03 August 2021

Dr. Paulette Gaynor
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

Dear Dr. Gaynor:

Re: GRAS Notice for Miracle Fruit Powder

In accordance with 21 CFR §170 Subpart E consisting of § 170.203 through 170.285, Miracle Fruit Farm, LLC, as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the conclusion that Miracle Fruit Powder, is GRAS on the basis of scientific procedures, for use across multiple beverage categories. These food uses of Miracle Fruit Powder are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Miracle Fruit Farm's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Erik Tietig
CEO
Miracle Fruit Farm, LLC

Email: erik@miraclefruitfarm.com
Tel: 305-345-8422

GRAS NOTICE FOR MIRACLE FRUIT POWDER

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:
Miracle Fruit Farm LLC
16300 SW 184th Street
Miami, FL
33187 USA

DATE:
03 August 2021

GRAS Notice for Miracle Fruit Powder

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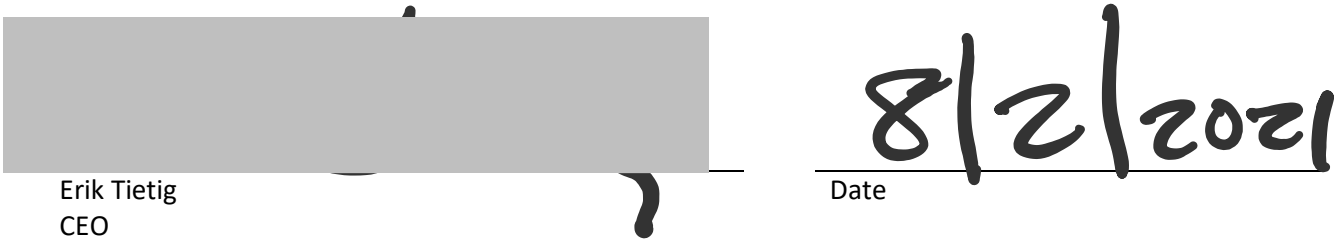
GRAS Notice for Miracle Fruit Powder

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Miracle Fruit Farm, LLC (Miracle Fruit Farm) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that miracle fruit powder, as manufactured by Miracle Fruit Farm, is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Miracle Fruit Farm's 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 Miracle Fruit Farm, 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 Miracle Fruit Farm and pertinent to the evaluation of the safety and GRAS status of miracle fruit powder as a food ingredient for use in various conventional beverages and fermented dairy products, as described herein.

Signed,



Erik Tietig
CEO
Miracle Fruit Farm, LLC

8/2/2021
Date

1.1 Name and Address of Notifier

Miracle Fruit Farm LLC
16300 SW 184th Street
Miami, FL
33187 USA

1.2 Common Name of Notified Substance

Miracle fruit powder

1.3 Conditions of Use

Miracle fruit powder is intended to be added as an ingredient to water-based beverages, carbonated beverages, fruit juices, fruit nectars, fruit-based smoothies, fruit drinks and ades, fermented dairy products (such as buttermilk, acidophilus milk, kefir, and yogurts), and ready to drink tea beverages (such as kombucha and iced tea) at use levels ranging from 1 to 6%. A summary of the food categories and use levels in which miracle fruit powder is intended for use is provided in Table 1.3-1 below. Food uses are organized according to 21 CFR §170.3 (U.S. FDA, 2020a). Miracle fruit powder is not intended for use in infant formula or infant food products, and the proposed food categories do not include food uses that are subject to the oversight by the U.S. Department of Agriculture (USDA) and the USDA Food Safety Inspection Service (FSIS).

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for Miracle Fruit Powder in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses[a]	Miracle Fruit Powder Use Levels (% weight/weight)
Beverages and Beverage Bases	Water-based beverages	1
	Carbonated beverages	1
Processed Fruits and Fruit Juices	Fruit juices	3
	Fruit nectars	3
	Fruit-based smoothies	6
	Fruit drinks and ades	3
Milk Products	Fermented dairy products (buttermilk, acidophilus milk, kefir) and yogurts	1
Coffee and Tea	Ready to drink tea beverages (*e.g.*, kombucha and iced tea)	2

CFR = *Code of Federal Regulations*; U.S. = United States.
[a] Miracle Berry Powder is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020b), Miracle Fruit Farm has concluded that the intended uses of miracle fruit powder 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 Notice 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:

Miracle Fruit Farm LLC
16300 SW 184th Street
Miami, FL
33187 USA

Should the U.S. FDA have any questions or additional information requests regarding this Notice, Miracle Fruit Farm will supply these data and information upon request.

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

It is Miracle Fruit Farm's 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 and Composition of the Ingredient

Synsepalum dulcificum is an evergreen bush or tree native to tropical West Africa that grows up to 18 ft. tall (Chen *et al*., 2006). The bright red fruit of this tree is small (2 to 3 cm) and is referred to as "miracle fruit", "miraculous berry", "sweet berry", or "miracle berry" (Lipatova and Campolattaro, 2016). The first historical record of human consumption of miracle fruit dates back to the early 1700s in Ghana (Roecklein and Leung, 1987). Miracle fruit was introduced to the U.S. from Africa by the USDA in 1917, and since then, cultivation of this fruit and its uses in the U.S. has steadily grown. Miracle fruit is commercially available in the U.S. in different forms, as a fresh berry, a freeze-dried powder, or tablet in a variety of dietary supplement products.

The notifiable ingredient, miracle fruit powder, is minimally processed and produced by pulping, maceration, and freeze-drying of de-seeded miracle fruit berries without the use of any solvents or chemical processing aids. Miracle fruit powder is a red/red-brown to pink powder with an odor that is characteristic of the fruit. Miracle fruit powder has been fully characterized and is primarily comprised of carbohydrates (~88% on dry basis), protein (~6% on dry basis), ash (~4% on dry basis), and moisture (~3.5%). The ingredient is intended for use in beverage and fermented dairy products for its taste-modifying effects, attributed to the active glycoprotein, miraculin, which accounts for approximately 0.1% (on dry basis) of the miracle fruit powder ingredient.

Miraculin is responsible for the taste-modifying effect of the miracle fruit by binding to the sweet receptors of the tongue, turning sour tastes into sweet (Morris, 1976). Miraculin is the largest known macromolecule that can affect taste perception (Lipatova and Campolattaro, 2016). Miraculin was first isolated in 1968 by researchers at Florida State University (Kurihara and Beidler, 1969), and was later purified and characterized by Theerasilp and Kurihara (1988). Miraculin exists naturally as a homodimer connected through a single interchain disulfide bond at Cys-138 and has a molecular weight of 24,600 Da (Theerasilp and Kurihara, 1988; Theerasilp *et al.*, 1989). Miraculin is expressed as a single polypeptide with 220 amino acids, containing 29 amino acid residues that are removed by post-translational processing. The peptide sequence of miraculin is publicly available on the UniProt/SwissProt database under Accession No. P13087.

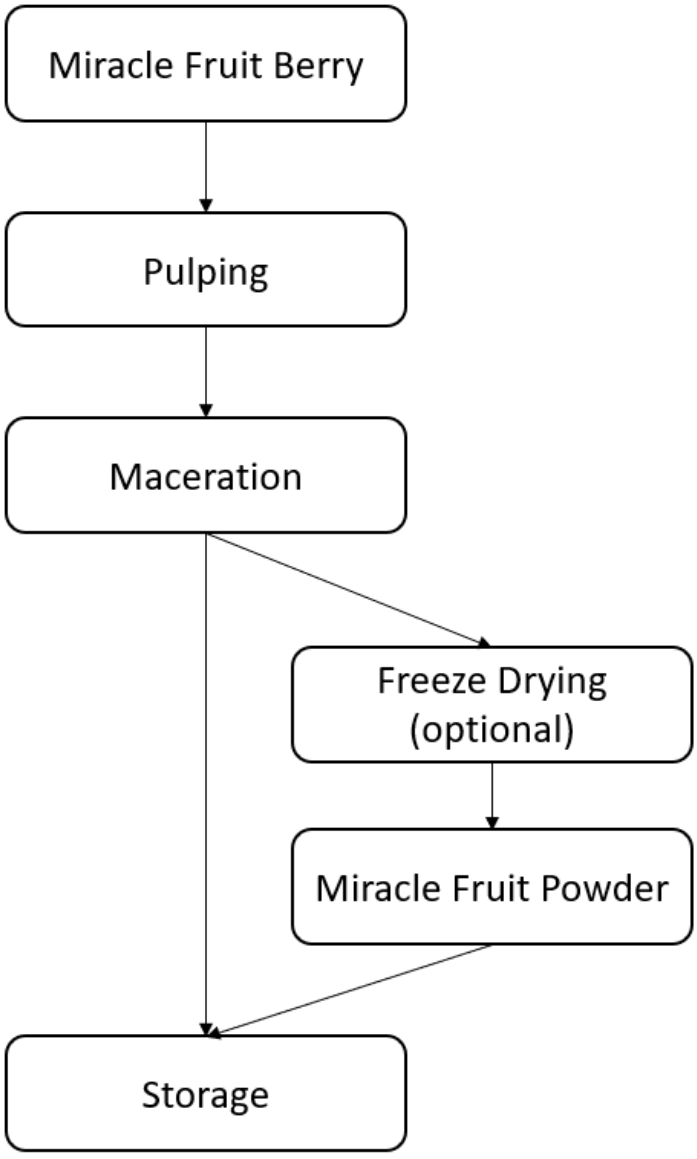
2.2 Method of Manufacture

Miracle fruit powder is manufactured in accordance with current Good Manufacturing Practice (cGMP) and complies with the principles of Hazard Analysis and Critical Control Points (HACCP). A schematic overview of the production process is provided in Figure 2.2-1 below. The miracle fruit is grown on a bushy shrub maintained at a height of approximately 6 ft. Plants are grown for 3 to 4 years before they produce commercial volumes of miracle fruit. The fruit is grown year-round and harvested by hand picking under good agricultural practices. Following picking, the miracle fruit is washed using chlorinated municipal water and a food-grade vegetable wash (Regal Veggie Wash from Chem-tel, Inc.).

In the first step of production process, the seed is removed from miracle fruit. The fruit is then pulped, macerated, freeze-dried, milled into a powder, and packaged. The production process includes quality control steps throughout to ensure that physical, chemical, and biological hazards are not introduced into the final product. For example, the process includes analysis during the freeze-drying step to ensure a reduction in water activity to control for microbiological hazards.

Analytical data on potential impurities that may be introduced from the manufacturing process or carried over from the starting material demonstrate the absence of any chemical, toxicological, or microbiological hazards arising from the production process of miracle fruit powder that would have an adverse effect on human health (see Sections 2.4 and 2.5).

Figure 2.2-1 Flowchart for the Production Process of Miracle Fruit Powder



2.3 Product Specifications

Food-grade specifications for physical, chemical, heavy metal, and microbiological parameters have been established for miracle fruit powder (see Table 2.3-1). All methods of analysis are internationally recognized [*e.g.*, Association of Official Analytical Chemists (AOAC), U.S. FDA Bacteriological Analytical Manual] or have been developed internally and validated. The microbiological specifications for miracle fruit powder include control for standard microbial contaminants (*e.g.*, total plate count, yeast and mold, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*), as well as additional specification limits for thermophilic acidophilic bacteria, guaiacol-producing bacteria, and heat-resistant mold for high acidic still beverages that are subject to heat treatment.

Table 2.3-1 Product Specifications for Miracle Fruit Powder

Specification Parameter	Specification Limit	Method of Analysis
Physical Parameters		
Appearance	Red/red-brown to pink powder	Visual
Odor	Characteristic of fruit	Sensory
Sensory (sweetness)	Sweetness induction of 0.15% citric acid solution equivalent to 3 to 7 Brix sucrose in water	Internal Method
Particle size	<420 µm	USP
Chemical Parameters		
Miraculin (dry basis)	≥0.048%	Internal Method (ELISA)
Carbohydrates (dry basis)	≥80%	Calculated
Total dietary fiber (dry basis)	≥5.5%	AOAC 991.43
Total fatty acids (dry basis)	≥0.4%	AOAC 996.06 AOCS Ce 2-66/Ce2b-11
Protein (dry basis)	≥4.5%	AOCS Ac 4-91
Ash (dry basis)	<5%	AOAC 923.03
Moisture	<6%	AOAC 934.03
Heavy Metals		
Arsenic	<0.05 ppm	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)
Cadmium	<0.5 ppm	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)
Lead	<0.1 ppm	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)
Mercury	<0.1 ppm	AOAC 2011.19 (ICP-MS) AOAC 993.14 (ICP-MS)
Microbiological Parameters		
Total count	<3,000 CFU/g	FDA BAM
Mold and yeast	<300 CFU/g	FDA BAM
Escherichia coli	Negative/g	FDA BAM
Salmonella	Negative/25 g	FDA BAM
Listeria monocytogenes	Negative/25 g	FDA BAM
Thermophilic acidophilic bacteria[a]	<1,000 CFU/g	IFU Method No. 12
Guaiacol-producing bacteria[a,b]	Absent	IFU Method No. 12

Table 2.3-1 Product Specifications for Miracle Fruit Powder

Specification Parameter	Specification Limit	Method of Analysis
Heat-resistant mold[a]	Absent	Chapter 22, Compendium of Methods for Microbiological Examination of Foods, 5th Ed, (2015).

AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemists' Society; BAM = Bacteriological Analytical Manual; CFU = colony forming units; ELISA = enzyme-linked immunosorbent assay; FDA = Food and Drug Administration; ICP-MS = inductively coupled plasma-mass spectrometry; IFU = International Fruit and Vegetable Juice Association; ppm = parts per million; USP = United States Pharmacopeia.

[a] Additional microbiological specification limit for high acidic still beverages subject to heat treatment.

[b] Testing performed only if there is positive thermophilic acidophilic bacteria growth.

2.4 Product Analysis of Miracle Fruit Powder

Three to 6 non-consecutive lots of miracle fruit powder were analyzed to determine conformance to the established physical, chemical, heavy metal and microbiological parameters, as presented in Section 2.3. As summarized in the sections that follow, the results demonstrate that the manufacturing process, as described in Section 2.2, produces a consistent product that meets the established product specifications.

2.4.1 Physical Parameters

Analysis of 3 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) demonstrates conformance to the established physical specification parameters (see Table 2.4.1-1).

Table 2.4.1-1 Analysis of Physical Parameters for 3 Non-Consecutive Batches of Miracle Fruit Powder

Specification Parameter	Specification Limit	Manufacturing Lot No.		
		2019-09-0060	2019-11-0060	2019-13-0060
Appearance	Red/red-brown to pink powder	Pink granular powder with black specks	Pink granular powder with black specks	Pink granular powder with black specks
Odor	Characteristic of fruit	Conforms	Conforms	Conforms
Sensory (sweetness)	Sweetness induction of 0.15% citric acid solution equivalent to 3 to 7 Brix sucrose in water	Conforms	Conforms	Conforms
Particle size	<420 µm	Conforms	Conforms	Conforms

2.4.2 Chemical Parameters

2.4.2.1 Proximates and Miraculin

Analysis of 6 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, 2019-13-0060, KVS20200506AD_Powder 2020-19-001A, KVS20200506AE_Powder 2020-19-002B, and KVS20200506AF_Powder 2020-19-003C) demonstrates conformance to the established specifications for proximates and miraculin content (see Table 2.4.2.1-1).

Table 2.4.2.1-1 Proximate Analysis of 6 Non-Consecutive Batches of Miracle Fruit Powder

Specification Parameter	Specification Limit	Manufacturing Lot No.					
		2019-09-0060*	2019-11-0060*	2019-13-0060*	KVS20200506AD_Powder 2020-19-001A	KVS20200506AE_Powder 2020-19-002B	KVS20200506AF_Powder 2020-19-003C
Carbohydrates (dry basis)	≥80%	90.8	90.7	90.8	86.2	85.4	86.9
Total dietary fiber (dry basis)	≥5.5%	13.0	13.6	13.3	10.9	11.8	10.2
Total fatty acids (dry basis)	≥0.4%	0.672	0.674	0.673	0.788	0.872	0.811
Protein (dry basis)	≥4.5%	5.16	5.23	5.14	7.56	8.34	7.22
Ash (dry basis)	<5%	3.38	3.38	3.40	5.45	5.41	5.11
Moisture	<6%	1.82	1.87	1.78	5.63	5.91	4.53
Miraculin (dry basis)	≥0.048%	0.055	0.052	0.054	0.28	0.24	0.32

*Values for Lots 2019-09-0060, 2019—11-0060 and 2019-13-0060 are corrected for moisture from “as is” data.

2.4.2.2 Heavy Metals

Analysis of 3 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) demonstrates conformance to the established heavy metal specification parameters (see Table 2.4.2.2-1).

Table 2.4.2.2-1 Analysis of Heavy Metals of 3 Non-Consecutive Batches of Miracle Fruit Powder

Specification Parameter	Specification Limit	Manufacturing Lot No.		
		2019-09-0060	2019-11-0060	2019-13-0060
Arsenic	<0.05 ppm	0.0359	0.0351	0.0353
Cadmium	<0.5 ppm	0.0480	0.0482	0.0476
Lead	<0.1 ppm	0.0110	0.00723	0.0105
Mercury	<0.1 ppm	<0.005	<0.005	<0.005

ppm = parts per million.

2.4.3 Microbiological Parameters

Analysis of 3 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) demonstrates conformance to the established microbiological specification parameters (see Table 2.4.3-1).

Table 2.4.3-1 Microbiological Analysis of 3 Non-Consecutive Batches of Miracle Fruit Powder

Specification Parameter	Specification Limit	Manufacturing Lot No.		
		2019-09-0060	2019-11-0060	2019-13-0060
Total count	<3,000 CFU/g	30	35	70
Yeast	<300 CFU/g	<10	40	40
Mold	<300 CFU/g	80	70	90
Escherichia coli	<3 MPN/g	<3 MPN/g	<3 MPN/g	<3 MPN/g
Salmonella	Negative/25 g	Negative/10 g	Negative/10 g	Negative/10 g

Table 2.4.3-1 Microbiological Analysis of 3 Non-Consecutive Batches of Miracle Fruit Powder

Specification Parameter	Specification Limit	Manufacturing Lot No.		
		2019-09-0060	2019-11-0060	2019-13-0060
Listeria monocytogenes	Negative/25 g	Negative	Negative	Negative
Thermophilic acidophilic bacteria[a]	<1,000 CFU/g	Negative	Negative	Negative
Guaiacol-producing bacteria[a]	Absent	Not performed[b]	Not performed[b]	Not performed[b]
Heat-resistant mold[a]	Absent	Not detected	Not detected	Not detected

CFU = colony forming units; MPN = most probable number.
[a] Additional microbiological analyses for high acidic still beverages subject to heat treatment.
[b] Analysis was not performed as the results for the presence of thermophilic acidophilic bacteria were negative.

2.5 Additional Chemical Characterization of Miracle Fruit Powder

2.5.1 Antinutrients

Three non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) were analyzed for total polyphenol content spectrophotometrically using the Folin-Ciocalteu method (Singleton *et al.*, 1999 [book edited by Abelson *et al.*, 1999]) (see Table 2.5.1-1). The results demonstrate that miracle fruit powder contains small amounts of total polyphenols ranging from 17.3 to 17.5 mg gallic acid equivalents (GAE)/g.

