Joywell

15 February 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 Pulp, Miracle Fruit Powder, and Miracle Fruit Protein

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Joywell Foods Inc., as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that Miracle Fruit Pulp, Miracle Fruit Powder and Miracle Fruit Protein, are GRAS on the basis of scientific procedures, for use in conventional food and beverage products across multiple categories; these food uses of Miracle Fruit Pulp, Miracle Fruit Powder and Miracle Fruit Protein, are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Joywell Food'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,

Jason Ryder, Ph.D.
CTO & Co-Founder
Joywell Foods, Inc.

Email: jason.ryder@joywellfoods.com
Tel: +1 (510) 684-5610

GRAS NOTICE FOR MIRACLE FRUIT PULP, MIRACLE FRUIT POWDER, AND MIRACLE FRUIT PROTEIN

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:
Joywell Foods Inc.
202 Cousteau Place, Suite 210
Davis, CA
95618 USA

DATE:
15 February 2021

GRAS Notice for Miracle Fruit Pulp, Miracle Fruit Powder, and Miracle Fruit Protein

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GRAS Notice for Miracle Fruit Pulp, Miracle Fruit Powder, and Miracle Fruit Protein

Part 1. § 170.225 Signed Statements and Certification

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

Signed,

15 February 2021

Jason Ryder
Chief Technology Officer & Co-Founder
Joywell Foods Inc.

Date

1.1 Name and Address of Notifier

Joywell Foods Inc.
202 Cousteau Place, Suite 210
Davis, CA
95618 USA

1.2 Common Name of Notified Substance

Miracle fruit pulp, miracle fruit powder, and miracle fruit protein

1.3 Conditions of Use

Miracle fruit powder, miracle fruit pulp, and miracle fruit protein are intended for use as taste modifiers in a variety of conventional food and beverages, as described in Table 1.3-1. Food uses are categorized according to 21 CFR §170.3 (U.S. FDA, 2020a). Miracle fruit powder and miracle fruit pulp are intended for use in all food and beverage categories at levels up to 0.70 g/serving or 5 g/serving, respectively, while miracle fruit protein is intended for use in a variety of food and beverage products at levels up to 5 mg/serving.

Table 1.3-1 Proposed Food Uses for Miracle Fruit Protein, Miracle Fruit Powder, and Miracle Fruit Pulp

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses	Miracle Fruit Protein Use Levels (%)	Miracle Fruit Powder Use Level (%)	Miracle Fruit Pulp Use Level (%)
Baked Goods and Baking Mixes	Cheesecake	0.004	0.56	4.00
Beverages, alcoholic	Cocktail drinks (pre-packaged)	0.00139	0.19	1.39
	Malt beverages	0.00141	0.20	1.41
	Distilled liquors	0.01136	1.59	11.36
	Wine	0.00338	0.47	3.38
Beverages and Beverages Bases, non-alcoholic	Packaged water-based beverages	0.00139	0.19	1.39
	Non-milk-based meal replacement beverages and protein drinks	0.00208	0.29	2.08
Chewing Gum	Chewing gum	0.06667	6.67	N/A
Coffee and Tea	Ready-to-drink coffee beverages	0.00139	0.19	1.39
	Ready-to-drink tea beverages	0.00139	0.19	1.39
Dairy Product Analogs	Milk analogs	0.00208	0.29	2.08
	Non-dairy yogurts	0.00294	0.41	2.94
Frozen Dairy Desserts and Mixes	Ice cream	0.00154	0.54	3.85
	Frozen yogurt	0.00222	0.78	5.56
	Frozen milk desserts and bars	0.00155	0.54	3.88
Fruit and Water Ices	Edible ices	0.00127	0.45	3.18
	Sherbet	0.002	0.70	5.00
	Sorbet	0.0015	0.53	3.76
Grain Products and Pastas	Cereal bars, granola bars, energy, protein, and meal replacement bars	0.0125	1.75	12.50
	Granola	0.0075	1.75	12.50
Milk Products	Packaged milk-based beverages	0.00208	0.29	2.08
	Yogurt	0.00294	0.41	2.94
	Yogurt drinks	0.00242	0.34	2.42
Processed Fruits and Fruit Juices	Packaged fruit juices, nectar, fruit drinks and ades, and fruit-based smoothies	0.00208	0.29	2.08
Processed Vegetables and Vegetable Juices	Packaged vegetable juices and blends	0.00208	0.29	2.08
Snack Foods	Fruit-based bars (without granola)	0.01667	2.33	16.67

Table 1.3-1 Proposed Food Uses for Miracle Fruit Protein, Miracle Fruit Powder, and Miracle Fruit Pulp

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses	Miracle Fruit Protein Use Levels (%)	Miracle Fruit Powder Use Level (%)	Miracle Fruit Pulp Use Level (%)
Soft Candy	Confectionery and chewy candy coatings and fillings	0.01333	2.33	16.67
	Gummy Candy	0.00667	2.33	16.67

CFR = *Code of Federal Regulations*.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020b), Joywell Foods has concluded that the intended uses of miracle fruit pulp, miracle fruit powder, and miracle fruit protein, as described herein, are GRAS on the basis of scientific procedures.

1.5 Availability of Information

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

Joywell Foods Inc.
202 Cousteau Place, Suite 210
Davis, CA
95618 USA

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

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

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

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

2.1 Identity of the Ingredients

Miracle fruit pulp, miracle fruit powder, and miracle fruit protein are obtained from the fruit of the miracle berry tree (*Synsepalum dulcificum* Daniell), an edible fruit tree native to west tropical Africa (Chen *et al*., 2006). The fruit of the tree is small (2 to 3 cm), bright red, and is known by different names, including "miraculous berry", "miracle fruit", "sweet berry", and "miracle berry". The human consumption of miracle fruit dates back to early 1700s in Ghana (Roecklein and Leung, 1987), long before its introduction to the U.S. in 1917 by the United States Department of Agriculture (USDA). Cultivation of miracle fruit, and its use in the U.S., has grown steadily over the years and its commercial use has expanded in the U.S. in the form of fresh berries, a freeze-dried powder, or tablet available in various dietary supplement-type products (see Section 3.1).

The taste-modifying effect of miracle berry is attributed to miraculin. This glycoprotein is expressed as a single polypeptide of 220 amino acid residues, including a 29 amino acid *N*-terminal signal peptide that is removed through post-translational processing. Miraculin has 2 glycosylation sites (Asn-42 and Asn-186), disulfide bond cross-linking, and a molecular weight of 25 kDa, with roughly 14% of the mass coming from the *N*-linked glycans (Theerasilp and Kurihara, 1988; Theerasilp *et al.*, 1989). Miraculin was first isolated from the fruit 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 with a molecular weight of roughly 50 kDa, connected through a single inter-chain disulfide bond at Cys-138. When consumed, miraculin imparts a taste-modifying effect through its interaction with the sweet receptors of the tongue, turning sour tastes into sweet (Morris, 1976).

The peptide sequence of single-chain miraculin is publicly available, as reported under Uniprot (Accession No. P13087), and consists of 220 amino acid sequences, with a signal peptide consisting of 29 amino acid residues (underlined):

MKELTMLSLSFFFVSALLAAAANPLLSAADSAPNPVLDIDGEKLRTGTNY
YIVPVLRDHGGGLTVSATTPNGTFVCPPRVVQTRKEVDHDRPLAFFPENP
KEDVVRVSTDLNINFSAFMPCRWTSSTVWRLDKYDESTGQYFVTIGGVKG
NPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYIDQKGR
RRLALSDKPFAFEFNKTVYF

The miracle fruit pulp ingredient is mainly comprised of moisture (on average 85%), with the remaining components being carbohydrates (on average 12%, as is), protein (on average 1%, as is), fat (on average 1%, as is), and ash (on average 0.4%, as is). The miracle fruit powder ingredient comprises carbohydrates (on average 78%, as is), fat (on average 10%, as is), protein (on average 6%, as is), ash (on average 4%, as is), and moisture (on average 1%). The miracle fruit protein ingredient comprises protein (on average 32%, as is), salt[1] (on average 46% NaCl), moisture (on average 5%), and carbohydrates (on average 5%, as is), with the remaining mass comprised of residual food-grade buffers (*i.e.*, citric acid) and minerals (on average 12%).

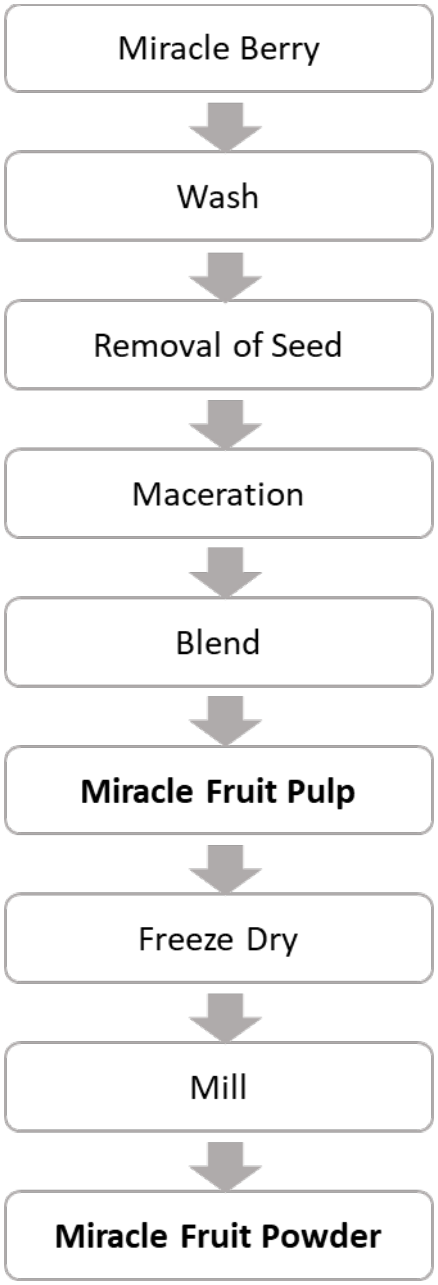
[1] This high salt concentration serves a dual purpose in the freeze-dried material. First, it acts as a bulking agent, making the ingredient easier to work with when formulating into food products. Second, when the ingredient is rehydrated, it acts as a stabilizer for the protein and maintains protein function.

2.2 Method of Manufacture

Miracle fruit pulp, miracle fruit powder, and miracle fruit protein are manufactured in accordance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis and Critical Control Points (HACCP). All processing aids and food contact materials used in the production process of miracle fruit pulp, miracle fruit powder, and miracle fruit protein are food-grade or have previously been determined to be GRAS for their intended uses. The berries are currently sourced within the U.S., though other international sources are available through commercial production in Taiwan.

A schematic of the production process of miracle fruit pulp and miracle fruit powder is provided in Figure 2.2-1 below. Fresh miracle berries are washed with water or a diluted bleach solution (*ca.* 1%). The washed berries are deseeded and macerated in an industrial de-stoner with wire screen (<2 mm). The resultant pulp is blended and stored frozen. The frozen pulp may be freeze-dried and milled into a fine powder that is less than 500 µm in size to obtain miracle fruit powder. This powder may then be stored at room temperature under low humidity conditions.

