).
Mercola, "Controversy over fake meat burger," 2011, https://articles.mercola.com/sites/articles/archive/2017/08/21/impossible-burger-meat-substitute.aspx (Year: 2011), 9 pages.
Moon et al., "Odour-active components of simulated beef flavour analyzed by solid phase microextraction and gas chromatography-mass spectrometry and -olfactometry," Food Research International, 39:294-308 (Apr. 2006).
Morita, "Comparison of aroma characteristics of 16 fish species by sensory evaluation and gas chromatographic analysis," J. Sci. Food Agric., 83:289-297 (2003).
Morningstar Farms Garden Veggie Patties Package Ingredients, posted on or before Jun. 26, 2013, accessed Jan. 7, 2014, https://www.morningstarfarms.com/products/burgers/garden-veggie-patties, 6 pages.
Mottram et al., "Formation of Sulfur Aroma Compounds in Reaction Mixtures Containing Cysteine and Three Different Forms of Ribose," J. Agric. Food Chem., 50:4080-4086 (2002).
Mottram, "Flavour formation in meat and meat products: a review," Food Chemistry, 62(4):415-24 (Aug. 1998).
Mottram,"An Overview of the Contribution of Sulfur-Containing Compounds to the Aroma in Heated Foods," Heteroatomic Aroma Compounds, American Chemical Society, pp. 73-92 (Aug. 2002).
Nacho Mom's Vegan Queso, Products and Nutrition Facts, posted on or before Sep. 20, 2010, retrieved on Feb. 7, 2014, http://fatgoblin.com/Home.html, 6 pages.
Naike, "Food Flavor Chemistry," 1st Edition China Light Industry Press, pp. 236-243 (1996) (English Translation).
Nielsen et al., "Improved Method for Determining Food Protein Degree of Hydrolysis", Journal of Food Science: Food Chemistry and Toxicology, vol. 66, 2001.
Nielson, Introduction to the Chemical Analysis of Foods, Jones & Bartlett Publishers, 1994.
Nutty Cow Nut Cheeses, Products and Nutrition Facts, posted on or before Jul. 23, 2012, retrieved Feb. 7, 2014, http://www.nuttycow.com/, 6 pages.
Oellingrath, "Heat Degradation of Heme In Met-hemoglobin and Met-myoglobin Model Systems Measured by Reversed-Phase Ion-Pair High Performance Liquid Chromatography," J Food Science, 1998, 53(1): 40-42.
Office Action in Chinese Application No. 201280041713.1, dated Jul. 13, 2017, 28 pages (English Translation).
Office Action in Chinese Application No. 201480013778.4, dated Aug. 8, 2017, 24 pages (English Translation).
Office Action in Chinese Application No. 201480014349.9, dated Jul. 24, 2017, 52 pages (English Translation).
Office Action in Japanese Patent Application No. 2015552865, dated Oct. 24, 2017, 15 pages (English Translation).
Office Action in Russian Patent Application No. 2014104812, dated May 23, 2017, 8 pages (English Translation).
Ofori and Hsieh, "The Use of Blood and Derived Products as Food Additives", Chapter 13 of book Food Additive Edited by Yehia El-Samragy Published: Feb. 22, 2012.
Oshodi et al., "In vitro protein digestibility, amino acid profile and available iron of infant-weaning food prepared from maize flour and bovine blood", Food Research International, vol. 30, No. 3-4, pp. 193-197, 1997.
PancakeNinja, "Beef and chicken cheese burgers"—Pancake Ninja, 2011, http://pancake-ninja.blogspot.com/2011/06/beef-and-chicken-cheese-burgers.html, downloaded Nov. 22, 2017, 8 pages.
Parmela Parmesan Style Aged Nut Cheese, Product and Nutrition Facts, 2012, retrieved Feb. 7, 2014, http://www.parmelafoods.com/your-health.html, 4 pages.
Pazos et al., "Effect of pH on Hemoglobin-Catalyzed Lipid Oxidation in Cod Muscle Membranes in Vitro and in Situ," J. Agric. Food Chem., 2005, 53:3605-3612.
Peace Cheese 100% Plant-based Cheese Alternative, Product and Nutrition Facts, posted on or before Jun. 6, 2012, retrieved Feb. 7, 2014, http://www.ilovepeacecheese.com/#/products/4571642621, 3 pages.
Peterson, "The Impact of the Enzymatic Hydrolysis Process on Recovery and Use of Proteins", In: Birch G.G., Blakebrough N., Parker K.J. (eds) Enzymes and Food Processing. Springer, Dordrecht (1981).
Proulx et al., "Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model," J. Agricultural and Food Chemistry, Feb. 2006, 54(4):1518-1522.
Proulx, "Diversified strategies for improving iron bioavailability of maize," Iowa State University—Retrospective Theses and Dissertations, 2007 retrieved on Sep. 19, 2016, retrieved from <http://lib.dr.iastate.edu/rtd/15852/>, 144 pages.
Punk Rawk Labs: an ongoing experiment in optimal health, Nut Milk Cheese Products, posted on or before Jun. 8, 2011, retrieved Feb. 7, 2014, http://www.punkrawklabs.net/cheeses.html, 4 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Ramos et al., "What is Masa?—Ingredient Intelligence," The Kitchen, retrieved on Dec. 1, 2016, http://www.thekitchn.com/whats-the-difference-between-masa-and-masa-harina-226434, 5 pages.
Rebellato et al., "Iron in fortified biscuits: A simple method for its quantification, bioaccessibility study and physicochemical quality," Food Research International, 2015, 77:385-391.
Reedy et al., "Development of a heme protein structure-electrochemical function database," Nucleic Acids Research, 2008, 36:307-313.
Richards et al., "Effects of Fish Heme Protein Structure and Lipid Substrate Composition on Hemoglobin-Mediated Lipid Oxidation," J. Agric. Food Chem., 2007, 55:3643-3654.
Richards et al., "Pro-oxidative Characteristics of Trout Hemoglobin and Myoglobin: A Role for Released Heme in Oxidation of Lipids," J. Agric. Food Chem., 2005, 53:10231-10238.
Richins et al., "Effect of Iron Source on Color and Appearance of Micronutrient-Fortified Corn Flour Tortillas," Cereal Chem., 85:561-5 (2008).
Road's End Organics, Cheese Sauce Mix Products and Nutrition Facts, posted on or before Oct. 28, 2009, retrieved Feb. 7, 2014, http://www.edwardandsons.com/reo_shop_chreese.itml, 6 pages.
Road's End Organics, Mac & Cheese Products and Nutrition Facts, posted on or before Oct. 28, 2009, retrieved Feb. 7, 2014, http://www.edwardandsons.com/reo_shop_pastas.itml, 7 pages.
Rochet and Chaintreau, "Carbonyl Odorants Contributing to the In-Oven Roast Beef Top Note," J. Agric. Food Chem., 53:9578-9585 (Nov. 2005).
Rowe, "Chemistry and technology of flavors and fragrances," Oxford:: Blackwell; 2005, 351 pages.
Rusli, "The Secret of These New Veggie Burgers: Plant Blood," The Wall Street Journal, Oct. 7, 2014, retrieved on Oct. 9, 2014, http://online.wsj.com/articles/the-secret-of-these-new-veggie-burgers-plant-blood-1412725267, 5 pages.
Schieberle et al., "Characterization of Key Odorants in Dry-Heated Cysteine-Carbohydrate Mixtures: Comparison with Aqueous Reaction Systems," Flavor Analysis, American Chemical Society, pp. 320-330 (Sep. 1998).
Schwartz, "Meet the Silicon Valley-Backed Vegan Cheese That You Might Actually Eat," Fast Company, Feb. 26, 2014, retrieved Sep. 11, 2015, http://www.fastcoexist.com/3025648/meet-the-silicon-valley-backed-vegan-cheese-that-you-might-actually-eat, 6 pages.
Segner, "Meatless burger made possible with local effort," Jul. 29, 2016, retrieved Aug. 1, 2016 <http://www.southernminn.com/owatonna_peoples_press/news/article_3d414149-1040-534d-blaf-bf4f8c486788.html>, 5 pages.
Selli et al., "Odour-active and off-odour components in rainbow trout (*Oncorhynchus mykiss*) extracts obtained by microwave assisted distillation-solvent extraction," Food Chemistry, 114:317-322 (2009).
Shahidi et al., "Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation," CRC Critical Reviews in Food Science and Nutrition, 24(2):141-243 (Jan. 1986).
Shi et al., "Identification of characteristic flavour precursors from enzymatic hydrolysis-mild thermal oxidation tallow by descriptive sensory analysis and gas chromatography-olfactometry and partial least squares regression," Journal of Chromatography B, 913-914:96-76 (Jan. 2013).
Shimbayashi et al., "Free Amino Acids and Phosphorylethanolamine in Milk Whey of Cow" Agr. Biol. Chem, 29(1):13-19, 1965.
Shu et al., "Parameter Effects on the Thermal Reaction of Cystine and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone," Thermal Generation of Aromas, American Chemical Society, pp. 229-241 (Oct. 1989).
Sister River Foods Parma!, Products and Nutrition Facts, Posted on or before Jun. 2, 2012, retrieved Feb. 11, 2014, http://www.veganstore.com/product/parma-vegan-parmesan/vegan-cheese-and-dairy-alternatives, 6 pages.
Soller, "The Impossible Burger is Ready for Its (Meatless) Close-Up," The Wall Street Journal, Jun. 14, 2016, retrieved Jun. 21, 2016 <http://www.wsj.com/articles/the-impossible-burger-is-ready-for-its-meatless-close-up-1465912323>, 8 pages.
Song, et al., "Contribution of oxidized tallow to aroma characteristics of beef-like process flavour assessed by gas chromatography-mass spectrometry and partial least squares regression," Journal of Chromatography A, 1254:115-124 (Sep. 2012).
Soy Kaas, Products, posted on or before Jan. 20, 2011, retrieved Feb. 11, 2014, http://www.soykaas.com/products, 1 page.
Soyco Cheese Products, Natural Pantry, retrieved Feb. 11, 2014, http://www.natural-pantry.com/search_results.asp?ct=All&site_search_qu=soyco&storeID=D92VLAQVMPDL9L5UHTS2WLU67NADEHUA, 10 pages.
Soymage Cheese Products, Good Earth Natural Foods, retrieved on Feb. 11, 2014, http://www.goodearthnaturalfoods.com/shop/brand2.asp?storeID=PJ102JRNHNGT8G0QMPEQ7LDC7GX6C2W2&alpha=S&brand=Soymage&brand_id=805, 6 pages.
Specht et al., "Identification of Volatile Flavor Compounds with High Aroma Values from Shallow-Fried Beef," J. Agric. Food Chem., 1994, 42:2246-2253.
Spence et al., "Multisensory Flavor Perception," Cell 161: 24-35 (2015).
Ste Martaen Cheese, Products and Nutrition Facts, posted on or before May 28, 2009, retrieved Feb. 11, 2014, http://stemartaen.bigcartel.com/, 14 pages.
Sterling, "Welcome to the Era of Plant-Based Meat," Food & Wine, Apr. 13, 2016, Retrieved Apr. 20, 2016, <http://www.foodandwine.com/blogs/welcome-era-plant-based-meat>, 3 pages.
Supplementary European Search Report for International No. EP 14737909.3, dated Oct. 7, 2016, 10 pages.
Supplementary European Search Report in European Application No. 15774164, dated Oct. 27, 2017, 11 pages.
Supplementary Partial European Search Report in European Application No. 14738061 dated Nov. 7, 2016, 11 pages.
Swanson, "Patenting the Quest for a More Perfect Veggie Burger," JDSUPRA Business Advisor, Jun. 21, 2016, retrieved Jun. 23, 2016 <http://www.jdsupra.com/legalnews/patenting-the-quest-for-a-more-perfect-72212/. 13 pages.
Tang et al., "Flavor chemistry of 2-methyl-3-furanthiol, an intense meaty aroma compound," Journal of Sulfur Chemistry, 11 pages, (2012).
The Daiya Advantage, Products and Nutrition Facts, posted on or before Jan. 26, 2010, retrieved on Feb. 7, 2014, http://us.daiyafoods.com/our-products, 126 pages.
The Good Scents Company, "The Good Scents Company Information System," 2015, retrieved on Dec. 1, 2016, http://www.thegoodscentscompany.com/, 2 pages.
The Vegetarian Express Parma Zaan Sprinkles, posted on or about Oct. 17, 2009, retrieved Feb. 11, 2014, http://www.thevegetarianexpress.com/cart/home.php?cat=250, 2 pages.
Tofu Rella Mozzarella Cheese, Natural Pantry, retrieved Feb. 11, 2014, http://www.natural-pantry.com/shop/product_view.asp?id=24684&StoreID=D92VLAQVMPDL9L5UHTS2WLU67NADEHUA&private_product=0, 2 pages.
Tofutti Cheese Products and Nutrition, posted on or before Jun. 26, 2013, retrieved Feb. 11, 2014, http://www.tofutti.com/dairy-free-cheeses/, 18 pages.
Tofutti Milk Free, "Premium Dairy Free Cheeses,", posted on or before Jun. 26, 2013, accessed Jan. 7, 2014, http://www.tofutti.com/dairy-free-cheeses/, 2 pages.
Tong et al. ,"Blood Composition of Different Beef Breed Types" Can. J. Anim. Sci, 66:915-924 (Dec. 1986).
Topunov, A.F., et al., "Hemoglobins: Evolution, Abundance, and Heterogeneity," Uspekhi Biologicheskoi Khimii, vol. 41, 2001, p. 207, partial translation, 1 page.
Trader Joe's Sliced Soy Cheese Alternative, Fotki, posted Oct. 27, 2008, retrieved Feb. 11, 2014, http://public.fotki.com/harwons/food/tj-sliced-soy-cheese.html, 1 pages.
Trader Joe's Vegan Mozzarella, A(soy) Bean, posted Jun. 7, 2013, retrieved Feb. 11, 2014, http://a-soy-bean.blogspot.com/2013/06/showdown-trader-joes-vegan-mozzarella.html, 13 pages.
Treeline Treenut Cheese, Products and Nutrition Facts, posted on or before Dec. 10, 2013, retrieved on Feb. 11, 2014, http://www.treelinecheese.com/treeline-cheese-products.html, 3 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Tressl et al., "Formation of Amino Acid Specific Maillard Products and Their Contribution to Thermally Generated Aromas," Thermal Generation of Aromas, American Chemical Society, pp. 156-171 (Oct. 1989).
Uauy et al., "Iron Fortification of Foods: Overcoming Technical and Practical Barriers," J. Nutr. 132:849S-852S (2002).
United States Environmental Protection Agency Document EPA-745-B-99-0145, 1999.
Usami, Aya et al., "Heme-mediated binding of a-casein to ferritin: evidence for preferential a-casein binding to ferrous iron" Biometals, vol. 24, 1217-1224, 2011.
Van Ba et al., "Principles of Meat Aroma flavors and Future Prospect," Intech Open Science, Open Minds, 2012, Chapter 7, 145-176.
Van Den Ouweland et al., "Process Meat Flavor Development and the Maillard Reaction," In Thermal Generation of Aromas, ACS Symposium Series, American Chemical Society, 1989, 433-441.
Vasilescu et al., "Chapter 6: Meat Freshness: Peroxynitrite's Oxidative Role, Its Natural Scavengers, and New Measuring Tools," American Chemical Society, Dec. 2014, 30 pages.
VBites, "Cheezly," posted on or before 2013 , accessed Jan. 7, 2014, http://www.vbitesfoods.com/meat-free/cheezly.html, 2 pages.
Vegan Sun Artisan Aged Raw Cheese, Vegan Essentials, retrieved Feb. 11, 2014, http://store.veganessentials.com/vegan-sun-artisan-aged-raw-cheese-p4201.aspx, 3 pages.
VegCuisine Soy Cheese Products, The Vegan Store, retrieved on Feb. 11, 2014, http://www.veganstore.com/category/s?keyword=vegcuisine, 5 pages.
Veggie Brothers Mozzarella Sticks, Vegan Essentials, Nov. 9, 2013, retrieved Feb. 11, 2014, http://store.veganessentials.com/vegan-mozzarella-sticks-by-veggie-brothers-p3761.aspx, 2 pages.
Victoria Vegan Sauces, Products and Nutrition Facts, posted on or about Sep. 16, 2012, retrieved Feb. 11, 2014, http://www.victoriafinefoods.com/products/specialty-sauces/victoria-vegan, 9 pages.
Walter et al, Effect of bovine-hemoglobin-fortified cookies on iron status of schoolchildren: a nationwide program in Child, Am J Clin Nutr, 1993, 57, pp. 190-194.
Wansink, B., "Overcoming the Taste Stigma of Soy" Journal of Food Science: Sensory and Nutritive Qualities of Food, vol. 68, 2604-2606, 2003.
Wayfare We Can't say It's Cheese Spread, Products and Nutrition Facts, posted on or about Oct. 12, 2013, retrieved Feb. 11, 2014, http://www.wayfarefoods.com/we-cant-say-its-cheese/, 5 pages.
Wisuthiphaet and Kongruang, "Production of Fish Protein Hydrolysates by Acid and Enzymatic Hydrolysis," J Med Bioeng, 2015, 4(6): 466-470.
Withycombe et al., "Identification of 2-Methyl-3-Furanthiol in the Steam Distillate from Canned Tuna Fish," Journal of Food Science, 53(2):658-660 (1988).
Wortham and Miller, "Venture Capitalists Are Making Bigger Bets on Food Start-Ups," The New York Times Online, Apr. 28, 2013, http://www.nytimes.com/2013/04/29/business/venture-capitalists-are-making-bigger-bets-on-food-start-ups.html?pagewanted=all&_r=1&, 4 pages.
Xiaoling, "Practical Technology and Quality Management of Deep Processing of Meat Products," China Textile & Apparel Press, pp. 9 and 10 (English Translation), 10 pages.
Yancey et al., "Effects of total iron, myoglobin, hemoglobin, and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks," Meat Science, 73:680-686 (2006).
Yves Veggie Cuisine The Good Slice, ShopWell, retrieved on Feb. 11, 2014, http://www.shopwell.com/yves-veggie-cuisine-the-good-slice-cheese-alternative-cheddar-style/soy-foods/p/6082260001, 1 page.
Zhengnong et al., "Cihai biological fascicle," Shanghai Lexicographical Publishing House, p. 243, Dec. 31, 1987 (English Translation).
Charkin, et al., "Fragmentation of heme and hemin+ with sequential loss of carboxymethyl groups: A DFT and mass-spectrometry study," Chemical Physics Letters, 2005, 415:362-369.
FDA Statement and Response, GRAS Notice 540, dated May 29, 2015, 13 pages.
FDA Statement, "Structural comparison of plant hemoglobins and animal myoglobins," annex to communication in opposition to EP Patent No. 2943072, 2019, 5 pages.
Fyrestam and Ostman, "Determination of heme in microorganisms using HPLC-MS/MS and cobalt(III) protoporphyirin IX inhibition of heme acquisition in *Escherichia coli*," Anal. Bioanal. Chem., 2017, 409:6999-7010.
GenBank Accession No. AFB70892.1, Sariam, "Non-symbiotic hemoglobin [*Vigna radiata*]," dated Feb. 26, 2012, 1 page.
Gledhill, "The detection of iron protoporphyrin (heme b) in phytoplankton and marine particulate material by electrospray ionisation mass spectrometry—comparison with diode array detection," Anal. Chim. Acta., 2014, 841:33-43.
Impossible Foods Response to Opponent's Reply in opposition to EP Patent No. 2943072, dated Nov. 21, 2019, 28 pages.
Jenschke, et al., "Unsaturated fatty acids and sodium affect the liver-like off-flavor in cooked beef," J. Anim. Sci., 2007, 85:3072-3078.
Jianjun Jiang, et al., "Food technology (vol. 1)," Higher Education Press, 2002, 1:31-32, English translation.
Kohler, et al., "Physiological Characterization of a Heme-Deficient Mutant of *Staphylococcus aureus* by a Proteomic Approach," Journal of Bacteriology 2003, 185:6928-6937.
Lane and Nursten, "The Variety of Odors Produced in Maillard Model Systems and How They Are Influenced by Reaction Conditions," in: The Maillard Reaction in Foods and Nutrition, Waller and Feather, eds., ACS Symposium Series, 1983, No. 215, pp. 141-158.
Opponent's Reply Letter in opposition to EP Patent. No. 2943072, dated Nov. 21, 2019, 15 pages.
Pashynska, et al., "Characterization of Noncovalent Complexes of Antimalarial Agents of the Artemisinin-Type and FE(III)-Heme by Electrospray Mass Spectrometry and Collisional Activation Tandem Mass Spectrometry," J. Am. Soc. Mass Spectrom., 2004, 15:1181-1190.
Song, et al., "Agricultural and sideline products processing value-added technology II: Processing of soybean products," Henan Science and Technology Press, 2009, 1:64-69, English translation.
Wang and Wang, "Principles and techniques of food nutrition and health care," China Light Industry Press, 1998, 1:446-449, English translation.
"happycow.net", [online], "For Love Of Sea Vegetables", dated Jul. 10, 2019, Internet URL: https://www.happycow.net/blog/for-love-of-sea-vegetables/, 5 pages.
"pacificharvest.com", [online], "Red Seaweeds—Nutritional Benefits & Quick Applications", dated Jul. 14, 2016, Retrieved from Internet on Jul. 26, 2019, Internet URL: https://www.pacificharvest.co.nz/blog/general-information/red-seaweeds, 11 pages.
"vitalitymagazine.com", [online], "Sprouts: The Miracle Food", Vitality Magazine, dated Feb. 1, 2005, Retrieved from Internet Jul. 29, 2019, Internet URL: https://vitalitymagazine.com/article/sprouts-the-miracle-food/, 10 pages.
"www.jackkruse.com", [online], "Brain Gut 6: Epi-Paleo Rx", dated Jul. 2012, Internet URL: https://jackkruse.com/brain-gut-6-epi-paleo-rx/?print=pdf, 12 pages.
"www.maraseaweed.com", [online], "Smoked Dulse", Applewood Smoked Dulse—Seaweed Flakes | Mara Seaweed, No Date, Retrieved from the Internet Jul. 15, 2019, Internet URL: https://maraseaweed.com/collections/products/products/smoked-dulse, 3 pages.
"www.myliquidsupplements.com", "Red seaweed is a rich source of nutrients, essential fatty acids, fiber, protein, complex cards, and minerals and now red sea vegetables are easy to find", Health Benefits of Red Seaweed, dated 2013, Retrieved from Internet Jul. 29, 2019, Internet URL: https//:www.myliquidsupplements.com/red-seaweed/, 10 pages.
"www.scotsman.com", [online], "Tom Kitchen: ideas for cooking with seaweed", dated Aug. 5, 2012, Retrieved from Internet Jul. 29, 2019, Internet URL: https://www.scotsman.com/news-2-15012/tom-kitchin-ideas-for-cooking-with-seaweed-1-2451932,12 pages.