Table 2.5.1-1 Analyses of 3 Non-Consecutive Batches of Miracle Fruit Powder for Total Polyphenols

Parameter	Manufacturing Lot No.		
	2019-09-0060	2019-11-0060	2019-13-0060
Total Polyphenols (mg/g as GAE)	17.5	17.3	17.3

GAE = gallic acid equivalents.

Three non-consecutive lots of miracle fruit powder (Lot Nos. KVS20200506AD_Powder 2020-19-001A, KVS20200506AE_Powder 2020-19-002B, and KVS20200506AF_Powder 2020-19-003C) were analyzed for antinutrient content (phytic acid, oxalic acid, trypsin inhibitors). As demonstrated in Table 2.5.1-2, phytic acid and trypsin inhibitor content were below the limit of quantitation (LOQ) across the 3 lots tested, while the oxalic acid content ranged from 1,170 to 1,350 ppm.

Table 2.5.1-2 Analyses of 3 Non-Consecutive Batches of Miracle Fruit Powder for Antinutrients

Parameter	Manufacturing Lot No.		
	KVS20200506AD_Powder 2020-19-001A	KVS20200506AE_Powder 2020-19-002B	KVS20200506AF_Powder 2020-19-003C
Oxalic acid (ppm, dry basis)[a]	1,180	1,350	1,170
Phytic acid (mg/g, dry basis)[b]	<1.06	<1.06	<1.05
Trypsin inhibitor (TIU/mg, dry basis)[c]	<0.530	<0.531	<0.524

AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemists' Society; ppm = parts per million; TIU = trypsin inhibitor units.
[a] Method of analysis: AOAC 986.13 (modified).
[b] Method of analysis: Lehrfeld (1989, 1994).
[c] Method of analysis: AOCS Ba 12-75, Hamerstrand *et al.* (1981) (modified).

Three non-consecutive lots of miracle fruit powder (Lot Nos. KVS20200506AD_Powder 2020-19-001A, KVS20200506AE_Powder 2020-19-002B, and KVS20200506AF_Powder 2020-19-003C) were analyzed for the presence of secondary plant compounds, specifically pyrrolizidine alkaloids or tropane alkaloids using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The reporting limit ranged from 1 to 2 µg/kg. The levels of each compound, including the sum of all pyrrolizidine alkaloids or tropane alkaloids, were below the reporting limit (see Appendix A for a complete list of analyzed alkaloids).

2.5.2 Fatty Acid Profile

The fatty acid profile of 6 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, 2019-13-0060, KVS20200506AD_Powder 2020-19-001A, KVS20200506AE_Powder 2020-19-002B, and KVS20200506AF_Powder 2020-19-003C) were characterized (see Table 2.5.2-1).

Table 2.5.2-1 Fatty Acid Profile of 6 Non-Consecutive Batches of Miracle Fruit Powder (Dry Basis)

Fatty Acid (%)	Manufacturing Lot No.					
	2019-09-0060*	2019-11-0060*	2019-13-0060*	KVS20200506AD_Powder 2020-19-001A	KVS20200506AE_Powder 2020-19-002B	KVS20200506AF_Powder 2020-19-003C
4:0 Butyric	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
6:0 Caproic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
8:0 Caprylic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
10:0 Capric	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
11C_18:1 Vaccenic	0.0074	0.0073	0.0077	NM	NM	NM
12:0 Lauric	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
14:0 Myristic	0.0224	0.0216	0.0214	0.017	0.019	0.018
14:1 Myristoleic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
15:0 Pentadecanoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
15:1 Pentadecenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
16:0 Palmitic	0.1507	0.1467	0.1476	0.162	0.18	0.165
16:1 Palmitoleic	<0.00713	<0.00713	<0.00713	0.008	0.01	0.008
17:0 Heptadecanoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
17:1 Heptadecanoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
18:0 Stearic	0.1120	0.1111	0.1100	0.137	0.147	0.141
18:2 Linoleic	0.2088	0.2130	0.2128	0.233	0.251	0.242
18:3 Gamma Linolenic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
18:3 Linolenic	0.0686	0.0707	0.0714	0.096	0.11	0.097
18:4 Octadecatetraenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:0 Arachidic	0.0090	0.0089	0.0089	0.011	0.013	0.011
20:1 Eicosenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:2 Eicosadienoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:3 Eicosatrienoic (n3)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:3 Homogamma Linolenic (n6)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:4 Arachidonic (n3)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:4 Arachidonic (n6)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
20:5 Eicosapentaenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
21:5 Heneicosapentaenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007

Table 2.5.2-1 Fatty Acid Profile of 6 Non-Consecutive Batches of Miracle Fruit Powder (Dry Basis)

Fatty Acid (%)	Manufacturing Lot No.					
	2019-09-0060*	2019-11-0060*	2019-13-0060*	KVS20200506 AD_Powder 2020-19-001A	KVS20200506 AE_Powder 2020-19-002B	KVS20200506 AF_Powder 2020-19-003C
22:0 Behenic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:1 Erucic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:2 Docosadienoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:3 Docosatrienoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:4 Docosatetraenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:5 Docosapentaenoic (n3)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:5 Docosapentaenoic (n6)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
22:6 Docosahexaenoic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
24:0 Lignoceric	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
24:1 Nervonic	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
9C_18:1 Oleic	0.0945	0.0956	0.0951	0.118	0.131	0.122
Monounsaturated Fatty Acids (Acid Form)	0.1002	0.1012	0.1011	0.128	0.144	0.131
Omega 3 Fatty Acids	0.0686	0.0707	0.0714	0.095	0.111	0.097
Omega 6 Fatty Acids	0.2088	0.2130	0.2128	0.233	0.252	0.242
Omega 7 Fatty Acids	0.0074	0.0073	0.0077	NM	NM	NM
Omega 9 Fatty Acids	0.0945	0.0956	0.0951	0.119	0.131	0.122
Polyunsaturated Fatty Acids (Acid Form)	0.2770	0.2833	0.2841	0.314	0.346	0.324
Saturated Fatty Acids (Acid Form)	0.2933	0.2884	0.2881	0.311	0.343	0.319
Total 18:1 cis	0.1002	0.1012	0.1011	0.126	0.14	0.129
Total 18:1 trans	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
Total 18:2 trans	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
Total 18:3 trans	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
Trans Fatty Acids (Acid Form)	<0.00713	<0.00713	<0.00713	<0.007	<0.007	<0.007
Unsaturated Fatty Acids (Acid Form)	0.3769	0.3842	0.3849	0.442	0.489	0.456

*Values for Lots 2019-09-0060, 2019—11-0060 and 2019-13-0060 were corrected for moisture from “as is” data.

2.5.3 Pesticides

Analysis for residual pesticides was conducted on 3 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) using a method based on AOAC 2007.01 and CEN Standard method EN 15662. The samples were prepared and analyzed by gas chromatography-tandem mass spectrometry or LC-MS/MS. The typical LOQs are in the range of 0.01 and 0.05 mg/kg. The pesticide content was below the LOQ across all tested batches, indicating the absence of pesticides in the final product.

2.5.4 Mycotoxins and Other Secondary Metabolites

Analysis for mycotoxins and other secondary metabolites was conducted on 3 non-consecutive lots of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) using ultra-high performance liquid chromatography–tandem mass-spectrometry (UHPLC-MS/MS). The UHPLC-MS/MS method was based on the method described by Varga *et al.* (2012). The results are summarized in Table 2.5.4-1 and demonstrate that the levels of these mycotoxins and other secondary metabolites were below each respective LOQ in the final product.

Table 2.5.4-1 Analysis for Mycotoxins and Other Secondary Metabolites of 3 Non-Consecutive Batches of Miracle Fruit Powder

Mycotoxin/Secondary Metabolite (ng/g)	LOQ (ng/g)	Manufacturing Lot No.		
		2019-09-0060	2019-11-0060	2019-13-0060
Aflatoxin B1	0.5	<0.5	<0.5	<0.5
Aflatoxin B2	0.5	<0.5	<0.5	<0.5
Aflatoxin G1	0.5	<0.5	<0.5	<0.5
Aflatoxin G2	0.5	<0.5	<0.5	<0.5
Aflatoxin M1	0.5	<0.5	<0.5	<0.5
Aflatoxin M2	0.5	<0.5	<0.5	<0.5
Deoxynivalenol	100	<100	<100	<100
T-2 Toxin	10	<10	<10	<10
HT-2 Toxin	100	<100	<100	<100
Fumonisin B1	25	<25	<25	<25
Fumonisin B2	25	<25	<25	<25
Ochratoxin A	1	<1	<1	<1
Zearalenone	30	<30.0	<30.0	<30.0

LOQ = limit of quantitation.

2.6 Chemical Characterization of Miracle Fruit Pulp

In order to demonstrate the compositional similarities between miracle fruit powder and miracle fruit pulp from which the powders were obtained, 3 non-consecutive lots of miracle fruit pulp (Lot Nos. KVS20200506AA_Pulp 050401A, KVS20200506AB_Pulp 050402B, and KVS20200506AC_Pulp 050403C) were analyzed for their proximate content, miraculin level, fatty acid composition, and antinutrient content. The results of these analyses are summarized in the sections that follow. The miracle fruit pulp that was produced as described in Section 2.2 was further processed into the 3 additional lots of miracle fruit powder described in the sections above (*i.e.*, Lot Nos. KVS20200506AD_Powder 2020-19-001A, KVS20200506AE_Powder 2020-19-002B, and KVS20200506AF_Powder 2020-19-003C). A comparison between the proximate analysis of miracle fruit powder with miracle fruit pulp, from which the powder is obtained, is provided in Section 6.3.1.

2.6.1 Proximate and Chemical Analysis

The proximate parameters and miraculin content of 3 non-consecutive lots of miracle fruit pulp (Lot Nos. KVS20200506AA_Pulp 050401A, KVS20200506AB_Pulp 050402B, and KVS20200506AC_Pulp 050403C) were measured (see Table 2.6.1-1).

Table 2.6.1-1 Proximates and Chemical Analysis of 3 Non-Consecutive Batches of Miracle Fruit Pulp

Parameter	Manufacturing Lot No.		
	KVS20200506AA_Pulp 050401A	KVS20200506AB_Pulp 050402B	KVS20200506AC_Pulp 050403C
Carbohydrates (%) (dry basis)	87.2	86.1	87.5
Total dietary fiber (%) (dry basis)	10.4	9.14	13.4
Total fatty acids (%) (dry basis	0.781	0.812	0.836
Protein (%) (dry basis)	8.85	9.72	8.43
Ash (%) (dry basis)	3.36	3.70	3.52
Moisture (%)	84.5	85.1	84.8
Miraculin (µg/g) (dry basis)*	3,138	2,991	3,294

*The reported "as is" values were corrected for moisture.

2.6.2 Fatty Acid Profile

The fatty acid profile of 3 non-consecutive lots of miracle fruit pulp (Lot Nos. KVS20200506AA_Pulp 050401A, KVS20200506AB_Pulp 050402B, and KVS20200506AC_Pulp 050403C) was characterized (see Table 2.6.2-1).

Table 2.6.2-1 Fatty Acid Profile of 3 Non-Consecutive Batches of Miracle Fruit Pulp (Dry Basis)

Fatty Acid (%)	Manufacturing Lot No.		
	KVS20200506AA_Pulp 050401A	KVS20200506AB_Pulp 050402B	KVS20200506AC_Pulp 050403C
4:0 Butyric	<0.013	<0.013	<0.013
6:0 Caproic	<0.013	<0.013	<0.013
8:0 Caprylic	<0.013	<0.013	<0.013
10:0 Capric	<0.013	<0.013	<0.013
12:0 Lauric	<0.013	<0.013	<0.013
14:0 Myristic	0.025	0.026	0.024
14:1 Myristoleic	<0.013	<0.013	<0.013
15:0 Pentadecanoic	<0.013	<0.013	<0.013
15:1 Pentadecenoic	<0.013	<0.013	<0.013
16:0 Palmitic	0.175	0.179	0.183
16:1 Palmitoleic	<0.013	<0.013	<0.013
17:0 Heptadecanoic	<0.013	<0.013	<0.013
17:1 Heptadecanoic	<0.013	<0.013	<0.013
18:0 Stearic	0.129	0.131	0.139
18:2 Linoleic	0.226	0.232	0.243
18:3 Gamma Linolenic	<0.013	<0.013	<0.013
18:3 Linolenic	0.099	0.099	0.108
18:4 Octadecatetraenoic	<0.013	<0.013	<0.013
20:0 Arachidic	<0.013	<0.013	<0.013
20:1 Eicosenoic	<0.013	<0.013	<0.013
20:2 Eicosadienoic	<0.013	<0.013	<0.013
20:3 Eicosatrienoic (n3)	<0.013	<0.013	<0.013

Table 2.6.2-1 Fatty Acid Profile of 3 Non-Consecutive Batches of Miracle Fruit Pulp (Dry Basis)

Fatty Acid (%)	Manufacturing Lot No.		
	KVS20200506AA_Pulp 050401A	KVS20200506AB_Pulp 050402B	KVS20200506AC_Pulp 050403C
20:3 Homogamma Linolenic (n6)	<0.013	<0.013	<0.013
20:4 Arachidonic (n3)	<0.013	<0.013	<0.013
20:4 Arachidonic (n6)	<0.013	<0.013	<0.013
20:5 Eicosapentaenoic	<0.013	<0.013	<0.013
21:5 Heneicosapentaenoic	<0.013	<0.013	<0.013
22:0 Behenic	<0.013	<0.013	<0.013
22:1 Erucic	<0.013	<0.013	<0.013
22:2 Docosadienoic	<0.013	<0.013	<0.013
22:3 Docosatrienoic	<0.013	<0.013	<0.013
22:4 Docosatetraenoic	<0.013	<0.013	<0.013
22:5 Docosapentaenoic (n3)	<0.013	<0.013	<0.013
22:5 Docosapentaenoic (n6)	<0.013	<0.013	<0.013
22:6 Docosahexaenoic	<0.013	<0.013	<0.013
24:0 Lignoceric	<0.013	<0.013	<0.013
24:1 Nervonic	<0.013	<0.013	<0.013
9C_18:1 Oleic	0.120	0.124	0.128
Monounsaturated Fatty Acids (Acid Form)	0.122	0.126	0.130
Omega 3 Fatty Acids	0.097	0.101	0.105
Omega 6 Fatty Acids	0.226	0.235	0.243
Omega 9 Fatty Acids	0.123	0.121	0.125
Polyunsaturated Fatty Acids (Acid Form)	0.311	0.317	0.336
Saturated Fatty Acids (Acid Form)	0.314	0.321	0.330
Total 18:1 cis	0.128	0.132	0.136
Total 18:1 trans	<0.013	<0.013	<0.013
Total 18:2 trans	<0.013	<0.013	<0.013
Total 18:3 trans	<0.013	<0.013	<0.013
Trans Fatty Acids (Acid Form)	<0.013	<0.013	<0.013
Unsaturated Fatty Acids (Acid Form)	0.432	0.443	0.467

2.6.3 Antinutrients

Three non-consecutive lots of miracle fruit pulp (Lot Nos. KVS20200506AA_Pulp 050401A, KVS20200506AB_Pulp 050402B, and KVS20200506AC_Pulp 050403C) were analyzed for antinutrient content (phytic acid, oxalic acid, trypsin inhibitors). As demonstrated in Table 2.6.3-1, oxalic acid, phytic acid and trypsin inhibitor content were below the LOQ for each respective compound.

Table 2.6.3-1 Analyses of 3 Non-Consecutive Batches of Miracle Fruit Pulp for Antinutrients

Parameter	Manufacturing Lot No.		
	KVS20200506AA_Pulp 050401A	KVS20200506AB_Pulp 050402B	KVS20200506AC_Pulp 050403C
Oxalic acid (ppm, dry basis)[a]	<2,680	<2,680	<2,630
Phytic acid (mg/g, dry basis)[b]	<6.45	<6.71	<6.58
Trypsin inhibitor (TIU/mg, dry basis)[c]	<3.23	<3.36	<3.29

AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemists' Society; LOQ = limit of quantitation; ppm = parts per million; TIU = trypsin inhibitor units.
[a] Method of analysis: AOAC 986.13 (modified). The same method was used for analysis of miracle fruit powder (see Table 2.5.1-2). The difference in LOQ is due to the higher water content of miracle fruit pulp (*ca.* 85%).
[b] Method of analysis: Lehrfeld (1989, 1994).
[c] Method of analysis: AOCS Ba 12-75, Hamerstrand *et al.* (1981) (modified).

Three non-consecutive lots of miracle fruit pulp (Lot Nos. KVS20200506AA_Pulp 050401A, KVS20200506AB_Pulp 050402B, and KVS20200506AC_Pulp 050403C) were analyzed for the presence of secondary plant compounds, specifically pyrrolizidine alkaloids or tropane alkaloids using LC-MS/MS. The reporting limit ranged from 1 to 2 µg/kg. The levels of each compound, including the sum of all pyrrolizidine alkaloids or tropane alkaloids, were below the reporting limit.

2.7 Stability of Miracle Fruit Powder

The stability of miracle fruit powder (Lot Nos. 2019-09-0060, 2019-11-0060, and 2019-13-0060) was investigated in a 52-week shelf-life stability study. Samples of miracle fruit powder were stored at 25±2°C and 60% relative humidity in metalized barrier pouches for 52 weeks. The miraculin content and moisture content of each sample were measured at 0, 2, 4, 8, 14, 26, 39, and 52 weeks. The data indicate that miracle fruit powder is stable for up to 52 weeks when stored at ambient temperature and humidity.