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

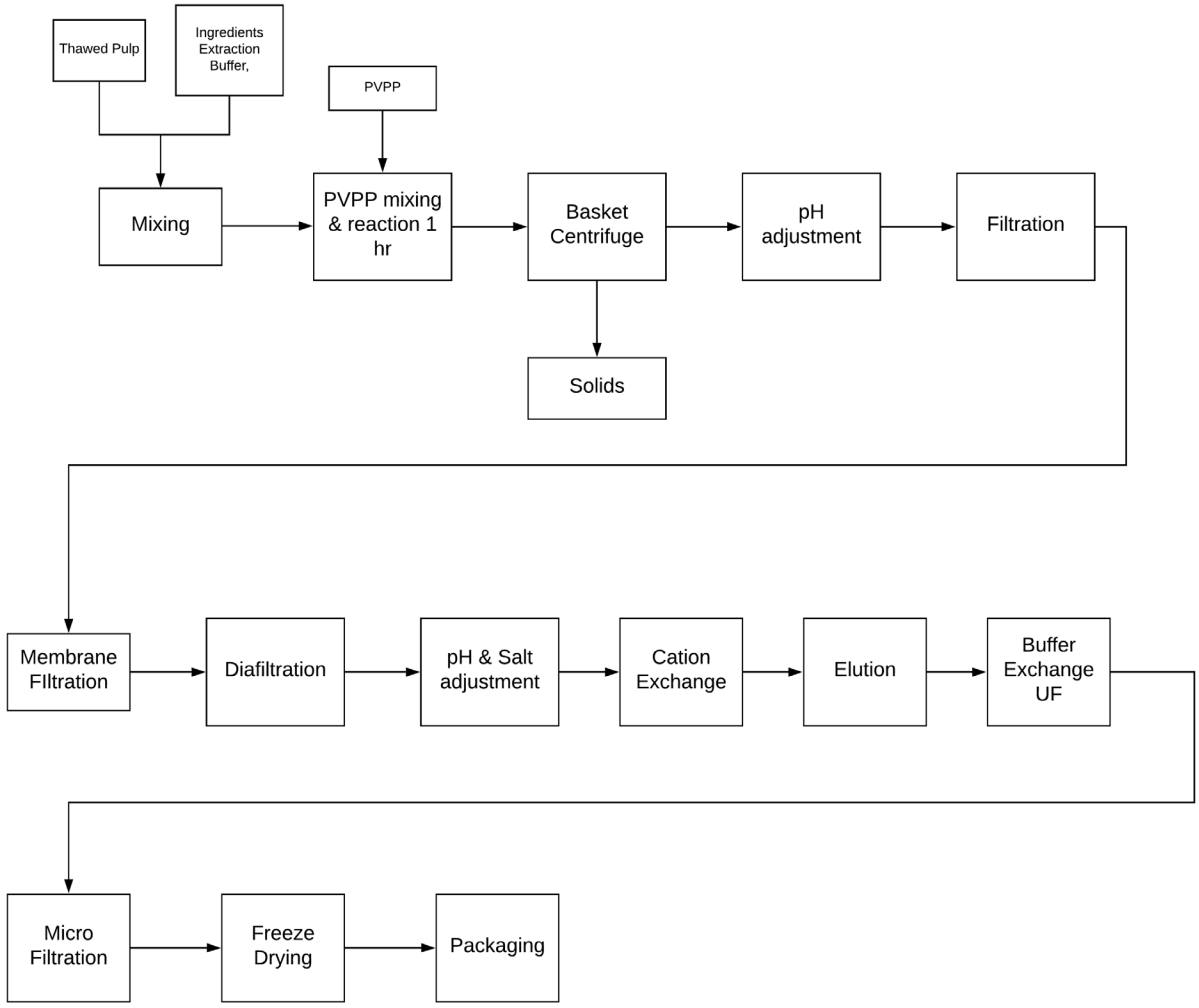


To produce miracle fruit protein, frozen miracle fruit pulp is thawed and mixed in jacketed tanks with an extraction buffer consisting of citric acid, sodium chloride, ethylenediaminetetraacetic acid (EDTA), and ascorbic acid. The pH is adjusted with sodium hydroxide and polyvinylpolypyrrolidone (PVPP) powder is used as a clarifying agent. The mixture is sheared at high speed to homogenize the ingredients. This shearing step also macerates the pulp to break down the particle size. The resultant slightly viscous mixture is then pumped into a basket centrifuge through a 3 to 5 µm cloth. The filtrate is recovered and drained by gravity or pumped; once all of the solution is processed, it is flushed with dialysis buffer (citric acid, sodium chloride, pH 5.0 adjusted with sodium hydroxide). The pH of the solution is then adjusted by addition of sodium hydroxide and is sheared at low speed. The resulting solution is clarified by sequential filtration from 20 µm down to 0.45 µm by either plate filter press, cartridge filtration, or disc-stack centrifugation. The solution is pumped through the apparatus and the filtrate is collected into a filtrate tank. Once all of

the pH-adjusted solution is pumped through and the filtrate collected, the system is flushed with dialysis buffer. The filtrate is then subjected to ultrafiltration through a M20 spiral wound membrane. The retentate is recirculated and the permeate is collected. The solution is then concentrated and the ultrafiltrate is subjected to diafiltration with a dialysis buffer in a "feed and bleed" set up. The flow rate into the feed tank is matched to the permeate flow rate, maintaining a constant level in the feed tank. The concentrate is drained from the unit and flushed with dialysis buffer to ensure maximum recovery of the protein. Next, the pH of the solution is adjusted for ion-exchange loading and subjected to ion exchange chromatography to obtain the miraculin protein.

As the elution buffer contains sodium and chloride salts, the concentrate is subjected to a second diafiltration step with an ultrafilter membrane and water. The concentrate is removed, and the membrane is flushed to recover as much protein as possible. The concentrate is subjected to a microfiltration step *via* a 0.45 µm filter to remove any microorganisms that may be present. The liquid concentrate is frozen in sanitized freezer-dryer pans or vessels then freeze-dried until the moisture content is "sufficiently low". After the freeze-drying step, the miracle fruit protein is packaged and stored at room temperature at low humidity. A schematic overview of the production process of miracle fruit protein is provided in Figure 2.2-2.

Figure 2.2-2 Schematic Overview of the Production Process of Miracle Fruit Protein



hr = hour(s); PVPP = polyvinylpolypyrrolidone; UF = ultrafiltration.

2.3 Product Specifications

2.3.1 Product Specifications for Miracle Fruit Pulp and Miracle Fruit Powder

Food-grade specifications have been established for miracle fruit pulp and miracle fruit powder (Table 2.3.1-1). All methods of analysis are internationally recognized [*e.g.*, Association of Official Analytical Chemists (AOAC)] or equivalent.

Table 2.3.1-1 Product Specifications for Miracle Fruit Pulp and Miracle Fruit Powder

Specification Parameter	Specification Limit		Method of Analysis
	Miracle Fruit Pulp	Miracle Fruit Powder	
Chemical			
Moisture (%)	<90	<5	AOAC 925.09 AOAC 926.08
Total protein (%) (as is)	<2	<10	AOAC 990.03 AOAC 968.06 AOAC 992.15
Ash (%) (as is)	<1	<5	AOAC 942.05
Carbohydrates (%) (as is)	<15	<85	Calculated
Fat (%) (as is)	<2	<15	AOAC 954.02
Heavy Metals			
Arsenic (ppm)	<0.05	<0.1	SAM 04001 (ICP-MS)
Cadmium (ppm)	<0.05	<0.1	SAM 04001 (ICP-MS)
Lead (ppm)	<0.05	<0.1	SAM 04001 (ICP-MS)
Mercury (ppm)	<0.05	<0.1	SAM 04001 (ICP-MS)
Microbiological Parameters			
Aerobic Plate Count	<100,000 CFU/g	<10,000 CFU/g	MFHPB-33
Escherichia coli	Negative	Negative	MFHPB-34
Salmonella spp.	Negative	Negative	MFHPB-20
Listeria spp.	Negative	Negative	MFHPB-30

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; ICP-MS = inductively coupled plasma mass spectroscopy; N/A = not available; ppm = parts per million.

2.3.2 Product Specifications for Miracle Fruit Protein

Food-grade specifications have been established for miracle fruit protein (Table 2.3.2-1). All methods of analysis are internationally recognized (*e.g.*, AOAC) or equivalent. The miraculin content is measured using a validated high-performance liquid chromatography (HPLC) method, developed by Joywell Foods.

Table 2.3.2-1 Product Specifications for Miracle Fruit Protein

Specification Parameter	Specification Limit	Method of Analysis
Chemical		
Moisture (%)	<7	AOAC 925.09 (mod.)
Total protein (%) (as is)	>20	AOAC 968.06 AOAC 990.03 AOAC 992.15
Sodium (% by mass)	<30%	AOAC 2015.01 / SAM 04001 (ICP-MS)

Table 2.3.2-1 Product Specifications for Miracle Fruit Protein

Specification Parameter	Specification Limit	Method of Analysis
Miraculin (% of total Protein)	>75% expected	HPLC (Validated Internal Method)
Heavy metals		
Arsenic (ppm)	<0.5	SAM 04001 (ICP-MS)
Cadmium (ppm)	<0.5	SAM 04001 (ICP-MS)
Lead (ppm)	<0.5	SAM 04001 (ICP-MS)
Mercury (ppm)	<0.5	SAM 04001 (ICP-MS)
Microbiological Parameters		
Aerobic Plate Count (CFU/g)	<10,000	MFHPB-33
Escherichia coli (/g)	Negative	MFHPB-34
Salmonella spp. (/g)	Negative	MFHPB-20
Listeria spp. (/g)	Negative	MFHPB-30

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; HPLC = high-performance liquid chromatography; ICP-MS = inductively coupled plasma mass spectroscopy; ppm = parts per million.

2.4 Batch Analyses

2.4.1 Batch Analyses for Miracle Fruit Pulp and Miracle Fruit Powder

Analysis of 3 non-consecutive lots of miracle fruit pulp (Lot Nos. 200219_523, 200224_045, 200224_532, and 200224_18) and 4 non-consecutive lots of miracle fruit powder (Lot Nos. 201911-0060, 201909-0060, 201915-0060, and 201913-0060) demonstrates that the manufacturing process as described in Section 2.2 produces a consistent product that meets the established product specifications. A summary of the batch analyses is presented in Table 2.4.1-1 below.

Table 2.4.1-1 Batch Analysis of Miracle Fruit Pulp and Miracle Fruit Powder

Parameter	Manufacturing Lot No.								
	Limit	Miracle Berry Pulp			Limit	Miracle Berry Powder			
		200219_523	200224_045	200224_18		201911-0060	201909-0060	201915-0060	201913-0060
Moisture (%)	<90	88.00	84.80	84.45	<5	0.6	0.7	1.2	0.6
Total protein (%)	<2	1.00	1.00	1.44	<10	5.38	7.13	6.94	5.31
Ash (%)	<1	0.41	0.43	0.49	<5	3.79	4.52	4.13	3.69
Carbohydrates (%)	<15	9.72	12.86	12.66	<85	79.29	77.47	76.75	80.84
Fat (%)	<2	0.87	0.91	0.96	<15	10.94	10.18	10.98	9.56
Arsenic (ppm)	<0.05	<0.01	<0.01	<0.01	<0.1	0.03	0.03	0.03	0.03
Cadmium (ppm)	<0.05	0.004	0.003	0.004	<0.1	0.053	0.062	0.065	0.054
Lead (ppm)	<0.05	<0.01	<0.01	<0.01	<0.1	<0.01	<0.01	<0.01	<0.01
Mercury (ppm)	<0.05	<0.005	<0.005	<0.005	<0.1	<0.005	<0.005	<0.005	<0.005
Aerobic Plate Count (CFU/g)	<100,000	930	8,700	8,900	<10,000	40	<10	20	<10
Coliforms (MPN/g)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 2.4.1-1 Batch Analysis of Miracle Fruit Pulp and Miracle Fruit Powder

Parameter	Manufacturing Lot No.								
	Limit	Miracle Berry Pulp			Limit	Miracle Berry Powder			
		200219_523	200224_045	200224_18		201911-0060	201909-0060	201915-0060	201913-0060
Escherichia coli	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Listeria spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units; MPN = most probable number; ppm = parts per million.

2.4.2 Batch Analyses for Miracle Fruit Protein

Analysis of 4 non-consecutive lots of miracle fruit protein (Lot Nos. PP01-2120, PP01-2320, PP01-2720, and PP12-1619) demonstrates that the manufacturing process as described in Section 2.2 produces a consistent product that meets the established product specifications. A summary of the batch analyses is presented in Table 2.4.2-1 below.