(56) **References Cited**

OTHER PUBLICATIONS

"www.shop.healthyhealing.com", [online], "What Are Sea Vegetables? (/healthyhealing-safety/what-are-sea-vegetables)", dated Oct. 25, 2012, Retrieved from the Internet Jul. 15, 2019, Internet URL: (https://shop.healthyhealing.com/Seaweed-Products_c_140.html), 5 pages.
"www.universal-tao.com", [online], "Sprouts", No Date, Retrieved from Internet on Jul. 29, 2019, Internet URL: https://www.universal-tao.com/article/sprouts/html, 9 pages.
"www.wildfoodplants.com", [online], "Teriyaki Weed", dated Sep. 9, 2007, Retrieved from Internet Jul. 15, 2019, Internet URL: https://wildfoodplants.com/2007/09/teriyaki-weed/, 2 pages.
Abou El-Ella, "Hard cheese substitute from soy milk", Journal of Food Science, Wiley-Blackwell Publishing, Inc, US, vol. 45, No. 6, Jan. 1, 1980.
Ames et al., "Volatile Components of a Yeast Extract Composition", J Food Sci 50, pp. 125-131, 1985.
Ames, "Volatile Sulfur Compounds in Yeast Extracts", Chapter 12, Sulfur compounds in foods. Washington DC: ACS, pp. 147-159, 1994.
Anderson et al., "A new hemoglobin gene from soybean: A role for hemoglobin in all plants", Proc. Natl. Acad. Sci. USA, 93, 5682-5687, 1996.
Baek et al., "Aroma Extract Dilution Analysis of a Beef like Process Flavor from Extruded Enzyme-Hydrolyzed Soybean Protein", J. Agric. Food Chem., 49, 790-793, 2001.
Bodnar, "Novel approaches to improving qualities of maize endosperm", Graduate Theses and Dissertations. Paper 10271. Ames, Iowa, USA: Iowa State University Digital Repository @ Iowa State University, 2011, 147 pages.
Buttery et al., "Thiamin Odor and Bis(2-methyl-3-furyl) Disulfide", J. Agri. Food Chem., vol. 32, No. 3, pp. 674-676, 1984.
Buttery, "Flavor Chemistry and Odor Thresholds", In: Teranishi, R, Wick, EL, Hornstein, I, editors. Flavor chemistry: thirty years of progress, pp. 353-365, 1999.
Chohan et al., "Catalytic Effect of Cobalt, Copper, Iron, Magnesium, Manganese, Nickel and Zinc Metal ions on the Conversion of Glucose into 5-Bydroxymethylfurfuraldehyde.", Jour. Chem. Soc. Pak. vol. 19, No. 3, pp. 221-223, 1997.
Collman et al., "Regioselective and Enantioselective Epoxidation Catalyzed by Metalloporphyrins", Science, vol. 261, pp. 1404-1411, 1993.
Derwent, "Cheese-like, soybean-fermented foodstuff prodn.—by inoculating soybean milk with lactic acid bacteria and protein decomposing yeast", 1979.
Duff et al., "Hemoglobin expression in germinating barley.", Seed Science Research, 431-436, 1998.
Erickson et al., "Lipid Oxidation of Muscle Foods", Chapter 12, Food Lipids Chemistry, Nutrition, and Biotechnology Second Edition,48 pages, 2002.
Evers et al., "Furans Substituted at the Three Position with Sulfur", American Chemical Society: Washington, pp. 184-119, 1976.
Everse, "Heme Proteins.", In Encyclopedia of Biological Chemistry, Elsevier Inc., vol. 2, pp. 354-361, 2004.
Extended European Search Report in Application No. 18203176.5, dated May 31, 2019.
Farmer et al., "Interaction of Lipid in the Maillard Reaction between Cysteine and Ribose: the Effect of a Triglyceride and Three Phospholipids on the Volatile Products", J Sci Food Agric., 53, 505-525, 1990.
Gasser et al., "Identification of volatile flavour compounds with high aroma values from cooked beef", Z Lebensm Unlcrs Forsch, 186: 489-494, 1988.
Gasser et al., "Primary odorants of chicken broth", Z Lebensm Unters Forsch, 190: 3-8, 1990.
Groves et al., "Asymmetric Hydroxylation, Epoxidation, and Sulfoxidation Catalyzed by Vaulted Binaphthyl Metalloporphyrins", J. Org. Chem., 55, 3628-3634, 1990.
Güntert et al., "Identification and Formation of Some Selected Sulfur-Containing Flavor Compounds in Various Meat Model Systems", J. Agri. Food Chem., 38, 2027-2041, 1990.
Gupta e al., "Plant hemoglobin's: Important players at the crossroads between oxygen and nitric oxide.", FEBS Letters, pp. 3843-3849, 2011.
Hardison, R., "Hemoglobin's from bacteria to man: Evolution of different patterns of gene expression.", The Journal of Experimental Biology, 1099-1117, 1998.
Hargrove et al., Characterization of Recombinant Soybean Leghemoglobin and Apolar Distal Histidine Mutants, Journal of molecular biology, 1032-42, 1997.
Hofmann et al., "Evaluation of the Key Odorants in a Thermally Treated Solution of Ribose and Cysteine by Aroma Extract Dilution Techniques", J. Agri. Food Chem., 43, 2187-2194, 1995.
Hofmann et al., "Lactic Fermentation of Ground Soybean for Use in Imitation Cream Cheese Products", Journal of Food Science, vol. 50, No. 2, pp. 325-329, 1985.
Hofmann et al., "Studies on the Formation and Stability of the Roast-Flavor Compound 2-Acetyl-2-thiazoline", J. Agri. Food Chem., 43, 2946-2950, 1995.
Jin et al., "Aroma Properties of Some Oxazoles", Perfumer & Flavorist, vol. 9, Aug./Sep., 4 pages, 1984.
Lee et al., "Cloning and Expression Analysis of 2-on-2 Hemoglobin from Soybean.", Journal of Plant Biology, 47(2), 92-98, 2004.
Lendl, "Untersuchung des Rsstzwiebelaromas", Z. Lebensm. Unters.-Forsch. 157, 229-234, 1975.
Lira-Ruan et al., "Expression of non-symbiotic hemoglobin 1 and 2 genes in rice (*Oryza sativa*) embryonic organs.", Communicative and Integrative Biology, 4(4), 457-458, 2011.
Lu et al., "Catalytic C—H functionalization by metalloporphyrins: recent developments and future directions", Chem. Soc. Rev., 40, 1899-1909, 2011.
MacLeod, "The Scientific and Technological Basis of Meat Flavours", 12, 36 pages, 1986.
Meunier et al., "Metal-oxo species in P450 enzymes and biomimetic models. Oxo-hydroxo tautomerism with water-soluble metalloporphyrins", Topics in Catalysis vol. 21, Nos. 1-3, pp. 47-54, 2002.
Mintel, [Online], "Greek Style Cultured Almond Milk Yogurt", retrieved from www.gnpd.com, Nov. 2012 Database accession No. 1943001, 5 pages.
Mottram, "Flavor Compounds Formed during the Maillard Reaction", Chapter 10, 23 pages, 1993.
Mouritsen et al., "Seaweeds for umami flavour in the New Nordic Cuisine", Flavour 1:4, 12 pages, 2012.
Pittet et al., "Comparative Study of Flavor Properties of Thiazole Derivatives", J. Agri. Food Chem., vol. 22, No. 2, pp. 264-269, 1974.
Shaw et al., "Bioavailability of Iron from Purple Laver (*Porphyra spp.*) Estimated in a Rat Hemoglobin Regeneration Bioassay", J. Agric. Food Chem., 48, pp. 1734-1737, 2000.
Strelec et al., "Aminopeptidases of Germinated and Non-Germinated Barley", Barley Aminopeptidases, Food Technol. Biotechnol. 47 (3), 296-303, 2009.
Van Den Ouweland et al., "Components Contributing to Beef Flavor. Volatile Compounds Produced by the Reaction of 4-Hydroxy-5-methyl-3(2.ff)-furanone and Its Thio Analog with Hydrogen Sulfide", J. Agri. Food Chem., vol. 23, No. 3, pp. 501-505, 1975.
Vernin et al., "Mechanisms of Formation of Heterocyclic Compounds in Maillard and Pyrolysis Reactions", Chapter III, Heterocycles in Maillard and Pyrolysis Reactions, pp. 151-217, 1982.
Wajcman et al., "L 'Hemoglobin, from microorganism to man: a single structural motif, multiple functions.", Comptes Rendus Biologies, 325(12), 1159-1174, 2002.
Weenen, "Process Flavourings", Flavourings, pp. 233-258, 1998.
Weinberger et al., "Different regulation of haloperoxidation during agar oligosaccharide-activated defense mechanisms in two related red algae, *Gracilaria* sp. and *Gracilaria chilensis*", Journal of Experimental Botany, vol. 58, No. 15/16, pp. 4365-4372, 2007.
Werkhoff et al., "Isolation and Characterization of Volatile Sulfur-Containing Meat Flavor Components in Model Systems", J. Agri. Food Chem., 38, 777-791, 1990.

(56) **References Cited**

OTHER PUBLICATIONS

Whitfield et al., "Effect of Phospholipid on the Formation of Volatile Heterocyclic Compounds in Heated Aqueous Solutions of Amino Acids and Ribose", 1. Sci. Food Agric., 42, 261-272, 1988.
Wu et al., "Characterization of the Aroma of a Meat like Process Flavoring from Soybean-Based Enzyme-Hydrolyzed Vegetable Protein", J. Agric. Food Chem., 50, 2900-2907, 2002.
Yaylayan et al., "Microwave and Thermally Induced Maillard Reactions", Chapter 38, Thermally Generated Flavors, 8 pages, 1993.
Zampronio et al., "Catalytic oxidation of cyclohexane and cyclooctene over a new metalloporphyrin supported on VOPO4 catalyst", Catalysis Letters vol. 104, Nos. 1-2, 4 pages, 2005.
Zhang et al., "Comparison of the Volatile Compounds Formed from the Thermal Reaction of Glucose with Cysteine and Glutathione", J. Agri. Food Chem., 39, 760-76, 1991.
"Peas, green raw," FoodData Central, US Department of Agriculture, retrieved from URL <https:/fdc.nal.usda.gov/fdc-app.html#/food-details/170419/nutrients>, Apr. 1, 2019, 8 pages.
Berrios, et al., "Carbohydrate composition of raw and extruded pulse flours," Food Research International, 2010, 43:531-536.
Dinh et al., "Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor," Meat Science, 2018, 146:122-130.
EP Opposition, in Application No. 14737766.7, dated Jul. 4, 2019, 8 pages.
Jahreis, et al., "Legume flours: nutritionally important sources of portein and dietary fiber," Ernahrungs Umschau, 2016, 63(2):36-42.
Liu, "Comparison of lipid content and fatty acid composition and their districtuion within seeds of 5 small grain species," J. Food Sco., 2011, 76(2):C334-C342.
Macnicol, "Developmental changes in the free amino acid pool and total protein amino acids of pea cotyledons (*Pisum sativum* L.)," Plant Physiol., 1983, 72:492-497.
Oldach, "Biochemistry of a Burger: Impossible Foods does science, makes food and tries to change the world," ASBMB Today, retrieved from URL <https://www.asbmb.org/asbmb-today/industry/100119/biochemistry-of-a-burger>, Oct. 1, 2019, 12 pages.
Opponent's Reply Letter in opposition to EP Patent. No. 2943072, dated Jan. 20, 2020, 18 pages.
Rebuttal Declaration of Dr. Jorge Ruiz-Carrascal and Supporting Exhibits, dated Jan. 20, 2020, 12 pages.
Warendorf, et al., "The flavour of bouillon: 2. Sensory analysis of non-volatiles and imitation of a bouillon," Z. Lebensm. Unters. Forsch., 1992, 195(3):215-223, English Abstract.
Wood, et al., "Fatty deposition, fatty acid composition and meat quality: a review," Meat Science, 2008, 78:343-358.
U.S. Appl. No. 14/796,970, filed Jul. 10, 2015, Marija Vrljic.
U.S. Appl. No. 15/786,776, filed Oct. 18, 2017, Marija Vrljic.
U.S. Appl. No. 16/238,802, filed Jan. 3, 2019, Marija Vrljic.
U.S. Appl. No. 16/238,769, filed Jan. 3, 2019, Marija Vrljic.
U.S. Appl. No. 14/152,499, filed Jan. 10, 2014, Patrick O'Reilly Brown M.D., Ph.D.
U.S. Appl. No. 14/752,540, filed Jun. 26, 2015, Patrick O'Reilly Brown M.D., Ph.D.
U.S. Appl. No. 15/985,694, filed May 21, 2018, Patrick O'Reilly Brown M.D., Ph.D.
U.S. Appl. No. 14/797,006, filed Jul. 10, 2015, Rachel Fraser.
U.S. Appl. No. 15/398,479, filed Jan. 4, 2017, Rachel Fraser.
U.S. Appl. No. 15/624,505, filed Jun. 15, 2017, Rachel Fraser.
U.S. Appl. No. 15/624,513, filed Jun. 15, 2017, Rachel Fraser.
U.S. Appl. No. 15/839,994, filed Dec. 13, 2017, Rachel Fraser.
U.S. Appl. No. 15/913,018, filed Mar. 6, 2018, Rachel Fraser.
U.S. Appl. No. 15/912,763, filed Mar. 6, 2018, Rachel Fraser.
U.S. Appl. No. 15/913,090, filed Mar. 6, 2018, Rachel Fraser.
U.S. Appl. No. 13/941,211, filed Jul. 12, 2013, Patrick O'Reilly Brown M.D., Ph.D.
U.S. Appl. No. 15/300,339, filed Sep. 29, 2016, Ranjani Varadan.
U.S. Appl. No. 16/238,698, filed Jan. 3, 2019, Ranjani Varadan.
U.S. Appl. No. 16/238,749, filed Jan. 3, 2019, Ranjani Varadan.