Table 2.7-1 Shelf-Life Stability Results of Miracle Fruit Powder (Lot Nos. 2019-09-0060, 2019-11-0060 and 2019-13-0060) Stored at 25±2°C/60% Relative Humidity for 52 Weeks

Parameter	Manufacturing Lot No.		
	2019-09-0060	2019-11-0060	2019-13-0060
Moisture (%)			
0	1.82	1.87	1.78
2	1.75	1.78	1.76
4	1.54	1.58	1.53
8	2.06	2.02	1.84
14	2.43	2.26	2.10
26	3.36	3.39	2.80
39	4.13	4.20	4.23
52	4.78	4.69	4.50
Miraculin (µg/g)[a]			
0	545	519	538
2	554	517	543
4	555	531	550
8	684	614	627

Table 2.7-1 Shelf-Life Stability Results of Miracle Fruit Powder (Lot Nos. 2019-09-0060, 2019-11-0060 and 2019-13-0060) Stored at 25±2°C/60% Relative Humidity for 52 Weeks

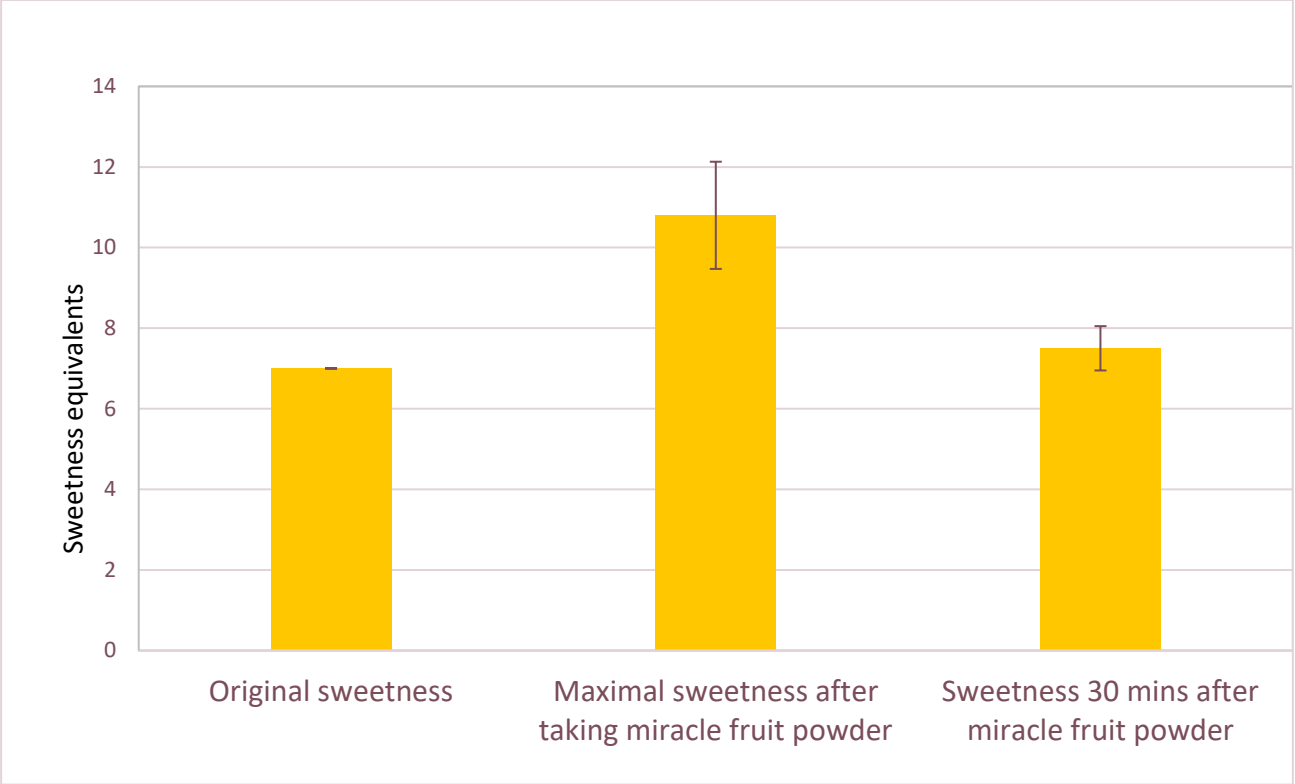
Parameter	Manufacturing Lot No.		
	2019-09-0060	2019-11-0060	2019-13-0060
14	645	595	612
26	581	592	679
39	524	545	557
52	487	495	519

[a] Average miraculin content based on 3 replicates. All values are on as is basis.

2.8 Technical Effect

Miracle fruit powder will be marketed in powdered form and added to beverage products and fermented dairy products for its ability to impart sweetness by modifying taste from sour to sweet due to the active glycoprotein miraculin. The sweetness profile of miracle fruit was evaluated by 6 trained panelists (Tafazoli *et al*., 2019). A baseline sweetness intensity was established with lemonade juice with a sweetness intensity of 7 Brix. Following establishment of a baseline sweetness intensity, each panelist consumed 0.08 g of miracle fruit powder and was instructed to hold the powder in the mouth for 1 minute before swallowing. Each panelist then consumed 60 mL of the original lemonade juice every 5 minutes for 30 minutes, and the sweetness of each cup was recorded. The results expressed as sweetness equivalency are summarized in Figure 2.8-1 below. The results indicate that miracle fruit significantly increased the perceived sweetness of lemonade juice, and sweetness of the juice returned to baseline levels in all subjects after 30 minutes. These results indicate the taste-modifying effect of miraculin is rapid with no lasting desensitization effect. In another published study evaluating the taste-modifying effect of miraculin, the maximum relative sweetening effect was achieved within 3 minutes of consumption, and rapidly declined after 30 minutes. These effects were concentration-dependent (Kurihara and Beidler, 1969).

Figure 2.8-1 Sweetness Intensity of Miracle Fruit Powder by Trained Panelists (n=6)



The taste-modifying effect of miracle fruit berries was also investigated as part of a *Sensation and Perception* course offered at Christopher Newport University (Lipatova and Campolattaro, 2016). Authors reported the findings from one semester involving 19 students. Each individual was provided 1 to 2 fresh berries obtained from Miracle Fruit Farm. One berry was reported to elicit an effect lasting approximately 30 minutes. Individuals were instructed to chew the berry thoroughly to coat the entire membrane of their tongue with the pulp of the fruit (approximately 30 seconds of chewing). The seed was discarded. Following consumption of the berries, each individual was provided 4 food products targeted for different taste receptors (sweet, sour, salty, and bitter). These included jellybeans (sweet), lemon wedges (sour), crackers (salty), raw broccoli (bitter). In addition to these food items, grapefruit, limes, green apple, sour candy, and apple cider were provided to further investigate the effect on the sour receptor. The taste perception was scored on a 0 to 10 scale. The palates of each individual were rinsed with water after each tasting. The authors reported one individual not experiencing any change in taste perception following consumption of miracle fruit. In the other individuals, miracle fruit did not significantly alter the perception of salty or bitter tastes. The perceived sweetness of each acidic food item (grapefruit, limes, green apple, sour candy, and apple cider) and lemon were significantly increased. The authors also reported a significant decrease in perception of sour intensity of the same food items following consumption of miracle fruit. The authors reported that miracle fruit does not have any physical effect on the sour taste receptor, and the change in perception of sourness are due to psychophysical nature of the human senses.

The taste-modifying effect of miracle fruit in cancer patients was investigated as part of a pilot study to improve dysgeusia (Wilken and Satiroff, 2012). Cancer patients (N=8) were provided either control (dried cranberries) or treatment (6 miracle fruits/day) for 14 days. Individuals were instructed to consume the fruit immediately prior to each meal. The study authors monitored eating habits (taste difference in foods and unpalatability of foods) and food intake throughout the study period. The authors reported that taste perception improved in all patients and food intake increased in "some" following consumption of the miracle fruit, and the fruit alleviated the adverse change in taste perception of radiation therapy (*i.e.*, "metallic" taste). One individual reported a sweet taste following consumption of a lemon wedge, with the effect lasting approximately 20 minutes after consuming the miracle fruit. The authors reported no significant changes in body weight in any individual. The taste-modifying effect of miracle fruit as reported in this pilot study were consistent with those reported by Lipatova and Campolattaro (2016) and Tafazoli *et al.* (2019).

Contrary to the way miracle fruit powder was tested and evaluated in the sensory trials described above, Miracle Fruit Farm's miracle fruit powder is intended for direct addition to various beverage and fermented dairy products. It is important to note that miracle fruit powder is not intended to be consumed prior to administration of these food products or any other sour-tasting product to specifically mask the sour properties of a food. As such, there will be no change or difference in the way products containing Miracle Fruit Farm's miracle fruit powder, as an ingredient, are consumed, compared to those conventional products listed in Section 1.3-1 that would not contain miracle fruit powder as an ingredient.

Part 3. § 170.235 Dietary Exposure

3.1 Functionality

Miracle fruit powder will be marketed in powdered form and added to beverage products and fermented dairy products for its sweetening and taste-modifying properties due to the active glycoprotein miraculin. Products to which miracle fruit powder will be added will not carry any structure/function or health claims and will be marketed similar to conventional products that do not contain this ingredient. The only difference between the products containing Miracle Fruit Farm's miracle fruit powder and the conventional products will be in the ingredient list. Therefore, no increases or changes in the consumption pattern of food products containing miracle fruit powder is expected compared to that from the conventional food products that do not contain the ingredient.

While addition of miracle fruit powder to beverages and dairy products is expected to change their sweetness profile, this ingredient will not have an impact on the pH of the foods to which it is added. Considering that miracle fruit powder-containing products will be marketed in a similar manner as any other conventional food products, there will be no risk to consumers that suffer from conditions such as acid-reflux or other digestive disorders; therefore, consumers who may experience digestive disorders such as acid-reflux would be expected to self-regulate products that contain Miracle Fruit Farm's miracle fruit powder in the same manner as any other acidic products and refrain from consuming them.

3.2 Estimated Dietary Intake of Miracle Fruit Powder

3.2.1 Methods

An assessment of the anticipated intake of miracle fruit powder as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (USDA, 2019; CDC, 2020a,b). This assessment was primarily conducted to evaluate exposure to antinutrient present in miracle fruit powder that could negatively affect the bioavailability of other nutrients in foods to which the ingredient is added (see Section 6.4). A summary of the results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2015-2016. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (USDA, 2019; CDC, 2020a,b). The NHANES data were employed to assess the mean and 90th percentile intake of miracle fruit powder for each of the following population groups:

- Children, ages 2 to 5 years;
- Children, ages 6 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (ages 2 years and older, gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of miracle fruit powder by the U.S. population[1]. Estimates for the daily intake of miracle fruit powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of miracle fruit powder averaged over all individuals surveyed, regardless of whether they consumed food products in which miracle fruit powder is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing miracle fruit powder during the 2 survey days). "Consumer-only" intake refers to the estimated intake of miracle fruit powder by those individuals who reported consuming food products in which the use of miracle fruit powder is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which miracle fruit powder is proposed for use on either Day 1 or Day 2 of the survey.

Estimates for the intake of miracle fruit powder were generated using the maximum use level indicated for each intended food use, as presented in Table 1.3-1, together with food consumption data available from the 2015-2016 NHANES dataset. The resulting intake estimates of miracle fruit powder are presented in Section 3.2.2.

3.2.2 Estimated Daily Intake of Miracle Fruit Powder

A summary of the estimated daily intake of miracle fruit powder from proposed food uses is provided in Table 3.2.2-1 on an absolute basis (g/person/day), and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 77.7% of the population groups consisted of consumers of food products in which miracle fruit powder is currently proposed for use (see Table 3.2.2-1). Children ages 6 to 11 had the greatest proportion of consumers at 89.9%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of miracle fruit powder were determined to be 8.4 and 17.4 g/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of miracle fruit powder on an absolute basis, at 9.7 and 20.1 g/person/day, respectively. Female teenagers had the lowest mean consumer-only intakes of 7.4 g/person/day, while children ages 2 to 5 had the lowest 90th percentile consumer-only intakes of 15.0 g/person/day, respectively (see Table 3.2.2-1).

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.2.2-1 Summary of the Estimated Daily Intake of Miracle Fruit Powder from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Children	2 to 5	6.6	14.8	87.6	482	7.5	15.0
Children	6 to 11	6.9	14.3	89.9	763	7.6	15.2
Female Teenagers	12 to 19	6.5	15.5	87.1	416	7.4	15.9
Male Teenagers	12 to 19	7.3	15.8	84.0	418	8.7	16.8
Female Adults	20 and up	5.9	14.9	77.9	1,767	7.5	16.8
Male Adults	20 and up	7.5	17.4	77.7	1,571	9.7	20.1
Total Population	2 and up	6.7	15.9	80.1	5,417	8.4	17.4

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (ages 2 years and older) mean and 90th percentile consumer-only intakes of miracle fruit powder were determined to be 140 and 298 mg/kg body weight/day, respectively. Among the individual population groups, children ages 2 to 5 years were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 446 and 951 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 99 and 219 mg/kg body weight/day, respectively (see Table 3.2.2-2).

Table 3.2.2-2 Summary of the Estimated Daily Intake Per Kilogram Body Weight of Miracle Fruit Powder from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Children	2 to 5	393	909	88.0	477	446	951
Children	6 to 11	224	499	89.9	761	249	515
Female Teenagers	12 to 19	106	256	87.0	409	122	258
Male Teenagers	12 to 19	110	241	84.2	418	131	257
Female Adults	20 and up	77	197	77.8	1,755	99	219
Male Adults	20 and up	86	202	77.5	1,551	111	229
Total Population	2 and up	112	262	80.0	5,371	140	298

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.3.3 Summary

Consumption data and information pertaining to the intended food uses of miracle fruit powder were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain miracle fruit powder at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that miracle fruit powder will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of miracle fruit powder by the total U.S. population (ages 2 years and older) from proposed food uses in the U.S. were estimated to be 8.4 g/person/day (140 mg/kg body weight/day) and 17.4 g/person/day (298 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of miracle fruit powder were determined to be 9.7 g/person/day (111 mg/kg body weight/day) and 20.1 g/person/day (229 mg/kg body weight/day), respectively, as identified among male adults. While children ages 2 to 5 had lower mean and 90th percentile consumer-only intakes of 7.5 and 15.0 g/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 446 and 951 mg/kg body weight/day at the mean and 90th percentile, respectively.

The information on the intakes of miracle fruit powder has been used to evaluate exposure to antinutrients present in the ingredient. The results of this assessment are provided in Section 6.4.

It should be noted that none of the ingredients are intended for use in food products consumed by infants and children up to 2 years of age.

Part 4. § 170.240 Self-Limiting Levels of Use

Miracle fruit powder is intended to be used as an ingredient for addition to beverage and fermented dairy products for its ability to impart sweetness by modifying taste from sour to sweet, due to the active glycoprotein miraculin. The taste-modifying effects of miraculin are limited by the capacity of the interaction with those receptors on the tongue. As such, the level of miracle fruit powder that is to be added to the various beverages and fermented dairy products will be limited to the achieving maximum tongue receptor interaction.

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 miracle fruit powder obtained by mechanical processing steps (*i.e.*, pulping, maceration, freeze-drying, milling) from miracle fruit as the starting material. Analytical composition data (see Section 6.3) demonstrated that there are no compositional differences between the miracle fruit powder and the starting material, aside from the moisture content considering that the miracle fruit powder undergoes freeze-drying to obtain the powder form. Mean values from the analysis of 6 production batches of miracle fruit powder (see Table 2.6.1-1) demonstrate that the ingredient is primarily composed of carbohydrates (~88% on dry basis), protein (~6% on dry basis), ash (~4% on dry basis), and moisture (~3.5%).

Miracle Fruit Farm's conclusion on the GRAS status of miracle fruit powder under its conditions of intended use, as an ingredient in various beverage products and fermented dairy products, is based on scientific procedures supported by the following:

1. Long history of consumption of miracle fruit globally;

2. Publicly available data related to the safety of miracle fruit and miracle fruit powder;

3. Compositional data on miracle fruit powder and pulp and comparison with other commonly consumed fruits;

4. Exposure to antinutrients from proposed uses of miracle fruit powder;

5. Publicly available safety data on miraculin; and

6. Consensus among a panel of experts (the GRAS Panel), qualified by scientific training and experience to evaluate the safety of food ingredients, namely the following scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine); Professor Emeritus George C. Fahey, Jr. (University of Illinois); and Professor Stephen L. Taylor (University of Nebraska).

6.1 Long History of Global Consumption of Miracle Fruit

Miracle fruit powder is minimally processed and has been demonstrated through analytical data to be compositionally similar to miracle fruit pulp, which is used as a starting material, with the only difference being in the moisture content. The first historical record of human consumption of miracle fruit dates back to the early 1700s in Ghana (Roecklein and Leung, 1987). In 1917, miracle fruit was introduced to the U.S. from Africa by the USDA. Since then, cultivation of miracle fruit and its use in the U.S. has steadily grown. Currently, a number of different products containing miracle fruit/miracle berry, and derivatives thereof, are marketed over the internet. Web searches for "miracle berry", "miracle fruit", and "miraculin" identified several dietary supplement-type products containing miracle berry or miracle fruit extract (mberry Miracle Fruit Tablets, My M Fruit LLC; MiraBurst Easy Melt Miracle Berry Tablets, MiraBurst; Miraculous Miracle Fruit Tablets, Miracle Fruit Farm; Miracle Frooties Miracle Fruit Tablets, Ruby Forest LLC) that are currently available on the U.S. market. The recommended serving size appears to be 100 to 175 mg miracle fruit extract/day. A Miracle Berry Diet Cookbook is also available instructing how to cook with and use the miracle berry within the daily diet, containing over 150 recipes to incorporate miracle berry in breakfast, lunch, and dinner options as well as cocktail recipes (Cantu, 2013). To date, there have been no adverse events resulting from consumption of miracle berry, miracle fruit, or miraculin products reported

through the U.S. FDA Adverse Event Reporting System or Center for Food Safety and Applied Nutrition Adverse Event Reporting System, suggesting that there is a history of safe use of miracle fruit in the U.S.

6.2 Publicly Available Data Related to Safety of Miracle Fruit and Miracle Fruit Powder

A comprehensive search of the scientific literature was conducted through May 2021 to identify publications related to the metabolism and safety of miracle fruit powder. The search was limited to full text articles within peer-reviewed scientific journals from the following literature databases: 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 search identified several repeated-dose studies on miracle fruit powder and fruits and leaf extracts of miracle fruit, which evaluated the effects on blood glucose, glucose tolerance, insulin resistance, hematology and blood chemistry of diabetic and non-diabetic rodents, and anti-hyperuricemic effects in mice. While these studies mainly evaluated efficacy-related endpoints of miracle fruit or extracts of the plant on diabetic and non-diabetic rats or rats consuming a high-fructose diet, the lack of adverse findings on the limited safety-related endpoints provide supporting evidence of the safety of miracle fruit powder. The results of these studies are summarized in Table 6.2-1 below.