Table 2.4.2-1 Batch Analysis of Miracle Fruit Protein

Specification Parameter	Specification Limit	Manufacturing Lot No.			
		PP01-2120	PP01-2320	PP01-2720	PP12-1619
Chemical					
Moisture (%)	<7%	5.55	5.47	4.93	5.03
Protein (%) (as is)	>20%	29.06	34.75	32.63	36.50
Miraculin (%) (as is)	>75%	89.1	88.5	83.1	81.5
Sodium (% by mass)	<30%	25.5	19.8	16.1	11.1
Heavy Metals					
Arsenic (ppm)	<0.5 ppm	<0.08	<0.08	<0.08	0.32
Cadmium (ppm)	<0.5 ppm	<0.08	<0.08	<0.08	<0.08
Lead (ppm)	<0.5 ppm	<0.08	<0.08	<0.08	<0.08
Mercury (ppm)	<0.5 ppm	0.40	0.32	0.16	0.16
Microbiological					
Aerobic plate count (CFU/g)	<10,000	8,700	5,300	80	50
Escherichia coli (/g)	Negative	Negative	Negative	Negative	Negative
Salmonella spp. (/g)	Negative	Negative	Negative	Negative	Negative
Total Coliforms (CFU/g)	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units; ND = not detected; ppm = parts per million; TBD = to be determined.

2.5 Additional Chemical Characterization

2.5.1 Miracle Fruit Pulp and Miracle Fruit Powder

2.5.1.1 Sugar Profile

The sugar profile of 4 non-consecutive batches of miracle fruit pulp (Lot Nos. 200219_523, 200224_045, 200224_532, and 200224_18) and 4 non-consecutive lots of miracle fruit powder (Lot Nos. 201911-0060, 201909-0060, 201915-0060, and 201913-0060) were measured using AOAC method 982.14. The results are summarized in Table 2.5.1.1-1 below and demonstrate the sugar content of the miracle fruit pulp and miracle fruit powder to be consistent across the production batches.

Table 2.5.1.1-1 Sugar Profile of 4 Non-Consecutive Batches of Miracle Fruit Pulp and Miracle Fruit Powder

Parameter	Manufacturing Lot No.							
	Miracle Fruit Pulp				Miracle Fruit Powder			
	200219_523	200224_532	200224_045	200224_18	201911-0060	201909-0060	201915-0060	201913-0060
Fructose (%)	3.30	3.12	3.35	3.38	29.37	26.69	27.90	30.97
Glucose (%)	2.74	2.61	2.78	2.85	27.00	25.73	26.12	27.80
Sucrose (%)	<0.15	0.74	0.92	0.77	1.67	1.58	1.63	1.77
Maltose (%)	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Lactose (%)	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Total sugars (%)	6.04	6.47	7.05	7.00	58.04	54.00	55.65	60.54
Total starch[a] (%)	2.2	2.0	1.9	2.0	19.00	20.00	19.00	20.00
Total dietary fiber (%)	1.6	1.6	0.7	1.3	14.2	13.5	13.8	14.0

[a] Including glucose.

2.5.1.2 Antinutrients

Four non-consecutive batches of miracle fruit pulp (Lot Nos. 200219_523, 200224_045, 200224_532, and 200224_18) and miracle fruit powder (Lot Nos. 201911-0060, 201909-0060, 201915-0060, and 201913-0060) were analyzed for phytic acid, oxalic acid, and trypsin inhibitors (Table 2.5.1.2-1). The levels of phytic acid and trypsin inhibitors were below the limit of detection, indicating the absence of these antinutrients in the miracle fruit pulp and miracle fruit powder. The oxalic acid content ranged from less than 400 to 1,170 ppm in miracle fruit pulp and 820 to 1,210 ppm in miracle fruit powder.

Table 2.5.1.2-1 Analysis for Antinutrients in 4 Non-Consecutive Batches of Miracle Fruit Pulp and Miracle Fruit Powder

Parameter	Manufacturing Lot No.							
	Miracle Fruit Pulp				Miracle Fruit Powder			
	200219_523	200224_532	200224_045	200224_18	201911-0060	201909-0060	201915-0060	201913-0060
Phytic acid (%)[a]	<0.14	<0.14	<0.14	<0.14	<0.14	<0.14	<0.14	<0.14
Oxalic acid (ppm)[b]	<400	NM	993	1,170	820	1,210	1,160	856
Trypsin inhibitors (TIU/mg)[c]	<0.5	NM	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

NM = not measured; ppm = parts per million; TIU = trypsin inhibitor units.
[a] Method of analysis: Ellis *et al.* (1977); Limit of detection: 0.14%.
[b] Method of analysis: AOAC 986.13 (modified).
[c] Method of analysis: AOCS Ba 12-75 (modified) and Hamerstrand *et al.* (1981) (modified); Limit of detection:0.5 TIU/mg

2.5.1.3 Polyphenol Content

One batch of miracle fruit pulp (Lot No. 200219_523) and 4 non-consecutive batches miracle fruit powder (Lot Nos. 201911-0060, 201909-0060, 201915-0060, and 201913-0060) were analyzed for polyphenol content as gallic acid equivalents (GAE), tannic acid equivalents (TAE), catechine equivalents (CE), or epicatechine equivalents (ECE) (Table 2.5.1.3-1). The results demonstrate that freeze-dried miracle fruit powder contains total polyphenols at levels of 21,700 to 32,600 mg/kg (as GAE), 26,200 to 39,200 mg/kg (as TAE), 17,000 to 26,000 mg/kg (as CE), or 13,200 to 19,800 mg/kg (as ECE). These values are in line with the values determined for miracle berry pulp, when factoring in moisture content.

Table 2.5.1.3-1 Polyphenol Content of 4 Non-Consecutive Batches of Miracle Fruit Pulp and Miracle Fruit Powder

Parameter	Manufacturing Lot No.					
	Miracle Berry Pulp		Miracle Berry Powder (As Is)			
	200219_523 (as is)	200219_523 (dry basis)	201911-0060	201909-0060	201915-0060	201913-0060
Polyphenols (as GAE) (mg/kg)	2,920	16,222	25,300	22,200	21,700	32,600
Polyphenols (as TAE) (mg/kg)	3,510	19,500	30,500	26,700	26,200	39,200
Polyphenols (as CE) (mg/kg)	2,320	12,889	20,200	17,700	17,300	26,000
Polyphenols (as ECE) (mg/kg)	1,770	9,833	15,400	13,500	13,200	19,800

CE = catechine equivalents; ECE = epicatechine equivalents; GAE = gallic acid equivalents; TAE = tannic acid equivalents.

2.5.1.4 Pesticides

Four non-consecutive batches of miracle fruit pulp (Lot Nos. 200219_523, 200224_045, 200224_532, and 200224_18) were analyzed for organochlorine pesticides and pyrethroids using gas chromatography-electron capture detector (GC-ECD). No pesticides were detected.

2.5.1.5 Minerals

The mineral content of 4 non-consecutive batches of miracle fruit pulp (Lot Nos. 200219_523, 200224_045, 200224_532, and 200224_18) and miracle fruit powder (Lot Nos. 201911-0060, 201909-0060, 201915-0060, and 201913-0060) were analyzed using inductively coupled plasma mass spectroscopy (ICP-MS) (Table 2.5.1.5-1).

Table 2.5.1.5-1 Mineral Content of 4 Non-Consecutive Batches of Miracle Fruit Pulp and Miracle Fruit Powder

Mineral (ppm)	Manufacturing Lot No.							
	Miracle Fruit Pulp				Miracle Fruit Powder			
	200219_523	200224_045	200224_532	200224_18	201911-0060	201909-0060	201915-0060	201913-0060
Aluminum	<0.2	0.3	<0.2	0.2	0.9	1.2	1.4	0.9
Antimony	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	<0.01	<0.01	<0.01	<0.01	0.03	0.03	0.03	0.03
Barium	0.03	0.02	0.04	0.03	0.75	0.22	0.21	0.21
Beryllium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bismuth	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Boron	0.8	0.7	0.8	1.0	5.8	7.9	8.4	6.0
Cadmium	0.004	0.003	0.004	0.004	0.053	0.062	0.065	0.054
Calcium	107	103	121	125	1,060	1,220	1,220	1,100
Chromium	0.09	0.06	0.07	0.10	0.18	0.24	0.26	0.17
Cobalt	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01	0.01
Copper	0.32	0.63	0.28	0.36	2.72	3.86	3.93	2.84
Iron	1.6	1.4	1.5	1.6	22.0	15.1	15.2	21.5
Lead	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Lithium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Magnesium	81.3	76.9	81.8	85.7	647	781	815	674
Manganese	3.36	2.39	2.53	2.81	22.9	22.7	22.0	23.8
Mercury	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Molybdenum	0.04	0.04	0.05	0.05	0.26	0.48	0.49	0.28
Nickel	0.05	0.75	0.06	0.09	0.15	0.25	0.27	0.16
Phosphorus	183	164	175	216	1,350	1,790	1,750	1,400
Potassium	2,050	2,100	2,150	2,170	14,000	16,100	16,300	14,300
Selenium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Silver	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Sodium	55	55	52	56	500	673	700	523
Strontium	0.52	0.44	0.64	0.73	4.83	5.34	5.55	5.12
Thallium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thorium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Tin	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	0.05	<0.01
Titanium	0.31	0.32	0.34	0.40	1.31	1.39	1.42	1.17
Uranium	<0.01	<0.01	<0.01	<0.01	0.02	0.02	0.02	0.02
Vanadium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2.5.1.5-1 Mineral Content of 4 Non-Consecutive Batches of Miracle Fruit Pulp and Miracle Fruit Powder

Mineral (ppm)	Manufacturing Lot No.							
	Miracle Fruit Pulp				Miracle Fruit Powder			
	200219_523	200224_045	200224_532	200224_18	201911-0060	201909-0060	201915-0060	201913-0060
Zinc	2.15	1.41	1.48	1.56	12.2	14.9	15.4	12.6
Zirconium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

ppm = parts per million.

2.5.2 Miracle Fruit Protein

2.5.2.1 Miracle Fruit Protein Purity

Analysis of 4 production batches of miracle fruit protein using Joywell Foods' validated HPLC method demonstrated the protein to be approximately 86% miraculin. Of the remaining 14%, peaks eluting close to the main peak of miraculin account for an additional 7% (on average). These peaks are suspected to be isoforms of miraculin, including different glycoforms. The remaining peak area was attributable to mostly low-level species (<1%), and another species accounting for about 3.2% on average.

2.5.2.2 Antinutrients

Analysis of 4 production batches of miracle fruit pulp and miracle fruit powder demonstrated the absence of phytic acid and trypsin inhibitors in the ingredient and low levels of oxalic acid (see Section 2.5.1.2). As the miracle fruit protein is produced by isolation and purification from the miracle fruit pulp, and considering the downstream production processes and purification steps, including microfiltration, diafiltration, it is expected that these antinutrients would not be present in miracle fruit protein.

2.5.2.3 Minerals

The mineral content of 4 non-consecutive batches of miracle fruit protein (Lot Nos. PP01-2120, PP01-2320, PP01-2720, and PP12-1619) were analyzed using ICP-MS (Table 2.5.2.3-1). Sodium and chloride are present in high amounts of the final product, approximately 181,324 and 280,691 ppm, respectively, due to the fact that sodium chloride is used as a processing aid in the buffer exchange step for miracle fruit protein and is thus concentrated in the final freeze-dried product. The chloride content was calculated using the molar equivalency of sodium chloride. The calculated sodium chloride content was approximately 46% on average (by mass), and the resulting calculated chloride content was 28% on average. The presence of high amounts of salt in the final freeze-dried material serves a dual purpose: functionality as a bulking agent and a stabilizer to maintain miraculin function.