* cited by examiner

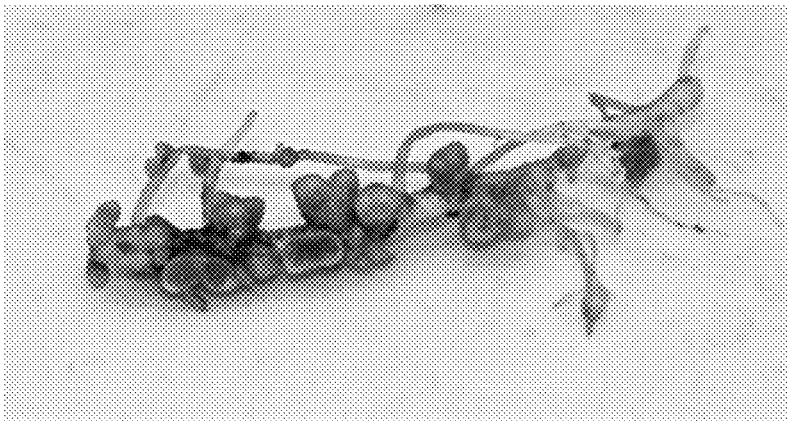


Figure 1

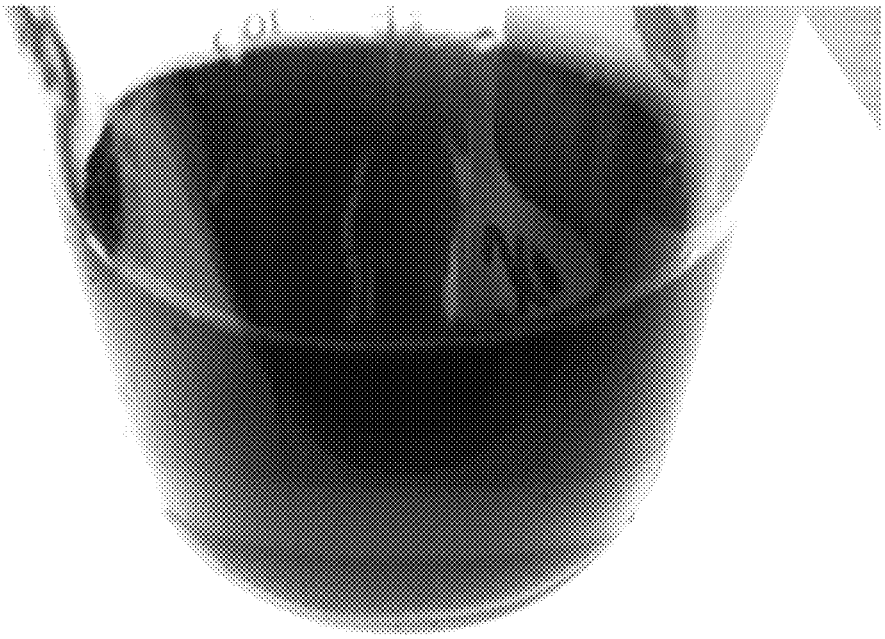


Figure 2

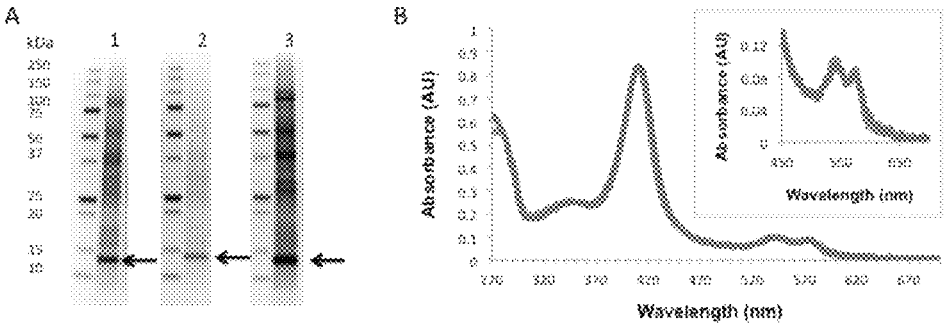


Figure 3

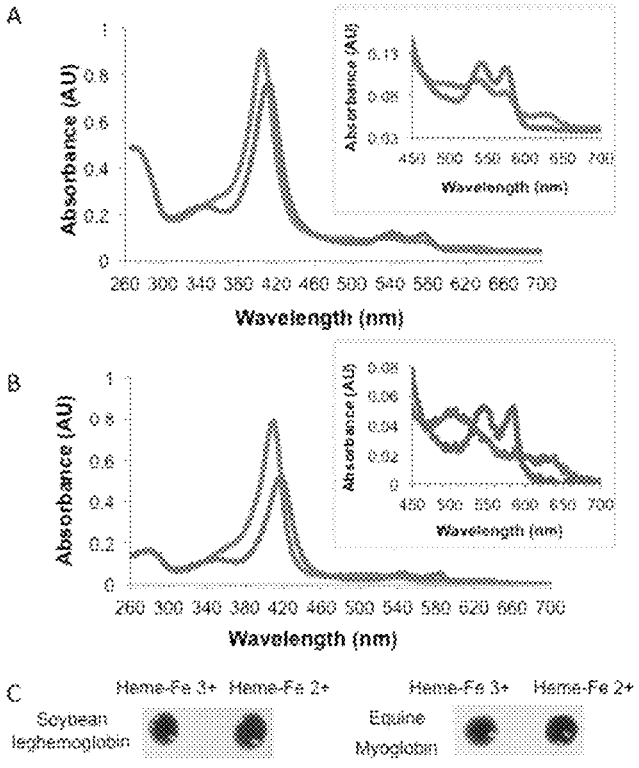


Figure 4

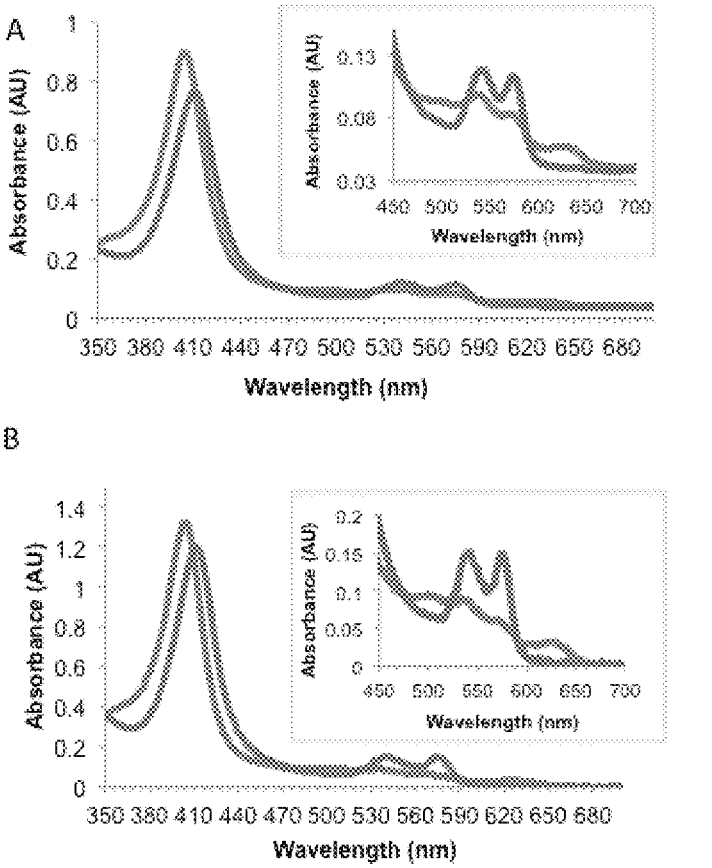


Figure 5

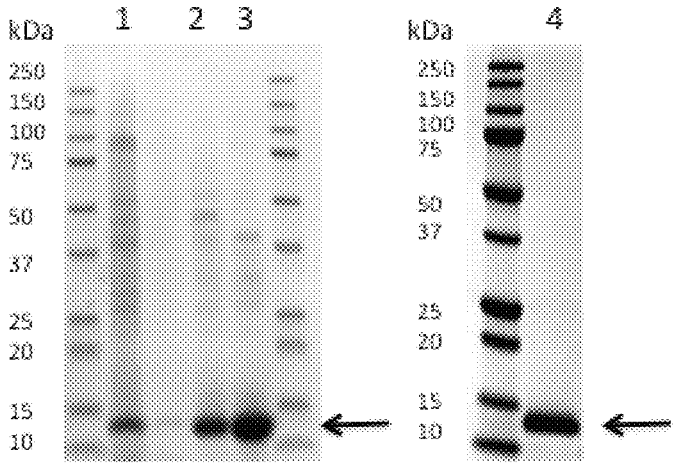


Figure 6

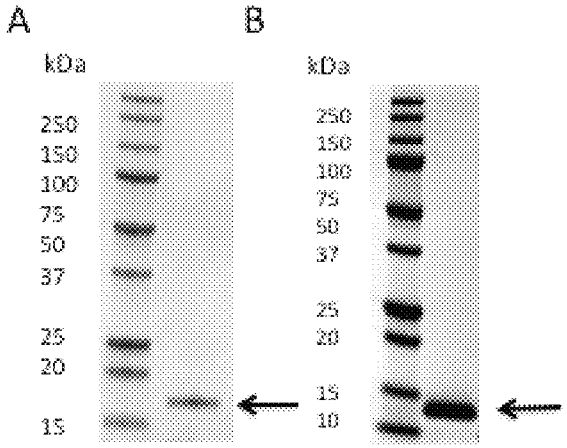

A
B
kDa
250
150
100
75
50
37
25
20
15
kDa
250
150
100
75
50
37
25
20
15
10

Figure 7

Figure 8

Figure 9

Figure 10

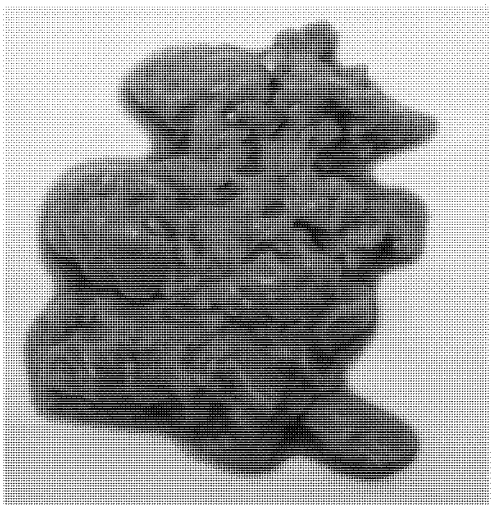


Figure 11

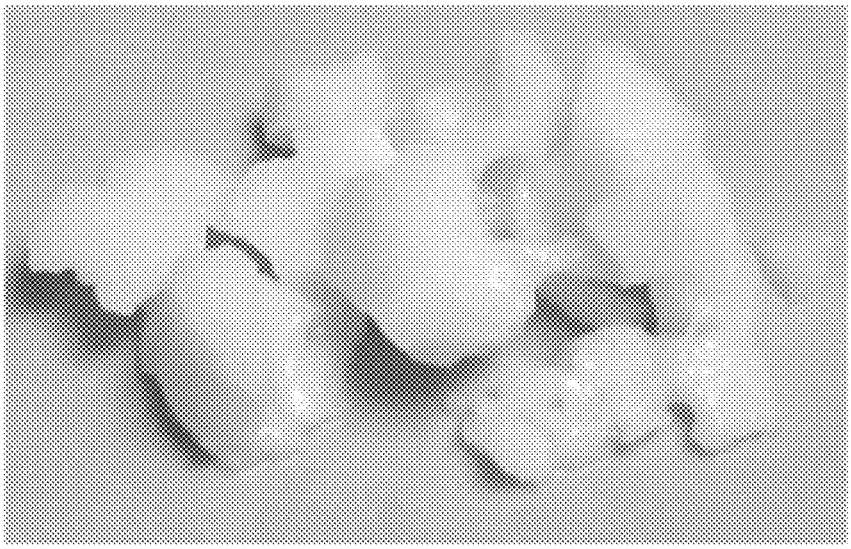


Figure 12

Figure 13

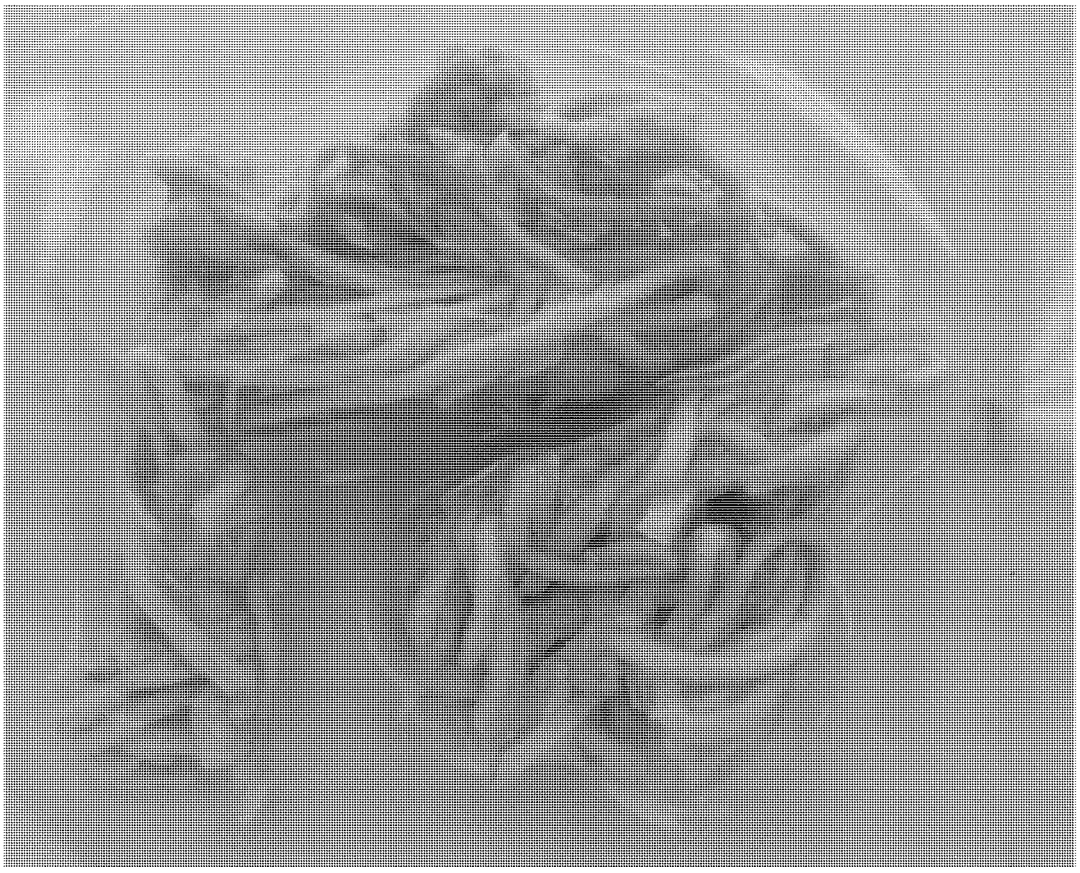


Figure 14

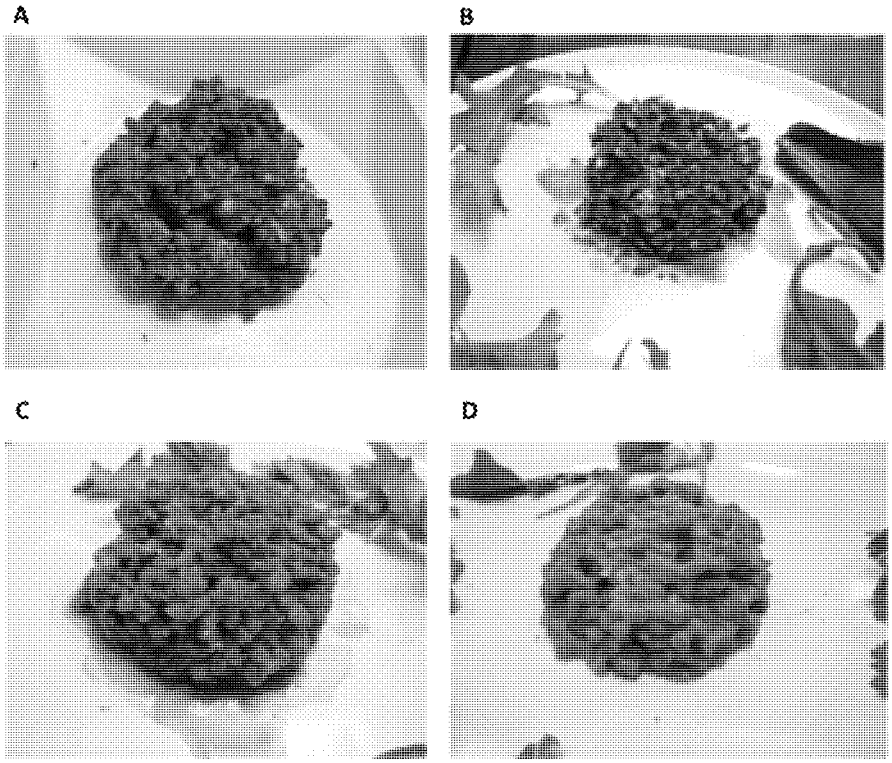


Figure 15

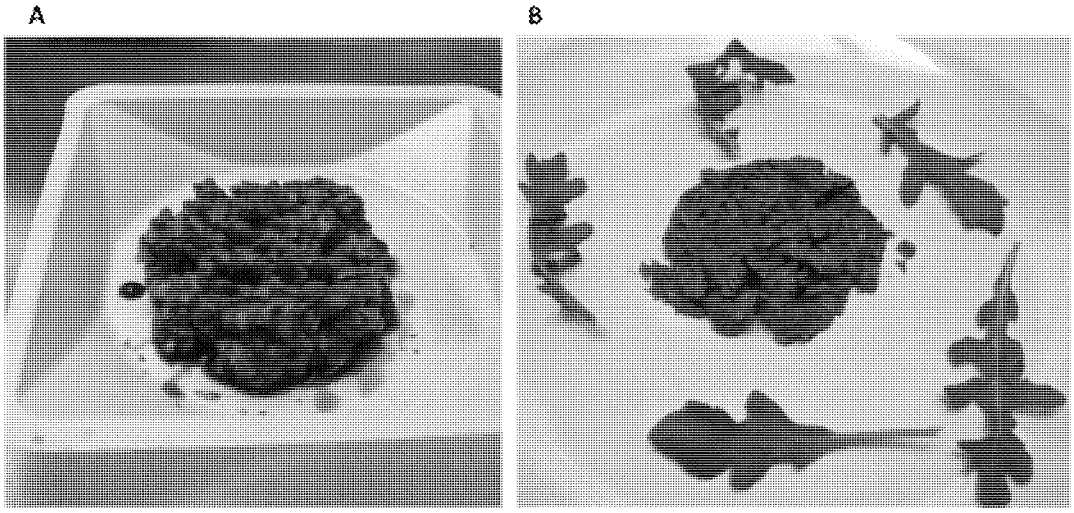


Figure 16

METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS REFERENCE

This application is a continuation of U.S. application Ser. No. 13/941,211 filed on Jul. 12, 2013, which is a continuation-in-part of International Application No. PCT/US2012/046560, filed Jul. 12, 2012, which claims the benefit of priority of U.S. Provisional Application 61/572,205, filed Jul. 12, 2011 and U.S. Provisional Application 61/671,069, filed Jul. 12, 2012, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. It has been estimated that if the worlds human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

The consumption of meat has a profound negative impact on human health. The heath benefits of a vegetarian diet are well established. If the human population would shift to a vegetarian diet the cost savings in health care would be significant.

Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products. Furthermore, as the products must largely be sold pre-cooked, with artificial flavors and aromas built in, they fail to replicate aromas, flavors, and other key features associated with cooking meat. As a result, these products appeal mainly to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat.

Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

SUMMARY OF THE INVENTION

In some aspects, the invention provides a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.

In one embodiment, the meat substitute composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.

In another embodiment, the one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.

In another embodiment, the one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.

In another embodiment, the one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.

In another embodiment, the one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.

In another embodiment, gluten does not account for 10% or more of said protein content by weight.

In another embodiment, each of said isolated, purified proteins is separately isolated and purified.

In another embodiment, the meat substitute composition comprises 1-7 isolated and purified proteins.

In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species.

In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.

In another embodiment, said protein content comprises no more than trace amounts of any other proteins derived from the one or more plant species.

In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

In another embodiment, said one or more isolated and purified proteins are not isolated from an animal.

In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source.

In another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources.

In another embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

In some embodiments, said genetically modified organism is a genetically modified bacteria or yeast organism.

In some embodiments, said isolated, purified protein has been formed into fibers.

In particular embodiments, said fibers resemble skeletal muscle fibers.

In yet more particular embodiments, said fibers are asymmetric fibers.

In some embodiments, the meat substitute composition further comprises one or more isolated and purified iron-containing proteins.

In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEKLFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELSRAWEVAYDELAAAIKKA.

In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQNPISSQ SRKLMQVLGI LVQGIDNLEG LIPTLQDLGR RHKQYGVVDS HYPLVGDCLL KSIQEYLGQG FTEEAKAAWT KVYGIAAQVM TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2.

In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEK LGGQAAMHAA VPLFYKKVLA DDRVKHYFKN TNMEHQAKQQ EDFLTMLLGG PNHYKGKNMA EAHKGMNLQN SHFDAIIENL AATLKELGVS DQIIGEAAKV IEHTRKDCLG K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3.

In some embodiments, the isolated and purified proteins are assembled into one or more gels.

In some embodiments, the meat substitute composition further comprises one or more fats.

In particular embodiments, said one or more fats are derived from a plant source.

In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.

In one embodiment, said color transition is from red to brown.

In one embodiment, said color transition is from pink to white or tan.

In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression.

In some embodiments, the indicator is an olfactory indicator that indicates cooking progression.

In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking.

In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins.

In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.

In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEKLFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELSRAWEVAYDELAAAIKKA.

In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal.

In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.

In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.

In other particular embodiments, said one or more plant sources are soy or pea plants.

In one embodiment, said one or more plant sources comprises one or more plants of the legume family.

In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.

In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified organism.

In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism.

In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

In a more particular embodiment, said meat substitute product contains no wheat gluten.

In other particular embodiments, said meat substitute product contains no soy protein isolate.

In other particular embodiments, said meat substitute product contains no soy protein concentrate.

In other particular embodiments, said meat substitute product contains no soy protein.

In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

In other particular embodiments, said meat substitute product contains no tofu.

In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

In some embodiments, said meat substitute product contains less than 1% cellulose.

In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

In another aspect, the invention provides a muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said protein content by weight.

In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 5% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 1% of said composition content by weight.

In some embodiments, said protein content is derived from one or more non-animal sources.

In particular embodiments, said one or more non-animal sources is a plant source.

In other particular embodiments, said one or more non-animal sources is a genetically modified yeast or bacteria.

In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

In one embodiment, said seed storage protein is moong bean 8S protein.

In some embodiments, said protein content is suspended in a gel.

In some embodiments, said protein content is in the form of a gel.

In one embodiment, said gel comprises an isolated, purified cross-linking enzyme.

In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

In a particular embodiment, said isolated, purified cross-linking enzyme is transglutaminase.

In some embodiments, said protein content has been assembled into fibers.

In particular embodiments, said fibers are arranged isotropically.

In one embodiment, said fibers are asymmetric fibers.

In some embodiments, the muscle tissue replica further comprises one or more isolated and purified iron-containing proteins.

In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLF SFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAAL-GSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid

sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 98% homology to SEQ ID NO 1.

In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross-linking reagents accounts for 0.1-35% of said protein content.

In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume.

In one embodiment, said one isolated and purified protein is moong bean 8S protein.

In one embodiment, said one or more isolated and purified iron-containing proteins is leghemoglobin.

In one embodiment, said one or more cross-linking reagents is transglutaminase.

In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten.

In a more particular embodiment, said muscle tissue replica contains no wheat gluten.

In other particular embodiments, said muscle tissue replica contains no soy protein isolate.

In other particular embodiments, said muscle tissue replica contains no soy protein concentrate.

In other particular embodiments, said muscle tissue replica contains no soy protein.

In a more particular embodiment, said muscle tissue replica contains less than 5% carbohydrates.

In other particular embodiments, said muscle tissue replica contains no tofu.

In some embodiments, said muscle tissue replica contains no tofu, and no wheat gluten.

In some embodiments, said muscle tissue replica contains no soy protein, and no wheat gluten.

In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates.

In some embodiments, said muscle tissue replica contains less than 1% cellulose.

In some embodiments, said muscle tissue replica contains less than 5% insoluble carbohydrates.

In some embodiments, said muscle tissue replica contains no soy protein, and less than 1% cellulose.

In some embodiments, said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 1% cellulose.

In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.

In a more particular embodiment, the meat replica contains no wheat gluten.

In other particular embodiments, the meat replica contains no soy protein isolate.

In other particular embodiments, the meat replica contains no soy protein concentrate.

In other particular embodiments, the meat replica contains no soy protein.

In a more particular embodiment, the meat replica contains less than 5% carbohydrates.

In other particular embodiments, the meat replica contains no tofu.

In some embodiments, the meat replica contains no tofu, and no wheat gluten.

In some embodiments, the meat replica contains no soy protein, and no wheat gluten.

In some embodiments, the meat replica contains no animal products and less than 5% carbohydrates.

In some embodiments, the meat replica contains less than 1% cellulose. In some embodiments, the meat replica contains less than 0.1% cellulose. In some embodiments, the meat replica contains less than 10% cellulose. In some embodiments, the meat replica contains less than 5% cellulose.

In some embodiments, the meat replica contains less than 5% insoluble carbohydrates.

In some embodiments, the meat replica contains no soy protein, and less than 1% cellulose.

In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.

In some embodiments, the meat replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

In another aspect, the invention provides a fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.

In some embodiments, said fat droplets are derived from a non-animal source.

In some embodiments, said fat droplets are comprised of one or more plant oils.

In some embodiments, said one or more plant oils is selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phophatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated

oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

In one embodiment, said one or more plant oils is rice bran oil or canola oil.

In some embodiments, said protein solution comprises one or more isolated, purified proteins.

In some embodiments, said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.

In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source.

In some embodiments, said non-animal source is a plant source.

In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

In some embodiments, said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.

In particular embodiments, said albumin protein is isolated pea albumin protein.

In some embodiments, said seed storage protein is moong bean 8S protein.

In some embodiments, said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

In other embodiments said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

In some embodiments, the fat replica further comprises a cross-linking enzyme.

In some embodiments, said cross-linking enzyme is transglutaminase.

In some embodiments, one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.

In particular embodiments, said plant oil is rice bran oil or canola oil.

In some embodiments, the fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the fat tissue replica additionally contains less than 1% wheat gluten.

In a more particular embodiment, said fat tissue replica contains no wheat gluten.

In other particular embodiments, said fat tissue replica contains no soy protein isolate.

In other particular embodiments, said fat tissue replica contains no soy protein concentrate.

In other particular embodiments, said fat tissue replica contains no soy protein.

In a more particular embodiment, said fat tissue replica contains less than 5% carbohydrates.

In other particular embodiments, said fat tissue replica contains no tofu.

In some embodiments, said fat tissue replica contains no tofu, and no wheat gluten.

In some embodiments, said fat tissue replica contains no soy protein, and no wheat gluten.

In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates.

In some embodiments, said fat tissue replica contains less than 1% cellulose.

In some embodiments, said fat tissue replica contains less than 5% insoluble carbohydrates.

In some embodiments, said fat tissue replica contains no soy protein, and less than 1% cellulose.

In some embodiments, said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, said fat tissue replica contains no wheat gluten, and less than 1% cellulose.

In some embodiments, said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin.

In some embodiments, said protein content is derived from non-animal source.

In some embodiments, said non-animal source is a plant source.

In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

In some embodiments, said one or more isolated proteins account for 50% or more of said protein content by weight.

In some embodiments, said one isolated and purified protein accounts for 90% or more of said protein content by weight.

In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

In some embodiments, said one or more isolated and purified proteins is a prolamin family protein.

In some embodiments, said one or more isolated and purified proteins is zein.

In some embodiments, said protein content is suspended in a gel.

In some embodiments, said gel comprises an isolated, purified cross-linking enzyme.

In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

In some embodiments, said protein content is formed into a fiber.

In some embodiments, said fiber is produced by an extrusion process.

In some embodiments, said fiber is stabilized by protein crosslinks.

In some embodiments, fiber contains an isolated, purified cross-linking enzyme.

In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

In another aspect, the invention provides a meat substitute product, comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

In some embodiments, the meat substitute product comprises two or more of said muscle replica, fat tissue replica, and connective tissue replica.

In some embodiments of the meat substitute product, said muscle replica accounts for 40-90% of said product by weight, said fat tissue replica accounts for 1-60% of said product by weight, and said connective tissue replica accounts for 1-30% of said product by weight.

In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein said protein content comprises one or more isolated, purified plant proteins.

In some embodiments, said protein content is derived from non-animal source.

In some embodiments, non-animal source is a plant source.

In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins.

In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.

In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

In some embodiments, the meat substitute product further comprises one or more isolated and purified iron-containing proteins.

In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,. In some embodiments, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1:

MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEK-APAAKDLFSFLANGVDPTNPKLTGHAEKLFALVRDS-AGQLKASGTVVADAALGSVHAQKAVTDPQFVVVK-EALLKTIKAAVGDKWSDELSRAWEVAYDELAAAIK-KA

In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

In a more particular embodiment, said meat substitute product contains no wheat gluten.

In other particular embodiments, said meat substitute product contains no soy protein isolate.

In other particular embodiments, said meat substitute product contains no soy protein concentrate.

In other particular embodiments, said meat substitute product contains no soy protein.

In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

In other particular embodiments, said meat substitute product contains no tofu.

In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

In some embodiments, said meat substitute product contains less than 1% cellulose.

In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

In some embodiments, said one or more isolated, purified iron-containing proteins is derived from a non-animal source.

In some embodiments, said non-animal source is a plant source.

In some embodiments, said plant source comprises one or more plants of the legume family.

In some embodiments, said one or more plants of the legume family is a soy or pea plant.

In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

In some embodiments, said iron-containing protein is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

In one embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1:

MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEK-APAAKDLFSFLANGVDPTNPKLTGHAEKLFALVRDS-AGQLKASGTVVADAALGSVHAQKAVTDPQFVVVK-EALLKTIKAAVGDKWSDELSRAWEVAYDELAAAIK-KA

In some embodiments, the food product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

In particular embodiments, the food product additionally contains less than 1% wheat gluten.

In a more particular embodiment, said food product contains no wheat gluten.

In other particular embodiments, said food product contains no soy protein isolate.

In other particular embodiments, said food product contains no soy protein concentrate.

In other particular embodiments, said food product contains no soy protein.

In a more particular embodiment, said food product contains less than 5% carbohydrates.

In other particular embodiments, said food product contains no tofu.

In some embodiments, said food product contains no tofu, and no wheat gluten.

In some embodiments, said food product contains no soy protein, and no wheat gluten.

In some embodiments, said food product contains no animal products and less than 5% carbohydrates.

In some embodiments, said food product contains less than 1% cellulose.

In some embodiments, said food product contains less than 5% insoluble carbohydrates.

In some embodiments, said food product contains no soy protein, and less than 1% cellulose.

In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.

In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates.

In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.

In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.

In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.

In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.

In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.

In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for con-

sumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

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 novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

FIG. **1** depicts a portion of the root of a pea plant (*Pisum sativum*) with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open rood nodule appears red.

FIG. **2** depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

FIG. **3** shows that leghemoglobins from different species are homologs and have similar color properties. In FIG. **3**, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).

FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line—the higher peak in FIGS. **4** and **5**) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. **4** panel C). Images of 10 μl liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

FIG. **5** depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

FIG. **6** depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~83% (lane 3), and size-exclusion chromatography to ~95% (lane 4).

FIG. **7** shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E.coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.

FIG. **8** shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. A deep pink color of the leghemo-

globin-infused cubes is apparent in color photos contrasting the pale tan color of the un-infused cubes.

FIG. **9** shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. In the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

FIG. **10** depicts 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

FIG. **11** depicts "White" muscle analog prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

FIG. **12** depicts a fat tissue analog based on moong beans and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

FIG. **13** depicts at tissue analog based on pea globulin and prepared in eppendorf tubes very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.

FIG. **14** shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

FIG. **15** depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **15** panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. **15** panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. **15** panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. **15**, panel D)

FIG. **16** depicts a ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog. The panel on the left (Panel A) shows the patty before cooking and the panel on the right (Panel B) shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

DETAILED DESCRIPTION OF THE INVENTION

Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The consumables can be made to mimic the cut or appearance of meat as it is currently sold. For instance a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. Alternatively, the consumables can be made with a unique look or appearance. For instance the consumable could contain patterns or lettering that is based upon the structure of the consumable. In some instances the consumables look like traditional meat products after they are prepared. For example a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked appears the same as a traditional cooked meet. In some embodiments the consumable may resemble a traditional meat shape in two dimensions, but not in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or thinker) than the traditional cut. So in some embodiments a composition that can be cut repeatedly into traditionally meat shaped products is provided.

The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides a method for producing a meat replica which does not contain products which have been shipped over 1, 10, 100, or 1000 miles prior to producing the meat replica.

The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. So, for example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture

as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments the same proteins can be isolated from different plant species. In some embodiments the invention provides for a method comprising isolating similar plant constituents from plant sources in different locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments the isolated constituents are from different plant populations in different locations. In some embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison beef can require over 2000 gallons of water per pound of meat.

The consumable may require less land are to produce than a meat product with similar protein content. For example the consumable may require 30% or less of the land area required to produce a meat product with similar protein content.

The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products.

The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance it may be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal-offspring interactions, or slaughter of animals for their meat.

The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces.

The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher pork chop.

The consumable can also be shipped in components and produced or assembled at a different location. When available local components can be used for production of the consumable. These can be supplemented with components which are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce shipping weight thereby reducing cost and environmental impact.

The consumables can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

The consumables are, in some embodiments, designed to replicate the experience of eating meat. The look, texture, and taste of the consumable can be such that it is similar or indistinguishable from meat. The invention therefore provides in certain embodiments methods for determining whether an animal or human can distinguish the consumable from meat.