Table 6.2-1 Summary of Repeated-Dose Studies of Miracle Fruit Powder or Extracts of Miracle Fruit and Miracle Fruit Leaf

Details of the Study Methodology	Reported Findings	Reference
Miracle Fruit Powder		
Test Animal: Male Wistar Rat (N=8/group) **Route of Administration:** Oral (gavage) **Duration:** Single dose[b] **Doses:** 0, 0.02, 0.04, 0.2 mg/kg	• Significant decrease in plasma glucose in all treatment groups (over 90 min)	Chen *et al.* (2006)
Test Animal: Male Wistar Rat (N=8/group) **Route of Administration:** Oral (gavage) **Duration:** 3 days[c] **Doses:** 0, 0.02, 0.04, 0.2 mg/kg	• Significant decrease in plasma glucose in IPGTT • Significant decrease in total AUC for glucose response and plasma insulin in all treatment groups • Significant decrease in glucose-insulin index in 0.2 mg/kg group	
Test Animal: Male Wistar Rat (N=8/group) **Route of Administration:** Oral (gavage) **Duration:** 28 days[d] **Doses:** 0 or 0.2 mg/kg	• Amelioration of plasma glucose lowering effect of tolbutamide in treatment groups compared to control	
Test Animal: Male Wistar Rat (N=8/group)[e] **Route of Administration:** Oral (gavage) **Duration:** 10 days[f] **Doses:** 0 or 0.2 mg/kg	• Significant increase in plasma glucose lowering activity • Reversal of hyperphagia effects • Significant decrease in food and water intake • No significant effect on body weight	
Extract of Miracle Fruit and Miracle Fruit Leaf		
Test Animal: Male albino rats[g] (N=5/group) **Route of Administration:** Oral (gavage) **Duration:** 4 weeks **Doses:** 0 or 200 mg/kg bw/day)[h]	• Significant decrease in blood glucose	Dioso *et al.* (2016)
Test Animal: Rat (sex and strain were not reported) (N=7/group) **Route of Administration:** Oral (drinking water) **Duration:** 21 days	• Significant increase in plasma Ca, Na, and K concentrations in diabetic control compared to non-diabetic control • Significant decrease in plasma calcium and potassium concentrations in all MSD and FSD groups compared to diabetic control but ↑ compared to non-diabetic control	Obafemi *et al.* (2016)

Table 6.2-1 Summary of Repeated-Dose Studies of Miracle Fruit Powder or Extracts of Miracle Fruit and Miracle Fruit Leaf

Details of the Study Methodology	Reported Findings	Reference
Doses: 0, 30, or 60 mg/kg methanolic (MSD) or flavonoid-rich (FSD) *Synsepalum dulcificum* leaf extract	• Significant decrease in plasma sodium concentration in MSD groups compared to diabetic control but ↑ compared to non-diabetic control • Significant decrease in plasma sodium concentration in FSD groups compared to diabetic control; no significant effect compared to non-diabetic control • Significant increase in WBC and neutrophil count in diabetic control compared to non-diabetic control • Significant decrease in PCV, hemoglobin concentration, and RBC in diabetic control compared to non-diabetic control • Significant decrease in WBC and neutrophil count in all MSD and FSD groups compared to diabetic control but ↑ compared to non-diabetic control • Significant increase in PCV, hemoglobin concentration, and RBC in all MSD and FSD groups compared to diabetic control but ↓ compared to non-diabetic control	
Test Animal: Rat (sex and strain were not reported) (N=7/group) **Route of Administration:** Oral (drinking water) **Duration:** 21 days **Doses:** 0, 30, or 60 mg/kg MSD or FSD *Synsepalum dulcificum* leaf extract	• Significant decrease in body weight in diabetic control compared to non-diabetic control • Significant increase in body weight in all MSD and FSD groups • Amelioration of serum glucose levels in all MSD and FSD diabetic animals • Significant decrease in ALP, AST, ALT, urea, and creatinine in all MSD and FSD diabetic groups compared to diabetic control; no significant effect in ALP and creatinine compared to non-diabetic control • Significant increase in AST and ALT in all MSD and FSD diabetic groups compared to normal control • Significant decrease in urea in high-dose MSD group compared to non-diabetic control • Significant increase in urea in high-dose FSD group compared to non-diabetic control • Significant decrease in ALP, AST, ALT, creatinine, urea in non-diabetic control compared to diabetic control • Significant increase in total protein in non-diabetic control compared to diabetic control • NSD in ALP or creatinine in non-diabetic MSD and FSD groups compared to non-diabetic control • NSD in AST or urea in non-diabetic MSD group compared to non-diabetic control • Significant increase in AST, ALT, urea in non-diabetic FSD group compared to non-diabetic control • Significant increase in total protein in all MSD and FSD diabetic groups compared to diabetic control; ↓ compared to non-diabetic control • Amelioration of liver, kidney, and pancreas lipid peroxidation biomarkers in all MSD and FSD groups	Obafemi *et al.* (2017)
Test Animal: Rat (sex and strain were not reported) **Route of Administration:** Oral **Duration:** 21 days **Doses:** 0, 30, or 60 mg/kg MSD or FSD *Synsepalum dulcificum* leaf extract	• Significant decrease in HbA1c, IL-6, TNF-α • Significant increase in serum insulin levels, hepatic hexokinase activity	Obafemi *et al.* (2019)

Table 6.2-1 Summary of Repeated-Dose Studies of Miracle Fruit Powder or Extracts of Miracle Fruit and Miracle Fruit Leaf

Details of the Study Methodology	Reported Findings	Reference
Test Animal: ICR Mice **Route of Administration:** Oral **Duration:** 7 days **Doses:** 0, 500, or 1,000 mg/kg body weight/day	• No significant effect relative-to-body liver and kidney weights, serum creatinine, or blood urea nitrogen	Shi *et al.* (2016)

↑ = increased; ↓ = decreased; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUC = area under the curve; bw = body weight; Ca = calcium; FSD = flavonoid-rich *Synsepalum dulcificum* leaf extract; HbA1c = hemoglobin A1c; IL-6 = interleukin-6; IPGTT = intraperitoneal glucose tolerance test; K = potassium; M = males; min = minutes; MSD = methanolic *Synsepalum dulcificum* leaf extract; Na = sodium; NSD = no significant difference; PCV = packed cell volume; RBC = red blood cells; TNF-α = tumor necrosis factor-α; WBC = white blood cells.

[a] All reported findings were statistically significant compared to respective controls unless otherwise noted.

[b] Animals were administered a single dose of miracle fruit powder and then provided a fructose-rich diet (60%) for 4 weeks.

[c] Animals were provided a fructose-rich diet (60%) for 4 weeks and were administered 3 doses per day of miracle fruit powder.

[d] Miracle fruit powder was administered every 8 hours, 3 doses per day, and 10 mg/kg tolbutamide was administered at 5 hours after miracle fruit powder treatment.

[e] Diabetes was induced by streptozocin injection.

[f] Miracle fruit powder was administered every 8 hours, 3 doses per day for 10 days, and then challenged with insulin injection.

[g] Diabetes was induced by alloxan injection.

[h] Extracted with ethanol.

Overall, the studies described in Table 6.2-1 are of limited relevance to the safety of Miracle Fruit Farm's miracle fruit powder considering that they are mainly efficacy-focused studies with some limited toxicological endpoints. In the study by Chen *et al*. (2006), the effects of lyophilized miracle fruit powder on insulin resistance of male Wistar rats consuming a fructose-rich diet was evaluated, which mainly focused on changes to the plasma glucose levels in rats fed a high-fructose diet, as compared to their respective control and were not reflective of such effects in comparison to animals that were fed standard rat chow alone. The potential effect of the lyophilized powder on dietary intake due to palatability was highlighted in the last experiment conducted in STZ-diabetic rats, wherein the authors reported that treatment with miracle fruit powder significantly reduced both food and water intake in comparison to those animals only receiving the dose vehicle (saline). If this reduction in food intake similarly occurred in the fructose-rich fed group that was fed miracle fruit powder, then this would have been a confounding factor in the overall results analysis thereby impacting the ability to accurately interpret the outcome of miracle fruit powder administration. The effect could have been amplified in the experiments with 60% fructose in the diet. Based upon the fact that the lyophilized miracle fruit powder used in the study included all parts of the miracle fruit, including seed, an argument can be made regarding the potential difference in material composition between the test material used by Chen *et al.* (2006) and Miracle Fruit Farm's miracle fruit powder. Furthermore, based upon a clear lack of detailed information on the study design and methodology, including the results from the control animals and the lack of information related to food and water intake and body weight data, it is difficult to interpret the impact of miracle fruit powder on insulin resistance. Based on these study limitations, the study by Chen *et al.* (2006) is of limited value to the risk assessment of Miracle Fruit Farm's miracle fruit powder.

In the studies conducted by Dioso *et al*. (2016), Obafemi *et al*. (2016, 2017, 2019), and Shi *et al*. (2016), the test articles were extracted from the leaf and fruits of miracle fruit through the use of various solvents including butanol, ethanol and methanol. These studies are considered of limited relevance to the safety of Miracle Fruit Farm's miracle fruit powder, which undergoes no extraction process, due to the compositional differences in the test articles. The effects reported on some serum biochemistry parameters may therefore be attributed to the concentrated fruit/leaf components following solvent extraction and/or any residual extraction solvents in the final product.

In addition to the published studies discussed above, the European Food Safety Authority (EFSA) reviewed a number of safety studies related to the dried fruits of *Synsepalum dulcificum* (*i.e.,* miracle fruit) as part of a novel food application (EFSA, 2021). The subject of the novel food application was Dried Miracle Berries, which are fruits of *S. dulcificum* that have been pitted and dried by lyophilization, similar to the process used to produce the Miracle Fruit Powder as described herein. The composition of the Dried Miracle Berries was approximately 4.4% moisture, 4.4% ash, 81% total carbohydrates, 5.1% total protein, and 2.6% total fat, which was generally similar to the Miracle Fruit Powder described herein (see Section 2.4.2.1-1 for further details). The miraculin content of the Dried Miracle Berries was reported to be 1.86%. The total polyphenol, oxalic acid, trypsin inhibitor, and sum of pyrrolizidine alkaloids content of the Dried Miracle Berries was reported to be approximately 4.33 mg GAE/g, between 0.05 and 0.1%, 0.80 to 0.97 TIU/mg dry weight, and not detected to 7.2 µg/kg, respectively. It was noted that the oxalic acid content was below the levels in fonio and wheat bran, the trypsin inhibitor content was below the levels reported in chia seeds and soy beans. The safety of Dried Miracle Berry was assessed using proprietary compositional and nutritional data, information on the allergenicity potential of the novel food, as well as a battery of safety studies, including genotoxicity, subchronic toxicity study, as well as data related to human exposure. A summary of the safety studies on Dried Miracle Berries reviewed by EFSA is provided in Table 6.2-2 below. EFSA concluded that Dried Miracle Berries does not have genotoxic potential based on the information on the ingredient, and the no-observed-adverse-effect-level (NOAEL) of Dried Miracle Berries was concluded to be 2,000 mg/kg body weight/day. The reported NOAEL is 14 times greater than the total population consumer-only mean intakes of Miracle Fruit Farm's miracle fruit powder, *i.e.*, 140 mg/kg body weight/day.

Table 6.2-2 Summary of Genotoxicity and Toxicity Studies on Dried Miracle Berries (EFSA, 2021)

Study	Test System	Concentration/Dose	Outcome
Bacterial reverse mutation test (OECD TG 471)	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 µg/plate (±S9)	Equivocal
Bacterial reverse mutation test (OECD TG 471)	*S*. Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 µg/plate (±S9)	Negative
In vivo mammalian erythrocyte micronucleus test (OECD TG 474)	Wistar rats	2,000 mg/kg bw/day	Undetermined (due to uncertainty whether the test article reached target cells)
In vitro mammalian cell micronucleus test (OECD TG 487)	Mouse lymphoma L5178Y TK+/– 3.7.2C cells	Up to 1,000 µg/mL	Negative
Acute oral toxicity study by Up-and-Down Procedure (OECD TG 425)	Wistar rats	5,000 mg/kg bw/day	No adverse effects reported
90-day repeated dose oral toxicity study with a 14-day recovery period (OECD TG 408, limit test)	Wistar rats	2,000 mg/kg bw/day	NOAEL = 2,000 mg/kg bw/day

bw = body weight; OECD = Organisation for Economic and Cooperative Development; TG = test guideline.

In addition to the animal toxicology studies, EFSA reviewed a number of human studies evaluating the taste altering properties of different products derived from *S. dulcificum* fruits (Capitanio *et al.,* 2011; Wong and Kern, 2011; Wilken and Satiroff, 2012; Igarashi *et al.,* 2013; Rodrigues *et al.,* 2016; Hudson *et al.,* 2018; Andrade *et al.,* 2019; Tafazoli *et al.,* 2019). EFSA noted that these studies did not evaluate safety-related parameters that would preclude their use in a safety assessment; however, no adverse effects regarding possible localized effects, such as irritation on the tongue and/or mouth, were reported following consumption of up to 600 mg for period of 2 weeks (EFSA, 2021). It was also noted by EFSA that chronic consumption of Dried Miracle Berries is not expected to pose any safety concern considering that the available sensory data indicate that the taste-altering effect of miraculin has a "*rapid onset and disappearance with no desensitizing impact on the receptors*", and that taste cells have regenerative capabilities with an approximate life span of 8 to 22 days (EFSA, 2021). EFSA concluded that the NOAEL determined from the 90-day oral toxicity study supports a daily intake of 0.7 g/day, considering a margin of exposure of 200 to ensure safety of the Dried Miracle Berries.

6.3 Compositional Analyses

6.3.1 Compositional Similarities between Miracle Fruit Powder and Miracle Fruit Pulp

Miracle fruit powder is minimally processed and is produced by pulping, maceration, and freeze-drying de-seeded miracle fruit berries without the use of any solvents or chemical processing aids. As such, the composition of miracle fruit powder is expected to be similar to miracle fruit berries/pulp, from which they are obtained. This is demonstrated through analytical data wherein, the chemical composition of 3 non-consecutive batches of miracle fruit powder was compared with 3 batches of miracle fruit pulp from which the powders where obtained. As presented in Table 6.3.1-1, the results of this analysis demonstrate that miracle fruit powder is compositionally similar to miracle fruit pulp from which it was obtained without the seed. The difference between the moisture content in miracle fruit pulp and powder is due to the removal of the moisture from pulp during the freeze-drying process involved in the production of miracle fruit powder.

Table 6.3.1-1 Analysis of 3 Non-Consecutive Batches of Miracle Fruit Powder and Miracle Fruit Pulp

Specification Parameter	Manufacturing Lot No.					
	KVS20200506AA_ Pulp 050401A	KVS20200506AD_ Powder 2020-19-001A	KVS20200506AB_ Pulp 050402B	KVS20200506AE_ Powder 2020-19-002B	KVS20200506AC_ Pulp 050403C	KVS20200506AF_ Powder 2020-19-003C
	Miracle Fruit Pulp	Miracle Fruit Powder	Miracle Fruit Pulp	Miracle Fruit Powder	Miracle Fruit Pulp	Miracle Fruit Powder
Carbohydrates (%, dry basis)	87.2	86.2	86.1	85.4	87.50	86.9
Total dietary fiber (%, dry basis)	10.4	10.9	9.14	11.8	13.4	10.2
Total fatty acids (%, dry basis)	0.78	0.788	0.812	0.872	0.836	0.811
Protein (%, dry basis)	8.85	7.56	9.72	8.34	8.43	7.22
Ash (%, dry basis)	3.36	5.45	3.70	5.41	3.52	5.11
Moisture (%)	84.5	5.63	85.1	5.91	84.8	4.53
Miraculin (%, dry basis)*	0.32	0.28	0.30	0.24	0.33	0.32

*The values are corrected for moisture content.

6.3.2 Compositional Similarities between Miracle Fruit Powder and Commonly Consumed Berries

The proximate analysis of miracle fruit powder has been compared with that of commonly consumed berries, such as blueberries, blackberries, and raspberries. As presented in Table 6.3.2-1, the proximate composition of miracle fruit powder is similar to other commonly consumed berries, with the exception of moisture content, considering that miracle fruit powder undergoes freeze-drying to obtain the powder form.

Table 6.3.2-1 A Proximate Analysis Comparison of Miracle Fruit Powder with Commonly Consumed Berries

Parameter	Miracle Fruit Powder[a]	Miracle Fruit Pulp[a]	Blueberries (USDA, 2020a)[b]	Blackberries (USDA, 2020b)[b]	Raspberries (USDA, 2020c)[b]
Carbohydrate (%, dry basis)	~86	~87	~90	80	~86
Protein (%, dry basis)	~8	~9	~4	~11	~8.5
Ash (%, dry basis)	~5	~3.5	~1	~3	N/A
Fat (%, dry basis)	~0.8	~0.8	~2	~4	5
Moisture (%)	~5.0	~85	~84	~88	~86

N/A = not available.

[a] The values are the mean of the 3 batches in Table 6.3.1-1.

[b] The "as is" values have been corrected for moisture content for ease of comparison.

6.4 Exposure to Antinutrients from Proposed Uses of Miracle Fruit Powder

Several production batches of miracle fruit powder have been analyzed for chemical, microbiological, and environmental contaminants originating from the manufacturing process or cultivation practices (see Section 2.5). The results of this analysis demonstrate that the final ingredient is absent of contaminants (*e.g.*, heavy metals, pesticides, and mycotoxins) and microbiological hazards, and the levels are in compliance with the established product specifications.

Analysis of 3 production batches of miracle fruit powder demonstrate the presence of low levels of oxalic acid (ranging between 1,170 to 1,350 ppm – see Section 2.5.1). Miracle fruit pulp does not contain any detectable levels of oxalic acid[2] (<2,630 ppm), and the miracle fruit powder and miracle fruit pulp does not contain any detectable levels of phytic acid or trypsin inhibitors. The oxalic acid content of miracle fruit powder was compared against the levels naturally occurring in several commonly consumed fruits. On a serving size basis, the proposed food uses of miracle fruit powder would provide approximately 12.0 mg/day (mean) or 24.8 mg/day (90th percentile) of oxalic acid in the highest consumer-only intake population group (*i.e.*, male adults). In comparison, one serving of black raspberries or concord grapes would provide approximately 82.5 or 37.5 mg/day of oxalic acid, which is appreciably greater than the amount of oxalic acid contributed by miracle fruit powder (USDA, 2020c,d).

[2] The same method of analysis was used to determine the oxalic acid content of the miracle fruit powder and miracle fruit pulp. The difference in the LOQ is due to the higher water content of the miracle fruit pulp (*ca.* 85%).

In a study by Du *et al*. (2014), the total phenolic content of the berry flesh was reported to be 1,448.3 mg GAE/100 g fresh fruit or 14.48 mg GAE/g. The level of total polyphenols in miracle berry flesh is comparable to those analyzed for 3 batches of Miracle Fruit Farm's miracle fruit powder (obtained from deseeded berries) (see Section 2.5.1) with values ranging from 17.3 to 17.5 mg GAE/g. The total polyphenol content of miracle berry reported in the Du *et al*., 2014 study is higher than that reported for other berry fruits, such as blackberry (435 mg GAE/100 g), blueberry (348 mg GAE/100 g), or strawberry (83.9 mg GAE/100 g) (Heinonen *et al*., 1998; León-González *et al*., 2013). While the level of total polyphenols per gram of fruit may be lower in standard berry fruits that are consumed on a daily basis, when compared from a serving size perspective, the total polyphenol intake level of miracle fruit powder is found to be lower than those reported for these berries. For example, the total polyphenol content of blackberries per serving size of 100 g (USDA, 2020b) would be 435 mg/serving, whereas the highest 90th percentile consumer-only intake of miracle fruit powder from all proposed uses of 20.1 g/day (male adults) (see Table 3.2.2-1) and a mean polyphenol content of 17.4 mg GAE/g (see Table 2.5.1-1) in miracle fruit powder, result in a total polyphenol intake of 350 mg/day, which is lower than the intakes of polyphenols from 1 serving size of blackberries (435 mg/serving). In comparison, the background dietary intakes of polyphenols that occur naturally in foods was addressed in GRN 497 (Amino Up Chemical Co., Ltd., 2013; U.S. FDA, 2014). The Applicant noted that consumption of the recommended 5 servings of fruits and vegetables on a daily basis would provide over 500 mg of polyphenols (Williamson and Holst, 2008; Martin and Appel, 2010), with total daily intake ranging between 100 to over 2,000 mg from a typical balanced diet (Clifford, 2004). Recent literature suggests the dietary intakes of polyphenols in the American population to be in the range of 900 mg/day or up to 900 mg per 1,000 kcal per day, which is in the range reported within GRN 497 (Del Bo' *et al*., 2019; Huang *et al*., 2020). In a publication investigating the toxicological profile of a water-soluble ingredient obtained from oil palm fruit, conducted in accordance with Organization for Economic Co-operation and Development (OECD) Test Guideline No. 408, a NOAEL of 2,000 mg/kg body weight/day was reported (Lynch *et al*., 2017). The test article was reported to contain approximately 4.4% polyphenols as GAE. The reported NOAEL of 2,000 mg/kg body weight/day supports a polyphenol intake of up to 6.2 g/day for a 70-kg individual. Therefore, considering the proposed food uses of the miracle fruit powder, as well as the background dietary intakes of polyphenols in a typical American diet, it is not expected that the levels of polyphenols from miracle fruit powder would pose any increased safety concern in the U.S. population.