Table 2.5.2.3-1 Mineral Content of 4 Non-Consecutive Batches of Miracle Fruit Protein

Mineral (ppm)	Manufacturing Lot No.			
	PP01-2120	PP01-2320	PP01-2720	PP12-1619
Aluminum	5.82	11.00	6.90	52.15
Antimony	<0.15	<0.15	<0.15	<0.15
Arsenic	<0.08	<0.08	<0.08	0.32
Barium	32.05	1.22	2.30	<0.15
Beryllium	<0.15	<0.15	<0.15	<0.15
Boron	<1	<1	<1	21.87
Cadmium	<0.08	<0.08	<0.08	<0.08
Calcium	211.24	203.59	155.45	444.14
Chromium	2.91	18.95	2.30	3.36
Cobalt	<0.15	<0.15	<0.15	<0.15
Copper	7.28	3.66	3.45	8.41
Iron	123.83	72.75	71.39	77.38
Lead	<0.08	<0.08	<0.08	<0.08
Magnesium	128.20	62.97	49.51	52.15
Manganese	2.91	1.83	2.30	1.68
Mercury	0.40	0.32	0.16	0.16
Molybdenum	<0.15	<0.15	<0.15	<0.15
Nickel	<0.15	11.61	<0.15	<0.15
Phosphorus	75.75	52.57	56.42	92.52
Potassium	<1.00	169.35	158.91	201.88
Selenium	4.37	<0.15	2.30	1.68
Silver	<0.15	<0.15	<0.15	<0.15
Sodium	254,952	198,090	161,216	111,036
Strontium	5.82	3.66	8.06	6.72
Thallium	<0.15	<0.15	<0.15	<0.15
Thorium	<0.15	<0.15	<0.15	<0.15
Tin	<0.15	<0.15	<0.15	<0.15
Titanium	1.45	0.61	1.15	1.68
Uranium	<0.15	<0.15	<0.15	<0.15
Vanadium	1.45	0.61	1.15	<0.15
Zinc	2.91	<0.15	<0.15	3.36
Chloride* (Calc)	394,675	306,643	249,562	171,884

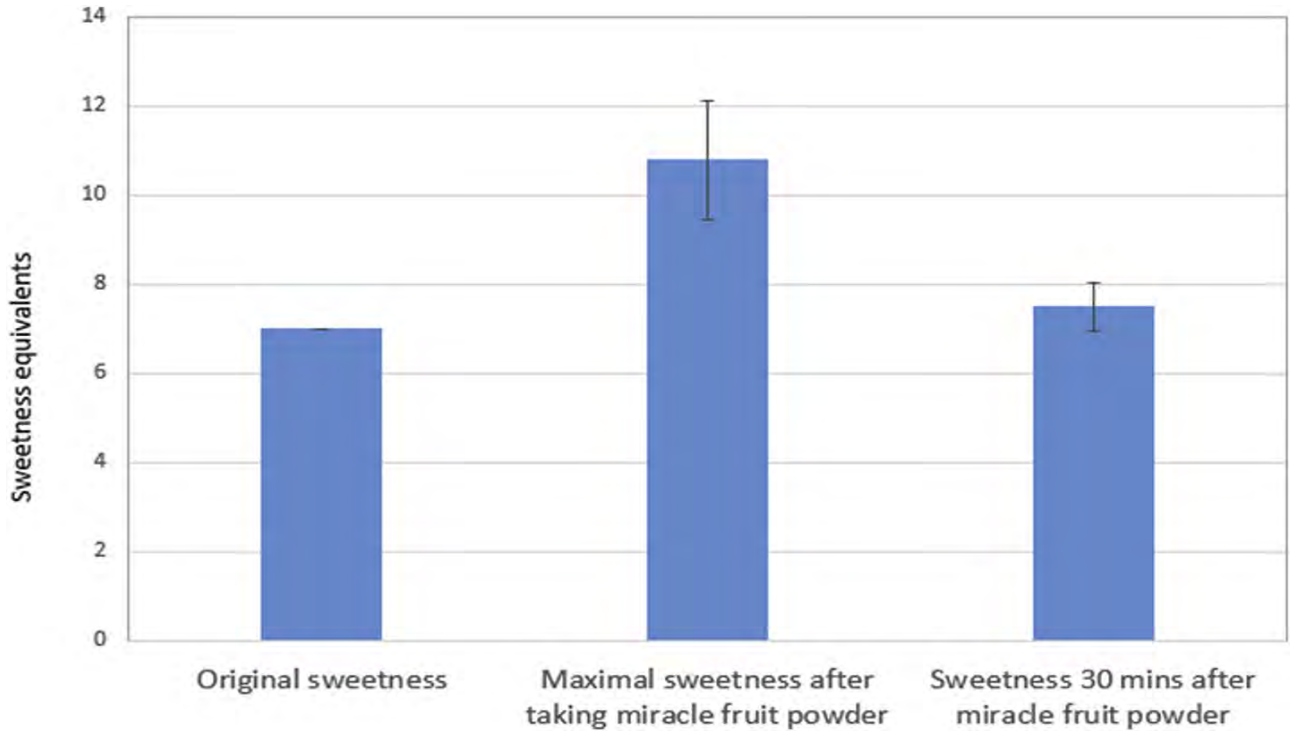
ppm = parts per million.
*Chloride calculated as molar equivalent to sodium (from NaCl).

2.6 Technical Effect

Miracle fruit powder, miracle fruit pulp and miracle fruit protein will be added to food and beverage products as taste modifiers and to impart sweetness by modifying the taste from sour to sweet. Kurihara and Beidler (1969) investigated the taste modifying effect of miraculin. The maximum relative sweetening effect was achieved within 3 minutes of consumption. The taste modification effect was concentration-dependent and declined rapidly after 30 minutes (Kurihara and Beidler, 1969). Similar

findings on the concentration-dependency of the taste modification effect were observed in the series of stability studies conducted with the miracle fruit pulp, miracle fruit powder, and miracle fruit protein (data not shown). Tafazoli *et al.* (2019) reported the taste modification effect of miracle fruit in a sensory panel test with 6 trained panelists. Each panelist was provided a lemonade juice with a sweetness intensity of 7 Brix to establish a baseline sweetness. Next, 0.08 g of miracle fruit powder was consumed by each panelist and held in the mouth for 1 minute before swallowing. Lemonade juice was consumed every 5 minutes for 30 minutes, and each panelist recorded the sweetness of each cup. The authors reported a significant increase in the perceived sweetness of lemonade juice, with sweetness returning to baseline after 30 minutes (Figure 2.6-1). These findings suggest a taste modification effect of miracle fruit (due to miraculin) with no lasting desensitization effect.

Figure 2.6-1 Sensory Evaluation of Lemonade Juice Following Consumption of Miracle Fruit Powder in Trained Panelists (N=6) (Taken from Tafazoli *et al.*, 2019)



mins = minute(s).

Miracle fruit powder and miracle fruit protein will be marketed in powdered form and these ingredients, along with miracle fruit pulp, will be added to a variety of food and beverage products, including alcoholic and non-alcoholic beverages, chewing gum, coffee and tea, dairy products, grain products, fruit-based and vegetable-based beverages, and confectionary products for its sweetening and taste-modifying properties due to the active glycoprotein, miraculin. There are no functional/health or nutrition claims associated with miracle fruit pulp, miracle fruit powder, and miracle fruit protein. Products containing miracle fruit pulp, miracle fruit powder or miracle fruit protein will be labelled and marketed in the same manner as conventional products. For example, an orange juice containing miracle fruit powder, pulp or protein will still be marketed as a "regular" orange juice, only the ingredient list will include an additional ingredient as "Miracle Fruit Powder", "Miracle Fruit Pulp", or "Miracle Fruit Protein". As such, it is not expected that an individual's consumption of a food product containing a miracle fruit-derived ingredient will increase compared to the consumption of the food product with no such ingredient, because both food products will be marketed and labelled in the same manner. Unlike the sensory study outlined above, the miracle fruit-

derived ingredients are to be added directly to the various food products, as outlined in Section 1.3, and are not intended to be consumed in the pulp, powder or protein form, prior to consuming sour foods, thus modifying their flavor profile. The miracle fruit ingredients will impart sweetness to the products to which they are added to, and do not change the pH of the food. As the food products containing miracle fruit products will be marketed in the same manner to the standard food products and that the sensory profile will not change, it is not expected that individuals who refrain from these foods would modify their consumption or diet accordingly.

As outlined above, while addition of miracle fruit powder, miracle fruit pulp, and miracle fruit protein to various food and beverage products will impart a sweetness, incorporation of Joywell Foods' miracle fruit-derived ingredients into the finished food or beverage product will not change the pH characteristics of the finished food. This has been demonstrated within 3 different product types, including flavored yogurt, an acidic beverage, and 3 flavors of popsicles, developed by Joywell foods. As presented in Table 2.7-1, addition of Joywell Foods' miracle fruit-derived ingredients will not impact the inherent pH of the finished food product.

Table 2.7-1 pH of Food Products with or Without Added Miracle Fruit Powder, Miracle Fruit Pulp, or Miracle Fruit Protein

Food Product	pH of the Food Product[a]			
	Control	Miracle Fruit Protein[b]	Miracle Fruit Pulp[c]	Miracle Fruit Powder[d]
Cherry Lemon Yogurt	4.40±0.01	4.41±0.01	4.40±0.01	4.39±0.01
Lemonade	2.77±0.01	2.89±0.01	2.77±0.01	2.76±0.01
Pop Lolly (Popsicle)				
Pineapple Lime Mint Flavor	3.38±0.00	NT	3.37±0.01	NT
Cherry Limeade	3.58±0.01	NT	3.58±0.01	NT
Mango Passionfruit	3.33±0.01	NT	3.36±0.00	NT

NT = not tested.

[a] Mean values and standard deviation of 3 tested samples for each food product.

[b] The levels of miracle fruit protein ranged from 1.5 mg in yogurt and 1.3 mg in lemonade samples.

[c] The levels of miracle fruit pulp ranged from 2.5 g in yogurt , 3 g in lemonade and 2 g in popsicle samples.

[d] The levels of miracle fruit powder ranged from 0.3 g in yogurt and 0.5 g in lemonade samples.

Considering that products containing Joywell Foods' ingredients will be marketed in the same manner as conventional food products and given the fact that addition of miracle fruit-derived ingredients were shown to not affect the pH of the various food products, it is unlikely that individuals who normally refrain from consuming acidic products, including those with digestive disorders such as acid reflux, would modify the consumption of these specific foods or their diet. As a result, individuals who suffer from acid reflux will continue to refrain from consuming such acidic foods and to self-regulate acidic food products that may contain Joywell Foods' ingredients in the same manner as conventional acidic foods. As such, there is little or no potential of these ingredients impacting individuals with acid reflux.

Part 3. § 170.235 Dietary Exposure

3.1 History of Use of the GRAS Substance and/or of its Source

Miracle fruit has reportedly been consumed in Ghana since the early 1700s (Roecklein and Leung, 1987) and was introduced to the U.S. in 1917 by the United States Department of Agriculture (USDA). Cultivation of miracle fruit has steadily grown over the years and several commercial products derived from miracle fruit are available on the U.S. marketplace. Brief general Internet searches for "miracle berry", "miracle fruit",

and “miraculin” identified several dietary supplement-type products containing miracle berry or miracle fruit extract that are currently available on the U.S. market [*e.g*., mberry Miracle Fruit Tablets, Richberry (freeze-dried miracle berries), MiraBurst Miracle Berry Tablets, Miraculous Melting Tablets, Miracle Frooties Miracle Fruit Tablets]. These products contain 100 to 200 mg of miracle fruit. Freeze-dried miracle berry/fruit also appear to be commercially available. 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 extract and/or miraculin in the U.S. It should be noted that exposures to miracle berry/fruit or miraculin is likely very limited based on the current availability of products containing these compounds and the nature of these types of products (*i.e*., dietary supplements). Therefore, the background dietary intake of miracle berry extract (and therefore miracle berry/fruit) and/or miraculin (as a purified protein) is likely negligible and unlikely to have an impact on its safe use as a food ingredient.