One method to determine whether the consumable is comparable to meat is to a) define the properties of meat and b) determine whether the consumable has similar properties. Properties of meat that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of meat that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. So, in one embodiment a group of people can be asked to rate a certain meat, for instance ground beef, according to properties which describe the meat. These ratings can be used as an indication of the properties of the meat. The consumables of the present invention can then be compared to the meat to determine how similar the consumable is to the meat. In some instances the properties of the consumables are then altered to make the consumable more similar to the meat. So, in some embodiments, the consumable is rated similar to meat according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

In some embodiments, subjects asked to identify the consumable identify it as a form of meat. In some embodiments one property of the compositions of the invention is that an animal, for example a human, will identify the composition as a meat. In some embodiments the human identifies the composition of the invention as having properties equivalent to meat. In some embodiments one or more properties of meat are equivalent according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than meat substitutes found in the art.

In embodiments an experiment can demonstrate that consumable s acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can tested multiple consumable samples, namely, natural meats vs. the consumable compositions described herein. Variables such as fat content can be standardized for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

Panelists can be served samples in booths, under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor, dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

The results of this experiment can indicate significant differences ($p<0.05$) or similarities between the traditional meats and the compositions of the invention.

These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally these results can demonstrate that compositions of the invention are preferred by panelist over other commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats.

Consumables of the invention can also have similar physical characteristics as traditional meat. In one embodiment the force required to pierce a 1 inch thick structure (e.g. a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar meat structure (e.g. a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

In some embodiments composition of the invention have a similar cook loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats.

In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile could be used to refine the consumable.

Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g, fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.

In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to

as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of said replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In yet other alternative embodiments, the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein said replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

Proteins and Protein Sources

In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated.

In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Non-limiting examples of non-animal sources include plants, funghi, bacteria, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In particular embodiments, the one or more isolated, purified proteins are derived from plant sources. Non-limiting examples of plant sources include grains such as, e.g., corn, maize, rice, wheat, barley, rye, triticale, teff, oilseeds including cottonseed, sunflower seed, safflower seed, rapeseed, leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, cabbage, green matter not ordinarily consumed by humans, including biomass crops, including switchgrass, miscanthus, sorghum, other grasses, alfalfa, corn stover, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, turnips, plants from the legume family, such as, e.g., clover, peas such as cowpeas, english peas, yellow peas, green peas, beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts, vetch (vicia), stylo (stylosanthes), arachis, indigofera, acacia, leucaena, cyamopsis, and sesbania. One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used in the present invention.

Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the muscle, fat, connective tissue replicas, or meat substitute products. Accordingly, in some embodiments, the one or more isolated proteins comprises an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, about 20-50% of the total weight of the dry matter of the source plant.

In particular embodiments, the one or more isolated proteins comprises an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco activase). Rubisco is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

In some embodiments, the one or more isolated proteins includes an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins

In some embodiments, the one or more isolated proteins includes proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean.

Muscle Replicas

A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which replicates or approximates animal skeletal muscle. Such a composition will be labeled herein as "muscle replica". In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an isotropic fashion. Accordingly, in some embodiments the muscle replica comprises fibers that are to some extent organized isotropically. In some embodiments the fibers comprise a protein component. In some embodiments, the fibers comprise about 1%, about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of a protein component.

In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds. These proteins provide examples of proteins with favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or fat tissue. Examples and embodiments of the one or more isolated, purified proteins are described herein. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight.

Skeletal muscle of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus in some embodiments, the muscle or meat replica includes a starch or pectin.

Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. Thus in some embodiments, the muscle replica can include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. In some embodiments the concentration of sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

In another aspect, the invention provides methods for making a muscle replica. In some embodiments, the composition is formed into asymmetric fibers prior to incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described in U.S. Pat. Nos. 6,379,738, 3,693,533, US20120093994, which are herein incorporated by reference.

In some embodiments extrusion can be conducted using an MPF19 twin-screw extruder (APV Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feed rate of 23 g/min, and water-flow rate of 11 g/min. Product

temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (<60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.

Fat Replica

Animal fat is important for the experience of eating cooked meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal fat. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates animal fat. Such a composition will be labeled herein as a "fat replica". In some embodiments, the fat replica and/or meat substitute product comprising the fat replica are partially derived from animal sources.

In some embodiments the meat substitute product has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments, the fat replica comprises a gel with droplets of fat suspended therein. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. In particular embodiments, the proteins used in the gel are plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.

The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources. In particular embodiments, the sources are plant sources. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phophatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be yeast or mold. For instance, in one embodiment the fat droplets comprise triglycerides derived from *Mortierella isabellina*.

In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

In some embodiments, the fat replica comprises a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In particular embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non-limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a particular embodiment, the isolated, purified protein is a purified pea albumin protein. In another particular embodiment, the isolated, purified protein is a purified pea globulin protein. In another particular embodiment the isolate purified protein is a Moong bean 8S globulin. In another particular embodiment, the isolated, purified protein is an oleosin. In another particular embodiment, the isolated, purified protein is a caloleosin. In another particular embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.

In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. Non-limiting examples of cross-linking enzymes include, e.g., transglutaminase, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl oxidase). In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein.

In some embodiments, the fat replica comprises at least 0.0001%, or at least 0.001%, or at least 0.01%, or at least 0.1%, or at least 1% (wt/vol) of a cross-linking enzyme. In particular embodiments, the cross-linking enzyme is transglutaminase.

In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution.

In some embodiments, the fat replica is assembled to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various embodiments the gel can be a proteinaceous gel, a hydrogel, an organogel, or a a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified pea globulin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified Moong bean 8S globulin enriched fraction. In yet more particular embodiments, the isolated, purified proteins comprise a Rubisco enriched fraction. In other particular embodiments, the one or more fats are derived from plant-based oils. In more particular embodiments, the one or more fats are derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phophatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross-linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross-linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

Connective Tissue Replica

Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates important textural and visual features of animal connective tissue. Such a composition will be labeled herein as “connective tissue replica”. In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water

content (ca. 50%), and low-to-none fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

The protein content of most fascia-type connective tissue is comprised mainly of collagen. Collagen is characterized by a high fraction of proline and alanine, and also is assembled into characteristic elongated fibrils or rod-like, flexible structures. Prolamins are one family of proteins found in non-animal sources, such as plant sources. Prolamins are highly abundant in plants and are similar in amino acid composition to collagen. Among proteins we tested for this purpose, prolamins were particularly favorable because of their low cost and their ability to readily form fibers or sheets when spun or extruded. Non-limiting examples of prolamin family proteins include, e.g., zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films and fibers. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal connective tissue. animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana*, monomers of which are "collagen-like" rod-like flexible molecules). The proteins may be artificially designed to emulate physical properties of animal connective tissue.

Methods for forming fascia-type connective tissue will be as those practiced in the art with a bias towards methods producing fibrous or fibrous-like structures by biological, chemical, or physical means, individually or in combination, serially or in parallel, before final forming. These methods may include extrusion or spinning.

Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each).

Compositionally, cartilage-type connective tissue will be very similar to fascia-type tissue with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue.

Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropically homogenous structures.

The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana*, monomers of which are "collagen-like" rod-like flexible molecules).

In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example transclutaminase, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art.

In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

In some embodiments the meat replica contains no animal products, less than 1% wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no soy protein isolate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no soy protein concentrate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no tofu, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products, no tofu, and no wheat gluten.

In some embodiments the meat replica contains no animal products, no soy protein, and no wheat gluten.

In some embodiments the meat replica contains no methylcellulose, no carrageenan, no caramel color, no Konjac flour, no gum Arabic, and no acacia gum.

In some embodiments the meat replica contains no animal products and less than 5% carbohydrates.

In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum and less than 5% carbohydrates.

In some embodiments the meat replica contains no animal products, and less than 1% cellulose.

In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates.

In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose.

In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.

In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose.

In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.

The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable.

In some embodiments the meat substitute compositions contains no animal protein, comprising between 10-30% protein, between 5-80% water, between 5-70% fat, comprising one or more isolated purified proteins. In particular embodiments, the meat substitute compositions comprise transglutaminase.

In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. F or example, in some embodiments the consumable is a plant-based meat replica can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in typically unnatural percentages. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked the consumable is similar to cooked meat. For example, a meat replica may be made

with a higher than typical water content for raw meat, but when cooked in a microwave the resulting product has percentages of components similar to meat cooked over a fire.

In some embodiments the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, is hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments the consumable contains a protein or proteins that have all of the amino acids found in proteins that are essential for human nutrition. In some embodiments the proteins added to the consumable are supplemented with amino acids.

Indicators of Cooking Meat

The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

Odorants released during cooking of meat are generated by reactions that can involve as reactants fats, protein, amino acids, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates. In some embodiments the odorants that combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions involving amino acids, fats and sugars found in plants as well as meat. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions involving one or more amino acids, fats, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus in some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein.

Evidence that the presence of leghemoglobin contributes favorably to aroma of meat replicas: A muscle replica comprising pea flour, sunflower oil, and glucose was heated for 10 minutes at 140 C in the presence of either reduced leghemoglobin (LHb) or a mixture of iron (Fe3+), sodium and EDTA (EFS) in sealed containers carrying solid phase microextraction (SPME) fibers. These fibers contain polydimethylsiloxane (PDMS) which adsorbs volatile compounds for analysis by GC-MS. Analysis of GC-MS data from multiple replicas reveal consistent differences between the LHb and EFS samples. Non-limiting examples of compounds found exclusively or more abundantly in the LHb samples are: 2-octanone, 2-methyl furan, which are often associated with the aroma of cooked meat, and many other unidentified compounds.

Color Indicators

The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transi-

tion to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. In some aspects, the invention provides a non-meat based meat substitute product that provides a visual indicator of cooking progression. In some embodiments, the visual indicator is a color indicator that undergoes a color transition during cooking. In particular embodiments, the color indicator recapitulates the color transition of a cut of meat as the meat progresses from a raw to a cooked state. In more particular embodiments, the color indicator colors the meat substitute product a red color before cooking to indicate a raw state and causes the meat substitute product to transition to a brown color during cooking progression. In other particular embodiments, the color indicator colors the meat substitute product a pink color before cooking to indicate a raw state and causes the meat substitute product to transition to a white or brown color during cooking progression.

The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein. In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, 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%, or more than about 2% of an iron-carrying protein by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organisms such as, e.g., bacteria or yeast. In some cases, the iron-carrying protein is myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin. In some cases, the iron-carrying protein is leghemoglobin. In some embodiments the consumable is a plant based meat replica that has leghemoglobin added. So, for example a replica of young beef can have about 0.4-1% leghemoglobin. In some cases, the iron-carrying protein is a cytochrome. In some embodiments the consumable is a plant based meat replica that has a cytochrome added. So, for example a replica of young beef can have about 0.4-1% of a cytochrome.

Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example blood is saved from a slaughter house, hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a meat replica that comprises an iron containing protein other than myoglobin or hemoglobin.

Examples of iron containing proteins include hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,).

Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is

finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

Food Products Comprising Isolated, Purified Leghemoglobin

In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghemglobin containing solution can be injected into raw or cooked meat. In another example a leghemoglobin solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with leghemoglobin.

Sources of Leghemoglobin

In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant, FIG. **1**). FIG. **2** shows 100 mls of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (FIG. **3**). In FIG. **3**, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10 mM sodium hydrosulfite in 20 mM potassium-phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel-exclusion chromatography. Inset shows a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. Some plant species express several leghemoglobin isoforms (for example soybean has four leghemoglobin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pH and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemoglobins can influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins.

Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1 mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. **4** panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

In other embodiments, leghemoglobin can be sourced from non-plant sources, such as from organisms such as bacteria or yeast which have been genetically modified to express high levels of leghemoglobin.

The oxidation state of the iron ion in leghemoglobin is important for its color. Leghemoglobin with the heme iron in the +2 oxidation state appears vivid red in color, while leghemoglobin with the heme iron in the +3 oxidation state appears brownish red. Thus, in using leghemoglobin as a source of red color in a meat replica, it is desireable to reduce the heme iron from the +3 state to the +2 state. Heme iron in leghemoglobin can be switched from oxidized (+3) state to reduced (+2) state with reducing reagents. Examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate are illustrated in FIG **5**. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel. The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (FIG. **1** shows Leghemoglobin isolated frompea root nodules). Root nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme-iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90%

ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20 mM potassium phosphate, 1 mM EDTA, 50 mM sodium chloride and ammonium sulfate was removed using dialysis or size-exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion-exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size-exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion exchange columns (FFQ or DEAE, GE Healthcare) in different buffers (20 mM potassium phosphate pH 7.4, containing 0 to 100 mM sodium chloride, 20 mM Tris pH 8 containing 0 to 100 mM sodium chloride, 20 mM sodium borax pH 9.8, 20 mM sodium chloride, 20 mM sodium carbonate pH 9, 20 mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in FIG. **6**. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flow-through is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (Bio-Rad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~83% (lane 3), and size-exclusion chromatography to ~95% (lane 4).

Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it. One illustrative example is shown in FIG. **7**. FIG. **7** shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E.coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.

Leghemoglobin purified from soybean and fava root nodules, respectively was tasted by a panel of volunteers and in each case described as tasting like blood.

Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin. (FIG. **1**, showing 100 mls of solution of leghemoglobin isolated from 30 grams of pea root nodules).

Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ed in a heme-protein containing solution, for instance a leghemoglobin solution. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

FIG. **8** shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. Note the deep pink color of the leghemoglobin-infused cubes in contrast to the pale tan color of the un-infused cubes.

FIG. **9** shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity-ligation assoacy or luninex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

EXAMPLES

An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.

Protein Purification for Components of the Replica

Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubsico was purified from fresh alfalfa plant. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-VIS and Pierce assay methods.

Moong bean 8S globulins: Moong bean flour was resuspended in in 50 mM potassium phosphate buffer pH 7 and 0.5M NaCl at 1:4 (wt/v) ratio, and mixture was incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at −20 C until further use.

Pea-albumins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 50 mM sodium acetate buffer pH 5 and incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at −20 C until further use.

Pea-globulins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 20 mM potassium phosphate buffer pH 8, 0.4M sodium chloride and stirred for 1 hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50% (wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at −20° C. until further use.

RuBisCO: RuBisCO was fractionated from alfalfa greens (or other green plants eg soybean plants, spinach etc) by first grinding leaves with 4 volumes of cold 50 mM KPhosphate buffer pH 7.4 buffer (with (in lab) or without (in field) 0.5M NaCl+2 mM DTT+1 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1 hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50% (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1 hr. The pellet from this step contains RuBisCO, and was kept at −20 C until used.

Obtaining Plant Proteins.

Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described. Pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000 g, 10 min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000 g, 10 min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000 g, 10 min and protein concentrated to desired concentration.

Constructing a Muscle Tissue Analog

Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above, For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaCl, pH 7.3) and the resulting solution dialyzed for 6 hours against 5 l of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 36 hours against 5 l of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.

Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using juicer blender. Unsoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for lhr, 4' C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and incubated for 12 hr, 4' C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20 mM potassium phosphate, 1 mM EDTA, 100 mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48 hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride. Total protein concentration was 57 mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme-iron oxidized state. Thus, leghemoglobin was incubated with 5 mM sodium hydrosulfite for 5 min and sodium hydrosulfite was removed using size-exclusion chromatography in 20 mM potassium phosphate, 100 mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV-VIS spectra analysis confirmed that leghemoglobin is in heme-iron reduced state.

Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer.

To prepare "dark" muscle tissue analog (FIG. **10**), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

"White" muscle analog (FIG. **11**) was prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were

63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (<1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood.

The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3-fold less strong against compression.

Fat Tissue Analog

Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95° C. for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.

Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90° C. for 30 min.

Fat tissue analog based on moong beans (FIG. **12**) and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

Fat tissue analog based on pea globulin (FIG. **13**) and prepared in eppendorf tubes was very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression. Fat tissue analog prepared in 50 ml Falcon tubes were similar in appearance, texture and aromas, but substantially softer (2-fold softer for canola oil, and 3-fold softer for rice bran oil, according to compressibility measurements).

Connective Tissue Analog

Connective tissue analog prototypes were developed using zein protein sourced from 100% yellow corn gluten meal, or from commercial sources, such as Amazein (Prairie Gold, Bloomington, Ill.). Zein proteins were solubilized in 70-90% ethanol with desired ratios at 1:3 to 1:5 (solids: solution). By precipitating zein proteins, for example by a change in pH, in a controlled manner, large zein structures result with physicochemical properties that can be manipulated as desired. For example, FIG. **14** shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

Ground beef replica prototypes made from gels of plant proteins and plant oils.

A ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **15** panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil) , 5% connective tissue analog (FIG. **15** panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog) , 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. **15** panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. **15**, panel D)

In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350° F. pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **16**). The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
<211> LENGTH: 144
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 1

Met Val Ala Phe Thr Glu Lys Gln Asp Ala Leu Val Ser Ser Ser Phe
1 5 10 15

Glu Ala Phe Lys Ala Asn Ile Pro Gln Tyr Ser Val Val Phe Tyr Thr
20 25 30

```
Ser Ile Leu Glu Lys Ala Pro Ala Ala Lys Asp Leu Phe Ser Phe Leu
        35                  40                  45

Ala Asn Gly Val Asp Pro Thr Asn Pro Lys Leu Thr Gly His Ala Glu
    50                  55                  60

Lys Leu Phe Ala Leu Val Arg Asp Ser Ala Gly Gln Leu Lys Ala Ser
65                  70                  75                  80

Gly Thr Val Val Ala Asp Ala Ala Leu Gly Ser Val His Ala Gln Lys
                85                  90                  95

Ala Val Thr Asp Pro Gln Phe Val Val Val Lys Glu Ala Leu Leu Lys
            100                 105                 110

Thr Ile Lys Ala Ala Val Gly Asp Lys Trp Ser Asp Glu Leu Ser Arg
        115                 120                 125

Ala Trp Glu Val Ala Tyr Asp Glu Leu Ala Ala Ala Ile Lys Lys Ala
    130                 135                 140


<210> SEQ ID NO 2
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Methylacidiphilum infernorum

<400> SEQUENCE: 2

Met Ile Asp Gln Lys Glu Lys Glu Leu Ile Lys Glu Ser Trp Lys Arg
1               5                   10                  15

Ile Glu Pro Asn Lys Asn Glu Ile Gly Leu Leu Phe Tyr Ala Asn Leu
            20                  25                  30

Phe Lys Glu Glu Pro Thr Val Ser Val Leu Phe Gln Asn Pro Ile Ser
        35                  40                  45

Ser Gln Ser Arg Lys Leu Met Gln Val Leu Gly Ile Leu Val Gln Gly
    50                  55                  60

Ile Asp Asn Leu Glu Gly Leu Ile Pro Thr Leu Gln Asp Leu Gly Arg
65                  70                  75                  80

Arg His Lys Gln Tyr Gly Val Val Asp Ser His Tyr Pro Leu Val Gly
                85                  90                  95

Asp Cys Leu Leu Lys Ser Ile Gln Glu Tyr Leu Gly Gln Gly Phe Thr
            100                 105                 110

Glu Glu Ala Lys Ala Ala Trp Thr Lys Val Tyr Gly Ile Ala Ala Gln
        115                 120                 125

Val Met Thr Ala Glu
    130


<210> SEQ ID NO 3
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena thermophile

<400> SEQUENCE: 3

Met Arg Lys Gln Pro Thr Val Phe Glu Lys Leu Gly Gly Gln Ala Ala
1               5                   10                  15

Met His Ala Ala Val Pro Leu Phe Tyr Lys Lys Val Leu Ala Asp Asp
            20                  25                  30

Arg Val Lys His Tyr Phe Lys Asn Thr Asn Met Glu His Gln Ala Lys
        35                  40                  45

Gln Gln Glu Asp Phe Leu Thr Met Leu Leu Gly Gly Pro Asn His Tyr
    50                  55                  60

Lys Gly Lys Asn Met Ala Glu Ala His Lys Gly Met Asn Leu Gln Asn
65                  70                  75                  80
```

-continued

```
Ser His Phe Asp Ala Ile Ile Glu Asn Leu Ala Ala Thr Leu Lys Glu
               85                  90                  95

Leu Gly Val Ser Asp Gln Ile Ile Gly Glu Ala Ala Lys Val Ile Glu
            100                 105                 110

His Thr Arg Lys Asp Cys Leu Gly Lys
        115                 120
```

What is claimed is:

1. A beef replica product, comprising:

a) a muscle replica comprising 0.1%-5% of a heme-containing protein, at least one sugar compound and at least one sulfur compound; and

b) a fat tissue replica comprising at least one plant oil and a denatured plant protein,

wherein said muscle replica and fat tissue replica are assembled in a manner that approximates the physical organization of meat.

2. The beef replica product of claim **1**, further comprising a connective tissue replica.

3. The beef replica product of claim **1**, wherein the denatured plant protein comprises one or more isolated non-heme-containing proteins.

4. The beef replica product of claim **3**, wherein each of said one or more isolated non-heme-containing proteins is derived from a different plant species.

5. The beef replica product of claim **3**, wherein said one or more isolated non-heme-containing proteins is selected from the group consisting of: ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), ribulose-1,5- bisphosphate carboxylase oxygenase activase (Rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin protein, proteinoplast, secalin, extensins, triticeae gluten, zein, a seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin.

6. The beef replica product of claim **1**, wherein said beef replica product does not contain one or more of methylcellulose, carrageenan, caramel color, konjac flour, gum arabic, and acacia gum.

7. The beef replica product of claim **1**, wherein said beef replica product contains less than 1% wheat gluten.

8. The beef replica product of claim **1**, wherein said beef replica product contains no wheat gluten.

9. The beef replica product of claim **1**, wherein said beef product does not contain one or more of soy protein isolate, soy protein concentrate, or tofu.

10. The beef replica product of claim **1**, wherein said beef product contains less than 5% carbohydrates.

11. The beef replica product of claim **1**, wherein said beef replica product is characterized by one or more of the following: contains no tofu, contains no soy protein, contains less than 1% cellulose, contains less than 5% insoluble carbohydrates, or contains no wheat gluten.

12. The beef replica product of claim **1**, wherein said beef replica product contains no animal products and less than 5% carbohydrates.

13. The beef replica product of claim **1**, wherein said beef replica product contains no wheat gluten and less than 5% insoluble carbohydrates.

14. The beef replica product of claim **3**, comprising:

a) 60-90% water;

b) 5-30% protein content;

c) 1-20% of a fat; wherein said one or more isolated non-heme-containing protein comprises one or more isolated plant proteins.

15. The beef replica product of claim **1**, wherein said plant protein is an oil body protein.

16. The beef replica product of claim **2**, wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight.

17. The beef replica product of claim **2**, wherein the connective tissue replica comprises a precipitated plant protein.

* * * * *

EXHIBIT 2

GRAS NOTICE FOR MYOGLOBIN PREPARATION

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Motif FoodWorks, Inc.
27 Drydock Avenue, 2nd Floor
Boston, Massachusetts
02210 USA

DATE:
April 14, 2021

GRAS Notice for Myoglobin Preparation

TABLE OF CONTENTS

List of Figures and Tables

GRAS Notice for Myoglobin Preparation

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Motif FoodWorks, Inc. (Motif FoodWorks) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that a Myoglobin Preparation, as manufactured by Motif FoodWorks, is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Motif FoodWorks' view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Motif FoodWorks the undersigned hereby certifies that all data and information presented in this Notice represents a complete, representative, and balanced submission, and considered all unfavorable as well as favorable information known to Motif FoodWorks and pertinent to the evaluation of the safety and GRAS status of the Myoglobin Preparation as a food ingredient for use in a variety of food products, as described herein.

DocuSigned by:
Janet E. Collins
2F33C163C3DE422...