6.5 Publicly Available Safety Data on Miraculin

The search of the scientific literature identified one publication related to the *in vitro* digestibility and safety of the glycoprotein miraculin (*i.e*., allergenicity, toxigenicity) using *in silico* tools (Tafazoli *et al*., 2019, 2020). The results of this study are discussed in further detail in the sections that follow.

6.5.1 Metabolic Data on Miraculin

6.5.1.1 In Silico Prediction of Digestibility of Miraculin

The digestibility of the 191 amino acid sequence of miraculin (*i.e*., without the signal peptide) was predicted using the PeptideCutter tool maintained by Expasy. PeptideCutter predicts possible cleavage sites within a peptide sequence by proteases under various gastric conditions. The *in silico* digestibility of the glycoprotein was predicted with pepsin (pH >2.0). PeptideCutter predicted 48 cleavage sites along the miraculin peptide sequence, with the resulting lengths of the peptide digest ranging between 1 and 19 amino acids (see Table 6.5.1.1-1).

Table 6.5.1.1-1 Results of *In Silico* Pepsin Digestion (pH 2.0) of Miracle Fruit Protein (Miraculin) Using PeptideCutter

Position of Cleavage Site	Enzyme (pH)	Resulting Peptide Sequence	Peptide Length (amino acids)	Peptide Mass (Da)
8	Pepsin (pH>2)	DSAPNPVL	8	811.890
14	Pepsin (pH>2)	DIDGEK	6	675.693
15	Pepsin (pH>2)	L	1	131.175
20	Pepsin (pH>2)	RTGTN	5	547.569
21	Pepsin (pH>2)	Y	1	181.191
22	Pepsin (pH>2)	Y	1	181.191
27	Pepsin (pH>2)	IVPVL	5	539.716
33	Pepsin (pH>2)	RDHGGG	6	597.588
34	Pepsin (pH>2)	L	1	131.175
44	Pepsin (pH>2)	TVSATTPNGT	10	947.998
45	Pepsin (pH>2)	F	1	165.192
63	Pepsin (pH>2)	VCPPRVVQTRKEVDHDRP	18	2131.441
65	Pepsin (pH>2)	LA	2	202.253
67	Pepsin (pH>2)	FF	2	312.368
81	Pepsin (pH>2)	PENPKEDVVRVSTD	14	1584.703
82	Pepsin (pH>2)	L	1	131.175
85	Pepsin (pH>2)	NIN	3	359.382
86	Pepsin (pH>2)	F	1	165.192
88	Pepsin (pH>2)	SA	2	176.172
94	Pepsin (pH>2)	FMPCRW	6	839.041
99	Pepsin (pH>2)	TSSTV	5	493.514
100	Pepsin (pH>2)	W	1	204.228
102	Pepsin (pH>2)	RL	2	287.362
104	Pepsin (pH>2)	DK	2	261.278
105	Pepsin (pH>2)	Y	1	181.191
111	Pepsin (pH>2)	DESTGQ	6	635.585
112	Pepsin (pH>2)	Y	1	181.191
113	Pepsin (pH>2)	F	1	165.192
130	Pepsin (pH>2)	VTIGGVKGNPGPETISS	17	1612.800
131	Pepsin (pH>2)	W	1	204.228
132	Pepsin (pH>2)	F	1	165.192
136	Pepsin (pH>2)	KIEE	4	517.580
137	Pepsin (pH>2)	F	1	165.192
141	Pepsin (pH>2)	CGSG	4	322.336
142	Pepsin (pH>2)	F	1	165.192
143	Pepsin (pH>2)	Y	1	181.191
144	Pepsin (pH>2)	K	1	146.189
145	Pepsin (pH>2)	L	1	131.175
164	Pepsin (pH>2)	VFCPTVCGSCKVKCGDVGI	19	1915.329
165	Pepsin (pH>2)	Y	1	181.191
176	Pepsin (pH>2)	IDQKGRRRLAL	11	1325.580
180	Pepsin (pH>2)	SDKP	4	445.473

Table 6.5.1.1-1 Results of *In Silico* Pepsin Digestion (pH 2.0) of Miracle Fruit Protein (Miraculin) Using PeptideCutter

Position of Cleavage Site	Enzyme (pH)	Resulting Peptide Sequence	Peptide Length (amino acids)	Peptide Mass (Da)
182	Pepsin (pH>2)	FA	2	236.271
183	Pepsin (pH>2)	F	1	165.192
184	Pepsin (pH>2)	E	1	147.131
185	Pepsin (pH>2)	F	1	165.192
190	Pepsin (pH>2)	NKTVY	5	623.707
191	**end of sequence**	F	1	165.192

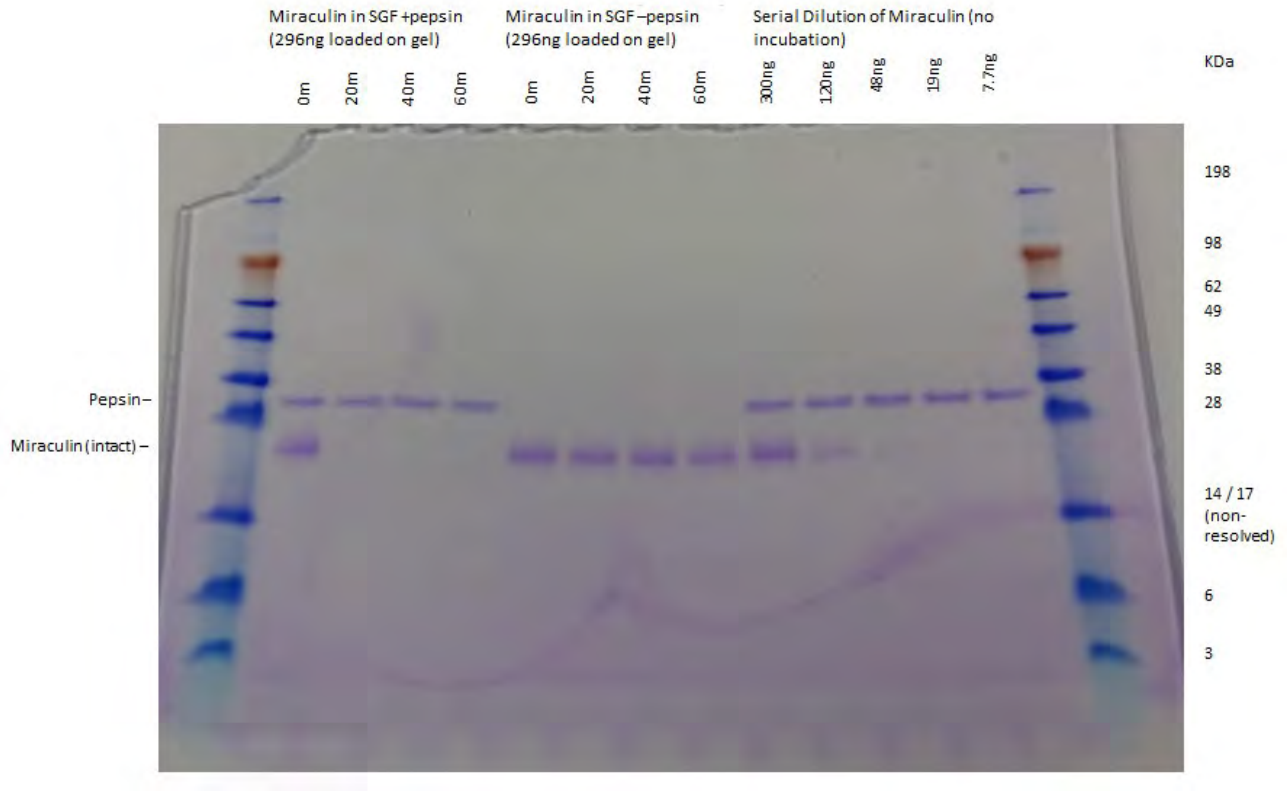
6.5.1.2 In Vitro Digestibility of Miraculin

The protein digestibility of miraculin was reported using an *in vitro* simulated gastric fluid (SGF) model (Tafazoli *et al*., 2019). Miraculin (0.08 mL; 2 mg/mL or 0.1 mg/mL final concentration in reaction) was added to a preincubation mixture consisting of SGF (10 U/µg pepsin) and incubated for up to 60 minutes. After 0, 20, 40, and 60 minutes of incubation, sample mixtures were quenched with sodium bicarbonate, tricine buffer solution, and a reducing agent, and heated at 85°C for 10 minutes. The digestibility of the protein was evaluated by gel electrophoresis after heating. The results of digestion are presented in Figure 6.5.1.2-1 and demonstrate that miraculin was completely digested within 20 minutes.

The effect of pepsin concentration on the digestion of miraculin was investigated in a second experiment in which miraculin was added to SGF containing 5.45 U/µg or 10 U/µg of pepsin, and incubated for 0, 1, 5, or 10 minutes. Miraculin was fully digested within 1 minute, indicating that the protein is rapidly metabolized, and the digestion kinetics are pepsin-dependent.

These results indicate that, following ingestion, miraculin would be completely and rapidly digested in the gastrointestinal tract.

Figure 6.5.1.2-1 Results of Digestion of Miraculin in Simulated Gastric Fluid at 37°C



The *in vitro* digestibility of miraculin (purity 85 to 90%) was investigated using the methods described by Thomas *et al.* (2004). The glycoprotein and digestion control proteins (pepsin sensitive[3] or resistant[4] proteins) were dissolved at 3.67 mg/mL in phosphate buffer saline (pH 7.0) or deionized water. For each protein, a sample tube containing 1.52 mL of SGF (1,600 U pepsin) was pre-heated to 37°C for 10 minutes prior to the addition of 0.08 mL of the glycoprotein or digestion control proteins. The test concentrations were 3.67 mg/mL or 2.00 mg/mL, providing either 5.45 U pepsin/µg protein or 10 U pepsin/µg protein, respectively. The samples were mixed by vortexing and placed in a 37°C water bath for up to 4 hours. At each timepoint, 100 µL of each sample was removed and quenched by sodium bicarbonate, buffer solution, and reducing agent. At time point 0, samples were quenched prior to the addition of test proteins. Quenched samples were heated for 10 minutes at 85°C and stored at -20°C. Samples were thawed and loaded onto tricine gels to run for 80 minutes at 125V. Serial dilutions of miraculin were prepared identical to time 0 samples. Gels were stained with Coomassie Blue or silver staining.

[3] Bovine serum albumin and peroxidase from horseradish served as pepsin-sensitive control proteins.
[4] Albumin from chicken egg white served as the pepsin-resistant control protein.

Miraculin was demonstrated to be completely digested within 20 minutes in the presence of SGF containing pepsin at a concentration of 10 U/µg protein (see Figure 6.5.1.2-1). No peptide fragments were detected. In a subsequent study with shorter digestion times (*i.e.*, 0, 1, 5, and 10 minutes), as well as a lower pepsin concentration (5.45 U/µg protein), similar results were reported (see Figure 6.5.1.2-2). The miraculin protein was rapidly digested within 1 minute. The digestion control proteins were digested as expected, and pepsin was reported to be intact throughout the incubation period. Miraculin was stable and intact at all timepoints in SGF without pepsin, suggesting that digestion of the glycoprotein is pepsin dependent. Fragments of the miraculin protein were not detected, indicating that digestion with pepsin is rapid and extensive.

Figure 6.5.1.2-2 Results of Digestion of Miraculin and Digestion Control Proteins at Different Concentrations of Pepsin (5.45 and 10 U/µg) for 0, 1, 5, and 10 Minutes (Coomassie Blue Staining)

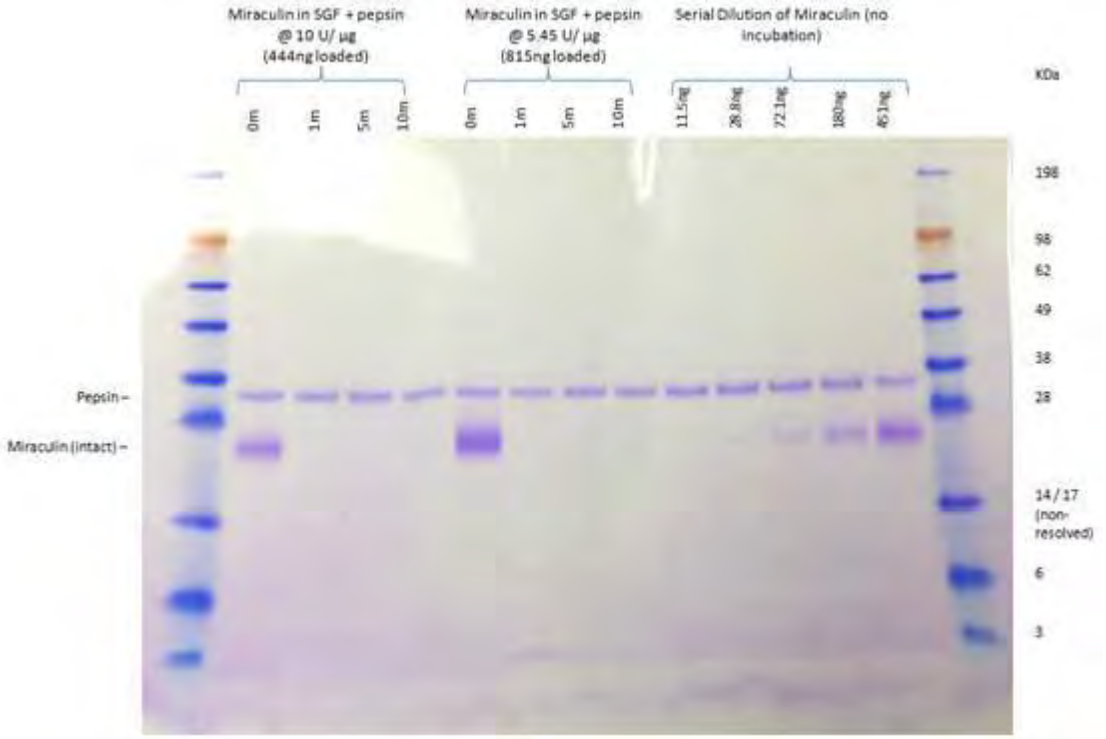
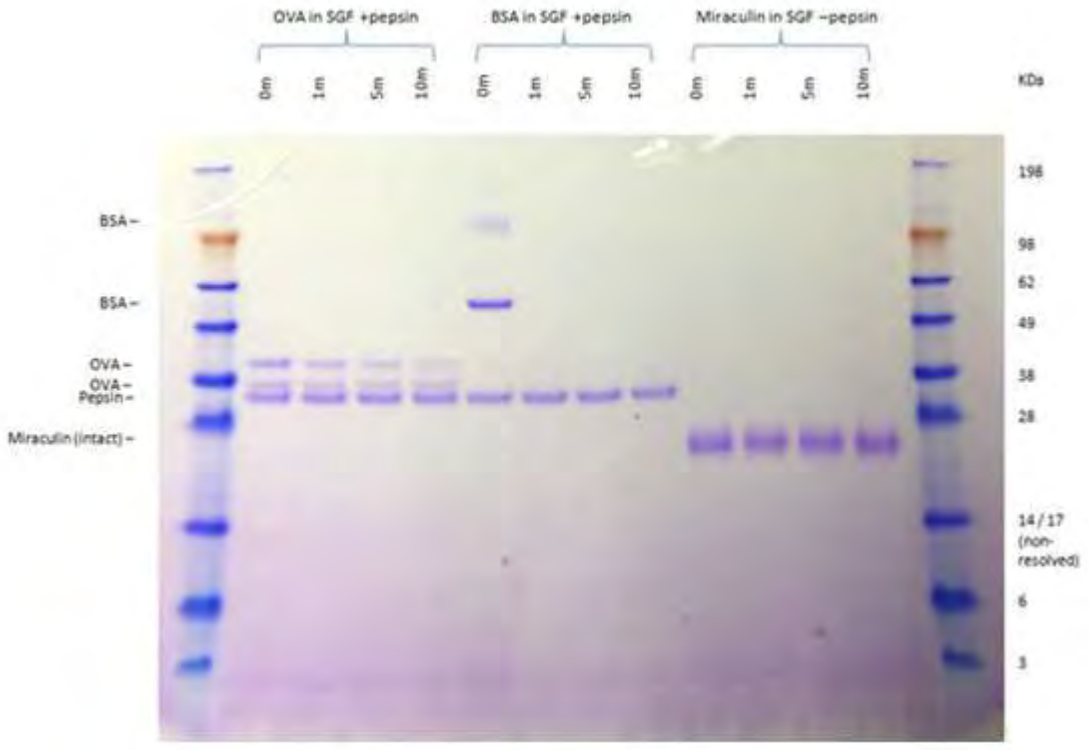
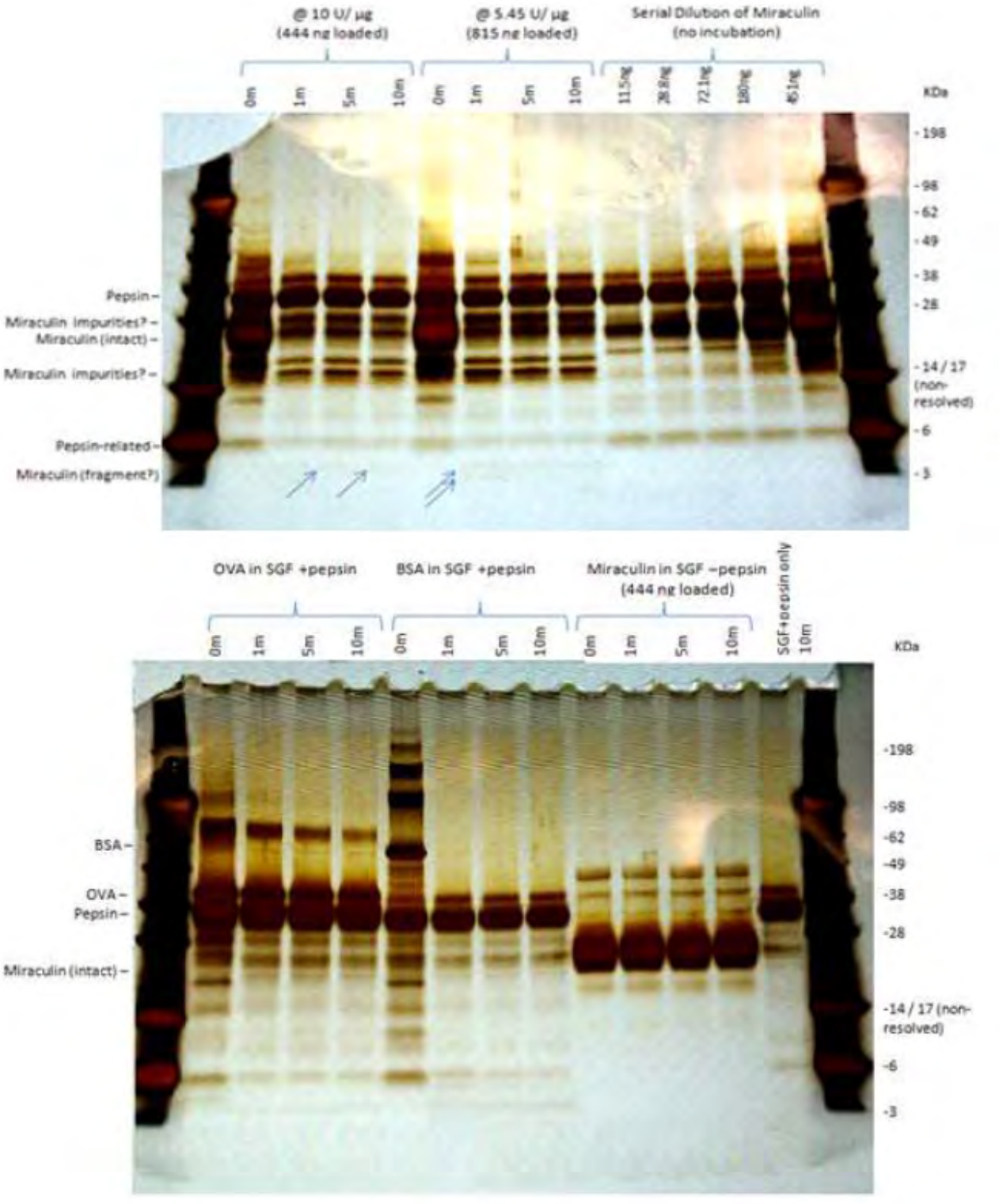