A GRAS affirmation petition and subsequent food additive petition for miracle fruit berries, concentrates, and extracts were denied in 1974 by the U.S. FDA based on the insufficiency of the available safety information at the time (U.S. FDA, 1977). In 2009, My M Fruit LLC submitted a New Dietary Ingredient (NDI) Notification to the FDA regarding the use of “miracle fruit extract” derived from the fruit of *S. dulcificum* in dietary supplements (NDI 574) (U.S. FDA, 2009). The recommended serving per day for Miracle Fruit Tablet is 1 tablet/day, or an effective serving size of 0.175 g miracle fruit extract/day. The tablet was intended to be dissolved completely on the tongue. Due to the fact that the effects of the tablets were to take place on the tongue and therefore not require ingestion, the FDA stated that its use would not be considered a dietary supplement.

While there is no formal regulatory status for miracle berry/fruit, miracle fruit protein, or products derived thereof in the U.S., the berry/fruit itself has been consumed as a conventional food both internationally and in the U.S. before 01 January 1958.

3.2 Estimated Dietary Intake of Miracle Fruit Protein, Miracle Fruit Powder, and Miracle Fruit Pulp for the Assessment of the Exposure to Antinutrients

3.2.1 Methods

An assessment of the anticipated intakes of miracle fruit protein, miracle fruit powder, and miracle fruit pulp for the determination of the exposure to antinutrients, as presented in Section 6.1.1 under the intended conditions of use 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) (CDC, 2020a,b; USDA, 2019a).

The NHANES data were employed to assess the mean and 90th percentile intakes of miracle fruit protein, miracle fruit powder, and miracle fruit pulp for each of the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 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, and both gender groups combined).

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). 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 intakes of miracle fruit protein, miracle fruit powder, and miracle fruit pulp by the U.S. population. Estimates for the daily intakes of these ingredients 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 the ingredient averaged over all individuals surveyed, regardless of whether they consumed food products in which these ingredients are proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing these ingredients during the 2 survey days). "Consumer-only" intake refers to the estimated intake of the ingredient by those individuals who reported consuming food products in which the use of these ingredients is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which these ingredients are proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intakes of miracle fruit powder and miracle fruit pulp were generated using the maximum use level indicated for each intended food use and food consumption data available from the 2015-2016 NHANES datasets, and are presented in Section 3.2.2.and 3.2.3.

3.2.2 Results of Intake Estimates

The percentage of consumers was high for all 3 ingredients and among all age groups evaluated in the current intake assessment; greater than 69.9% of the population groups consisted of consumers of food products in which miracle fruit protein, miracle fruit powder, and miracle fruit pulp are currently proposed for use (Table 1.3-1). Children had the greatest proportion of consumers at up to 98.2%. 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.

3.2.2.1 Miracle Fruit Powder

A summary of the estimated daily intake of miracle fruit powder from proposed food uses is provided in Table 3.2.2.1-1 on an absolute basis (g/day) and on a body weight basis (mg/kg body weight/day).

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of miracle fruit powder were determined to be 1.70 and 3.38 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 2.25 and 4.31 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 0.90 and 1.88 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 determined to be 27 and 54 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 any population group, of 73 and 155 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 19 and 38 mg/kg body weight/day, respectively.

Table 3.2.2.1-1 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)	Consumer-Only Intake (g/day)		Consumer-Only Intake (mg/kg bw/day)	
		Mean	90th Percentile	Mean	90th Percentile
Infants and Young Children	0 to 2	0.90	1.88	73	155
Children	3 to 11	1.28	2.35	50	99
Female Teenagers	12 to 19	1.30	2.51	22	45
Male Teenagers	12 to 19	1.51	3.13	23	49
Female Adults	20 and up	1.42	2.75	19	38
Male Adults	20 and up	2.25	4.31	26	50
Total Population	2 and up	1.70	3.38	27	54

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

3.2.2.2 Miracle Fruit Pulp

A summary of the estimated daily intake of miracle fruit pulp from proposed food uses is provided in Table 3.2.2.2-1 on an absolute basis (g/day), and on a body weight basis (g/kg body weight/day).

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of miracle fruit pulp were determined to be 12.24 and 24.27 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 pulp on an absolute basis, at 16.18 and 30.92 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.45 and 13.48 g/person/day, respectively. On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of miracle fruit pulp were determined to be 0.19 and 0.39 g/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 any population group, of 0.52 and 1.11 g/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 0.14 and 0.27 g/kg body weight/day, respectively.

Table 3.2.2.2-1 Summary of the Estimated Daily Intake of Miracle Fruit Pulp from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	Consumer-Only Intake (g/day)		Consumer-Only Intake (g/kg bw/day)	
		Mean	90th Percentile	Mean	90th Percentile
Infants and Young Children	0 to 2	6.45	13.48	0.52	1.11
Children	3 to 11	9.20	16.88	0.36	0.71
Female Teenagers	12 to 19	9.36	18.19	0.16	0.32
Male Teenagers	12 to 19	10.89	22.83	0.16	0.35
Female Adults	20 and up	10.22	20.07	0.14	0.27
Male Adults	20 and up	16.18	30.92	0.19	0.35
Total Population	2 and up	12.24	24.27	0.19	0.39

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

Overall, consumption data and information pertaining to the intended food uses of miracle fruit powder and miracle fruit pulp were used to estimate the *per capita* and consumer-only intakes of these ingredients for specific demographic groups and for the total U.S. population, so as to evaluate exposure to anti-nutrients from miracle fruit pulp and powder under the intended conditions of use, as discussed in Section 6.1.1. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain miracle fruit protein, miracle berry powder, or miracle fruit pulp 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 the ingredients will have 100% market penetration in all identified food categories.

For miracle fruit powder, on a consumer-only basis, the resulting mean and 90th percentile intakes by the total U.S. population from proposed food uses in the U.S. were estimated to be 1.70 g/person/day (27 mg/kg body weight/day) and 3.38 g/person/day (54 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 2.25 g/person/day (26 mg/kg body weight/day) and 4.31 g/person/day (50 mg/kg body weight/day), as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 0.90 and 1.88 g/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 73 and 155 mg/kg body weight/day and the mean and 90th percentile intake.

For miracle fruit pulp, on a consumer-only basis, the resulting mean and 90th percentile intakes by the total U.S. population from proposed food uses in the U.S. were estimated to be 12.24 g/person/day (0.19 g/kg body weight/day) and 24.27 g/person/day (0.39 g/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of miracle fruit pulp were determined to be 16.18 g/person/day (0.19 mg/kg body weight/day) and 30.92 g/person/day (0.35 mg/kg body weight/day), as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.45 and 13.48 g/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 0.52 and 1.11 g/kg body weight/day and the mean and 90th percentile intake.

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 pulp, miracle fruit powder, and miracle fruit protein are intended as ingredients for use in conventional food and beverage products. The active glycoprotein in all these ingredients is miraculin. Miraculin exerts its taste modifying effects by binding to the taste receptors of the tongue to change taste from sour to sweet. There will be a limitation of this modification effect due to the saturation of the receptors on the tongue, thus, limiting the levels of each miracle fruit ingredient that can be added in various food and beverage products.

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

Although there exists a long and wide history of consumption of miracle berry in the U.S. and globally, the statutory basis for Joywell Foods' conclusion of the GRAS status of miracle fruit powder, miracle fruit pulp and miracle fruit protein is based on scientific procedures and not common use in food.

Part 6. § 170.250 Narrative and Safety Information

6.1 Safety Narrative

A comprehensive search of the scientific literature relevant to the safety of miracle fruit pulp, miracle fruit powder, and miracle fruit protein was conducted through February 2021 using the electronic search tool ProQuest Dialog™[2]. Search terms were prepared to reflect the compound of interest with metabolism and preclinical/clinical endpoints.

Joywell Foods has concluded that miracle fruit powder, miracle fruit pulp, and miracle fruit protein are GRAS for use in various conventional food and beverage products, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of miracle fruit powder, miracle fruit pulp, and miracle fruit protein, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Associate Professor Joseph Baumert (University of Nebraska-Lincoln), Professor Emeritus Robert J. Nicolosi (University of Massachusetts Lowell), and Professor Emeritus I. Glenn Sipes (University of Arizona).

The GRAS Panel independently and critically evaluated all data and information presented herein, and concluded that miracle fruit powder, miracle fruit pulp, and miracle fruit protein is GRAS for use in various conventional food and beverage products, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and the conclusions of the GRAS Panel are provided in Appendix A.

6.1.1 Safety of Miracle Fruit Powder and Miracle Fruit Pulp

6.1.1.1 Publicly Available Data Relevant to Safety of Miracle Fruit Powder and Miracle Fruit Pulp

The results of the literature search identified a number of studies conducted with miracle fruit powder or the fruit or leaf extracts of miracle fruit. These studies were mainly efficacy-focused with some limited toxicity-related endpoints and evaluated the effects of miracle fruit powder or fruit and extracts thereof on blood glucose, glucose tolerance, insulin resistance, hematology and blood chemistry of diabetic and non-diabetic rats, and anti-hyperuricemic effects in mice. These studies included evaluation of 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). A summary of these studies is provided in Table 6.1.1.1-1 below.

[2] The following databases were searched: 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®.

Table 6.1.1.1-1 Summary of Studies Conducted with Miracle Fruit Powder or Fruits and Leaf Extracts of Miracle Fruit

Species (Strain), Sex, Number of Animals	Route of Administration and Study Duration	Dose/Concentration	Parameters Evaluated	Significant Findings[a]	Reference
Rat (Wistar) M N=8/group	Oral (gavage) Single dose[b]	0, 0.02, 0.04, 0.2 mg/kg miracle fruit powder	Plasma glucose at 0, 90, 120, 150, 180 min	• ↓ plasma glucose at ≥90 min in all treatment groups	Chen *et al.* (2006)
Rat (Wistar) M N=8/group	Oral (gavage) 3 days[c]	0, 0.02, 0.04, 0.2 mg/kg miracle fruit powder	IPGTT, plasma glucose at 0, 30, 60, or 120 min	• Dose-dependent ↓ in plasma glucose in IPGTT • ↓ total AUC for glucose response and plasma insulin in all treatment groups • ↓ glucose-insulin index in 0.2 mg/kg group	
Rat (Wistar) M N=8/group	Oral (gavage) 28 days[d]	0 or 0.2 mg/kg miracle fruit powder	Plasma glucose	• Amelioration of plasma glucose lowering effect of tolbutamide in treatment groups compared to control	
Rat (Wistar)[e] M N=8/group	Oral (gavage) 10 days[f]	0 or 0.2 mg/kg miracle fruit powder	Plasma glucose, bw, food and water consumption	• ↑ plasma glucose lowering activity • Reversal of hyperphagia effects • ↓ food and water intake • NSE on body weight	
Albino rats[g] M N=5/group	Oral (gavage) 4 weeks	0, 50, or 100% leaf extract or fruit extract (approximately 200 mg/kg bw/day)[h]	Blood glucose	• ↓ blood glucose	Dioso *et al.* (2016)
Rat (strain NR) Sex NR N=7/group	Oral (drinking water) 21 days	0, 30, or 60 mg/kg methanolic (MSD) or flavonoid-rich (FSD) *Synsepalum dulcificum* leaf extract	Hematology (RBC, WBC, PCV, hemoglobin concentration, neutrophil count), plasma sodium, calcium, and potassium concentrations	• ↑ plasma calcium, sodium, and potassium concentrations in diabetic control compared to non-diabetic control • ↓ plasma calcium and potassium concentrations in all MSD and FSD groups compared to diabetic control but ↑ compared to non-diabetic control • ↓ plasma sodium concentration in MSD groups compared to diabetic control but ↑ compared to non-diabetic control • ↓ plasma sodium concentration in FSD groups compared to diabetic control; NSD compared to non-diabetic control • ↑ WBC and neutrophil count in diabetic control compared to non-diabetic control • ↓ PCV, hemoglobin concentration, and RBC in diabetic control compared to non-diabetic control	Obafemi *et al.* (2016)