4/15/2021

Janet E. Collins, Ph.D., R.D.
Vice President, Regulatory Government and Industry Affairs
Motif FoodWorks, Inc.
jcollins@motiffoodworks.com

Date

1.1 Name and Address of Notifier

Motif FoodWorks, Inc.
27 Drydock Avenue, 2nd Floor
Boston, Massachusetts
02210 USA

1.2 Common Name of Notified Substance

Yeast-derived heme protein (non-animal)

1.3 Conditions of Use

Motif FoodWorks' Myoglobin Preparation is intended for use in plant-based ground meat analogue products at levels providing ≤2% myoglobin protein to contribute to the flavor and aroma in ground meat analogues to mimic flavors associated with cooked ground meat. Examples of meat analogue products include burgers, patties, sausages, and other plant-based meat analogues, including fresh and/or frozen entrées or meals, where ground meat or poultry is typically the principal ingredient.

The use of Myoglobin Preparation in ground meat analogues is self-limiting based on acceptable organoleptic (flavor and aroma) properties of the final food products.

The Myoglobin Preparation is intended for use in food products consumed by the general population. As Myoglobin Preparation will be used in meat alternative products, substituting 1:1 for conventional meat and poultry products, consumption patterns for food products containing Myoglobin Preparation as an ingredient are anticipated to be similar to those for meat and poultry in a typical American diet. The Myoglobin Preparation is not intended for use in infant formula and is not intended for addition to meat and poultry products regulated by the United States Department of Agriculture (USDA). Motif notes that myoglobin imparts a red coloration when exposed to oxygen and simulated ready-to-cook meat products that incorporate myoglobin will typically have a red to pink coloration similar to meat. Although the primary function of myoglobin in food is for flavor, a secondary effect of the ingredient on the coloring of some food applications is recognized and a Color Additive Petition will be submitted to the Agency to support such uses.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020a), Motif FoodWorks has concluded that the intended uses of Myoglobin Preparation as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Motif FoodWorks, Inc.
27 Drydock Avenue, 2nd Floor
Boston, Massachusetts
02210 USA

Should the U.S. FDA have any questions or additional information requests regarding this Notification, Motif FoodWorks will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Motif FoodWorks' view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the *Freedom of Information Act*, 5 U.S.C. 552.

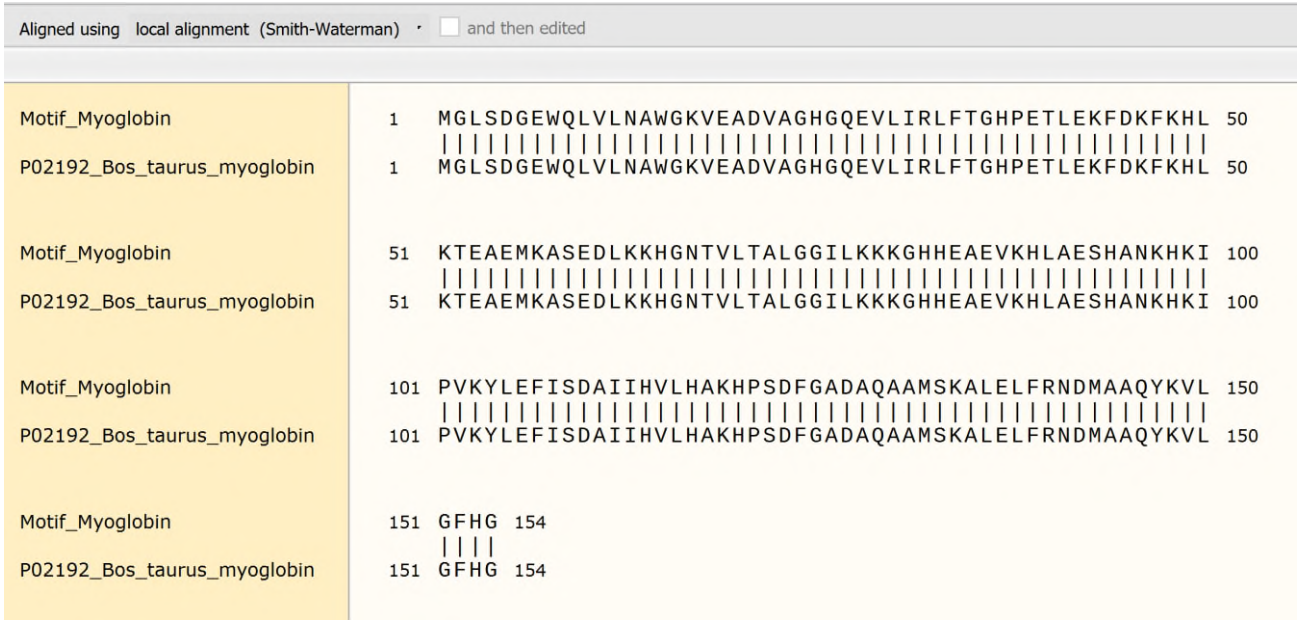
Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity of the Ingredient

Motif FoodWorks' myoglobin ingredient is a liquid flavoring preparation (herein referred to as Myoglobin Preparation) containing myoglobin produced by fermentation from a modified strain of *Pichia pastoris* expressing the myoglobin gene from *Bos taurus*. The ingredient has a moisture content of ≥92.5%, a myoglobin content of ≥3%, and a myoglobin protein purity of ≥65%. The remaining components of Myoglobin Preparation include water, ash (≤1.5% w/w), fat (≤1% w/w), and carbohydrate (≤0.5% w/w) and has a total organic solids (TOC) content of ≤7.5%[1]. Myoglobin Preparation is formulated with food-grade excipients, stabilizers, preservatives (*e.g.*, sodium phosphate, sodium ascorbate, sodium chloride), and antimicrobial agents, depending on storage conditions.

Myoglobin[2] (UniProtKB/Swiss-Prot No. P02192, GeneID: 280695, VGNC Symbol: MB) is the characterizing component of the Myoglobin Preparation. As shown in Figure 2.1-1 below, the myoglobin protein in the Myoglobin Preparation has 100% sequence homology to myoglobin protein from *Bos taurus*. Bovine myoglobin has a high level of homology to hemoglobin proteins from porcine and ovine species, as well as from birds.

Figure 2.1-1 Amino Acid Sequence Homology of Motif FoodWorks' Myoglobin to Bovine Myoglobin



[1] The Myoglobin Preparation may contain ≤0.2 mg/L *Pichia* protein.
[2] UniProtKB/Swiss-Prot No. P02192. GeneID No. 280695. VGNC Symbol: MB.

The myoglobin present in Motif FoodWorks' Myoglobin Preparation has been characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion chromatography (SEC), and peptide mass spectrometry. As shown in Figure 2.1-2, myoglobin obtained from fermentation of *P. pastoris* strain t838417 (Lane 2) has a molecular weight of approximately 17 kDa, corresponding to its predicted molecular weight, and displays a similar gel migration pattern to a commercial bovine muscle-derived myoglobin standard (Innovative Research; Catalog # IBOMBLY250MG). The slight differences in the migration patterns are explained by the apparent low-level O-glycosylation within the bovine standard identified during proteomic analyses. Similar findings were observed in the SEC profiles when Motif FoodWorks' Myoglobin Preparation was compared to a commercial standard demonstrating a high purity of the ingredient and slight differences in retention times due to the absence of glycosylation in myoglobin from *P. pastoris* (Figure 2.1-3). The results of the proteomics analyses corroborate the identity of the protein as bovine myoglobin relative to a commercial standard and demonstrate that the material contains negligible glycosylation compared to 3 potential O-glycosylation sites identified in the bovine myoglobin standard (Table 2.1-4). Low level residues (≤0.2 mg/L) of native proteins from the fermentation organism are expected to be present in the myoglobin preparation. *Pichia* yeast has a long history of safe use in food biotechnology for production of food enzymes (EFSA, 2017, Spohner *et al*., 2015) and the production strain has been used previously for the manufacture of soybean leghemoglobin, a similar protein used for flavoring (FDA, 2018a).

A discussion of the historical use and safety of *Pichia* yeast as a food processing organism is presented in Sections 2.2.1 and 6.1. Based on the long history of safe use of *Pichia pastoris* in food production and recent characterization of native proteins from the same production strain as reported by Jin *et al*., (2018), further characterization of the residual *Pichia* proteins was not considered necessary for the GRAS evaluation.

Figure 2.1-2 **Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Results of Myoglobin from Myoglobin Preparation with Lane 2 Showing Motif FoodWorks' Myoglobin Preparation and Lane 3 Showing Bovine Myoglobin Standard**

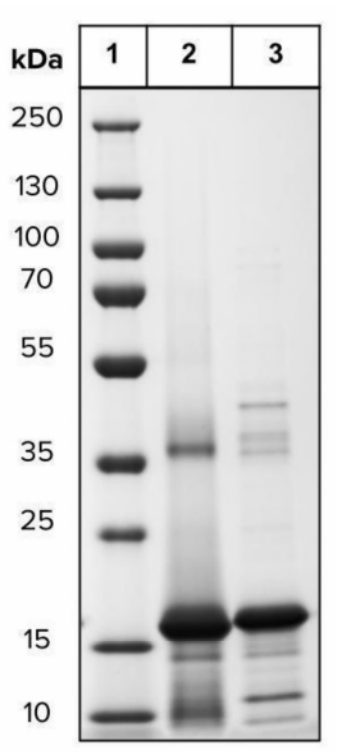
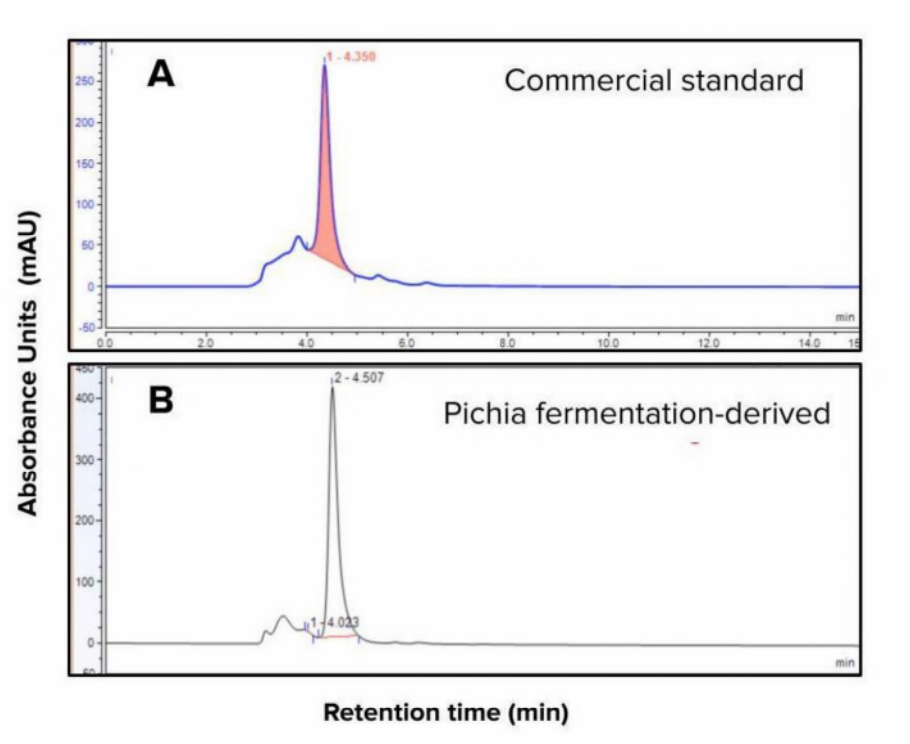


Figure 2.1-3 **Characterization of Myoglobin from Myoglobin Preparation and Bovine Muscle-Derived Myoglobin Standard by Size Exclusion Chromatography (A and B) and Ultraviolet-Visible Spectroscopy (C and D)**



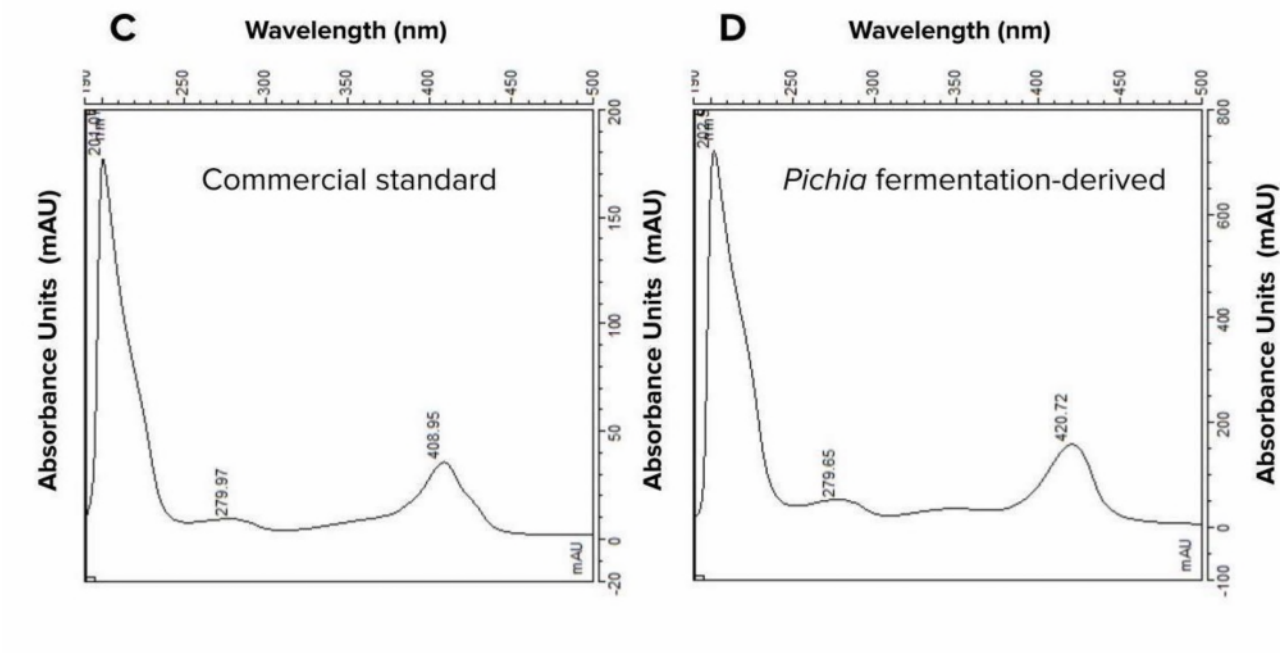
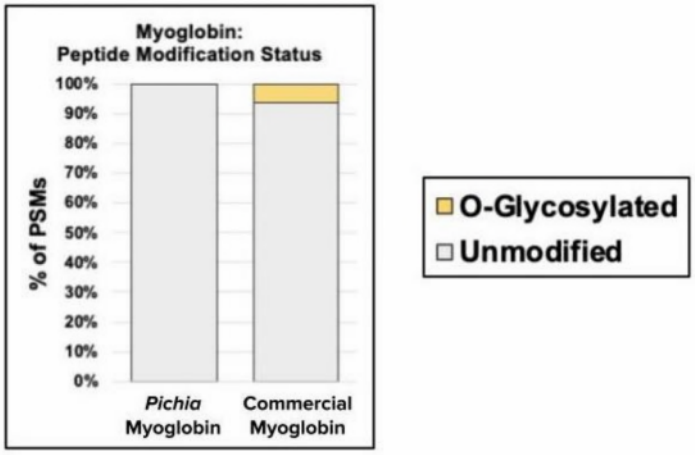


Figure 2.1-4 **Predicted Glycosylation Pattern Using Liquid Chromatography Mass Spectroscopy (LC-MS/MS) Analyses of Commercial Bovine Myoglobin Standard (Right) Compared to Myoglobin from *Pichia* (Left)**



2.2 Method of Manufacture

2.2.1 Description of the Production Microorganism

2.2.1.1 Host (Parental) Organism

Pichia pastoris[3] is a eukaryotic, methylotrophic, non-pathogenic, and non-toxigenic microorganism widely used by the biotechnology industry for production of recombinant proteins and food enzymes (Balamurugan *et al.*, 2007; Kurtzman, 2009). The genome of *P. pastoris* was sequenced in 2009 (De Schutter *et al*., 2009). *P. pastoris* was first used in the commercial preparation of a single cell protein for use in animal feed, and since then has been extensively used in food production and human pharmaceutical products (Ahmad *et al*., 2014; Brady *et al*., 2020). According to Brady *et al.* (2020), since 2003, *P. pastoris* has been used as a host organism in over 7,000 research articles and accounted for approximately 17% of the total recombinant genes produced in 2009 (Sørensen, 2010). *P. pastoris* is a well characterized microorganism and has an established history of safe use in food production. A detailed description of *P. pastoris* was discussed in GRAS Notice (GRN) 737. In the European Union (EU), *P. pastoris* (*K. phaffiii*) was granted qualified presumption of safety (QPS) status by the European Food Safety Authority (EFSA) Panel on Biological Hazards for use in enzyme production (EFSA, 2017).

[3] *Pichia pastoris* was reassigned to the genus *Komagataella* following phylogenetic analysis of gene sequences, and important strains of '*Pichia pastoris*' commonly used in biotechnology are members of *Komagatealla phaffii*. (Kurtzman *et al*., 2020).

The taxonomic identity of *P. pastoris* is presented in Table 2.2.1.1-1.

Table 2.2.1.1-1 Taxonomic Identity of *Pichia pastoris*

Kingdom	Fungi
Phylum	Ascomycota
Class	Saccharomycetes
Order	Saccharomycetales
Family	Phaffomycetaceae
Genus	*Komagataella*
Species	*phaffi (pseudonym = Pichia pastoris)*

The host organism, *P. pastoris* NRRL Y-7556, used for construction of the production strain (t303048), is a methylotrophic yeast capable of using methanol as the sole carbon source. The lineage of this organism was discussed by Brady *et al.* (2020). Genetic typing of the strain has resulted in re-naming of the strain from *Pichia pastoris* to *Komagatella phaffii* (Kurtzman, 2009); however, the strain is still often referred to as *Pichia pastoris,* and for simplicity the name *P. pastoris* will be used throughout the Notification.

As reported by Braun-Galleani *et al.* (2019) almost all research on *K. phaffii* (*P. pastoris*) has been conducted using the genetic background of strain CBS7435 (synonymous with NRRL Y-11430). The origin of this strain was previously unclear since the strain was first deposited in the CBS and NRRL culture collections in connection with a US patent granted to Phillips Petroleum; however, Braun-Galleani *et al.* (2019) have demonstrated that CBS7435 (NRRL Y-11430) is identical to the type strain of *K. phaffii* (NRRL Y-7556), which was isolated from an oak tree. Corroborating these conclusions, genotypic analyses by Brady *et al.,* (2020) have demonstrated that strain NRRL Y-11430 and NRRL Y-7556 differ by a single nucleotide polymorphism (SNP). Motif FoodWorks has therefore concluded that the *P. pastoris* NRRL Y-7556 host strain is from the same lineage as *P. pastoris* NRRL Y-11430, which served as the host organism for production of soybean leghemoglobin (Impossible Foods, Inc., 2017; U.S. FDA, 2018a).

2.2.1.2 Construction of the Production Organism

The production strain, *P. pastoris* t838417, was constructed obtained from a genetically modified strain of *P. pastoris*, using the principles described by the Organisation for Economic Co-operation and Development (OECD) criteria for Good Industrial Large-Scale Practice (GILSP) microorganisms (OECD, 1992, 1993), as well as criteria for safe production microorganisms (Pariza and Foster, 1983; Pariza and Johnson, 2001).

The parental organism, *P. pastoris* t303048, is genetically modified to overexpress the proteins of the native heme biosynthetic pathway of *P. pastoris*. The heme biosynthetic pathway consists of 8 steps, each catalyzed by an enzyme that is highly conserved across plant, animal, and fungal species. Genes encoding all 8 enzymes were generated by DNA synthesis and transformed into *P. pastoris* t303048 using antibiotic resistance cassettes. The antibiotic resistance cassettes were removed from the strains after each round of transformation. This process yielded a stable intermediate strain, *P. pastoris* t486367, containing extra copies of each of the native *Pichia* heme biosynthesis enzymes.

P. pastoris t486367 was then modified to express *Bos taurus* (bovine myoglobin) protein. The *Bos taurus* myoglobin gene was codon-optimized for expression in *P. pastoris* and generated by DNA synthesis; multiple copies of the gene were stably integrated, along with an antibiotic resistance cassette, into *P. pastoris* t486367, using standard biotechnology practices. Subsequently, the antibiotic resistance cassette was removed. The resulting strain was identified as *P. pastoris* t830652. The gene encoding for bovine myoglobin is the only recombinant protein-encoding DNA inserted into the host organism.

To support optimal expression of the promoters utilized in the previous steps, *P. pastoris* t830652 was modified by inserting an additional copy of the gene encoding a transcription factor native to *P. pastoris*, along with an antibiotic resistance cassette. Following introduction of the transcription factor gene and removal of the antibiotic resistance gene, the production strain *P. pastoris* t838417 was obtained.

The production strain does not contain any antibiotic resistance genes or plasmid sequences, and therefore, does not pose any risk of transferring antibiotic resistance to non-related organisms. Similarly, no antibiotic resistance genes/DNA are present in the Myoglobin Preparation. Removal of all antibiotic resistance genes introduced during construction of the production strain was confirmed phenotypically and by whole genome sequencing. Myoglobin Preparation does not contain viable cells of the production strain, as they are lysed during the manufacturing process and removed by centrifugation and microfiltration. The Myoglobin Preparation may contain residual *Pichia* proteins at levels ≤0.2 mg/L.

All changes introduced into the production strain *P. pastoris* t838417 are stably integrated in the genome and confirmed to be present after growth on non-selective fermentation media during and after a round of fermentation. No plasmid sequences are present in the production strain, and therefore no plasmid sequences are expected to be capable of being transferred from the production strain to non-related organisms.

2.2.2 Description of the Manufacturing Process

2.2.2.1 Raw Materials and Processing Aids

All raw materials and processing aids comply with food-grade specifications, as established in the *Food Chemicals Codex* (FCC) or equivalent international food or pharmacopeia standard (*e.g.*, United States Pharmacopeia), and are permitted for use in food by U.S. federal regulations or are GRAS for their respective uses. All filtration aids are those commonly used by the food industry in the purification of food ingredients.

2.2.2.2 Production Process

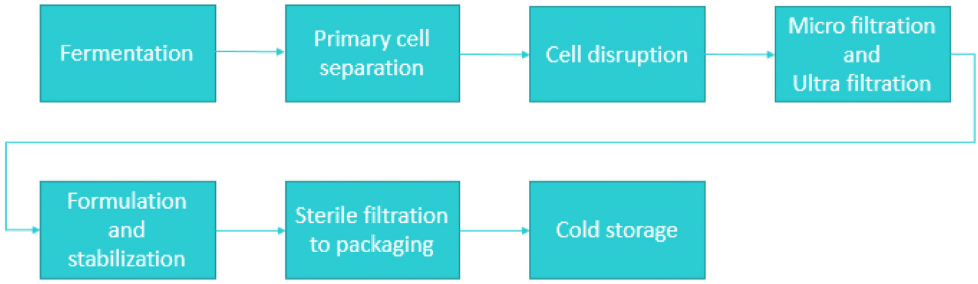
The myoglobin protein is prepared in stages: expression of myoglobin protein by the production organism following submerged fermentation (Fermentation Process), then enrichment and stabilization of the expressed myoglobin protein (Recovery Process). The Myoglobin Preparation is standardized to a concentration of about 3% myoglobin protein (purity ≥65%). The production process of the Myoglobin Preparation is discussed in further detail below.

In the Fermentation Process, the production strain *P. pastoris* t838417 is fermented by submerged fed-batch fermentation for the expression of myoglobin protein. The cells of the production strain are kept at -80°C in 20% (v/v) glycerol as the source inoculum. Working cell banks are prepared from the master cell bank after testing for microbial purity, specific growth rate, and yield prior to production fermentation. The fermentation broth is periodically analyzed microscopically to ensure culture purity. Process parameters including pH, temperature, agitation, dissolved oxygen, methanol concentration, and glycerol concentration are routinely monitored through fermentation following methods consistent with GRN 737 (Impossible Foods, Inc., 2017; U.S. FDA, 2018a). If microbial contamination is detected or other process deviations impacting safety and/or quality of the final product are identified, the fermentation broth is sterilized by steam in place and discarded.