Figure 6.5.1.2-2 Results of Digestion of Miraculin and Digestion Control Proteins at Different Concentrations of Pepsin (5.45 and 10 U/µg) for 0, 1, 5, and 10 Minutes (Coomassie Blue Staining)



Due to the low sensitivity of Coomassie Blue staining and in order to enhance detection of lower abundance peptides, the above experiment was repeated using the more sensitive Silver Staining approach. Following digestion by pepsin for 0, 1, 5, and 10 minutes, the results of the Silver Staining corroborated the previous findings that miraculin was rapidly digested at both 5.45 and 10 U pepsin/µg protein. Minor peptide fragments were reported at approximately 4 kDa at both concentrations within 10 minutes of digestion. The other bands were reported to be pepsin or miraculin impurities, as similar bands were observed at time point 0 and serial dilutions of miraculin, which were not digested (see Figure 6.5.1.2-3).

Figure 6.5.1.2-3 ***In Vitro*** **Digestion of Miraculin and Digestion Control Proteins at Different Concentrations of Pepsin (5.45 and 10 U/µg) for 0, 1, 5, and 10 Minutes (Silver Staining)**



Arrows denote minor potential miraculin digestion fragments.

6.5.1.3 Proteolytic Fate of Miraculin

Using the same digestion method as described in Section 6.2.1, Tafazoli *et al.* (2019) reported the proteolytic fate of the miraculin protein following pepsin digestion. The digested peptides of miraculin were evaluated using LC-MS/MS. Miraculin was added to SGF containing 5.45 U/µg pepsin and incubated for up to 10 minutes at 37°C. Digest samples were collected after 0, 0.5, 1, and 10 minutes. Tafazoli *et al.* (2019) reported that miraculin was increasingly digested with a longer digestion time (*i.e.*, number of unique peptides increased with longer digestion periods). After 0 minutes of digestion, the authors reported 5 unique peptides while the number of unique peptides after 0.5, 1, and 10 minutes of digestion were 33, 54, and 61, respectively. The number of unique peptides after 10 minutes of pepsin digestion encompassed approximately 75% of the amino acid sequence. The authors reported that the only peptides that were not identified after 10 minutes of digestion were peptides with cysteine residues (*i.e.*, disulfide bonds) that may be resistant to digestion. The authors further evaluated the unique peptides from miraculin digestion for their allergenic potential (see Section 6.3 for further details). The authors concluded that the *in vitro* digestibility studies suggest that miraculin, following ingestion, would be rapidly and completely metabolized into small peptides, and ultimately its amino acid components. This was further supported by the results of the Silver Staining method over a longer digestion period of up to 240 min, demonstrating that in the *in vitro* digestibility study, miraculin was rapidly digested to fragments that had a molecular weight below the detection limit of the gel electrophoresis method (*i.e.*, 2 to 5 kDa), suggesting the complete digestion of the protein.

6.5.2 Bioinformatics Assessment of Miraculin

The amino acid sequence of miraculin (UniProt Accession No. P13087) was subject to bioinformatic analyses to predict the potential allergenicity of miraculin and to determine whether the candidate protein shares any structural homology to known toxins in the absence of sufficient *in vivo* toxicological data. Structural homology between the candidate proteins and known allergens or toxins may suggest the candidate protein has allergenic or toxic potential. Interpretation of the sequence alignment data involved an evaluation of the percent identity (*i.e.*, quantification of amino acid alignment between the queried protein and a known allergen or toxin) and the expectation value (E-value) and query cover. Based upon the information from these bioinformatics searches, additional information and experimental approaches can be directed, as might be necessary, to reach conclusions on the allergenic or toxic potential of a candidate protein. The results of these searches are summarized in the sections that follow.

6.5.2.1 Allergenicity of Miraculin

The potential allergenicity of miraculin (UniProt Accession No. P13087) was investigated using bioinformatics searches on the AllergenOnline Database[5] (version 21; updated 14 February 2021) (FARRP, 2021) and Allermatch Database[6] (updated 04 July 2019) to determine whether the amino acid sequence of miraculin shares similarity to known allergens. The databases contain a comprehensive list of putative allergenic proteins developed *via* a peer reviewed process for the purpose of assessing the potential allergenicity of novel proteins. In addition, the Allermatch database was constructed using the allergenic proteins from the COMPARE[7], UniProt[8], and WHO/IUIS[9] allergen databases. Sequence homology

[5] http://www.allergenonline.org/.
[6] http://www.allermatch.org/.
[7] https://comparedatabase.org/.
[8] https://www.uniprot.org/docs/allergen.
[9] http://www.allergen.org/.

searches of the full-length amino acid sequence and 80-amino acid sliding window alignment were conducted using FASTA. The results are summarized in Table 6.5.2.1-1 below.

In the full-length amino acid sequence search, an identity cut-off value of 50% was used as allergic cross-reactivity may occur at matches greater than 50% (Aalberse, 2000). However, it is noted that cross-reactivity is rare at 50%, and, in fact, allergic cross-reactivity may require greater than 70% identity over the full-length sequence (Aalberse, 2000). Nevertheless, in the full-length amino acid sequence search using the AllergenOnline and Allermatch databases, no hits greater than 50% identity were identified, suggesting the unlikely potential for cross-reactivity to putative allergens.

In the 80-amino acid sliding window alignment search, segments of 80-amino acids (*e.g.*, 1–80, 2–81, 3–82, *etc.*) derived from each full-length amino acid sequence were searched in accordance with the methodology described by the FAO/WHO (2001) and Codex Alimentarius (2003, 2009). Significant homology was defined as an identity match of greater than 35% in accordance with the FAO/WHO (2001) and Codex Alimentarius (2003, 2009) criteria. Immunoglobulin E (IgE) cross-reactivity to putative allergens may be considered a possibility at matches greater than 35% identity. A number of sequences with identity matches ranging from 36 to 39% with known allergens from commonly consumed agricultural products, *Solanum tuberosum* (potato) and *Glycine max* (soybean), were identified using AllergenOnline (see Table 6.5.2.1-1). The clinical significance of low identity matches (35 to 40% over 80 amino acid windows) is questionable; the recommended criterion of >35% identity over 80 amino acid windows is quite conservative and other factors should be considered when the percent identity is low (Goodman, 2006).

Table 6.5.2.1-1 Search Results of AllergenOnline (Version 21) and Allermatch

Sequence Identifier	Source	Description	80 mer		Full Length		
			% Identity	# Hits (>35%)	Length	E-value	% Identity
AllergenOnline							
994779	*Solanum tuberosum*	Proteinase inhibitor	39.30	23/141	227	$4.0x10^{-12}$	28.60
124148	*Solanum tuberosum*	Aspartic protease inhibitor 11	35.80	6/141	194	$1.6x10^{-11}$	29.9
256429	*Glycine max*	Kunitz trypsin inhibitor KTi	37.54	12/141	215	$1.8x10^{-11}$	31.6
18770	*Glycine max*	Trypsin inhibitor subtype A	37.5	12/141	215	$1.8x10^{-11}$	31.6
256635	*Glycine max*	Kunitz trypsin inhibitor KTi1	37.54	14/141	212	$1.6x10^{-6}$	33.5
18772	*Glycine max*	Trypsin inhibitor subtype B	37.5	12/141	215	$4.5x10^{-8}$	31.6
256636	*Glycine max*	Kunitz trypsin inhibitor KTi2	37.54	8/141	213	$3.2x10^{-5}$	32.4
Allermatch							
P25273	*Glycine max*	Kunitz-type trypsin inhibitor KTI2	37.52	8	187	-	33.7
Q39899	*Glycine max*	Kunitz trypsin inhibitor	35.04	2	189	-	32.8
Q39898	*Glycine max*	Kunitz trypsin inhibitor	35.04	2	189	-	32.8
P01070	*Glycine max*	Trypsin inhibitor A	35.04	2	189	-	32.8
P25272	*Glycine max*	Kunitz-type trypsin inhibitor KTI1	36.23	7	186	-	34.9

The potential for cross-reactivity between miraculin and these potato and soybean trypsin inhibitors is low based on the lack of significant full-length identity and the rather low (35 to 39%) identities over sliding 80-mer windows between the potato and soybean trypsin inhibitors and miraculin. Also, although these trypsin inhibitors are recognized as known allergens, neither the potato proteinase inhibitors nor the soy Kunitz trypsin inhibitor are considered as important food allergens from a clinical context (Taylor *et al.*, 2015).

While the soybean Kunitz trypsin inhibitor is one of the known allergens from soy, this protein is very infrequently identified as an allergen in investigations conducted with soy-allergic patients. The soybean Kunitz trypsin inhibitor (SKTI) consists of 181 amino acids and represents 4 to 7% of the total extractable protein in soy. SKTI is a tightly packed protein with 2 disulfide bonds between Cys39-Cys86 and Cys138-Cys145, both of which are critical for trypsin inhibition and resistance to denaturation (Sessa and Ghantous, 1987). SKTI is not glycosylated and trypsin inhibition is achieved through reversible binding of SKTI to the trypsin enzyme (Kunitz, 1947; Friedman and Brandon, 2001; Barać *et al*., 2004; Mikić *et al.*, 2009). SKTI is an inhalation allergen associated with occupational exposure to flour dust in bakers (Baur *et al*., 1996; Quirce *et al.*, 2006). As an allergen, SKTI primarily affects bakers exposed to large amounts of inhaled soy flour. Individuals with SKTI-induced baker's asthma have serum IgE specific for binding to SKTI, positive skin prick test to extracts of SKTI, and reactions during a specific inhalation challenge with purified SKTI (Baur *et al.*, 1996; Quirce *et al.*, 2006). The incidence of inhaled SKTI related allergic reactions is very low. Ingestion of SKTI has only been confirmed to cause an allergic reaction in 1 individual, although symptoms were severe in this case (Moroz and Yang, 1980). This patient's sensitization to SKTI may not have occurred through the ingestion of soybeans because the patient worked in a biochemical laboratory that used SKTI in experiments. Although this report in 1980 was one of the first identifications of a soybean allergen, no other cases of food-related soybean allergies have been attributable to SKTI in the intervening years. Instead, the major soy allergens have been identified as Gly m 5 (conglycinin), Gly m 6 (glycinin), Gly m 4 (a starvation associated message protein cross-reactive to the major birch tree pollen allergen, Bet v 1), and perhaps Gly m 8 (a 2S albumin) (Kattan and Sampson, 2015; Taylor *et al*., 2015).

The major allergen in potatoes is Sol t 1, a 43 kDa protein known as patatin (Seppälä *et al*., 1999; Astwood *et al*., 2000; Majamaa *et al*., 2001). Patatin is the main storage protein of the potato tuber. Potatoes also contain several proteinase inhibitors that have been identified as allergens by Seppälä *et al*., 2000, 2001. The initial study investigated 12 patients sensitized to raw potato. On subsequent investigation, only 7 of these 12 subjects reacted to oral challenges with cooked potato (Seppälä *et al.*, 2001); the other patients had positive reactions when their skin was rubbed with raw potato. Seven of these 12 potato-allergic patients showed IgE binding to proteins in the 20kD region and 3 of 12 to proteins in the 16kD and 18kD regions after electrophoretic separation of potato proteins (Seppälä *et al*., 2000). N-Terminal sequencing of the purified proteins showed that all belonged to the family of Kunitz-type trypsin inhibitors (Seppälä *et al*., 2000). Subsequently, Seppälä *et al.*, 2001 described the 20kD protein as Sol t 2, the 18kD protein as Sol t 3, and the 16 kD protein as Sol t 4. Sol t 2 was further identified as a cathepsin D proteinase inhibitor with an IgE-binding N-glycan region that was identical to the major grass allergen, Lol p 11 (Seppälä *et al*., 2001). In an enzyme-linked immunosorbent assay (ELISA), 43 to 67% of 39 allergic children showed specific IgE binding to the new allergens, Sol t 2–4 (Seppälä *et al*., 2001). However, oral challenges were not conducted on the additional 27 potato-sensitized subjects to demonstrate that they would actually react upon consumption of potato. While Seppälä *et al*. (2001) characterized Sol t 2-4 as major potato allergens, the allergenicity of these proteins has not subsequently been corroborated by other clinical investigators. The importance of these proteinase inhibitors from potato as allergens remains uncertain. Together with the low identity of these proteins with miraculin, the likelihood of cross-reactivity between miraculin and potato seems remote.

The allergenic profile of the glycoprotein miraculin was investigated using AlgPred, which utilizes a support-vector machine (SVM) analysis. The *in silico* search with AlgPred identified the following on the miraculin protein:

1. Non-allergen based on algorithms for IgE epitopes, motif alignment and search tool (MAST), and allergen representative peptides (ARP); and

2. Allergen based on SVM analysis of the amino acid composition and dipeptide composition.

The results are summarized in Table 6.5.2.1-2. AlgPred was used to evaluate the allergenicity of soy leghemoglobin, and it was concluded that the reliability of this tool was questionable due to a reported high false positive rate (GRN 737 – U.S. FDA, 2018). Furthermore, it was concluded that allergenicity assessments using established databases, such as AllergenOnline, was "*more than adequate to demonstrate that* […] *have little or no allergenic potential*" (GRN 737 – U.S. FDA, 2018). Considering the multitude of results from established methodologies that have been successfully used in the assessment of allergenicity potential of proteinaceous compounds (FAO/WHO, 2001; Codex Alimentarius, 2009; EFSA, 2017), it can be concluded that miraculin has a low risk of allergenicity.

The allergenicity potential of miraculin was also evaluated using AllerTOP (version 2.0), which is based on auto cross covariance (ACC) transformation of protein sequences into uniform equal-length vectors (Wold *et al*., 1993). AllerTOP predicted the miraculin sequence to be a "probable non-allergen", with the nearest protein to be *beta*-galactosidase (Accession No. P48980), a non-allergen.

Table 6.5.2.1-2 Assessment of the Allergenicity Potential of Miraculin Using AlgPred

Algorithm	Result	Sensitivity (True Allergen)	Specificity (True Non-Allergen)	Error Rate (False Allergen)	Analysis Type
IgE Epitopes	Non-Allergen	10.84%	98.25%	1.75%	Sequence Motif
Motif Alignment and Search Tool (MAST)	Non-Allergen	22.05%	86.68%	13.32%	Sequence Motif
Allergen Representative Peptides (ARP)	Non-Allergen	66.56%	97.97%	2.03%	Sequence Motif
Support Vector Machine (SVM) Amino Acid Composition	Allergen	84.21%	56.07%	43.93%	Amino Acid Composition
Support Vector Machine (SVM) Dipeptide Composition	Allergen	84.83%	61.09%	38.91%	Amino Acid Composition

As part of the novel food application for Dried Miracle Berries, EFSA evaluated the allergenicity potential of Dried Miracle Berries (EFSA, 2021). The total protein fraction of Dried Miracle Berries was between 5 and 6%, of which, the glycoprotein miraculin comprised 15 to 40% of this amount (*i.e.,* between 0.75 to 2.4% miraculin in the final product). In the EFSA opinion, it was noted that no allergic reactions have been reported in the scientific literature following consumption of miracle fruits/berries. EFSA (2021) reported the results of an *in silico* search of miraculin (UnitProt No. P13087) against the National Center for Biotechnology Information (NCBI) amino acid database, and matches greater than 50% identity were identified to belonging to proteins from peach, sesame, and bitter lemon (Kunitz trypsin inhibitor 2) and miraculin precursor from tomato. In addition, the sequential identity between miraculin and widely known protein allergens from other plants was evaluated by EFSA, and the results indicated sequence identities of 83% with latex, 50% with peach, 53% with soy, and 80% with peanuts, with low query coverage across all matches (EFSA, 2021). It was concluded that there are "*no significant homology between miraculin and*

widely known pan-allergens" using the Basic Local Alignment Search Tool (BLAST) maintained by the NCBI (EFSA, 2021). The EFSA Panel concluded that these *in silico* findings are preliminary and do not allow to draw definitive conclusions on cross-reactivity. The results of Tafazoli *et al.* (2019, 2020) were also reviewed and discussed by EFSA, as well as a preliminary ELISA screening of the novel food (Dried Miracle Berries) with the major food allergens, in which positive results for peanut allergens were identified.

A BLAST search against the NCBI database with the results limited to proteins originating from *Arachis hypogaea* (peanut) did not identify any significant sequence homology to known peanut allergens. The highest identity match (50.52%) was to miraculin from peanuts; the query coverage was 85% and E-value of 10^{-53} suggest this match to be significant. It is noted that peanuts are one of the most well characterized foods with respect to allergenic proteins; miraculin from peanuts has not been identified as a major allergenic protein. A search of the AllergenOnline database confirm that miraculin from peanuts is not an allergenic protein. Likewise, the search results using internationally recognized guidelines for *in silico* allergenicity assessments revealed sequence homology matches to proteins from soybean and potatoes, with no matches to proteins from peanuts (see Table 6.5.2.1-1). It is also noted that the details of the *in silico* searches performed by the applicant of Dried Miracle Berries, as well as the methodology of the ELISA test are scarce, and would be difficult to ascertain and replicate the findings (EFSA, 2021). Therefore, based on the totality of the evidence discussed herein, and on the basis of the results from various *in silico* assessments, it can be concluded that miracle fruit powder has low risk for allergenicity or cross-reactivity with any major protein allergens, including peanuts.