Table 6.1.1.1-1 Summary of Studies Conducted with Miracle Fruit Powder or Fruits and Leaf Extracts of Miracle Fruit

Species (Strain), Sex, Number of Animals	Route of Administration and Study Duration	Dose/Concentration	Parameters Evaluated	Significant Findings[a]	Reference
				• ↓ WBC and neutrophil count in all MSD and FSD groups compared to diabetic control but ↑ compared to non-diabetic control • ↑ PCV, hemoglobin concentration, and RBC in all MSD and FSD groups compared to diabetic control but ↓ compared to non-diabetic control	
Rat (strain NR) Sex NR N=7/group	Oral (drinking water) 21 days	0, 30, or 60 mg/kg methanolic (MSD) or flavonoid-rich (FSD) *Synsepalum dulcificum* leaf extract	Body weight, serum biochemistry (glucose, urea, ALT, AST, ALP, HDL-cholesterol, total cholesterol, triglycerides, total protein), liver, kidney, and pancreas lipid peroxidation (MDA, SOD, GST, GPx, catalase)	• ↓ body weight in diabetic control compared to non-diabetic control • ↑ body weight in all MSD and FSD groups • Amelioration of serum glucose levels in all MSD and FSD diabetic animals • ↓ ALP, AST, ALT, urea, and creatinine in all MSD and FSD diabetic groups compared to diabetic control; NSD in ALP and creatinine compared to non-diabetic control • ↑ AST and ALT in all MSD and FSD diabetic groups compared to normal control • ↓ urea in high-dose MSD group compared to non-diabetic control • ↑ urea in high-dose FSD group compared to non-diabetic control • ↓ ALP, AST, ALT, creatinine, urea in non-diabetic control compared to diabetic control • ↑ 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 • ↑ AST, ALT, urea in non-diabetic FSD group compared to non-diabetic control • ↑ 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)

Table 6.1.1.1-1 Summary of Studies Conducted with Miracle Fruit Powder or Fruits and Leaf Extracts of Miracle Fruit

Species (Strain), Sex, Number of Animals	Route of Administration and Study Duration	Dose/Concentration	Parameters Evaluated	Significant Findings[a]	Reference
Rat (strain NR) Sex NR	Oral (NFS) 21 days	0, 30, or 60 mg/kg MSD or FSD *Synsepalum dulcificum* leaf extract	HbA1c, IL-6, TNF-α, serum insulin levels, hepatic hexokinase activity	• ↓ HbA1c, IL-6, TNF-α • ↑ serum insulin levels, hepatic hexokinase activity	Obafemi *et al.* (2019)
Mouse (ICR)[i] Sex NR	Oral (NFS) 7 days	0, 500, or 1,000 mg/kg body weight/day of miracle fruit butanol extract	Liver and kidney weight, serum creatinine and blood urea nitrogen	• NSD relative-to-body liver and kidney weights, serum creatinine, or blood urea nitrogen	Shi *et al.* (2016)

↑ = increase(d); ↓ = decrease(d); ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUC = area under the curve; bw = body weight; FSD = flavonoid-rich *Synsepalum dulcificum* leaf extract; GPx = glutathione peroxidase; GST = glutathione-S-transferase; HbA1c = hemoglobin A1c; HDL-cholesterol = high-density lipoprotein cholesterol; IL-6 = interleukin-6; IPGTT = intraperitoneal glucose tolerance test; M = males; MDA = malondialdehyde; min = minute(s); MSD = methanolic *Synsepalum dulcificum* leaf extract; NFS = not further specified; NR = not reported; NSD = no significant difference; NSE = no significant effect; PCV = packed cell volume; RBC = red blood cells; SOD = superoxide dismutase; 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.

[i] Animals were treated with oxonic acid potassium salt to induce hyperuricaemia.

The relevance of these studies to the safety of Joywell Foods' miracle fruit pulp/powder or miracle fruit protein is limited due to the following:

- The studies conducted were non-standard toxicological studies, which mainly focused on efficacy related endpoints with some limited toxicological assessments.

- Detailed compositional analysis of the dosage materials and levels of miraculin protein were not provided.

- The test articles in the studies by Dioso *et al*. (2016), Obafemi *et al*. (2016, 2017, 2019), and Shi *et al*. (2016) were extracted from the fruit using various solvents including methanol, ethanol, or butanol resulting in compositional differences from miracle fruit pulp. As such, these test articles are not representative of Joywell Foods' miracle fruit pulp, miracle fruit powder or miracle fruit protein. Furthermore, due to the compositional differences between the test articles and miracle fruit pulp, the observed effects can be due to a concentrated component in the fruit/leaf or residual extraction solvents. Considering the foregoing, these studies are not considered relevant to the safety of miracle fruit pulp/powder or miracle fruit protein.

- In the study by Chen *et al.* (2006), which evaluated the effects of lyophilized miracle fruit powder on insulin resistance of male Wistar rats consuming a fructose-rich diet, the findings of the study appear to mainly reflect changes to those rats fed the fructose-rich diet and not the "control" animals that were fed standard rat chow. In addition, no analysis on food/water intake or bodyweight was conducted in test animals in the fructose-rich experiments. Therefore, it is difficult to determine whether the test article that was administered 3 times daily was having an impact on food intake due to sensory/palatability issues, thereby reducing dietary consumption. This could have led to a decrease in the amount of fructose available for absorption from the fructose-rich diet, and its eventual impact on the blood glucose and consequently insulin levels.

Although the above-described studies are of limited value to the safety of miracle fruit pulp or miracle fruit powder, nonetheless, the results of these studies do not raise any safety concerns with consumption of miracle fruit-derived ingredients.

A search of the European Union Novel Food catalog identified an application for authorization to place on the market of the dried fruit of *S. dulcificum* Daniell (referred to as "Dried Miracle Berry") for use as an ingredient in food supplements (European Commission, 2018). The application has been prepared and submitted by Baïa Food Co, in line with the administrative and scientific requirements of Commission Implementing Regulation (EU) 2017/2469 laying down for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, and specifically relates to the category covered in Article 2 (iv) "*food consisting of, isolated from or produced from plants or their parts, except when the food has a history of safe food use within the Union and is consisting of, isolated from or produced from a plant or a variety of the same species obtained by traditional propagating practices which have been used for food production within the Union before 15 May 1997*", and is currently under review by the European Food Safety Authority (EFSA) (EFSA-Q-2018-1032) (EFSA, 2020).

The subject of the Novel Food application, Dried Miracle Berry, is produced through blending and dehydrating the pulp and skin of the miracle fruit (*S. dulcificum*), which is similar to the manufacturing process of Joywell Foods' miracle fruit powder. As such, the safety of Dried Miracle Berry can be considered relevant to the safety of Joywell Foods' ingredients. The novel food ingredient is intended to be used in food supplements at a maximum daily dose of 0.9 g/day. In the Novel Food application, the safety of Dried Miracle Berry was evaluated based on proprietary compositional analytical studies, data on the level

of nutrients, potential toxicants, and contaminants, as well as information on the potential allergenicity, genotoxicity, subchronic toxicity, and human exposure to Dried Miracle Berry. The applicant has requested protection of proprietary data according to Article 26 of Regulation (EU) 2015/2283, as such, access to the full safety data is not available; however, in the summary document referenced by the European Commission (European Commission, 2018), it is stated that:

> "*The toxicological in vitro and in vivo studies performed by Baïa Food Co. showed no acute or sub-chronic adverse effects and hence, no NOAEL or ADI values could be determined accurately but only an indicative value could be provided (NOAEL 2000 mg/kg/day). To date, no adverse events have been described in the clinical trials and sensory analysis published in the literature*".

In comparison, the established no-observed-adverse-effect level (NOAEL) in the subchronic toxicity study conducted with Dried Miracle Berry is 37 times greater than the total population consumer-only intakes of Joywell Foods' miracle fruit powder, *i.e.*, 54 mg/kg body weight/day, and 5 times greater than the total population consumer-only intakes of Joywell Foods' miracle fruit pulp (*i.e*., 390 mg/kg body weight/day).

6.1.1.2 Compositional Data Supporting the Safety of Miracle Fruit Powder and Miracle Fruit Pulp

The safety of miracle fruit powder and miracle fruit pulp is further supported by the compositional analysis demonstrating that these ingredients are well characterized and are devoid of microbiological contaminants and heavy metals or environmental contaminants originating from the cultivation process such as pesticides (see Sections 2.4.1 and 2.5.1). The composition of miracle fruit pulp is essentially similar to miracle berries minus the seeds, and likewise, the use of miracle fruit pulp as a taste modifier is directly comparable to the addition of miracle fruit to various food products without the seed.

The proximate composition of miracle fruit pulp is comparable to the proximate analysis of several commonly consumed fruits, including blackberries, blueberries, raspberries, and sweet cherries, as presented in Table 6.1.1.2-1.

Table 6.1.1.2-1 Proximate Analysis of Miracle Fruit Pulp *Versus* Other Commonly Consumed Fruits

Parameter	Miracle Fruit Pulp	Blackberries (USDA, 2020a)	Blueberries (USDA, 2019b)	Raspberries (USDA, 2020b)	Sweet Cherries (USDA, 2019b)	Sour Cherries (USDA, 2019b)
Moisture (%)	~85	~88	~84	~86	~82	~86
Carbohydrate (%, as is)	~12	~9.6	~14.5	~12	~16	~12
Protein (%, as is)	~1	~1.4	~0.7	~1.2	~1.1	~1
Fat (%, as is)	~1	~0.5	~0.3	~0.7	~0.2	~0.3
Ash (%, as is)	~0.4	~0.4	~0.2	N/A	~0.5	~0.4

N/A = not available.

Considering that miracle fruit pulp is freeze-dried to produce the powder, the composition of miracle fruit powder is similar to that of miracle fruit pulp, when corrected for moisture (see Table 2.3.1-1). In addition to moisture, any minor potential variations in the composition of the pulp and powder are due to the fact that the powder form is milled after freeze-drying, which can contribute to small shifts in the composition.

The levels of antinutrients (trypsin inhibitors, oxalic acid, phytic acid, and polyphenols) in miracle fruit powder and miracle fruit pulp are unlikely to negatively affect the bioavailability of other nutrients in foods to which the ingredients are added and are of no safety concern. The levels of polyphenols in miracle fruit pulp and miracle fruit powder and the resultant daily intakes are compared to those from commonly consumed fruits (see Table 6.1.1.2-2). Although the levels of polyphenols in miracle fruit pulp and powder are higher than those reported for other fruits, when compared from a serving size perspective, the total polyphenol intake levels of miracle fruit powder and miracle fruit pulp are calculated to be half of that resulting from 1 serving of blackberries and comparable to those from other berries.

Table 6.1.1.2-2 Content and Daily Intakes of Polyphenols from Miracle Fruit Pulp and Miracle Fruit Powder *Versus* Other Commonly Consumed Fruits

Fruit	Polyphenols as GAE (mg/kg)	Fresh Fruit Daily Serving Size	Daily Intakes of Polyphenols (mg/day)
Miracle Fruit Powder	25,450[a]	-	86.02[b]
Miracle Fruit Pulp[c]	16,222	-	393.70[d]
Blackberries (FAO/IZiNCG, 2018)	5,694.3	150 g (USDA, 2020a)	854.1
Blueberries (FAO/IZiNCG, 2018)	2234.1	150 g (USDA, 2019b)	335.1
Raspberries (FAO/IZiNCG, 2018)	1546.5	150 g (USDA, 2020b)	232
Sweet Cherries (FAO/IZiNCG, 2018)	1,749	138 g (USDA, 2019c)	241.4
Sour Cherries (FAO/IZiNCG, 2018)	3,521.6	103 g (USDA,2019d)	362.7

GAE = gallic acid equivalent.
[a] Mean of 4 lots (see Table 2.5.1.3-1).
[b] Calculated taking into account the total population 90th percentile consumer-only intakes of miracle fruit powder on an absolute basis (see Table 3.2.2.1-1).
[c] Dry basis.
[d] Calculated taking into account the total population 90th percentile consumer-only intakes of miracle fruit pulp on an absolute basis (see Table 3.2.2.2-1).