Following the fermentation process, the production strain cells in the fermentation broth are washed and lysed. Insoluble material within the lysate is removed by centrifugation and microfiltration. Ultrafiltration is used to concentrate the myoglobin protein. The resulting concentrate is formulated with sodium chloride, sodium phosphate, sodium ascorbate, and may include other food-grade antimicrobials and antioxidants to stabilize the formulation, which is stored as a frozen liquid (-20°C).

A schematic overview of the recovery process is provided in Figure 2.2.2.2-1.

Figure 2.2.2.2-1 Schematic Overview of the Recovery Process Steps to Obtain the Myoglobin Protein



2.3 Product Specifications and Batch Analysis

Food-grade specifications for physical, chemical, and microbiological parameters have been established for the Myoglobin Preparation. All methods of analysis are internationally recognized (*e.g.*, Association of Official Analytical Chemists, U.S. FDA Bacteriological Analytical Manual). The chemical, physical, and microbiological specifications of the product are presented in Table 2.3-1. Pathogen presence or greater than 10^4 CFU/mL aerobic count, or failure to comply with the specifications would result in batch discard; execution of additional sanitization standard operating procedures in compliance with internal food-safety standards, and a root cause analysis.

Table 2.3-1 Product Specifications and Analysis of 3 Production Batches of Myoglobin Preparation

Specification Parameter	Specification Limit	Method of Analysis	Manufacturing Lot No.		
			M2- 001/2021	M2- 002/2021	M2-003/2021
Physico-Chemical Parameters					
Myoglobin Protein (% w/w)[a]	≥3	SEC HPLC	3	3	3
Myoglobin Protein Purity (% w/w)[b]	≥65	Chromatographic purity (at 280 nM)	98	98	97
Protein (% w/w)[c]	≤4.62	Calculated	3.06	3.06	3.09
Fat (% w/w)	≤1	AOAC 933.05	<0.08	<0.08	0.29
Moisture (% w/w)	≥92.5	AOAC 925.40	95.60	96.04	95.93
Total Organic Solids (% w/w)[d]	≤7.5	Difference	4.40	3.96	4.07
Ash (% w/w)	≤1.5	AOAC 945.46	1.27	1.36	1.6
Carbohydrate (% w/w)[e]	≤0.5	Difference	0.01	0.00	0.00
pH	6.5 to 8.5	AOAC 981.12	6.88	7.11	7.32
Heavy Metals					
Lead (ppm)	<0.4	AOAC 2015.01 Mod 2232	<0.01	<0.01	<0.01
Arsenic (ppm)	<0.05	AOAC 934.03	<0.01	<0.01	<0.01
Mercury (ppm)	<0.05	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)	<0.005	<0.005	<0.005
Cadmium (ppm)	<0.2	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)	<0.001	<0.001	<0.001
Microbiological Parameters					
Aerobic plate count (CFU/g)	$<10^4$	AOAC 966.23	<100	<100	<100
Yeast (per g)	<10	FDA-BAM, 7th ed.	<10	<10	<10
Mold (per g)	<10	FDA-BAM, 7th ed.	<10	<10	<10
Escherichia coli (3 tubes MPN) (per g)	<3	AOAC 966.24	<3	<3	<3
Salmonella spp. (per 25g)	Negative	AOAC RI 100201	Negative	Negative	Negative
Listeria monocytogenes (per 25 g)	Negative	AOAC 2003.12	Negative	Negative	Negative

AOAC = Association of Official Analytical Chemists; BAM = Bacteriological Analytical Manual; CFU = colony forming units; FDA = Food and Drug Administration; HPLC = high-performance liquid chromatography; ICP-MS = inductively coupled plasma-mass spectrometry; MPN = most probable number; ppm = parts per million; SEC = size exclusion chromatography.

[a] Myoglobin protein may exceed 3%, if additional moisture is removed during the concentration step of the manufacturing process (Figure 2.2.2.2-1).

[b] The balance of protein in the Myoglobin Preparation is residual *Pichia* protein.

[c] Protein (% w/w) content calculated as follows: Protein (% w/w) = Myoglobin Protein (% w/w)/Myoglobin Protein Purity (%).

[d] Solids (% w/w) calculated by difference as follows, Solids (% w/w) = 100-Moisture (% w/w).

[e] Carbohydrates (% w/w) calculated by difference as follows, Carbohydrate (% w/w) = Solids (% w/w) – Fat (% w/w) – Ash (% w/w) – Protein (% w/w).

Part 3. § 170.235 Dietary Exposure

3.1 Background Intake of Myoglobin

No federal regulations permitting the addition of myoglobin to the U.S. food supply have been promulgated, and Motif FoodWorks is not aware of other GRAS sources of myoglobin in the U.S. marketplace. Current background intakes of bovine myoglobin are solely contributed from the consumption of beef; however, myoglobin isoforms are also present in pork products and poultry. Reported concentrations of myoglobin in various meat products consumed in the diet vary based on species, muscle tissue and animal age. Myoglobin imparts a characteristic red coloration to raw meat products and myoglobin levels are accordingly highest in red meats such as beef and lowest in white meats such as poultry (see Table 3.1-1 below). The variability of myoglobin concentrations in meat products complicates estimation of dietary intakes from background foods; conservative estimates of 0.5% myoglobin have been reported for "meat" (Yip and Dallman, 1996).

Table 3.1-1 Concentrations of Myoglobin in Meat and Poultry Products

Meat Source	Myoglobin Concentration	Reference
Beef	0.02 to 0.18%	Texas A&M (2021)
	0.243%	Fleming *et al.* (1960)
	0.4 to 1%	Clydesdale and Francis (1971)
	0.199 to 0.364%	Rickansrud and Hendrickson (1967)
Pork	0.062% to 0.095%	Newcom *et al*. (2004)
	0.1 to 0.3%	Clydesdale and Francis (1971)
	0.079% to 0.16%	Lawrie (1950)
Chicken	0 to 0.582%	Kranen *et al*. (1999)

Dietary intakes of meat in the U.S. population have been estimated by the Economic Research Service (ERS) of the USDA. *Per capita* intakes of meat are reported as part of the ERS Food Availability Data System (FADS), which includes 3 data series on food and nutrient availability for consumption: food availability data, loss-adjusted food availability data, and nutrient availability data. The ERS considers these data to serve as proxies for actual consumption of food commodities at the national level. The food availability data series includes estimates for loss-adjusted food availability data (LAFA) to adjust for food spoilage, plate waste, and other losses thereby more closely approximating actual consumption (USDA-ERS, 2021). Data for loss-adjusted food availability data for red meat, poultry and fish are shown in Table 3.1-2 below. *Per capita* total intake estimates for red meat, poultry and fish were 180g/person per day. Using a mean estimated myoglobin concentration of 0.5% as reported by Yip and Dallman (1996), the total estimated dietary intake of myoglobin is *ca*. 1 g/person per day.

Table 3.1-2 USDA-ERS *Per Capita* Consumption Estimates for Various Meat Sources Adjusted for Loss[a]

Meat Type	*Per Capita* Intake (g/person/day)	Myoglobin Concentration (% wt/wt)[b]	*Per Capita* Myoglobin Intake (g/person/day)
Red Meat	94.4	0.5%	0.472
Poultry	77.6	0.5%	0.388
Total Fish	8.3	0.5%	0.06
Total Meat	180.3	0.5%	0.90

Conc. = concentration; ERS = Economic Research Service; USDA = United States Department of Agriculture.
[a] USDA, Economic Research Service - based on data from various sources as documented on the Food Availability Data System home page. Data last updated June 1, 2020.
[b] Mean concentration of myoglobin based on reported estimates from Yip and Dallman (1996).

Assuming that the proposed food uses of myoglobin in meat alternative products would substitute for various meat products on a 1:1 basis, the introduction of Motif FoodWorks' Myoglobin Preparation to the U.S. marketplace would not change background intakes of myoglobin in the U.S. population. Motif FoodWorks notes that the intended use levels provided here, of up to 2%, are higher than anticipated concentrations naturally occurring in meat products; however, typical use levels in foods are expected to be closer to 1.0 to 1.25% for most food categories. Motif FoodWorks also notes that estimated dietary intakes of Myoglobin Preparation from the proposed food uses will be limited to a large extent by the current market availability of meat alternative products. Although there is limited information available on the current food supply of meat alternative products, it has been estimated that meat alternative products may capture up to 20% of the market for conventional protein sources from animals in North America (Gourévitch *et al.,* 2021). It can be concluded that a maximum use level of up to 2% myoglobin in the diet and 1:1 substitution of meat alternative products for conventional meat products will not increase total dietary intake of myoglobin in the U.S. population.

3.2 Estimated Dietary Intake of Myoglobin Preparation Using NHANES

In addition to the 1:1 substitutional approach described above using *per capita* estimates, Motif FoodWorks has also conducted dietary estimates for the company's Myoglobin Preparation *via* dietary intake modeling using survey data provided by the National Health and Nutrition Examination Surveys (NHANES). As discussed, Motif FoodWorks' Myoglobin Preparation is intended to be used in meat analogue products that will substitute for conventional meat-based products currently in the marketplace. Currently, the majority of such foods in the U.S. marketplace are plant-based products that simulate ground meat (*e.g.*, plant-based burgers, sausages and "meat" snacks, frozen entrees).

Using the NHANES Data Derivation [2013-2018 (CDC, 2019)], the U.S. dietary exposure to plant-based meat and poultry analogues was estimated. One-day dietary intake data were analyzed from a population of 22,818 individuals over the age of 2 years, excluding incomplete data and individuals pregnant or lactating, in the data set, using SAS 9.4[4]. The population of individuals in the survey reporting consumption of plant-based meat analogues was extremely small in the survey population [116 participants in the subgroup of a population of 22,818 (NHANES, 2013-2018)]. Therefore, exposure data was limited to consumers only to understand the current consumption patterns of frequent consumers of these products.

Food codes for various meat analogue products (soy-based burger, grain-based sausages, vegetarian hot dog) were selected and dietary intake estimates for total consumers were obtained for myoglobin based on

[4] Fulgoni V (2021) [Personal communication. RE: NHANES data: Dietary exposure to myoglobin added at various levels to plant-based meat analogues. NHANES (2013-2018) data].

incorporation levels of 1%, 1.5%, and 2% for consumers only (N=116). Estimated daily intakes of myoglobin from proposed food uses of the Myoglobin Preparation in meat alternative products is presented in Table 3.2-1. Due to the small sample size, data are limited to mean intakes for consumers only as the 90th percentile estimates were considered unreliable. Mean intakes for consumers aged 2+ years was 0.714 g per person per day based on a myoglobin use level of 1%. At the highest use level of 2% the mean dietary intake of myoglobin was 1.43 g.

Table 3.2-1 Estimated Mean Daily Intake of Myoglobin from Meat Alternative Products in the U.S. for "Consumers Only" Aged 2+ Years (2013-2018 NHANES Data)

Population	Myoglobin Inclusion Level (% wt/wt basis)		
	1%	1.5%	2%
	Mean Intakes of Myoglobin (g/person/day)		
Consumers (2+ years) (N=116)	0.71	1.07	1.43
Male Consumers	0.69	1.03	1.38
Female Consumers	0.73	1.09	1.46

NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
Due to the small sample size data 90th percentile data was statistically unreliable and therefore is not reported .

As discussed, typical food use applications will incorporate a use level of between 1 to 1.25%, and therefore the estimated dietary intakes of *ca*. 1 g per person per day are largely in-line with background consumption of myoglobin in the diet from conventional meat sources (see Section 3.2). As food uses of the Myoglobin Preparation will be substitutional for conventional meat on a 1:1 basis, no change in total population intakes of myoglobin is expected from the introduction of the ingredient to the U.S. marketplace.

Motif FoodWorks notes that the dietary intake estimates reported in Table 3.2-1 below will over-estimate dietary intakes as it assumes that all potential foods to which Myoglobin Preparation may be added are consumed in a given day. Motif FoodWorks also notes that the general category of meat alternative products is a rapidly growing area of food technology and that current food codes represented within the NHANES databases are unlikely to be inclusive of the broad variety of foods now and soon-to-be available to U.S. consumers. Consumer demand for such products also is growing and therefore limitations in extrapolating the small sample size of consumers (N=116) to the U.S. population of meat analogue consumers should be recognized. Accordingly, Motif FoodWorks placed an emphasis on the dietary intake calculations presented in Section 3.2 where a 1:1 substitution for conventional meat products is assumed relative to the anticipated market share for such products in the immediate and foreseeable future.

Part 4. § 170.240 Self-Limiting Levels of Use

The use of myoglobin in plant-based meat analogues has self-limiting levels of use due to changes in sensory characteristics associated with cooked meat that appear to peak at an inclusion level of around 1.0 to 1.25% of the formulation and may negatively impact flavor and aroma at an inclusion level close to 2% of the formulation. The amount of myoglobin added above the inclusion level of 1.25% in a food formulation limits the sensory acceptability of the plant-based meat analogues.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. § 170.250 Narrative and Safety Information

The subject of this GRAS Notice is Myoglobin Preparation containing myoglobin, a heme protein obtained through fermentation of a genetically modified strain of *P. pastoris*. The Myoglobin Preparation is a liquid mixture containing bovine myoglobin (≥3% w/w) with a purity of at least 65%. Residual proteins from *Pichia* are <0.2%. The safety assessment of the Myoglobin Preparation therefore focused on hazard characterization of the production organism, and hazard characterization of the protein expression product (*i.e.*, myoglobin) under its conditions of intended use. Motif FoodWorks has applied the safety assessment practices used for biotechnology-derived food enzymes outlined by Pariza and Johnson (2001) for the Myoglobin Preparation. Under this safety assessment paradigm, the need for toxicological investigations of enzyme preparations produced using biotechnology is determined on the basis of 2 primary considerations: (1) the availability of data and information substantiating that the production organism is from a safe lineage that has been the subject of previous toxicological evaluations; and (2) that there is evidence to support the safety of the introduced protein expression product(s) (*i.e.*, myoglobin). Motif FoodWorks also considered the science-based 2-tiered, weight-of-evidence strategy to assess the safety of novel proteins used in the context of agricultural biotechnology developed by the International Life Sciences Institute (ILSI) International Food Biotechnology Committee (Delaney *et al.*, 2008). Under this paradigm, the safety assessment draws upon knowledge of the biological and chemical characteristics of the protein for analyses of hazard at the Tier I level and includes an assessment of the biological function or mode of action and intended application of the protein, history of safe use, comparison of the amino acid sequence of the protein to other proteins, as well as the biochemical and physico-chemical properties of the proteins. Only proteins that cannot be adequately characterized under the Tier I evaluation would proceed to toxicological evaluation under Tier II.

With respect to the safety of the production organism, as discussed in further detail in Section 6.2, the production strain, *P. pastoris* t838417, is a non-pathogenic and non-toxigenic yeast species with a long history of safe use in food production (U.S. FDA, 2018b). The production strain has been genetically modified to express a synthetic gene encoding for bovine myoglobin. Other than the gene encoding for bovine myoglobin, the production strain does not contain any other exogenous DNA, and the final Myoglobin Preparation is absent of detectable levels of the production strain. Successful integration of the myoglobin gene has been confirmed using whole genome sequencing. The introduced nucleotide sequences are codon optimized for expression in *Pichia* are confirmed to encode for a protein sequence that is identical to bovine myoglobin. Motif FoodWorks has demonstrated that the production strain (NRRL Y-7556) is genetically identical to the strain host (NRRL Y-11430) used for the manufacture of soybean leghemoglobin described by Impossible Foods in GRN 737 and therefore is from a safe strain lineage with a history of food use. Bioinformatic evaluations conducted on the production strain by Jin *et al.*, (2018) and Reyes *et al.*, (2021), have demonstrated that residual proteins from the production strain are non-toxigenic and of low allergenic potential for cross-reactivity to major food allergens.

For safety evaluation of the myoglobin protein, Tier I evaluation leveraged the history of safe consumption of myoglobin from meat, and therefore emphasis was placed on demonstrating qualitative equivalence of myoglobin in Motif FoodWorks' flavor preparation to myoglobin from meat. If it could be demonstrated that Motif FoodWorks' myoglobin is qualitatively equivalent to myoglobin in meat, then it could be concluded that a 1:1 substitutional use of the Myoglobin Preparation in meat analogue products would be as safe as dietary intake of myoglobin from current food consumption patterns of meat and other myoglobin containing foods. In this regard, Motif FoodWorks has presented analytical data confirming the identity of the myoglobin synthesized by the production strain using qualitative comparisons of the myoglobin preparation relative to a commercial bovine myoglobin standard using SDS-PAGE, SEC, and proteomic mass spectrometry. The only qualitative difference between myoglobin expressed by *Pichia* and bovine derived myoglobin is the relative absence of glycosylation in *Pichia* expressed myoglobin, which compares to an observed low-level O-glycosylation of bovine myoglobin in at least 3 residues. Motif FoodWorks has therefore concluded that there are no qualitative differences between myoglobin expressed by *Pichia* relative to native bovine myoglobin that is present in meat. Accordingly, the long history of safe consumption of myoglobin from consumption of meat products can be extended to Motif FoodWorks' myoglobin ingredient. As the outcome the Tier I hazard assessment was sufficient to conclude on safety of the ingredient under its intended conditions of use, it was concluded that further hazard characterization *via* toxicology testing under the ILSI Tier II testing scheme was not required.

Myoglobin is present in all commonly consumed meat sources, such as beef, pork, and poultry, and has an extensive history of safe consumption by the global population. There is a common knowledge of the history of consumption of myoglobin from animal sources. In order to corroborate the history of safe consumption of myoglobin, a comprehensive search of the scientific literature was conducted through March 2021. The literature search was completed using ProQuest and included searches of the following databases for pertinent literature on the safety of bovine myoglobin or myoglobin from *Bos taurus*: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. The relevance and specificity of the literature search was increased through the implementation of search terms "bovine myoglobin" or "myoglobin from *Bos taurus*" to reflect the compound of interest in combination with preclinical/clinical endpoints. The search results were retrieved and reviewed in 2 stages (titles and abstracts). The search did not identify any publications, relevant to the safety of bovine myoglobin.

The safety of the bovine myoglobin present in Motif FoodWorks' Myoglobin Preparation is supported by its long history of safe consumption, as well as bioinformatics searches of the protein evaluating its lack of allergenicity and toxigenicity potential. The results of these searches are discussed in Sections 6.3.2 and 6.3.3, respectively, and indicate that Motif FoodWorks' Myoglobin Preparation would not pose an allergenic or toxigenic risk to U.S. consumers.

6.1 Safety of the Production Strain

The safety of the production strain used in the production of Motif FoodWorks' myoglobin was assessed using the same principles for assessing the safety of microbially-derived enzymes for use in food production (Pariza and Foster, 1983; IFBC, 1990; Pariza and Johnson, 2001; Sewalt *et al*., 2016; FAO/WHO, 2020). This approach to the safety evaluation of food enzymes is widely accepted by the scientific community and regulatory agencies and includes an evaluation of the pathogenicity, toxigenicity, and antimicrobial resistance of the production strain, as well as the genetic modification techniques. These points are discussed herein.

P. pastoris is a well characterized non-toxigenic and non-pathogenic microorganism that has a recognized history of safe use in food production. Current *P. pastoris* laboratory strains are from lineages isolated from an oak tree and a chestnut tree and were deposited in a culture collection at the NRRL[5] (www.biogrammatics.com).

Information on the non-pathogenicity and non-toxigenicity properties of *P. pastoris* was discussed in GRN 737 and is incorporated by reference to Section 6.1.3 of the Notice. *P. pastoris* is recognized as a non-toxin producing microorganism and is classified as a biosafety level 1 (BSL1) organism by the ATCC. This species has QPS status in the EU for use in enzyme production (EFSA, 2017), corroborating that *P. pastoris* is a safe and suitable source organism for production of food ingredients and is not capable of producing toxic metabolites when used for food protein production. *P. pastoris* is widely used by the biotechnology industry for the production of recombinant proteins and food enzymes (Cereghino and Cregg, 2000; Cregg *et al.*, 2000; Balamurugan *et al.*, 2007; Kurtzman, 2009; Reyes *et al.*, 2021), with over 300 recombinant proteins produced from this species since the 1980s (Diversa Corporation, 2006; U.S. FDA, 2006). Dried *P. pastoris* is also permitted for the addition to chicken feed as a source of protein under 21 CFR § 573.750 (U.S. FDA, 2020b). This information suggests that *P. pastoris* is non-pathogenic to humans and non-toxigenic and would therefore be a safe and suitable source organism for production of myoglobin (Pariza and Johnson, 2001).

The production strain used in the production of Motif FoodWorks' myoglobin is a genetically modified strain of *P. pastoris*. The production strain was constructed in a similar manner as described in GRN 737 (Impossible Foods, Inc., 2017; U.S. FDA, 2018a) using the principles described by OECD GILSP (OECD, 1992, 1993). Motif FoodWorks' strain of *P. pastoris* (strain t486367) meets the criteria for a safe and suitable source organism described by Pariza and Johnson (2001). The production strain was obtained from a wildtype strain of *P. pastoris* (t303048). A synthetic gene encoding for bovine myoglobin was inserted into the wildtype strain; this strain also contains extra copies of the heme biosynthetic enzymes native to *P. pastoris*. The synthetic gene encoding for bovine myoglobin is the only non-native gene present in the production strain and has been confirmed by bioinformatics to not confer any pathogenic, virulent, or toxigenic factors to the production strain. The genetic stability of the production strain was confirmed after growth on non-selective fermentation media. The production strain does not contain any plasmids or antibiotic resistance genes, as confirmed by phenotyping and whole genome sequencing of the production strain. Overall, it can be concluded that Motif FoodWorks' *P. pastoris* production strain is derived from a strain lineage with a long history of safe use.

Motif FoodWorks' Myoglobin Preparation may contain ≤0.2 mg/L *Pichia* protein. The toxigenicity of *Pichia* proteins was recently discussed by Jin *et al.* (2018) and Reyes *et al.* (2021) following a proteomics assessment of the native proteins expressed by *P. pastoris*. It was concluded that the native *Pichia* proteins do not share structural homology with known toxins and would not cause a toxigenicity concern (Jin *et al.*, 2018; Reyes *et al.*, 2021). Therefore, on the basis that Motif's production strain was derived from the same host strain lineage as that investigated by Jin *et al.*, (2018) and Reyes *et al.*, (2021), Motif has concluded that the small concentrations of residual *Pichia* proteins present in the ingredient would not pose a safety concern.

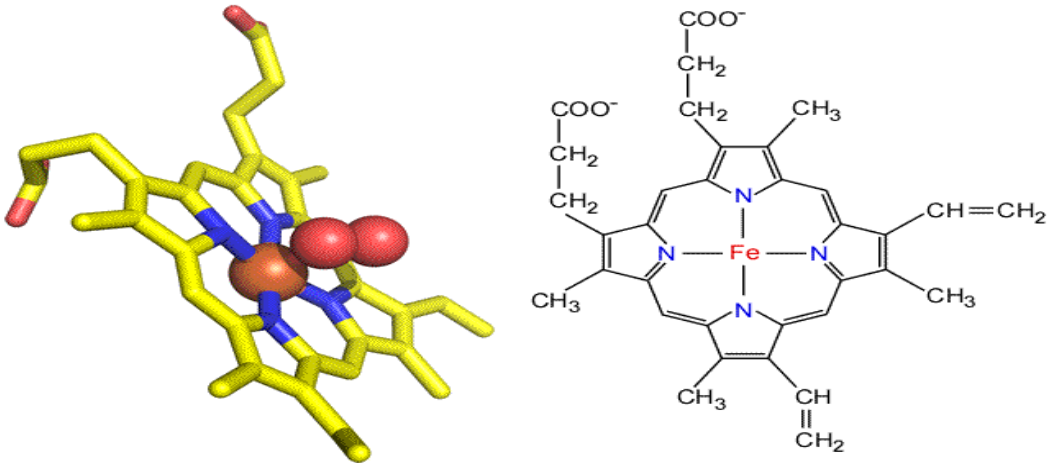
[5] The NRRL collection has been renamed to the Agriculture Research Service Culture Collection and is maintained by the Microbial Genomics and Bioprocessing Research Unit (MGB) of the National Center for Agricultural Utilization Research (NCAUR).