6.5.2.2 Allergenicity Assessment of Protein Digests of Miraculin

The allergenic potential of peptide digests of miraculin was evaluated in a recent publication (Tafazoli *et al*., 2019, 2020). As discussed in the publication and also Section 6.2.2, miraculin was digested with SGF containing pepsin for up to 10 minutes and the resulting peptides were characterized by LC-MS/MS. The authors reported 61 unique peptides from the digested miraculin protein. The 61 identified peptides were evaluated for allergenicity potential using a similar approach as described above (*i.e*., full-length amino acid sequence and 80-amino acid sliding window). The full-length search of each peptide digest revealed a number of matches with known allergens, with identity scores ranging from 36 to 67% and similarity scores ranging from 60 to 100%. The corresponding E-values ranged from 0.00067 to 0.95 with an amino acid overlap of 8 to 25. Therefore, considering the high E-values and an identity match of less than 67% over a short amino acid coverage (<25), it is unlikely that these peptide digests would pose any allergenic risk (Aalberse, 2000). The 80-amino acid sliding window searches with each peptide digest did not reveal any significant structural homology with any known allergens. The authors concluded that the results of the *in silico* searches with the peptide digests do not suggest that miraculin will pose a risk of cross-reactivity with known allergens (Tafazoli *et al*., 2019, 2020).

In order to enhance detection of lower abundance peptide fragments that were detected within 10 min of digestion in an *in vitro* digestibility model with SGF, a follow-up experiment was conducted using the more sensitive Silver Staining method over a longer digestion period of 240 min. The results of the Silver Staining demonstrated complete and rapid digestion of miraculin within 20 min to fragments that had a molecular weight below the detection limit of gel electrophoresis method (*i.e.*, 2 to 5 kDa). The weight of the available evidence and the nature of the potential matches to trypsin inhibitors from soy and potato, including *in silico* results from the allergenicity assessment, suggest that miraculin is unlikely to have potential for allergenicity. These conclusions are further corroborated with the fact that exposures to miraculin *via* miracle berry and miracle fruit in commercial products in the U.S. have not been reported to be associated with any allergenic reactions.

6.5.2.3 Toxigenicity of Miraculin

A sequence alignment query was conducted for the miraculin amino acid sequence against downloaded protein sequences obtained from a curated database of animal venom proteins and toxins maintained in the UniProtKB/Swiss-Prot Tox-Prot[10] database. The amino acid sequences were compared using BLAST. The BLAST search results are summarized in Table 6.5.2.3-1 below. Several matches to animal toxins/venoms were identified, with sequence identities ranging from 25 to 54% and corresponding E-values of 0.61 to 9.3 with generally low query coverage (<25%). While there are no formal guidelines established for what constitutes a significant sequence similarity between an introduced protein and protein toxins (Hammond *et al*., 2013), considering the low query coverage and high E-values/scores (Pearson, 2000; Bushey *et al*., 2014) identified for the alignments between miraculin and the animal toxins/venoms, as presented in Table 6.5.2.3-1, the results of the full-length sequence alignment search of miraculin suggest that it does not share homology or structural similarity to any animal venom protein, toxins, virulence factors or harbors any toxic potential.

Table 6.5.2.3-1 Search Results of UniProtKB/Swiss-Prot Tox-Prot with Miraculin

Organism	Description	Sequence Length	Query Cover	E-value	% Identity
Daboia siamensis	Snaclec 5	148	19%	0.61	29%
Daboia siamensis	Snaclec 3	148	19%	0.58	29%
Stichodactyla helianthus	Kappa-stichotoxin-She3a	35	5%	1.8	54%
Lychas mucronatus	Lipolysis-activating peptide 1-alpha chain	83	25%	1.9	25%
Conus leopardus	Alpha-conotoxin-like Lp1.6a	61	9%	6.1	50%
Apis cerana cerana	Mast cell degranulating peptide	50	6%	6.6	53%
Dendroaspis angusticeps	Muscarinic toxin 1	66	23%	7.6	33%
Tityus obscurus	Potassium channel toxin alpha-KTx 13.1	23	8%	7.7	44%
Stichodactyla haddoni	Kappa-stichotoxin-Shd5a	74	14%	8.2	32%
Heterodactyla hemprichii	Kappa-thalatoxin-Hhe2a	75	14%	8.4	32%
Thalassianthus aster	Kappa-thalatoxin-Tas2a	75	5%	8.5	54%
Dendroaspis angusticeps	Muscarinic toxin 4	66	16%	8.6	42%
Ethmostigmus rubripes	U-scoloptoxin(16)-Er4a	130	20%	8.9	37%
Stichodactyla gigantea	Kappa-stichotoxin-Sgt4a	74	14%	9.3	32%

[10] The UniProtKB/Swiss-Prot Tox-Prot database is available at: http://www.uniprot.org/uniprot/?query=taxonomy%3A%22Metazoa+[33208]%22+AND+%28keyword%3Atoxin++OR+annotation%3A%28type%3A%22tissue+specificity%22+AND+venom%29%29+AND+reviewed%3Ayes&sort=score.

6.6 Conclusions

Based on the above data and information presented herein, Miracle Fruit Farm has concluded that the intended uses of miracle fruit powder in beverage and fermented dairy products, as described in Section 1.3, is GRAS based on scientific procedures. General recognition of Miracle Fruit Farms' 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 miracle fruit powder, as described herein is GRAS.

Miracle Fruit Farms' miracle fruit powder; 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*. The GRAS Panel Consensus Statement is provided in Appendix B.

Part 7. § 170.255 List of Supporting Data and Information

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APPENDIX A – LIST OF TESTED ALKALOIDS

List of Tested Alkaloids and their Reporting Limits

Compound	Reporting Limit
Echimidine	1 µg/kg
Echimidine N-oxide	1 µg/kg
Erucifoline	1 µg/kg
Erucifoline N-oxide	1 µg/kg
Europine	1 µg/kg
Europine N-oxide	1 µg/kg
Heliotrine	1 µg/kg
Heliotrine N-oxide	1 µg/kg
Intermedine	1 µg/kg
Intermedine-N-oxide/Indicine-N-oxide	1 µg/kg
Jacobine	1 µg/kg
Jacobine N-oxide	1 µg/kg
Lasiocarpine	1 µg/kg
Lasiocarpine N-oxide	1 µg/kg
Lycopsamine/Indicine	1 µg/kg
Lycopsamine N-oxide	1 µg/kg
Monocrotaline	1 µg/kg
Monocrotaline N-oxide	1 µg/kg
Retrorsine	1 µg/kg
Retrorsine N-oxide	1 µg/kg
Senecionine	1 µg/kg
Senecionine N-oxide	1 µg/kg
Seneciphylline	1 µg/kg
Seneciphylline N-oxide	1 µg/kg
Senecivernine	1 µg/kg
Senecivernine N-oxide	1 µg/kg
Senkirkine	1 µg/kg
Trichodesmine	1 µg/kg
Sum of all positive pyrrolizidinalkaloids	-
Anisodamine	2 µg/kg
Atropine	1 µg/kg
Atropmine-NOx	2 µg/kg
Homatropine	2 µg/kg
Littorin	2 µg/kg
Norscopolamine	2 µg/kg
Scopolamine	1 µg/kg
Scopolamine-N-oxide	1 µg/kg
Sum of Atropin/Scopolamin	-
Sum of Atropin/Scopolamin and their N-oxides	-
Sum of all positive tropanalkaloids	-

APPENDIX B – GRAS PANEL CONSENSUS STATEMENT

GRAS Panel Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Uses of Miracle Fruit Powder

16 September 2020

INTRODUCTION

At the request of Miracle Fruit Farm, LLC. (Miracle Fruit Farm), an Expert Panel (the "GRAS Panel") of independent scientists, qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, conducted a critical and comprehensive assessment of data and information pertinent to the safety miracle fruit powder to determine whether the intended uses of miracle fruit powder as an ingredient in conventional beverages and fermented dairy products, as described in Table A-1, would be Generally Recognized as Safe (GRAS) based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine); Professor Emeritus George C. Fahey, Jr. (University of Illinois); and Professor Stephen L. Taylor (University of Nebraska).

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of publicly available scientific data and information compiled from the literature and summarized in a dossier titled "*Documentation Supporting the Generally Recognized as Safe (GRAS) Status of the Proposed Uses of Miracle Fruit*" (dated 16 April 2020, revised 02 September 2020), which included an evaluation of available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended uses of miracle fruit powder. This dossier was prepared in part from a comprehensive search of the scientific literature performed at the request of Miracle Fruit Farm through February 2020 and included information characterizing the identity and purity of the ingredient, the manufacture of the ingredient, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, and the safety of miracle fruit powder and its active glycoprotein component, miraculin.

Following its independent and collective critical evaluation, and on the basis of scientific procedures, the GRAS Panel unanimously concluded that Miracle Fruit Farm's miracle fruit powder, meeting food-grade specifications and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS for use as an ingredient, as described in Table A-1. A summary of the information critically evaluated by the GRAS Panel is presented below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

Miracle fruit powder is a reddish brown to pink powder obtained from de-seeded miracle fruit berries. Miracle fruit, which is also referred to as “miraculous berry”, “sweet berry”, or “miracle berry”, is a small (2 to 3 cm) bright red fruit from *Synsepalum dulcificum*, an evergreen bush or tree that is native to tropical West Africa, but also grown in Florida. Miracle fruit powder is intended for use as an ingredient for addition to beverages and fermented dairy products for its ability to impart sweetness by modifying taste from sour to sweet due to the active glycoprotein, miraculin.

Miraculin, present within the thin-layered pulp of the miracle fruit berries, is a single polypeptide with 191 amino acid residues that was isolated in 1968 by researchers at Florida State University (Theerasilp and Kurihara, 1988). Miraculin imparts a taste-modifying effect when consumed by binding to the sweet receptors of the tongue, turning sour tastes into sweet (Morris, 1976). The protein has 2 glycosylated sites (Asn-42 and Asn-186) crosslinked by disulfide bonds with a molecular weight of 28 kDa (Theerasilp and Kurihara, 1988; Theerasilp *et al.*, 1989). The amino acid sequence of miraculin is publicly available on the Uniprot/Swissprot database under Accession No. P13087.

Miracle fruit powder is manufactured consistent with cGMP, and the production process complies with the principles of Hazard Analysis and Critical Control Points (HACCP). The finished ingredient is produced by pulping, maceration, and freeze-drying the de-seeded-miracle fruit berries and does not involve the use of any solvent or chemical processing aid.

Miracle Fruit Farm has established food-grade specifications for miracle fruit powder which include organoleptic and compositional parameters, including heavy metals and microbiological contaminants. All analytical methods are internationally recognized or have been developed internally and validated. The GRAS Panel reviewed the results from 3 non-consecutive batches of miracle fruit powder and concluded that the manufacturing process produces a consistent product that conforms to the established specifications.

Miracle fruit powder has been fully characterized and is primarily comprised of carbohydrates (*ca.* 89% on dry weight basis), protein (*ca.* 5% on dry weight basis), ash (*ca.* 3.3% dry weight basis), moisture (*ca.* 1.8%), and fatty acids (<1% dry weight basis), with a minimum miraculin content of 480 ppm (~0.05% on dry basis). The proximate composition of miracle fruit powder was demonstrated through analytical data to be comparable with the composition of miracle fruit pulp (carbohydrate *ca.* 87% on dry weight basis), protein (*ca.* 9% on dry weight basis), ash (*ca.* 3.5% on dry weight basis), moisture (*ca.* 85%), and fatty acids (<1% on dry weight basis), from which they were obtained, with the only difference being in the moisture content, considering that miracle fruit powder is freeze-dried. Detailed analyses of antinutrients including oxalic acid, phytic acid, trypsin inhibitors, and polyphenols demonstrated that the levels of these antinutrients in miracle fruit powder are comparable with those occurring in miracle fruit pulp; they occur at low or non-detectable levels and are not associated with any safety concerns.

The GRAS Panel also reviewed the results of a 52-week shelf-life stability study. Samples from 3 non-consecutive lots of miracle fruit powder were stored at 25±2°C and 60% relative humidity in metalized barrier pouches for 52 weeks. The miraculin and moisture contents of each sample were measured at 0, 2, 4, 8, 14, 26, and 52 weeks. The results indicate that miracle fruit powder is stable for up to 52 weeks when stored at ambient temperature and humidity.

TECHNICAL EFFECT

Miracle fruit powder is intended for addition to beverages and fermented dairy products for its ability to impart sweetness by modifying taste from sour to sweet due to the active glycoprotein, miraculin. The sweetness profile of miracle fruit was evaluated by 6 trained panelists (Tafazoli *et al.*, 2019). A baseline sweetness intensity was established with lemonade juice with a sweetness intensity of 7 Brix. Following establishment of a baseline sweetness intensity, each panelist consumed 0.08 g of miracle fruit and was instructed to hold the powder in the mouth for 1 minute before swallowing. Each panelist consumed 60 mL of lemonade juice every 5 minutes for 30 minutes, and the sweetness intensity of each cup consumed was recorded. The results indicate that miracle fruit significantly increased the perceived sweetness of lemonade juice initially upon testing after miracle fruit consumption and that sweetness returned to baseline levels in all subjects after 30 minutes. The sensory data support the rapid taste modifying effect of miraculin, with no lasting sweetness desensitization effect. In another study evaluating the taste modifying effect of miraculin, the maximum relative sweetening effect was achieved within 3 minutes of consumption, with the effects reported to be concentration-dependent. As outlined within Tafazoli *et al.* (2019), the sensory response rapidly declined after 30 minutes (Kurihara and Beidler, 1969).

INTENDED USE AND ESTIMATED EXPOSURE

Miracle fruit powder is intended to be added as an ingredient to water-based beverages, carbonated beverages, fruit juices, fruit nectars, fruit-based smoothies, fruit drinks and ades, fermented dairy products (such as buttermilk, acidophilus milk, kefir, and yogurts), and ready to drink tea beverages (such as kombucha and iced tea) at use levels ranging from 1 to 6% (see Table A-1). Miracle fruit powder is intended to be used for its ability to impart sweetness by modifying taste from sour to sweet. The GRAS Panel reviewed data related to the estimated dietary exposure to miracle fruit powder based on an assessment of its anticipated intake as an ingredient under the intended conditions of use and use levels using the information from the 2015-2016 cycle of the National Health and Nutrition Examination Survey (NHANES).

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of miracle fruit powder were determined to be 8.3 and 17.4 g/person/day, respectively. Of the individual population groups, male adults had the highest mean and 90th percentile consumer-only intakes of miracle fruit powder on an absolute basis, at 9.7 and 20.1 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.5 and 14.0 g/person/day, respectively. On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of miracle fruit powder were 145 and 311 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes, of 525 and 1,137 mg/kg body weight/day, respectively. It should be noted that foods containing miracle fruit powder are not intended for infants and young children. Female adults had the lowest mean and 90th percentile consumer-only intakes of 99 and 219 mg/kg body weight/day, respectively.

The GRAS Panel also reviewed estimated exposure to the active glycoprotein miraculin from the proposed food uses of miracle fruit powder. Corresponding intakes of miraculin were calculated based on the previously mean concentration of 534 µg/g. Among the total population (all ages), the mean and 90th percentile consumer-only intakes of miraculin were 4.4 and 9.3 mg/person/day, respectively. Of the individual population groups, male adults had the highest mean and 90th percentile consumer-only intakes of miraculin on an absolute basis, at 5.2 and 10.7 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 3.5 and 7.4 mg/person/day,

respectively. On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of miraculin were 77 and 166 µg/kg body weight/day, respectively. Among the individual population groups, infants and young children had the highest mean and 90th percentile consumer-only intakes of any population group, 280 and 607 µg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 53 and 117 µg/kg body weight/day, respectively.

SAFETY NARRATIVE

A comprehensive search of the scientific literature was conducted through February 2020 to identify publications related to the safety of miracle berry powder and miraculin. The search was limited to full text articles within peer-reviewed scientific journals from the following literature databases: 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 search identified several repeated-dose studies that primarily evaluated efficacy-related endpoints of miracle fruit/miracle berry or extracts thereof (Chen *et al.*, 2006; Dioso *et al*., 2016; Obafemi *et al*., 2016, 2017, 2019; Shi *et al*., 2016).

Miracle fruit (*S. dulcificum*) has been consumed in West Africa since at least the 1700s, and in the United States (U.S.) since its introduction in 1917. The fruit itself and numerous supplement-type products containing miracle fruit extract are commercially available in the U.S., suggesting that there exists a history of safe consumption of miracle fruit (*S. dulcificum*) by U.S. consumers. It may be concluded that the lack of reported adverse reports supporting a history of safe use of miracle fruit, the minimal processing (*i.e.*, its production does not involve the use of extraction solvents or processing chemicals) of Miracle Fruit Farm's miracle fruit and the very low levels of antinutrients including oxalic acid, phytic acid, trypsin inhibitors, and polyphenols support the safety of the proposed uses of this product.

The safety of miracle fruit powder as an ingredient for use in beverage products is further predicated upon a detailed understanding of the manufacturing process and its impact on the ingredient composition (*i.e*., the potential to concentrate natural toxins or antinutrients), as well as a full characterization of the ingredient and the safety of its active glycoprotein component, miraculin. The miraculin protein has been the subject of *in vitro* digestibility testing and *in silico* safety evaluation, wherein the miraculin amino acid sequence obtained from the GenBank database (Uniprot Accession No. P13087) was investigated with respect to allergenicity and toxigenicity potential (Tafazoli *et al.*, 2019, 2020). The results of the *in vitro* digestibility study and bioinformatic searches indicate that the miraculin protein is rapidly enzymatically hydrolyzed by gastric juice and would not pose an allergenic or toxigenic risk to consumers. The results of the *in vitro* digestibility and *in silico* bioinformatics searches on miraculin, as the active component of miracle fruit powder, have been published by Tafazoli *et al.* (2019, 2020), and are considered pivotal in the safety assessment of miracle fruit powder.

Each of these safety considerations is discussed in detail below.

Compositional Analyses

Miracle fruit powder has been fully characterized and is primarily comprised of carbohydrates (*ca.* 89% on dry weight basis), protein (*ca.* 5% on dry weight basis), ash (*ca.* 3.3% on dry weight basis), moisture (*ca.* 1.8%), and fatty acids (<1% on dry weight basis), with a minimum miraculin content of 480 ppm (~0.05% on dry weight basis). These standard dietary components will undergo normal digestive physiological processes and therefore raise no safety concerns. The article of commerce is produced by pulping, maceration, and freeze-drying of de-seeded-miracle fruit berries and does not involve the use of any solvent or chemical processing aid. Miracle fruit powder is minimally processed and its chemical composition is similar to that of the miracle fruit. This was demonstrated by comparing the chemical composition of 3 non-consecutive batches of miracle fruit powder with 3 batches of miracle fruit pulp from which the powders were obtained. The results of these analyses demonstrate that miracle fruit powder is compositionally similar to the miracle fruit pulp from which it was obtained, thus demonstrating that the manufacturing process for miracle fruit powder does not introduce any impurities. The only difference is the moisture content in miracle fruit pulp and powder which is due to the removal of the moisture from pulp during the freeze-drying process involved in the production of miracle fruit powder.