The oxalic acid contents of miracle fruit pulp and miracle fruit powder (see Table 2.5.1.2-1) are compared with those of the commonly consumed fruits. As presented in Table 6.1.1.2-3, although the oxalic acid levels of miracle fruit pulp and miracle fruit powder are higher than those reported for other commonly consumed fruits, from a serving size perspective, the total oxalic acid intakes of miracle fruit powder and miracle fruit pulp are much lower than that resulting from 1 serving of black raspberries or concord grapes and comparable to those from other berries.

Table 6.1.1.2-3 Content and Daily Intakes of Oxalic Acid from Miracle Fruit Pulp and Miracle Fruit Powder *Versus* Other Commonly Consumed Fruits

Fruit	Oxalic Acid Content (mg/g)	Fresh Fruit Daily Serving Size	Daily Intakes of Oxalic Acid (mg/day)
Miracle Fruit Powder[a]	1.0115[a]	-	3.42
Miracle Fruit Pulp	0.8543	-	20.73
Blueberries[b]	0.15	150 g (USDA, 2019b)	22.50
Black Raspberries[b]	0.55	150 g (USDA, 2020c)	82.50
Concord Grapes[b]	0.25	150 g (USDA, 2020d)	37.50
Strawberries[b]	0.15	150 g (USDA, 2020e)	22.50

Table 6.1.1.2-3 Content and Daily Intakes of Oxalic Acid from Miracle Fruit Pulp and Miracle Fruit Powder *Versus* Other Commonly Consumed Fruits

Fruit	Oxalic Acid Content (mg/g)	Fresh Fruit Daily Serving Size	Daily Intakes of Oxalic Acid (mg/day)
Miracle Fruit Powder[a]	1.0115[a]	-	3.42
Miracle Fruit Pulp	0.8543	-	20.73

[a] Mean of 4 lots (see Table 2.5.1.2-1).
[b] (Han *et al.*, 2015).

6.1.2 Safety of Miracle Fruit Protein

6.1.2.1 Publicly Available Data Relevant to Safety of Miracle Fruit Protein

Following a comprehensive search of the scientific literature, 1 study was identified that evaluated the *in vitro* digestibility and *in silico* safety (allergenicity and toxigenicity) of miraculin (Tafazoli *et al*., 2019, 2020). This study serves as pivotal evidence of safety for Joywell Foods' miracle fruit protein. The *in vitro* digestibility study of miraculin demonstrated that the glycoprotein is rapidly and completely digested by gastric proteases following ingestion (see Section 6.1.2.1 and 6.1.2.2 for further details). The miraculin amino acid sequence obtained from the GenBank database (Accession No. P13087) was subjected to *in silico* testing to investigate the allergenicity and toxigenicity potential of the glycoprotein (see Section 6.1.2.3 and 6.1.2.4). The results of these bioinformatic searches indicate that miraculin would not pose an allergenic or toxigenic risk to consumers of products to which Joywell Foods' miracle fruit protein is added.

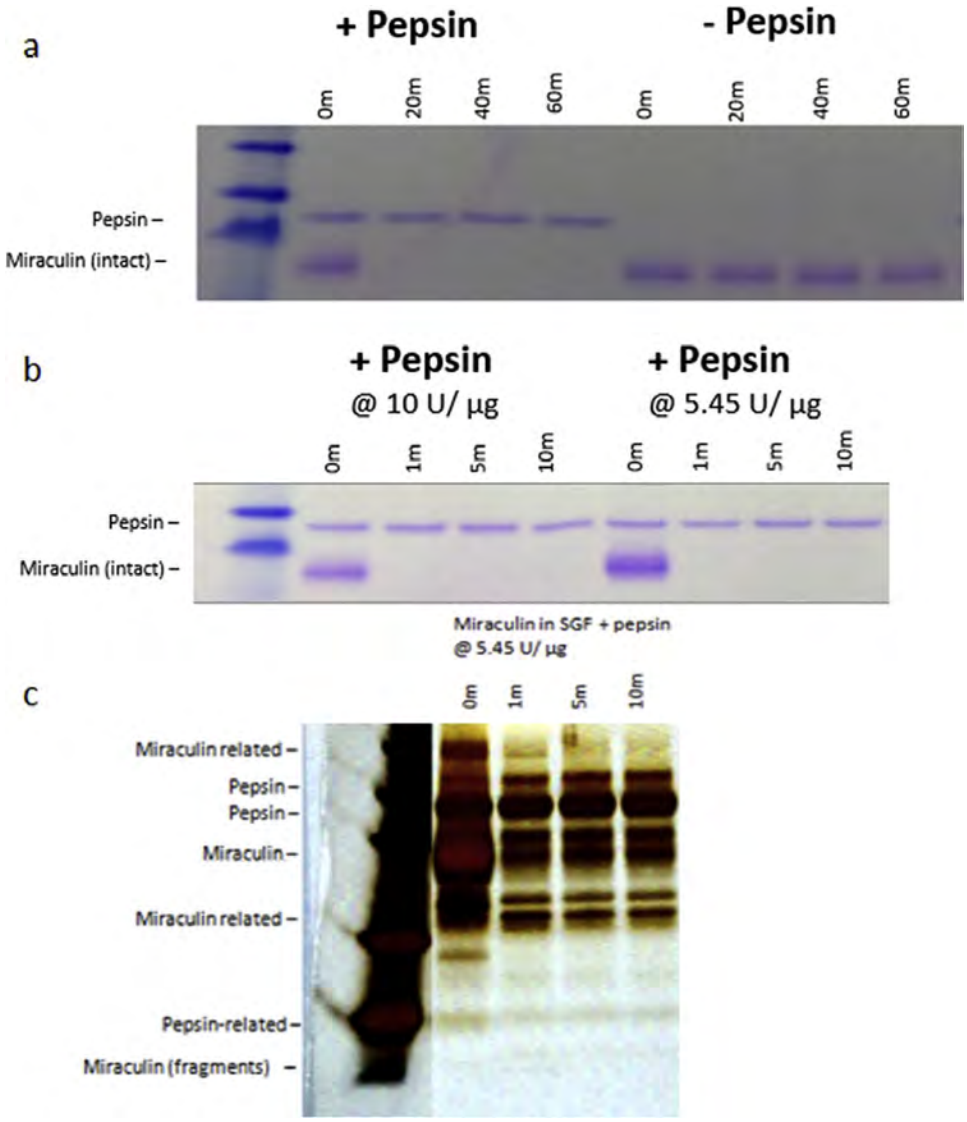
6.1.2.2 In Vitro Digestibility of Miraculin

The protein digestibility of miraculin was investigated in an *in vitro* simulated gastric fluid (SGF) model (Tafazoli *et al*., 2019, 2020). This study was conducted according to the methodology described by Thomas *et al.* (2004) and was designed to mimic the conditions of the human stomach. 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. At various timepoints during incubation (0, 20, 40, and 60 minutes), sample mixtures were quenched with sodium bicarbonate, tricine buffer solution, and a reducing agent, and heated at 85°C for 10 minutes. After the heating process, the protein digestibility was evaluated by gel electrophoresis using Coomassie blue and silver stains. The results of digestion demonstrate that miraculin was completely digested within 20 minutes (Figure 6.1.2.2-1a). In the silver staining, minor peptide fragments were reported at approximately 4 kDa within 10 minutes of digestion (Figure 6.1.2.2-1c).

The effect of pepsin concentration on the digestion of miraculin was evaluated in an SGF model containing pepsin at concentrations of 5.45 U/µg or 10 U/µg, incubated for up to 10 minutes. The results demonstrated that miraculin was readily digested within 1 minute of incubation with pepsin and that the digestion kinetics is pepsin dependent (Figure 6.1.2.2-1b).

Overall, the findings of Tafazoli *et al.* (2019, 2020) demonstrate that miraculin is expected to be readily digested in the gastrointestinal tract following ingestion.

Figure 6.1.2.2-1 ***In vitro*** **Digestibility of Miraculin (a) in the Presence of SGF with and without Pepsin for 60 Minutes, (b) in the Presence of SGF with Pepsin at Concentrations of 5.45 U/g and 10 U/g for 10 Minutes, (c) in Silver Stained Gels After Digestion with 5.45 U/g Pepsin for 10 Minutes (Taken from Tafazoli *et al*., 2019)**



SGF = simulated gastric fluid.

6.1.2.3 Pepsin Digest Mapping of Miraculin

The proteolytic fate of miraculin following pepsin digestion using liquid chromatography coupled with tandem mass spectroscopy was reported by Tafazoli *et al.* (2019, 2020). In this study, 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 at 0, 0.5, 1, and 10 minutes. The authors reported miraculin to be increasingly digested with longer digestion time; the number of unique peptides were reported to be 5, 33, 54, and 61 after 0, 0.5, 1, and 10 minutes of digestion, respectively. The number of unique peptides encompassed approximately 75% of the entire amino acid sequence after 10 minutes of pepsin digestion. The only peptides that were not identified after 10 minutes of digestion were peptides with cysteine residues (*i.e*., disulfide bonds) that were likely resistant to digestion (Tafazoli *et al.*, 2019). Based on the results of this study, the authors

concluded that following ingestion, miraculin would be readily digested into small peptide fragments and its amino acid components.

The authors provided the sequences of the pepsin digests, which were each further evaluated for their allergenicity potential (see Section 6.1.2.5).

6.1.2.4 In Silico Digestibility of Miracle Fruit Protein

The digestibility of miraculin was investigated using PeptideCutter[3], a publicly available bioinformatics tool, which predicts potential cleavage sites in a peptide sequence by proteases. The tool predicts the possible cleavage sites, as well as a map and table of each position. The full-length peptide sequence of miraculin without the signal peptide (1-29) was searched with PeptideCutter for pepsin digestion (pH >2.0), in order to corroborate the results of the *in vitro* digestibility study discussed in Section 6.1.2.2. Pepsin was predicted to cleave the 191 amino acid sequence of miraculin at 48 sites providing corroborating evidence that the protein is readily digested by gastric enzymes (Table 6.1.2.4-1).

Table 6.1.2.4-1 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

[3] https://web.expasy.org/peptide_cutter/.

Table 6.1.2.4-1 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)
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
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

6.1.2.5 Allergenicity Assessment of Miraculin

The allergenicity potential of miraculin (Accession No. P13087) was investigated through a search of the scientific literature to identify publications on the allergenicity of miracle fruit or miraculin, as well as a widely accepted bioinformatics approach recommended by the FAO/WHO and Codex Alimentarius, as well as EFSA (FAO/WHO, 2001; Codex Alimentarius, 2009; EFSA, 2017).

In the first step in the evaluation of the potential allergenicity of miraculin, a search of the scientific literature was conducted using the PubMed database to identify publications on allergenicity of miracle fruit or miracle fruit protein (miraculin). Search terms were used to focus the relevancy of the results to potential allergenicity of miracle fruit or miracle fruit protein (miraculin). Publications were reviewed for *in vitro* mechanistic studies, clinical studies or case reports on allergenic reactions in humans due to ingestion of the miracle fruit or its protein. No publications were identified which reported any type of allergenic reaction to miracle fruit consumption. One publication (Tafazoli *et al*., 2019, 2020) discussed the allergenic potential of miraculin, as well as the peptide digests of the glycoprotein. This study is discussed in further detail below.