6.2 Safety of Myoglobin

6.2.1 History of Safe Consumption of Myoglobin

Myoglobin is part of a superfamily of heme-containing globular proteins involved in binding iron and/or transportation of oxygen. This globular heme protein is ubiquitous in nature and present in most organisms including bacteria, protozoa, fungi, plants, and animals (Hardison, 1998). Hemoglobin and myoglobin are structurally similar to other heme proteins and contain the identical heme B cofactor. The chemical structure of myoglobin is presented in Figure 6.2.1-1. Consumption of the heme B cofactor is widespread in humans and other animals as heme proteins, such as myoglobins and hemoglobins are abundant in animal tissues where they are consumed as meat. Heme proteins, specifically myoglobins, have been present in the human diet since the beginning of recorded history. Heat treatment of these iron-binding proteins potentiates the meat-like, serum and metallic flavors typically associated with cooked muscle tissue (AMSA, 2015). The Myoglobin Preparation is standardized to contain ≥3% heme protein (see Section 2.3 for further details).

Figure 6.2.1-1 Chemical Structure of Myoglobin



The myoglobin present in Motif FoodWorks' Myoglobin Preparation is 100% identical to bovine myoglobin and shares structural homology to hemoglobin proteins from other commonly consumed animal sources of meat. Considering myoglobin is widely distributed in commonly consumed meats, such as beef and pork, the protein itself has an apparent long history of safe consumption in the human diet. As discussed in Section 3.1, myoglobin is currently consumed at a level of approximately *ca*. 1 g/person per day from red meat, poultry and fish sources in the U.S. diet. In addition, meat extracts and concentrates produced using meats from sources such as bovine, containing myoglobin, are widely consumed in the U.S. population.

Meat extracts, meat protein extracts, and beef protein, which include myoglobin, are considered safe and suitable ingredients for use in the production of meat, poultry, and egg products under FSIS Directive 7120.1 (USDA-FSIS, 2021). The GRAS status of beef protein when used as a binding agent at levels up to 0.89% was filed by the U.S. FDA without objection under GRN 313 (U.S. FDA, 2010).

Therefore, there is a long history of safe consumption of bovine myoglobin in the human diet. To date, there have been no reports of adverse effects following consumption of bovine myoglobin. Similarly, although allergenicity to red meat has been reported in the scientific literature, only 1 case has been associated with myoglobin (see Section 6.2.2 for further details). The amino acid sequence of bovine myoglobin does not contain significant sequence homology to known toxins or allergens, and therefore, would not raise toxigenicity or allergenicity concerns.

6.2.2 Allergenicity of Myoglobin

Food allergies reportedly occur in about 8% of children and less than 2% of adults in the U.S. population, and are most frequently associated with one of the "Big Eight" major allergens [*i.e*., milk, egg, fish (*e.g*., bass, flounder, or cod), Crustacean shellfish (*e.g*., crab, lobster, or shrimp), tree nuts (*e.g*., almonds, pecans, or walnuts), wheat, peanuts, and soybeans] that require allergen labeling under the *Food Allergen Labeling and Consumer Protection Act* of 2004 [(FALCPA) U.S. FDA, 2018b; National Academy of Medicine, 2016]. Although not considered one of the major allergens, red meat allergies have been reported in some individuals, but these cases are rare. The incidence rate of beef allergy was reported to be between 3.28% and 6.52% among children with atopic dermatitis, and about 0.3% in the general population (Fiocchi *et al*., 2000). Most reported cases of allergenic responses to meat, specifically beef, involve sensitization to bovine serum albumin (BSA), and, to a lesser extent, bovine gamma globulin (BGG) (Werfel *et al*., 1997; Fiocchi *et al*., 2000; Vazquez Fuertes *et al*., 2013). BSA and, to a lesser degree, BGG, also are identified as allergenic proteins from cows' milk. BSA and BGG are heat-labile proteins and meat-allergic individuals are typically reactive to undercooked meat. Nevertheless, Fuentes *et al.* (2004) reported a singular case of a 35-year-old woman having allergic episodes after exposure to beef, lamb, and fish and without allergic response to milk. Negative skin prick tests were reported from the subject's assessment while IgE responses differed among proteins presented under differing environmental conditions. Largely degraded proteins were identified in heated meat extracts except a heat-stable, 17 kDa protein, identified as bovine myoglobin, which stayed in solution. While researchers reported significant amino acid sequence homology among myoglobins from different species, the amino acid sequences are not identical. Nevertheless, the evidence indicates that myoglobin was the probable cause of the allergic reaction occurring in this patient. The relevance of bovine myoglobin in this allergenic case report has been disputed (Fiocchi *et al*., 2005) and reviewed in GRN 737 (Impossible Foods, Inc., 2017; U.S. FDA, 2018a). Bioinformatics on myoglobin from different animal species, including cow, pigs, sheep, goats, and chicken, indicates that bovine myoglobin shares structural similarities with myoglobin from goat, sheep, and pig meat, and may cause allergenicity (Chakraborty *et al*., 2014). These findings may explain the reported allergic responses after exposure to beef and lamb in the 35-year-old woman reported by Fuentes *et al.* (2004). Nevertheless, it should be reiterated that beef allergy is rare considering widespread consumption of beef and other meats containing oxygen-binding globin proteins in the global population, and a search of the scientific literature[6] indicates other cases of myoglobin allergy have not been reported since 2004.

[6] Databases searched included: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®.

Over the past decade, meat allergy also has been associated with a carbohydrate, α-Gal (galactose-α-1,3-galactose) linked to meat proteins (Platts-Mills et al., 2020). Affected individuals are typically sensitized to α-galactose from being bitten by one of several species of ticks (ACAAI, 2014). The symptoms of this specific type of meat allergy, known as "α-Gal syndrome," have a delayed onset time of several hours and occur with all species of mammalian meats. According to Kuehn (2018), an unusual set of human physiological associations exist with α-Gal mammalian meat allergy, including subject blood type, past infection, co-existing allergy, and a previous tick bite (often Lone Star Tick). Not all individuals who are sensitized to galactose-α-1,3-galactose have reported allergic reactions to meat. Platts-Mills et al. (2020) reported that of 300 hunters and forest workers in Germany, 58 individuals were positive for α-Gal syndrome, of which, only 5 individuals had allergic reactions to mammalian meat or innards. The causes of the α-Gal allergy are not known but the presence of galactose-α-1,3-galactose in the human body initiates an antigen reaction. Galactose-α-1,3-galactose exists in all mammalian species except humans where it is not naturally present and can be transmitted to humans *via* ticks. The only apparent treatment of α-Gal syndrome is elimination of red meat from the diet. As described in Section 2.2, Motif FoodWorks' myoglobin is not glycosylated, and as a taxonomically distant species *P. pastoris* would not produce endogenous *alpha*-gal epitopes.

A sequence homology search was conducted using the AllergenOnline database version 21 (updated 14 February 2021) maintained by the Food Allergy Research and Resource Program of the University of Nebraska (FARRP, 2021) to determine whether the bovine myoglobin[7] shares significant sequence homology to known allergens. The database contains a comprehensive list of putative allergenic proteins developed *via* a peer reviewed process for the purpose of evaluating food safety. A sequence homology search was conducted according to the approach outlined by the FAO/WHO (2001) and the WHO/FAO (Codex Alimentarius, 2009). In accordance with this guideline, the AllergenOnline database was searched using a sliding window of 80-amino acid sequences (segments 1–80, 2–81, 3–82, *etc*.) derived from the full-length bovine myoglobin amino acid sequence. The 80 amino acid alignment search was conducted using default settings (*E* value cutoff = 1 and maximum alignments of 20). Significant homology is defined as an identity match of greater than 35%, and in such instances, cross-reactivity with the known allergen should be considered a possibility (FAO/WHO, 2001). Using this search strategy, no matches were identified. A sequence homology search conducted using the exact 8-mer approach did not produce any matches. The results of the sequence homology search indicate that bovine myoglobin present in Motif FoodWorks' Myoglobin Preparation does not pose an allergenic risk to consumers.

Based on the totality of evidence, meat allergy is rare and is not associated with consumption of myoglobin in the human diet. Allergic responses to meat consumption, specifically red meat from bovine sources, have been associated with the heat-labile proteins BSA and BGG; only 1 case of allergic reaction to myoglobin has been reported in the scientific literature in 2004. Likewise, Motif FoodWorks' Myoglobin Preparation does not contain galactose-α-1,3-galactose and would not pose any risk of α-Gal syndrome. Bioinformatics on the amino acid sequence of bovine myoglobin suggest that the protein does not share significant sequence homology with known allergens, and cross-reactivity of the protein to known allergens is unlikely. Therefore, the available evidence suggests that consumption of Myoglobin Preparation would not pose any significant allergenic risk in the U.S. population.

[7] The allergenicity search was performed using the amino acid sequence of bovine myoglobin available under UniProt Accession No. P02192.

In addition to myoglobin, the Myoglobin Preparation may contain residual levels of native proteins from *P. pastoris*. The potential allergenicity of *Pichia* proteins was addressed in publications by Jin *et al.* (2018) and Reyes *et al.* (2021). LC-MS/MS Proteomics was used to identify and semi-quantify residual *Pichia* proteins in a leghemoglobin preparation, the subject of GRN 737, which were then investigated for potential allergenicity using *in silico*-based methods based on recommendations by the Codex Alimentarius (2009). The authors concluded that residual proteins originating from the source organism that may be present do not share significant sequence homology with known allergens, and therefore, do not pose a risk of cross-reactivity (Jin *et al*., 2018; Reyes *et al*., 2021). Conversely, the *Pichia* proteins share significant sequence homology with proteins from common yeasts, such as *Saccharomyces* spp. Based on the available data, the authors concluded that *Pichia* proteins are unlikely to present a risk of allergenicity. As the *Pichia* proteins evaluated by Jin *et al*., (2018) and Reyes *et al*., (2021) were derived from the same host strain lineage as that used by Motif, conclusions that residual *Pichia* proteins are of low toxicity risk can be extended to Motif's Myoglobin Preparation.

6.2.3 Toxigenicity of Myoglobin

The myoglobin present in Motif FoodWorks' Myoglobin Preparation is 100% identical to bovine myoglobin (UniProt Accession No. P02192). The amino acid sequence of the bovine myoglobin was compared against downloaded protein sequences obtained from a curated database of animal venom proteins and toxins maintained in the UniProtKB/Swiss-Prot Tox-Prot database[8] (Jungo *et al*., 2012) using the Basic Local Alignment Search Tool (BLAST) maintained by the National Center for Biotechnology Information. Searches were performed using the default search parameters (E-value threshold = 0.05; BLOSUM62). The search was conducted on March 23, 2021 and the toxin database included 7,271 proteins. One match to a delta-conotoxin from *Conus gloriamaris* was identified with an identity score of 28% and E-value of 0.02. The query cover was 41% and maximum bit-score was 29.3. Currently, there are no formal guidelines established for what would constitute a significant sequence similarity between a query protein and a known protein toxin (Hammond *et al*., 2013). However, Pearson (2013) reported for protein alignments, an E-value or E-score of <0.001 can reliably be used to infer homology, and alternatively, the bit-score may be used to infer homology and is considered to be a more reliable indicator of significant sequence homology. A bit-score of 50 is "*almost always significant*", while a bit-score of 40 is only significant (E-value <0.001) in searches of protein databases with less than 7,000 entries (Pearson, 2013). Furthermore, Pearson (2013) reported that "*homologous sequences that share more than 40% identity are very likely to share functional similarity*" and the E-value or E-score is commonly used to determine the statistical significance of excess similarity. Therefore, based on these criteria, the identified match with delta-conotoxin from *C. gloriamaris* is not considered to be suggestive of significant sequence homology, and bovine myoglobin does not share structural homology or similarity to any known animal venom protein or toxin, and would not harbor any toxic potential on the basis of the *in silico* search.

Myoglobin Preparation may contain ≤0.2 mg/L of proteins from the source organism, *Pichia pastoris*. The production strain is genetically modified to express a gene encoding for bovine myoglobin and does not contain any other exogenous sources of DNA; with the exception of the synthetic DNA encoding for bovine myoglobin, the production strain only contains DNA that is native to *P. pastoris*. The toxigenicity of native *Pichia* protein was discussed in 2 separate publications on a genetically modified strain of *P. pastoris* used as a production strain for soy leghemoglobin (Jin *et al*., 2018; Reyes *et al*., 2021). In these publications, it was concluded that the native *Pichia* proteins do not share sequence homology with any toxins, thus maintaining that *P. pastoris* is a non-toxigenic organism. The toxigenicity of the same *P. pastoris* production strain was addressed in GRN 737 in which the U.S. FDA did not raise any safety concerns with the

[8] Available at: https://www.uniprot.org/program/Toxins.

production organism (Impossible Foods, Inc., 2017; U.S. FDA, 2018a). As previously discussed, *P. pastoris* has a long history of safe use in food production, and to date, no toxic effects of this species have been reported in the scientific literature. Analytical data demonstrated that Myoglobin Preparation is a highly purified ingredient (purity >98%), in which the production strain is removed from the final product. Therefore, Motif FoodWorks' Myoglobin Preparation is not anticipated to pose any toxigenic concern from either the myoglobin itself or arising from the manufacturing process.

6.3 General Recognition of Safety

Motif FoodWorks has concluded that Myoglobin Preparation containing bovine myoglobin is GRAS for use in meat analogue products, as described in Section 1.3, based on scientific procedures. The safety of *P. pastoris* was evaluated using generally recognized safety assessment practices applied to food enzymes under the Pariza Johnson decision tree (Pariza and Johnson, 2001). In this regard Motif FoodWorks has demonstrated that the production strain is from a safe lineage of *P. pastoris* that has been previously demonstrated to be safe for use in food production of similar food ingredients (*e.g.*, soybean leghemoglobin).

The safety of myoglobin was evaluated using the 2-tier testing paradigm developed by the ILSI for evaluation of proteins used in the context of agricultural biotechnology (Delaney *et al.*, 2008). Bovine myoglobin has a long history of apparent safe consumption from ingestion of beef. Myoglobins are highly conserved among animal species and therefore are also consumed from ingestion of other red meats, poultry, and fish. Although bovine myoglobin from *P. pastoris* does not have a history of consumption, Motif FoodWorks has demonstrated that bovine myoglobin expressed by *P. pastoris* is qualitatively highly identical to bovine myoglobin that is present in red meat. Bovine myoglobin is intended for use in meat analogue products that will substitute 1:1 for meat products on the marketplace and in the absence of qualitatively meaningful differences in the identities of bovine myoglobin from *Pichia* to bovine myoglobin from beef, the history of safe consumption of myoglobin from meat consumption can be extended to Motif FoodWorks' ingredient.

Motif FoodWorks has concluded that use of the company's Myoglobin Preparation in meat alternative products, as described in Section 1.3, are GRAS on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of myoglobin and *P. pastoris*, as discussed herein, and on consensus among a panel of experts qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Professor Emeritus Stephen L. Taylor, Ph.D. (GRAS Panel Chair), (University of Nebraska; Professor Emeritus Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine); and Professor Emeritus Michael W. Pariza, Ph.D., (Food Research Institute University of Wisconsin-Madison).

The GRAS Panel, convened by Motif, independently and critically evaluated all data and information presented herein, and also concluded that Motif's Myoglobin Preparation is GRAS for use as a 1:1 substitution for meat in meat alternative products, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel is presented in Appendix A.

6.4 Conclusion

Based on the above data and information presented herein, Motif FoodWorks has concluded that the intended uses of Myoglobin Preparation in meat alternative products intended to substitute for current red meat and poultry sources on a 1:1 basis, as described in Section 1.3, is GRAS based on scientific procedures.

General recognition of Motif FoodWorks' GRAS conclusion is supported by the unanimous consensus rendered by an independent panel of experts, qualified by experience and scientific training, to evaluate the use of Myoglobin Preparation in food, who similarly concluded that the intended use of Myoglobin Preparation as described herein is GRAS.

Motif FoodWorks' Myoglobin Preparation therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the *Code of Federal Regulations*.

Part 7. § 170.255 List of Supporting Data and Information

ACAAI (2014). *Meat Allergy*. American College of Allergy, Asthma & Immunology (ACAAI). Available at: https://acaai.org/allergies/types/food-allergies/types-food-allergy/meat-allergy [©2014].

Ahmad M, Hirz M, Pichler H, Schwab H (2014). Protein expression in *Pichia pastoris*: recent achievements and perspectives for heterologous protein production. Appl Microbiol Biotechnol 98(12):5301-5317. DOI:10.1007/s00253-014-5732-5.

AMSA (2015). *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat, 2nd edition, version 1.01*. Champaign (IL): American Meat Science Association (AMSA). Available at: https://meatscience.org/publications-resources/printed-publications/sensory-and-tenderness-evaluation-guidelines.

Balamurugan V, Reddy GR, Suryanarayana VVS (2007). *Pichia pastoris*: a notable heterologous expression system for the production of foreign proteins—vaccines. Indian J Biotechnol 6(2):175-186.

Brady JR, Whittaker CA, Tan MC, Kristensen II DL, Ma D, Dalvie NC, et al. (2020). Comparative genome-scale analysis of *Pichia pastoris* variants informs selection of an optimal base strain. Biotechnol Bioeng 117(2):543-555. DOI:10.1002/bit.27209.

Braun-Galleani S, Dias JA, Coughlan AY, Ryan AP, Byrne KP, Wolfe KH (2019). Genomic diversity and meiotic recombination among isolates of the biotech yeast *Komagataella phaffii* (*Pichia pastoris*). Microb Cell Fact 18(1):211 [13pp, plus supplementary figures]. DOI:10.1186/s12934-019-1260-4.

CDC (2019). *[NHANES Data Derivation (2013-2018)].* (National Health and Nutrition Examination Survey/NHANES). Washington (DC): Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/nchs/nhanes/index.htm.

Cereghino JL, Cregg JM (2000). Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. FEMS Microbiol Rev 24(1):45-66. DOI:10.1111/j.1574-6976.2000.tb00532.x.

Chakraborty S, Foysal SH, Hasan MN, Khan N (2014). In silico characterization and comparative analysis of allergenicity of allergic proteins from different food sources. Am J Biochem Biotechnol 11(1):17-24. DOI:10.3844/ajbbsp.2015.17.24.

Clydesdale, F.M. and F.J. Francis. 1971. Color Measurement of Foods: XXVII. The chemistry of meat color. Food Product Development. 5(4):81-90. Cited In: Shellabarger Nocito, 1972.

Codex Alimentarius (2009). *Foods Derived from Modern Biotechnology, 2nd edition*. (Codex Alimentarius). Geneva, Switzerland: World Health Organization (WHO) / Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.fao.org/docrep/011/a1554e/a1554e00.htm.

Cregg JM, Cereghino JL, Shi J, Higgins DR (2000). Recombinant protein expression in *Pichia pastoris*. Mol Biotechnol 16(1):23-52. DOI:10.1385/MB:16:1:23.

De Schutter K, Lin YC, Tiels P, Van Hecke A, Glinka S, Weber-Lehmann J, et al. (2009). Genome sequence of the recombinant protein production host *Pichia pastoris*. Nat Biotechnol 27(6):561-566. DOI:10.1038/nbt.1544.

Delaney B, Astwood JD, Cunny H, Conn RE, Herouet-Guicheney C, MacIntosh S, et al. (2008). Evaluation of protein safety in the context of agricultural biotechnology (ILSI International Food Biotechnology Committee Task Force on Protein Safety). Food Chem Toxicol 46(Suppl. 2):S71-S97. DOI:10.1016/j.fct.2008.01.045.

Diversa Corporation (2006). *GRAS Notification Concerning BD16449 Phospholipase C Enzyme Preparation from Pichia pastoris*. (GRN No. 204). Submitted by San Francisco (CA): Morin & Associates for San Diego (CA): Diversa Corporation to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety, Division of Biotechnology and GRAS Notice Review. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=204 [Dec. 4, 2006 - FDA response - no questions].

EFSA (2017). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 6: suitability of taxonomic units notified to EFSA until March 2017 (EFSA Panel on Biological Hazards/BIOHAZ) (Question no EFSA-Q-2016-00827, adopted: 7 June 2017 by European Food Safety Authority). EFSA J 15(7):4884 [32pp]. DOI:10.2903/j.efsa.2017.4884. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/4884.

FAO/WHO (2001). *Evaluation of Allergenicity of Genetically Modified Foods*. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22-25 January 2001. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/foodsafety/publications/gmo-allergenicity/en.

FAO/WHO (2020). Section 9.1.4.2. Enzymes. In: . *Principles and Methods for the Risk Assessment of Chemicals in Food, 2nd edition*. (Environmental Health Criteria, No. 240). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switzerland: World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: https://www.who.int/publications/i/item/9789241572408 [section 9.1.4.2 revised from 2009 edition].

FARRP (2021). *AllergenOnline Version 21: Home of the FARRP Allergen Protein Database*. Lincoln (NE): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: http://www.allergenonline.org [February 14, 2021].

Fiocchi A, Restani P, Riva E (2000). Beef allergy in children. Nutrition 16(6):454-457. DOI:10.1016/S0899-9007(00)00285-9.

Fiocchi A, Restani P, Bouygue GR, Martelli A (2005). Beef allergy in adults and children. Allergy 60(1):126. DOI:10.1111/j.1398-9995.2005.00647.x.

Fleming HP, Blumer TN, Craig HB (1960). Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. J Anim Sci 19(4):1164-1171. DOI:10.2527/jas1960.1941164x.

Fuentes MM, Palacios R, Garcés M, Caballero ML, Moneo I (2004). Isolation and characterization of a heat-resistant beef allergen: myoglobin. Allergy 59(3):327-331. DOI:10.1111/j.1398-9995.2004.00266.x.

Gourévitch A, Portincaso M, de la Tour A, Goeldel N, Legris A (2021). *Looking to Nature for the Next Industrial Revolution*. Boston (MA): Boston Consulting Group (BCG). Available at: https://www.bcg.com/publications/2021/why-nature-co-design-will-be-so-important-for-the-next-industrial-revolution.

Hammond B, Kough J, Herouet-Guicheney C, Jez JM (2013). Toxicological evaluation of proteins introduced into food crops (ILSI International Food Biotechnology Committee Task Force on Use of Mammalian Toxicology Studies in Safety Assessment of GM Foods). Crit Rev Toxicol 43(Suppl. 2):25-42. DOI:10.3109/10408444.2013.842956.

Hardison R (1998). Hemoglobins from bacteria to man: evolution of different patterns of gene expression. J Exp Biol 201(8):1099-1117.

IFBC (1990). Chapter 4: Safety evaluation of foods and food ingredients derived from microorganisms (International Food Biotechnology Council/IFBC). Regul Toxicol Pharmacol 12(3, Part 2):S114-S128. DOI:10.1016/S0273-2300(05)80080-7.

Impossible Foods Inc. (2017). *GRAS Notification for Soy Leghemoglobin Protein Preparation Derived from Pichia Pastoris*. (GRN No. 737). Submitted by Redwood City (CA): Impossible Foods Inc. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=737 [Jul. 23, 2018 - FDA response - no questions].

Jin Y, He X, Andoh-Kumi K, Fraser RZ, Lu M, Goodman RE (2018). Evaluating potential risks of food allergy and toxicity of soy leghemoglobin expressed in *Pichia pastoris*. Mol Nutr Food Res 62(1):1700297 [13pp]. DOI:10.1002/mnfr.201700297.