Analysis of several production batches of the final product demonstrated an absence of environmental contaminants (*e.g.*, heavy metals, mycotoxins, and pesticides) and microbiological hazards that may have originated from the cultivation practices or manufacturing process, and low levels of antinutrients (*e.g.*, polyphenols, oxalic acid, phytic acid, trypsin inhibitors, and alkaloids). The levels of antinutrients in miracle fruit powder were shown to be comparable to the levels occurring in miracle fruit pulp. In a study by Du *et al*. (2014), the total phenolic content of the berry flesh was reported to be 1,448.3 mg gallic acid equivalents (GAE)/100 g fresh fruit or 14.48 mg GAE/g. The level of total polyphenols in miracle berry flesh is comparable to those analyzed for 3 batches of Miracle Fruit Farm's miracle fruit powder (obtained from de-seeded berries) with values ranging from 17.3 to 17.5 mg GAE/g. The total polyphenol content of miracle berry reported in the Du *et al*. (2014) study is higher than that reported for other berry fruits, such as blackberry (435.0 mg GAE/100 g), blueberry (348.0 mg GAE/100 g), and strawberry (83.9 mg GAE/100 g) (Heinonen *et al*., 1998; León-González *et al*., 2013). While the level of total polyphenols per gram of fruit may be lower in standard berry fruits that are consumed on a daily basis, when compared from a serving size perspective, the total polyphenol intake level of miracle fruit powder is found to be lower than those reported for these berries. For example, the total polyphenol content of blackberries per serving size of 100 g (USDA, 2019) would be 435 mg/serving, whereas the highest 90th percentile consumer-only intake of miracle fruit powder from all proposed uses of 20.1 g/day (male adults) and a mean polyphenol content of 17.7 mg GAE/g in miracle fruit powder, result in a total polyphenol intake of 356 mg/day, which is lower than the intakes of polyphenols from 1 serving size of blackberries (435 mg/serving). The exposure to antinutrients within miracle fruit powder raise no safety concerns.

Studies on Miracle Fruit Powder and Miracle Fruit/Leaf Extract

The publicly available efficacy-based studies related to the safety of miracle fruit powder included evaluation of either solvent extracts or studies involving diabetic rats, or rats consuming fructose-rich diets. Specifically, the studies evaluated the effects of miracle fruit and leaf ethanol extracts on blood glucose of diabetic rats (Dioso *et al*., 2016); effects of miracle berry leaf methanolic and flavonoid-rich extracts on hematological parameters and serum electrolytes of diabetic and non-diabetic rats (Obafemi *et al*., 2016, 2019) or glucose tolerance, serum biochemistry, and liver, pancreas, and kidney histopathology of diabetic and non-diabetic rats (Obafemi *et al*., 2017); anti-hyperuricemia effects of miracle berry leaf butanol extracts in ICR mice (Shi *et al*., 2016); and effects on insulin resistance of rats consuming a fructose-rich diet (Chen *et al.*, 2006). These studies were conducted for periods up to 4 weeks. While these studies were

conducted to evaluate the efficacy of miracle fruit/berry or extracts thereof, some limited safety-related endpoints were also assessed. The effects on some serum biochemistry parameters noted in Obafemi *et al.* (2017) were not consistent or dose-dependent. The test articles in this study were methanolic extracts, and the composition of the test article in comparison to a standard miracle berry powder could not be determined; therefore, the observed effects could be due to a concentrated berry component or the residual solvent and are not considered relevant to miracle fruit powder.

Chen *et al.* (2006) reported dosing of the lyophilized miracle fruit powder dissolved in saline at 0.02, 0.04, and 0.2 mg/kg body weight as a single dose or thrice daily in the repeat dose experiments (4-week fructose chow experiments and a 10-day STZ rat experiment). The authors did not indicate whether the test article was fully dissolved, whether the test article was dosed as a suspension, or whether the seeds were removed from the fruit prior to processing. If the test article was the lyophilized miracle fruit powder, it is not comparable to the miracle fruit powder as described herein. The powder used in the study included all parts of the miracle fruit including seed and is not similar to miracle food product described herein. This study is not relevant for the risk assessment of Miracle Fruit Farm's miracle fruit powder.

Safety Information Related to the Active Glycoprotein, Miraculin

In Vitro Digestibility of Miraculin

The protein digestibility of miraculin was reported using an *in vitro* simulated gastric fluid (SGF) model (Tafazoli *et al.*, 2019). Miraculin (0.08 mL; 2 mg/mL or 0.1 mg/mL final concentration in reaction) was added to a preincubation mixture consisting of SGF (10 U/µg pepsin) and incubated for up to 60 minutes. After 0, 20, 40, and 60 minutes of incubation, sample mixtures were quenched with sodium bicarbonate, tricine buffer solution, and a reducing agent, and heated at 85°C for 10 minutes. The digestibility of the protein was evaluated by gel electrophoresis using Coomassie Blue after heating. The results demonstrate that miraculin was completely digested within 20 minutes. The *in vitro* digestibility of miraculin (purity 85 to 90%) was further investigated using the methods described by Thomas *et al.* (2004). The glycoprotein and digestion control proteins (pepsin sensitive[1] or resistant[2] proteins) were dissolved at 3.67 mg/mL in phosphate buffer saline (pH 7.0) or deionized water. Miraculin was completely digested within 20 minutes in the presence of SGF containing pepsin at a concentration of 10 U/µg protein. No peptide fragments were reported. Similar results were reported in a subsequent study with shorter digestion times (*i.e.*, 0, 1, 5, and 10 minutes) and a lower pepsin concentration (5.45 U/µg protein). The miraculin protein was rapidly digested within 1 minute. The digestion control proteins were digested as expected, and pepsin was reported to be intact throughout the incubation period. Miraculin was stable and intact at all timepoints in SGF without pepsin, reported, indicating that digestion with pepsin is rapid and extensive (Tafazoli *et al.*, 2019).

Due to the low sensitivity of Coomassie Blue staining, and in order to enhance detection of lower abundance peptides, the above experiment was repeated using the more sensitive Silver Staining approach. The GRAS Panel reviewed the results of the digestion study conducted with Silver Staining, which demonstrated that following digestion by pepsin for 0, 1, 5, and 10 minutes, the results of the Silver Staining corroborated the previous findings that miraculin was rapidly digested at both 5.45 and 10 U pepsin/µg protein. Minor peptide fragments were reported at approximately 4 kDa at both concentrations within

[1] Bovine serum albumin and peroxidase from horseradish served as pepsin-sensitive control proteins.
[2] Albumin from chicken egg white served as the pepsin-resistant control protein.

10 minutes of digestion. The other bands were reported to be pepsin or miraculin impurities, as similar bands were observed at time point 0 and serial dilutions of miraculin, which were not digested.

Since the short digestion time period of 10 minutes still showed the presence of peptide fragments using Silver Staining, the *in vitro* digestibility study was repeated under similar conditions testing a higher purity miraculin product (purity 98.24%) and longer digestion times (*i.e.*, 0, 20, 40, 60, 120, and 240 minutes). The results demonstrate that the levels of intact miraculin were below the limit of detection of 10 ng within 20 minutes of digestion. This represents about ≤1.2 to ≤2.3% of the original amount of miraculin remaining. Furthermore, no miraculin digest fragments were reported at any timepoint. Low levels of potential minor fragments (around 4 kDa) that were noted within 10 minutes of digestion during the first experiment were not reported in the follow-up experiment up to 4 hours of digestion.

The GRAS Panel noted the absence of detectable miraculin fragments in the experiments conducted with Silver Staining that corroborate the findings of Coomassie Blue staining supporting the conclusion that the miraculin protein is readily digested using a standard *in vitro* digestion protocol. The size of the peptide fragments after 20 minutes of digestion were demonstrated to be lower in molecular weight than the ranges that are detectable using a gel electrophoresis method (*i.e.*, 2 to 5 kDa), supporting the rapid and full digestion of miraculin.

Proteolytic Fate of Miraculin

Tafazoli *et al.* (2019) reported the proteolytic fate of the miraculin protein following pepsin digestion. The digested peptides of miraculin were evaluated using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Miraculin was added to SGF containing 5.45 U/µg pepsin and incubated for up to 10 minutes at 37°C. Digest samples were collected after 0, 0.5, 1, and 10 minutes. Tafazoli *et al.* (2019) reported that miraculin was increasingly digested with a longer digestion time (*i.e.*, number of unique peptides increased with longer digestion periods). After 0 minutes of digestion, the authors reported 5 unique peptides while the number of unique peptides after 0.5, 1, and 10 minutes of digestion were 33, 54, and 61, respectively. The number of unique peptides after 10 minutes of pepsin digestion encompassed approximately 75% of the amino acid sequence. The authors concluded that the *in vitro* digestibility studies suggest that miraculin, following ingestion, would be rapidly and completely metabolized into small peptides, and ultimately its amino acid components. This was further supported by the results of the Silver Staining method over longer digestion period of up to 240 minutes, demonstrating that in the *in vitro* digestibility study, miraculin was rapidly digested to fragments that had a molecular weight below the detection limit of the gel electrophoresis method (*i.e.*, 2 to 5 kDa), suggesting the complete digestion of the protein.

Allergenicity of Miraculin

The potential allergenicity of miraculin (Uniprot Accession No. P13087) was investigated using the AllergenOnline Database[3] (version 20; updated 10 February 2020) (FARRP, 2020) and Allermatch Database[4] (updated 04 July 2019) to determine whether the amino acid sequence of miraculin shares similarity to known allergens that might produce an allergenic response. The databases contain a comprehensive list of putative allergenic proteins developed *via* a peer-reviewed process for the purpose of assessing the potential allergenicity of novel proteins. In addition, the Allermatch database was constructed using the allergenic proteins from the COMPARE[5], UniProt[6], and WHO/IUIS[7] allergen databases. Sequence homology searches of the full-length amino acid sequence and 80-amino acid sliding window alignment were conducted using FASTA.

In the full-length amino acid sequence search, an identity cut-off value of 50% was used, as allergic cross-reactivity may occur at matches greater than 50% (Aalberse, 2000). However, it is noted that cross-reactivity is rare at 50%, and, in fact, allergic cross-reactivity may require greater than 70% identity over the full-length sequence (Aalberse, 2000). Nevertheless, in the full-length amino acid sequence search using the AllergenOnline and Allermatch databases, no hits greater than 50% identity were identified, suggesting the unlikely potential for cross-reactivity to putative allergens.

In the 80-amino acid sliding window alignment search, segments of 80 amino acids (*e.g.*, 1–80, 2–81, 3–82, *etc.*) derived from each full-length amino acid sequence were searched in accordance with the methodology described by the FAO/WHO (2001) and Codex Alimentarius (2003, 2009). Significant homology was defined as an identity match of greater than 35% in accordance with the FAO/WHO (2001) and Codex Alimentarius (2003, 2009) criteria. Immunoglobulin E (IgE) cross-reactivity to putative allergens may be considered a possibility at matches greater than 35% identity. A number of sequences with identity matches ranging from 36 to 39% with known allergens from commonly consumed agricultural products, *Solanum tuberosum* (potato) and *Glycine max* (soybean), were identified using AllergenOnline. The clinical significance of low identity matches (35 to 40% over 80 amino acid windows) is questionable; the recommended criterion of >35% identity over 80 amino acid windows is quite conservative and other factors should be considered when the percent identity is low (Goodman, 2006).

The potential for cross-reactivity between miraculin and these potato and soybean trypsin inhibitors is low based on the lack of significant full-length identity and the rather low (35 to 39%) identities over sliding 80-mer windows between the potato and soybean trypsin inhibitors and miraculin. Also, although these trypsin inhibitors are recognized as known allergens, neither the potato proteinase inhibitors nor the soy Kunitz trypsin inhibitor are considered as important food allergens from a clinical context (Taylor *et al.*, 2014).

The allergenic potential of peptide digests of miraculin was evaluated in a recent publication (Tafazoli *et al.*, 2019, 2020). As discussed in the publication, miraculin was digested with SGF containing pepsin for up to 10 minutes and the resulting peptides were characterized by LC-MS/MS. The authors reported 61 unique peptides from the digested miraculin protein. The 61 identified peptides were evaluated for allergenicity potential using a similar approach as described above (*i.e.*, full-length amino acid sequence and 80-amino acid sliding window). The full-length search of each peptide digest revealed a number of matches with

[3] http://www.allergenonline.org/.
[4] http://www.allermatch.org/.
[5] https://comparedatabase.org/.
[6] https://www.uniprot.org/docs/allergen.
[7] http://www.allergen.org/.

known allergens, with identity scores ranging from 36 to 67% and similarity scores ranging from 60 to 100%. The corresponding E-values ranged from 0.00067 to 0.95 with an amino acid overlap of 8 to 25. Therefore, considering the high E-values and an identity match of less than 67% over a short amino acid coverage (<25), it is unlikely that these peptide digests would pose any allergenic risk (Aalberse, 2000). The 80-amino acid sliding window searches with each peptide digest did not reveal any significant structural homology with any known allergens. The authors concluded that the results of the *in silico* searches with the peptide digests do not suggest that miraculin will pose a risk of cross-reactivity with known allergens (Tafazoli *et al.*, 2019, 2020).

In order to enhance detection of lower abundance peptide fragments that were detected within 10 minutes of digestion in an *in vitro* digestibility model with SGF, a follow-up experiment was conducted using the more sensitive Silver Staining method over a longer digestion period of 240 minutes. The results of the Silver Staining demonstrated complete and rapid digestion of miraculin within 20 minutes to fragments that had a molecular weight below the detection limit of gel electrophoresis method (*i.e.*, 2 to 5 kDa). The weight of the available evidence and the nature of the potential matches to trypsin inhibitors from soy and potato, including *in silico* results from the allergenicity assessment, suggest that miraculin is unlikely to have potential for allergenicity. These conclusions are further corroborated with the fact that exposures to miraculin *via* miracle berry and miracle fruit in commercial products in the U.S. have not been reported.

Toxigenicity of Miraculin

A sequence alignment query was conducted for the miraculin amino acid sequence against downloaded protein sequences obtained from a curated database of animal venom proteins and toxins maintained in the UniProtKB/Swiss-Prot Tox-Prot[8] database (Tafazoli *et al*., 2019, 2020). The amino acid sequences were compared using the Basic Local Alignment Search Tool (BLAST) maintained by the National Center for Biotechnology Information. Several matches to animal toxins/venoms were identified, with sequence identities ranging from 25 to 54% and corresponding E-values of 0.61 to 9.3 with generally low query coverage (<25%). While there are no formal guidelines established for what constitutes a significant sequence similarity between an introduced protein and protein toxins (Hammond *et al.*, 2013), considering the low query coverage and high E-values/scores (Pearson, 2000; Bushey *et al.*, 2014) identified for the alignments between miraculin and the animal toxins/venoms, the results of the full-length sequence alignment search of miraculin suggest that it does not share homology or structural similarity to any animal venom protein, toxins, or virulence factors, and does not harbor any toxic potential.

[8] The UniProtKB/Swiss-Prot Tox-Prot database is available at: http://www.uniprot.org/uniprot/?query=taxonomy%3A%22Metazoa+[33208]%22+AND+%28keyword%3Atoxin++OR+annotation%3A%28type%3A%22tissue+specificity%22+AND+venom%29%29+AND+reviewed%3Ayes&sort=score.

Summary and Basis for GRAS

Miracle fruit has a history of safe consumption both internationally and in the U.S., and its uses have steadily grown. Various forms of miracle fruit such as fresh berry, freeze-dried powder, or tablets are commercially available. No adverse events or serious side effects have been reported based on consumption of these commercial forms of miracle fruit, supporting the general safety of the ingredient. The miracle fruit powder is mainly comprised of carbohydrates (*ca.* 89% on dry weight basis), protein (*ca.* 5% on dry weight basis), ash (*ca.* 3.3% on dry weight basis), moisture (*ca.* 1.8%), and fatty acids (<1% on dry weight basis), with a minimum miraculin content of 480 ppm (~0.05% on dry weight basis), and is compositionally similar to miracle fruit pulp, with the only difference being in the moisture content. These standard dietary components will undergo normal digestive physiological processes upon consumption and therefore do not raise any safety concerns. Detailed analyses of the levels of anti-nutrients including oxalic acid, phytic acid, trypsin inhibitors, and polyphenols demonstrated that the levels of these antinutrients in miracle fruit powder are comparable with those occurring in miracle fruit pulp. The level of polyphenols in miracle fruit powder is comparable to that in the miracle fruit, and the exposure resulting from those polyphenols in miracle fruit powder is lower than that from other commonly consumed berries on a per serving size basis. The manufacturing process does not introduce any impurities that are safety concern.

Among the total population, the mean and 90th percentile consumer-only intakes of miracle fruit powder were determined to be 8.3 and 17.4 g/person/day or 525 and 1,137 mg/kg body weight/day, respectively. Of the individual population groups, male adults had the highest mean and 90th percentile consumer-only intakes of miracle fruit powder on an absolute basis, at 9.7 and 20.1 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.5 and 14.0 g/person/day, respectively. On a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of any population group at 525 and 1,137 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes at 99 and 219 mg/kg body weight/day, respectively.

The glycoprotein, miraculin, constitutes a small portion of miracle fruit powder (approximately 0.05 to 0.3%). Purified miraculin protein was reported to be rapidly enzymatically hydrolyzed by gastric juice with limited time response for the molecule receptor interaction. Sensory data suggest that the impact on sour taste reverts back to normal within 30 minutes of consumption. The *in silico* analysis coupled with the rapid hydrolysis indicates a lack of cross-reactivity with any known allergen. Published studies that reported on the potential health effects of the miracle berry did not use the berry fruit powder and were considered irrelevant for the safety assessment of miracle berry powder.

CONCLUSION

We, the members of the GRAS Panel, have, independently and collectively, critically evaluated the data and information summarized above, and unanimously conclude that the proposed uses of Miracle Fruit Farm's miracle fruit powder are safe.

We further unanimously conclude that the proposed uses of Miracle Fruit Farm's miracle fruit powder, meeting appropriate food-grade specifications and produced in accordance with current Good Manufacturing Practice (cGMP), are Generally Recognized as Safe (GRAS) under conditions of intended use based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

[redacted]

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18 September 20
Date

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22 September 2020
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Table A-1 Summary of the Individual Proposed Food Uses and Use Levels for Miracle Fruit Powder in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Food Uses[a]	Miracle Fruit Powder Use Levels (%)
Beverages and Beverage Bases	Water-based beverages	1
	Carbonated beverages	1
Processed fruits and Fruit Juices	Fruit juices	3
	Fruit nectars	3
	Fruit-based smoothies	6
	Fruit drinks and ades	3
Milk Products	Fermented dairy products (buttermilk, acidophilus milk, kefir, and yogurts)	1
Coffee and Tea	Ready to drink tea beverages (*e.g.*, kombucha and iced tea)	2

CFR = Code of Federal Regulations; U.S. = United States.

[a] Miracle Berry Powder is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.