Jungo F, Bougueleret L, Xenarios I, Poux S (2012). The UniProtKB/Swiss-Prot Tox-Prot program: a central hub of integrated venom protein data. Toxicon 60(4):551-557. DOI:10.1016/j.toxicon.2012.03.010.

Kranen RW, van Kuppevelt TH, Goedhart HA, Veerkamp CH, Lambooy E, Veerkamp JH (1999). Hemoglobin and myoglobin content in muscles of broiler chickens. Poult Sci 78(3):467-476. DOI:10.1093/ps/78.3.467.

Kuehn BM (2018). Tick bite linked to red meat allergy. JAMA 319(4):332-332. DOI:10.1001/jama.2017.20802.

Kurtzman CP (2009). Biotechnological strains of *Komagataella* (*Pichia*) *pastoris* are *Komagataella phaffii* as determined from multigene sequence analysis. J Ind Microbiol Biotechnol 36(11):1435-1438. DOI:10.1007/s10295-009-0638-4.

Lawrie RA (1950). Some observations on factors affecting myoglobin concentrations in muscle. The Journal of Agricultural Science 40(4):356-366. DOI:10.1017/S0021859600046116.

National Academy of Medicine (2016). *Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy.* National Academies Press. Washington, D.C., USA. https://www.nationalacademies.org/news/2016/11/new-report-outlines-steps-to-address-public-health-concerns-of-food-allergy-safety

Newcom DW, Stalder KJ, Baas TJ, Goodwin RN, Parrish FC, Wiegand BR (2004). Breed differences and genetic parameters of myoglobin concentration in porcine longissimus muscle. J Anim Sci 82(8):2264-2268. DOI:10.2527/2004.8282264x.

OECD (1992). *Safety Considerations for Biotechnology 1992*. Paris, France: Organization for Economic Cooperation and Development (OECD). Available at: http://www.oecd.org/science/emerging-tech/2375496.pdf.

OECD (1993). *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles.* Organization for Economic Cooperation and Development (OECD). Available at: https://www.oecd.org/science/biotrack/41036698.pdf.

Pariza MW, Foster EM (1983). Determining the safety of enzymes used in food processing. J Food Prot 46(5):453-468 [plus addendum]. DOI:10.4315/0362-028X-46.5.453.

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul Toxicol Pharmacol 33(2):173-186. DOI:10.1006/rtph.2001.1466.

Pearson WR (2013). An introduction to sequence similarity ("homology") searching. Curr Protoc Bioinformatics 42(1):3.1.1-3.1.8 [Chapter 3, Unit 3.1]. DOI:10.1002/0471250953.bi0301s42.

Platts-Mills TAE, Commins SP, Biedermann T, van Hage M, Levin M, Beck LA, et al. (2020). On the cause and consequences of IgE to galactose-α-1,3-galactose: A report from the National Institute of Allergy and Infectious Diseases Workshop on Understanding IgE-Mediated Mammalian Meat Allergy. J Allergy Clin Immunol 145(4):1061-1071. DOI:10.1016/j.jaci.2020.01.047.

Reyes TF, Chen Y, Fraser RZ, Chan T, Li X (2021). Assessment of the potential allergenicity and toxicity of *Pichia* proteins in a novel leghemoglobin preparation. Regul Toxicol Pharmacol 119:104817 [15pp, plus supplementary data]. DOI:10.1016/j.yrtph.2020.104817.

Rickansrud DA, Henrickson RL (1967). Total pigments and myoglobin concentration in four bovine muscles. J Food Sci 32(1):57-61. DOI:10.1111/j.1365-2621.1967.tb01957.x.

Sewalt V, Shanahan D, Gregg L, La Marta J, Carrillo R (2016). The Generally Recognized as Safe (GRAS) process for industrial microbial enzymes. Ind Biotechnol 12(5):295-302. DOI:10.1089/ind.2016.0011.

Shellabarger Nocito J (1972). *Myoglobin Content and Color of Raw Pork Loin Roasts as Affected by Freezing at Two Rates*. [M.Sc. Thesis]. Knoxville (TN): University of Tennessee. Available at: https://trace.tennessee.edu/cgi/viewcontent.cgi?article=5357&context=utk_gradthes.

Sørensen HP (2010). Towards universal systems for recombinant gene expression. Microb Cell Fact 9:27. DOI:10.1186/1475-2859-9-27.

Spohner SC, Müller H, Quitmann H, Czermak P (2015). Expression of enzymes for the usage in food and feed industry with Pichia pastoris. J Biotechnol 202:118-34.

Texas A&M (2021). *Meat Science*. College Station (TX): Texas A&M University. Available at: https://meat.tamu.edu/ansc-307-honors/meat-color/ [last accessed: March 29, 2021].

U.S. FDA (2006). *Agency Response Letter GRAS Notice No. GRN 204 [Phospholipase C enzyme preparation from Pichia pastoris expressing a heterologous phospholipase C gene, San Diego (CA): Diversa Corporation]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=205 [Dec. 4, 2006 - FDA response - no questions].

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 313 [Beef protein, Gloucester (MA): Proteus Industries, Inc].* Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=313 [Original FDA letter dated Aug. 11, 2010, corrected Dec. 13, 2010 – FDA response – no questions].

U.S. FDA (2018a). *Agency Response Letter GRAS Notice No. GRN 737 [Soy leghemoglobin preparation from a strain of Pichia pastoris, Redwood City (CA): Impossible Foods Inc]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=737 [Jul. 23, 2018 - FDA response - no questions].

U.S. FDA (2018b). *Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA).* (Public Law 108-282, Title II). Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: https://www.fda.gov/food/food-allergensgluten-free-guidance-documents-regulatory-information/food-allergen-labeling-and-consumer-protection-act-2004-falcpa [current as of 07/16/2018].

U.S. FDA (2020a). Part 170—Food additives. §170.30—Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2020/title21 [last amended: 4-1-19].

U.S. FDA (2020b). Part 573—Food additives permitted in feed and drinking water of animals. §573-750—Pichia pastoris dried yeast. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2020/title21 [last amended: 4-1-20].

USDA-ERS (2021). *Food Availability (Per Capita) Data System*. Washington (DC): U.S. Department of Agriculture, Economic Research Service (USDA-ERS). Available at: https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/ [Last updated: March 29, 2021].

USDA-FSIS (2021). *Safe and Suitable Ingredients Used in the Production of Meat, Poultry and Egg Products - Revision 55*. (FSIS Directive 7120.1). Washington (DC): U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). Available at: https://www.fsis.usda.gov/policy/fsis-directives/7120.1.

Vazquez Fuertes L, Perez Bustamante M, Bueso Fernandez A, Gonzalez Pino A, Pineda De La Losa F (2013). A case of eosinophilic esophagitis, with sensitization to BSA (meat allergy) and rhinoconjunctivitis due to dog epithelium. Clin Transl Allergy 3(3):P166 [2pp]. DOI:10.1186/2045-7022-3-S3-P166.

Werfel SJ, Cooke SK, Sampson HA (1997). Clinical reactivity to beef in children allergic to cow's milk. J Allergy Clin Immunol 99(3):293-300. DOI:10.1016/s0091-6749(97)70045-9.

Yip R, Dallman PR (1996). Iron. In: Ziegler EE, Filer LJ Jr, editors. *Present Knowledge in Nutrition, 7th edition*. Washington (DC): International Life Sciences Institute (ILSI), pp. 277-292.

JS 44 (Rev. 09/19)

CIVIL COVER SHEET

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. *(SEE INSTRUCTIONS ON NEXT PAGE OF THIS FORM.)*

I. (a) PLAINTIFFS

IMPOSSIBLE FOODS INC.

(b) County of Residence of First Listed Plaintiff _____
(EXCEPT IN U.S. PLAINTIFF CASES)

(c) Attorneys *(Firm Name, Address, and Telephone Number)*

Ian R. Liston, Esq., Wilson Sonsini Goodrich & Rosati, P.C.
222 Delaware Ave., Suite 800, Wilmington, DE 19801 (302) 304-7600

DEFENDANTS

MOTIF FOODWORKS, INC.

County of Residence of First Listed Defendant _____
(IN U.S. PLAINTIFF CASES ONLY)

NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE TRACT OF LAND INVOLVED.

Attorneys *(If Known)*

II. BASIS OF JURISDICTION *(Place an "X" in One Box Only)*

- ❒ 1 U.S. Government Plaintiff
- ☒ 3 Federal Question *(U.S. Government Not a Party)*
- ❒ 2 U.S. Government Defendant
- ❒ 4 Diversity *(Indicate Citizenship of Parties in Item III)*

III. CITIZENSHIP OF PRINCIPAL PARTIES *(Place an "X" in One Box for Plaintiff and One Box for Defendant)*

(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	❒ 1	❒ 1	Incorporated *or* Principal Place of Business In This State	❒ 4	❒ 4
Citizen of Another State	❒ 2	❒ 2	Incorporated *and* Principal Place of Business In Another State	❒ 5	❒ 5
Citizen or Subject of a Foreign Country	❒ 3	❒ 3	Foreign Nation	❒ 6	❒ 6

IV. NATURE OF SUIT *(Place an "X" in One Box Only)*

Click here for: Nature of Suit Code Descriptions.

CONTRACT
- ❒ 110 Insurance
- ❒ 120 Marine
- ❒ 130 Miller Act
- ❒ 140 Negotiable Instrument
- ❒ 150 Recovery of Overpayment & Enforcement of Judgment
- ❒ 151 Medicare Act
- ❒ 152 Recovery of Defaulted Student Loans (Excludes Veterans)
- ❒ 153 Recovery of Overpayment of Veteran's Benefits
- ❒ 160 Stockholders' Suits
- ❒ 190 Other Contract
- ❒ 195 Contract Product Liability
- ❒ 196 Franchise

REAL PROPERTY
- ❒ 210 Land Condemnation
- ❒ 220 Foreclosure
- ❒ 230 Rent Lease & Ejectment
- ❒ 240 Torts to Land
- ❒ 245 Tort Product Liability
- ❒ 290 All Other Real Property

TORTS

PERSONAL INJURY
- ❒ 310 Airplane
- ❒ 315 Airplane Product Liability
- ❒ 320 Assault, Libel & Slander
- ❒ 330 Federal Employers' Liability
- ❒ 340 Marine
- ❒ 345 Marine Product Liability
- ❒ 350 Motor Vehicle
- ❒ 355 Motor Vehicle Product Liability
- ❒ 360 Other Personal Injury
- ❒ 362 Personal Injury - Medical Malpractice

PERSONAL INJURY
- ❒ 365 Personal Injury - Product Liability
- ❒ 367 Health Care/ Pharmaceutical Personal Injury Product Liability
- ❒ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ❒ 370 Other Fraud
- ❒ 371 Truth in Lending
- ❒ 380 Other Personal Property Damage
- ❒ 385 Property Damage Product Liability

CIVIL RIGHTS
- ❒ 440 Other Civil Rights
- ❒ 441 Voting
- ❒ 442 Employment
- ❒ 443 Housing/ Accommodations
- ❒ 445 Amer. w/Disabilities - Employment
- ❒ 446 Amer. w/Disabilities - Other
- ❒ 448 Education

PRISONER PETITIONS

Habeas Corpus:
- ❒ 463 Alien Detainee
- ❒ 510 Motions to Vacate Sentence
- ❒ 530 General
- ❒ 535 Death Penalty

Other:
- ❒ 540 Mandamus & Other
- ❒ 550 Civil Rights
- ❒ 555 Prison Condition
- ❒ 560 Civil Detainee - Conditions of Confinement

FORFEITURE/PENALTY
- ❒ 625 Drug Related Seizure of Property 21 USC 881
- ❒ 690 Other

LABOR
- ❒ 710 Fair Labor Standards Act
- ❒ 720 Labor/Management Relations
- ❒ 740 Railway Labor Act
- ❒ 751 Family and Medical Leave Act
- ❒ 790 Other Labor Litigation
- ❒ 791 Employee Retirement Income Security Act

IMMIGRATION
- ❒ 462 Naturalization Application
- ❒ 465 Other Immigration Actions

BANKRUPTCY
- ❒ 422 Appeal 28 USC 158
- ❒ 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- ❒ 820 Copyrights
- ☒ 830 Patent
- ❒ 835 Patent - Abbreviated New Drug Application
- ❒ 840 Trademark

SOCIAL SECURITY
- ❒ 861 HIA (1395ff)
- ❒ 862 Black Lung (923)
- ❒ 863 DIWC/DIWW (405(g))
- ❒ 864 SSID Title XVI
- ❒ 865 RSI (405(g))

FEDERAL TAX SUITS
- ❒ 870 Taxes (U.S. Plaintiff or Defendant)
- ❒ 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- ❒ 375 False Claims Act
- ❒ 376 Qui Tam (31 USC 3729(a))
- ❒ 400 State Reapportionment
- ❒ 410 Antitrust
- ❒ 430 Banks and Banking
- ❒ 450 Commerce
- ❒ 460 Deportation
- ❒ 470 Racketeer Influenced and Corrupt Organizations
- ❒ 480 Consumer Credit (15 USC 1681 or 1692)
- ❒ 485 Telephone Consumer Protection Act
- ❒ 490 Cable/Sat TV
- ❒ 850 Securities/Commodities/ Exchange
- ❒ 890 Other Statutory Actions
- ❒ 891 Agricultural Acts
- ❒ 893 Environmental Matters
- ❒ 895 Freedom of Information Act
- ❒ 896 Arbitration
- ❒ 899 Administrative Procedure Act/Review or Appeal of Agency Decision
- ❒ 950 Constitutionality of State Statutes

V. ORIGIN *(Place an "X" in One Box Only)*

☒ 1 Original Proceeding ❒ 2 Removed from State Court ❒ 3 Remanded from Appellate Court ❒ 4 Reinstated or Reopened ❒ 5 Transferred from Another District *(specify)* ❒ 6 Multidistrict Litigation - Transfer ❒ 8 Multidistrict Litigation - Direct File

VI. CAUSE OF ACTION

Cite the U.S. Civil Statute under which you are filing ***(Do not cite jurisdictional statutes unless diversity)***:
35 U.S.C. § 1, et seq.

Brief description of cause:
Patent Infringement

VII. REQUESTED IN COMPLAINT:

❒ CHECK IF THIS IS A **CLASS ACTION** UNDER RULE 23, F.R.Cv.P.

DEMAND $

CHECK YES only if demanded in complaint:

JURY DEMAND: ☒ Yes ❒ No

VIII. RELATED CASE(S) IF ANY

(See instructions):

JUDGE _____ DOCKET NUMBER _____

DATE
03/09/2022

SIGNATURE OF ATTORNEY OF RECORD
/s/ Ian R. Liston

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

INSTRUCTIONS FOR ATTORNEYS COMPLETING CIVIL COVER SHEET FORM JS 44

Authority For Civil Cover Sheet

The JS 44 civil cover sheet and the information contained herein neither replaces nor supplements the filings and service of pleading or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. Consequently, a civil cover sheet is submitted to the Clerk of Court for each civil complaint filed. The attorney filing a case should complete the form as follows:

I.(a) **Plaintiffs-Defendants.** Enter names (last, first, middle initial) of plaintiff and defendant. If the plaintiff or defendant is a government agency, use only the full name or standard abbreviations. If the plaintiff or defendant is an official within a government agency, identify first the agency and then the official, giving both name and title.

(b) **County of Residence.** For each civil case filed, except U.S. plaintiff cases, enter the name of the county where the first listed plaintiff resides at the time of filing. In U.S. plaintiff cases, enter the name of the county in which the first listed defendant resides at the time of filing. (NOTE: In land condemnation cases, the county of residence of the "defendant" is the location of the tract of land involved.)

(c) **Attorneys.** Enter the firm name, address, telephone number, and attorney of record. If there are several attorneys, list them on an attachment, noting in this section "(see attachment)".

II. **Jurisdiction.** The basis of jurisdiction is set forth under Rule 8(a), F.R.Cv.P., which requires that jurisdictions be shown in pleadings. Place an "X" in one of the boxes. If there is more than one basis of jurisdiction, precedence is given in the order shown below.
United States plaintiff. (1) Jurisdiction based on 28 U.S.C. 1345 and 1348. Suits by agencies and officers of the United States are included here.
United States defendant. (2) When the plaintiff is suing the United States, its officers or agencies, place an "X" in this box.
Federal question. (3) This refers to suits under 28 U.S.C. 1331, where jurisdiction arises under the Constitution of the United States, an amendment to the Constitution, an act of Congress or a treaty of the United States. In cases where the U.S. is a party, the U.S. plaintiff or defendant code takes precedence, and box 1 or 2 should be marked.
Diversity of citizenship. (4) This refers to suits under 28 U.S.C. 1332, where parties are citizens of different states. When Box 4 is checked, the citizenship of the different parties must be checked. (See Section III below**; NOTE: federal question actions take precedence over diversity cases.**)

III. **Residence (citizenship) of Principal Parties.** This section of the JS 44 is to be completed if diversity of citizenship was indicated above. Mark this section for each principal party.

IV. **Nature of Suit.** Place an "X" in the appropriate box. If there are multiple nature of suit codes associated with the case, pick the nature of suit code that is most applicable. Click here for: Nature of Suit Code Descriptions.

V. **Origin.** Place an "X" in one of the seven boxes.
Original Proceedings. (1) Cases which originate in the United States district courts.
Removed from State Court. (2) Proceedings initiated in state courts may be removed to the district courts under Title 28 U.S.C., Section 1441.
Remanded from Appellate Court. (3) Check this box for cases remanded to the district court for further action. Use the date of remand as the filing date.
Reinstated or Reopened. (4) Check this box for cases reinstated or reopened in the district court. Use the reopening date as the filing date.
Transferred from Another District. (5) For cases transferred under Title 28 U.S.C. Section 1404(a). Do not use this for within district transfers or multidistrict litigation transfers.
Multidistrict Litigation – Transfer. (6) Check this box when a multidistrict case is transferred into the district under authority of Title 28 U.S.C. Section 1407.
Multidistrict Litigation – Direct File. (8) Check this box when a multidistrict case is filed in the same district as the Master MDL docket.
PLEASE NOTE THAT THERE IS NOT AN ORIGIN CODE 7. Origin Code 7 was used for historical records and is no longer relevant due to changes in statue.

VI. **Cause of Action.** Report the civil statute directly related to the cause of action and give a brief description of the cause. **Do not cite jurisdictional statutes unless diversity.** Example: U.S. Civil Statute: 47 USC 553 Brief Description: Unauthorized reception of cable service

VII. **Requested in Complaint.** Class Action. Place an "X" in this box if you are filing a class action under Rule 23, F.R.Cv.P.
Demand. In this space enter the actual dollar amount being demanded or indicate other demand, such as a preliminary injunction.
Jury Demand. Check the appropriate box to indicate whether or not a jury is being demanded.

VIII. **Related Cases.** This section of the JS 44 is used to reference related pending cases, if any. If there are related pending cases, insert the docket numbers and the corresponding judge names for such cases.

Date and Attorney Signature. Date and sign the civil cover sheet.

AO 120 (Rev. 08/10)

TO:	**Mail Stop 8** **Director of the U.S. Patent and Trademark Office** **P.O. Box 1450** **Alexandria, VA 22313-1450**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK**

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	3/9/2022	for the District of Delaware

PLAINTIFF	DEFENDANT
IMPOSSIBLE FOODS INC.	MOTIF FOODWORKS, INC.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	10,863,761	12/15/2020	IMPOSSIBLE FOODS INC.
2			
3			
4			
5			

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

Copy 1—Upon initiation of action, mail this copy to Director **Copy 3—Upon termination of action, mail this copy to Director**
Copy 2—Upon filing document adding patent(s), mail this copy to Director **Copy 4—Case file copy**

AO 440 (Rev. 06/12) Summons in a Civil Action

UNITED STATES DISTRICT COURT

for the

District of Delaware

IMPOSSIBLE FOODS INC.

Plaintiff(s)

v.

MOTIF FOODWORKS, INC.

Defendant(s)

Civil Action No.

SUMMONS IN A CIVIL ACTION

To: *(Defendant's name and address)* MOTIF FOODWORKS, INC.
c/o The Corporation Trust Company
Corporation Trust Center
1209 Orange Street
Wilmington, DE 19801

A lawsuit has been filed against you.

Within 21 days after service of this summons on you (not counting the day you received it) — or 60 days if you are the United States or a United States agency, or an officer or employee of the United States described in Fed. R. Civ. P. 12 (a)(2) or (3) — you must serve on the plaintiff an answer to the attached complaint or a motion under Rule 12 of the Federal Rules of Civil Procedure. The answer or motion must be served on the plaintiff or plaintiff's attorney, whose name and address are:

Ian R. Liston
WILSON SONSINI GOODRICH & ROSATI, P.C.
222 Delaware Ave., Suite 800
Wilmington, DE 19801

If you fail to respond, judgment by default will be entered against you for the relief demanded in the complaint. You also must file your answer or motion with the court.

Date: 03/09/2022

CLERK OF COURT

/s/ John A. Cerino

Signature of Clerk or Deputy Clerk

Civil Action No.

PROOF OF SERVICE

(This section should not be filed with the court unless required by Fed. R. Civ. P. 4 (l))

This summons for *(name of individual and title, if any)* _____ was received by me on *(date)* _____ .

❒ I personally served the summons on the individual at *(place)* _____ on *(date)* _____ ; or

❒ I left the summons at the individual's residence or usual place of abode with *(name)* _____ , a person of suitable age and discretion who resides there, on *(date)* _____ , and mailed a copy to the individual's last known address; or

❒ I served the summons on *(name of individual)* _____ , who is designated by law to accept service of process on behalf of *(name of organization)* _____ on *(date)* _____ ; or

❒ I returned the summons unexecuted because _____ ; or

❒ Other *(specify):*

My fees are $ _____ for travel and $ _____ for services, for a total of $ 0.00 .

I declare under penalty of perjury that this information is true.

Date: _____

_____
Server's signature

_____
Printed name and title

_____
Server's address

Additional information regarding attempted service, etc:

IN THE UNITED STATES DISTRICT COURT
FOR DISTRICT OF DELAWARE

IMPOSSIBLE FOODS INC., Plaintiff, v. MOTIF FOODWORKS, INC., Defendant.	)	CASE NO.:

PLAINTIFF IMPOSSIBLE FOODS INC.'S RULE 7.1 DISCLOSURE STATEMENT

Pursuant to Rule 7.1 of the Federal Rules of Civil Procedure, Plaintiff Impossible Foods Inc. states that it does not have a parent corporation and that no publicly held corporation owns 10% or more of its stock.

WILSON SONSINI GOODRICH & ROSATI, P.C.

/s/ Ian R. Liston
Ian R. Liston (#5507)
Jennifer A. Ward (#6476)
222 Delaware Avenue, Suite 800
Wilmington, DE 19801
(302) 304-7600
iliston@wsgr.com
jward@wsgr.com

Counsel for Plaintiff Impossible Foods Inc.

OF COUNSEL:

Matthew R. Reed
WILSON SONSINI GOODRICH & ROSATI, P.C.
650 Page Mill Road
Palo Alto, CA 94304
(650) 493-9300
mreed@wsgr.com

Wendy L. Devine
WILSON SONSINI GOODRICH & ROSATI, P.C.
One Market Plaza
Spear Tower, Suite 3300
San Francisco, CA 94105
(415) 947-2000
wdevine@wsgr.com

Lori P. Westin
WILSON SONSINI GOODRICH & ROSATI, P.C.
12235 El Camino Real
San Diego, CA 92130
(858) 350-2300
lwestin@wsgr.com

G. Edward Powell III
WILSON SONSINI GOODRICH & ROSATI, P.C.
1700 K Street NW, Fifth Floor
Washington, DC 20006
(202) 973-8800
epowell@wsgr.com

Dated: March 9, 2022