In the second step, a sequence alignment search was conducted using the peptide sequence of the miraculin protein and the AllergenOnline database (Version 21) with the approach outlined by FAO/WHO (2001), Codex Alimentarius (2009), and EFSA (2017). In accordance with these guidelines, a "sliding window" of 80-amino acid sequences (*e.g*., segments 1–80, 2–81, 3–82, *etc*.) derived from the miraculin peptide sequence was searched against the AllergenOnline database[4]. The 80-amino acid alignment searches were conducted using default settings (E-value cut-off = 1 and maximum alignments of 20) and the FASTA36 algorithm. Significant sequence homology was defined as an identity match of greater than 35%; matches greater than 35% are suggestive of potential immunoglobulin E (IgE) cross-reactivity with putative allergens. Matches with an E-value greater than 10^{-7} were not considered to be significant (Hileman *et al.*, 2002; Song *et al*., 2015). If the sequence alignments were not significant, then no significant homology is expected to known allergens. 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 (Table 6.1.2.5-1).

It should be noted that although trypsin inhibitors from potato and soybean are considered known allergens, from a clinical perspective, neither the potato proteinase inhibitors nor the soy Kunitz trypsin inhibitor are considered as important food allergens (Taylor *et al.*, 2015). Soybeans contain multiple allergenic proteins and are considered one of the most common allergenic foods in the world (Kattan and Sampson, 2015; Taylor *et al*., 2015). 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 contribute to the trypsin inhibitory effect and resistance to denaturation (Sessa and Ghantous, 1987). SKTI is considered an inhalation allergen associated with occupational exposure to flour dust in bakers, affecting bakers exposed to large amounts of inhaled soy flour (Baur *et al*., 1996; Quirce *et al.*, 2006). The incidence of allergic reactions related to inhaled SKTI is very low (Moroz and Yang, 1980). Instead, the major soy allergens have been reported to be 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), as well as Gly m 8 (a 2S albumin) (Kattan and Sampson, 2015; Taylor *et al*., 2015).

Allergic reactions following the ingestion of potatoes have been infrequently described. Several allergenic proteins have been identified in potato with the major allergen in potatoes being reported, as Sola t 1, a 43 kDa protein known as patatin, the main storage protein of the potato tuber (Seppälä *et al*., 1999; Astwood *et al*., 2000; Majamaa *et al*., 2001). The importance of proteinase inhibitors from potato as allergens is unclear. Considering the low identity of these proteins with miraculin, and the fact that potatoes are not a major food allergen suggests the risk of cross-reactivity of miraculin to potato allergens is of low concern to human health.

In addition to the 80-amino acid sequence alignment search, a search using the full-length amino acid sequence was performed with the miraculin peptide sequence. An identity cut-off value of 50% was used, considering that allergic cross-reactivity may occur at matches greater than 50% (Aalberse, 2000). However, cross-reactivity at 50% identity is rare, and generally allergic cross-reactivity requires greater than 70% identity over the full-length sequence (Aalberse, 2000). No hits greater than 50% identity were identified, suggesting that the potential for cross-reactivity to putative allergens is very unlikely (Table 6.1.2.5-1).

[4] http://www.allergenonline.org/

Table 6.1.2.5-1 Search Results of AllergenOnline (Version 21)

Sequence Identifier	Source	Description	80 mer		Full Length		
			% Identity	# Hits (>35%)	Length	E-value	% Identity
994779	*Solanum tuberosum*	Proteinase inhibitor	39.30	23/141	227	4.0×10^{-12}	28.60
124148	*Solanum tuberosum*	Aspartic protease inhibitor 11	35.80	6/141	194	1.6×10^{-11}	29.9
256429	*Glycine max*	Kunitz trypsin inhibitor KTi	37.54	12/141	215	1.8×10^{-11}	31.6
18770	*Glycine max*	Trypsin inhibitor subtype A	37.50	12/141	215	1.8×10^{-11}	31.6
256635	*Glycine max*	Kunitz trypsin inhibitor KTi1	37.54	14/141	212	1.6×10^{-6}	33.5
18772	*Glycine max*	Trypsin inhibitor subtype B	37.50	12/141	215	4.5×10^{-8}	31.6
256636	*Glycine max*	Kunitz trypsin inhibitor KTi2	37.54	8/141	213	3.2×10^{-5}	32.4

In the publication by Tafazoli *et al.* (2019, 2020), the allergenic potential of the peptide digests of miraculin was evaluated using a bioinformatics approach as described above. Miraculin was digested with SGF containing pepsin for up to 10 minutes and the resulting peptides were characterized by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The authors reported 61 unique peptides from the digested miraculin protein which were each evaluated for allergenicity potential through a search of the full-length amino acid sequence and 80-amino acid sliding window. The complete list of pepsin digests and the results of the allergenicity assessment are provided in Appendix B. The full-length search of each peptide digest revealed a number of matches with known allergens, with identity scores ranging from 33 to 100% and similarity scores ranging from 60 to 100%. The corresponding E-values ranged from 0.00036 to 0.95 with an amino acid overlap of 5 to 25. Considering the high E-values and low identity matches over a short amino acid coverage (<25 amino acids), it is unlikely that these peptide digests would raise any allergenic risk (Aalberse, 2000). The 80-amino acid sliding window searches with each peptide digest did not identify 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).

The allergenicity potential of miraculin was further investigated using a support-vector machine (SVM) analysis from AlgPred[5]. Information on the sensitivity, specificity and error rate of AlgPred, as well as the results of the analysis are summarized in Table 6.1.2.5-2. The results of AlgPred identified mixed results: the miraculin protein was predicted to be a non-allergen based on algorithms for IgE epitopes, motif alignment and search tool (MAST), and allergen representative peptides (ARP), and was predicted to be an allergen based on SVM analysis of the amino acid composition and dipeptide composition. The SVM analysis suggest that miraculin is a potential allergen based on its amino acid composition. AlgPred has been used previously for the allergenicity assessment of soy leghemoglobin obtained from a genetically modified strain of *Pichia pastoris*, which has GRAS status for use in meat-analogue products as described in GRN 737 (U.S. FDA, 2018). The notifier reported that the allergenicity assessment using AllergenOnline[6], similar as described above, was "*more than adequate to demonstrate that* […] *have little or no allergenic potential*". Furthermore, the applicant stated that SVM-based analysis is controversial as the reliability of this method is questionable. AlgPred predicted the soy leghemoglobin to be a potential allergen; however, it was noted that this methodology has a high false positive rate. The applicant stated that AlgPred identified 46% of all proteins in the SwissProt to be potential allergens, even after all known allergens and related proteins were removed (Saha and Raghana, 2006; Impossible Foods Inc., 2018). It is known that only a small portion of

[5] http://crdd.osdd.net/raghava/algpred/index.html.
[6] http://www.allergenonline.org/

proteins are potential allergens. Taking into account the results from well-established methodologies for assessing the allergenicity potential of proteins, as outlined by FAO/WHO (2001), Codex Alimentarius (2009), and EFSA (2017), which have been used successfully in the allergenicity assessment of numerous novel proteins such as food enzymes and soy leghemoglobin (Impossible Foods Inc., 2018; U.S. FDA, 2018), miraculin has a low potential risk of allergenicity.

Table 6.1.2.5-2 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

The allergenicity potential of miraculin was also considered through a search using AllerTOP (version 2.0)[7], a bioinformatics tool for prediction of allergenicity. The method is based on auto cross covariance (ACC) transformation of protein sequences into uniform equal-length vectors as developed by 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), which is defined as a non-allergen.

6.1.2.6 Toxigenicity of Miraculin

The miraculin amino acid sequence was searched 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 using BLASTp. As described above, the search was conducted using default search parameters (E-value <0.05, BLOSUM62). Significant sequence homology for full-length sequence alignments is defined as a percent identity greater than 50%, an E-value below 0.001 ($1x10^{-3}$), and a bit-score greater than 50 for databases of 7,000 to 7,000,000 proteins (Pearson, 2013). No significant sequence homology was identified, suggesting that miraculin does not share homology or structural similarity to any animal venom protein or toxin, or harbors any toxic potential. Similar findings with respect to toxigenicity of miraculin were reported by Tafazoli *et al.* (2019, 2020).

6.2 Conclusions on the Safety of Miracle Fruit Pulp, Miracle Fruit Powder and Miracle Fruit Protein

Joywell Foods' miracle fruit powder and miracle fruit pulp are minimally processed ingredients produced from miracle fruit, which has a widespread history of consumption in the U.S. since 1917 following its introduction by the USDA, and globally since the 1700s. Cultivation of miracle fruit has steadily grown over

[7] https://www.ddg-pharmfac.net/AllerTOP/index.html.
[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.

the years and several commercial products derived from miracle fruit are currently available on the U.S. marketplace. Miracle fruit pulp is mainly comprised of moisture (on average ~85%) with the remaining components being carbohydrates (on average ~12%, as is), protein (on average ~1%, as is), fat (on average ~1%, as is) and ash (on average ~0.4%, as is), while miracle fruit powder mainly consists of carbohydrates (on average ~ 78%, as is), fat (on average ~10%, as is), protein (on average ~6%, as is), ash (on average ~4%, as is) and moisture (on average ~1%). The composition of miracle fruit pulp is similar to miracle berries without the seed, and also similar to other commonly consumed berries, including blackberries, blueberries, raspberries and cherries from both a proximate analysis and a detailed antinutrient analysis. Likewise, the composition of miracle fruit powder is similar to that of miracle fruit pulp, from which it is derived following a freeze-drying process. Both ingredients have been well-characterized with respect to the levels of microbiological contaminants, heavy metals, sugars, minerals, antinutrients and pesticides, and no safety concerns are expected from these contaminants. The level of antinutrients such as phytic acid and trypsin inhibitors in miracle fruit pulp and miracle fruit powder were negligible and below the experimental limit of detection. Exposure to antinutrients, such as polyphenols, present in miracle fruit powder and miracle fruit pulp used as ingredients in food products are no different from eating a serving size of blueberries and sour cherries and much lower than exposure to polyphenols from blackberries. Similarly, exposure to oxalic acid from the use of miracle fruit powder and miracle fruit pulp as ingredients are similar to exposure from 1 serving of blueberries and strawberries and much lower than exposure to oxalic acid from 1 serving of black raspberries. As such, the levels of antinutrients in miracle fruit powder and miracle fruit pulp are unlikely to negatively affect the bioavailability of other nutrients in foods to which the ingredients are added and are of no safety concern. Miracle fruit pulp and powder are intended for use in a variety of food and beverage products to impart sweetness. These products will be labelled and marketed in the same manner as conventional products and there will be no changes in the pH profile of the finished food product. As such, it is unlikely that individuals who normally refrain from consuming acidic products, including those with digestive disorders such as acid reflux, would modify the consumption of these specific foods or their diet. As a result, individuals with digestive disorders, such as acid reflux will continue to refrain from consuming such acidic foods and to self-regulate acidic food products that may contain Joywell Foods' ingredients in the same manner as conventional acidic foods. Therefore, there is little or no potential of Joywell Foods' ingredients to exacerbate or create a potential public health problem for individuals with acid reflux. Furthermore, the available data in the literature on miracle fruit or its leaf extracts or miracle fruit powder, although mainly efficacy-based, do not raise any safety concerns with respect to the consumption of Joywell Foods' miracle fruit powder or miracle fruit pulp. While the consumption of the miracle fruit and resultant supplement products has continued to rise over the last century, there has been no reported incidence of any adverse effects or allergenic response.

Joywell Foods' miracle fruit protein has been characterized to be comprised of protein (on average ~32%, as is), salt (on average ~46% NaCl), carbohydrates (on average ~5%, as is), and moisture (on average ~5%). The safety of miracle fruit protein was evaluated based on the publicly available *in vitro* digestibility and *in silico* safety (allergenicity and toxigenicity) assessment of miraculin (Tafazoli *et al.,* 2019, 2020). Miraculin has been demonstrated to be rapidly and fully digested in an *in vitro* digestibility model with SGF, suggesting that the glycoprotein will be readily digested into small peptides that are considered transient in nature and, ultimately, will be broken down into individual amino acids, as such, reducing the potential for absorption to elicit an allergenic response. The totality of the available evidence, including *in silico* results from the allergenicity and toxigenicity assessments using several publicly available bioinformatics tools, suggest that miraculin is unlikely to have potential for allergenicity or toxigenicity. These conclusions are further corroborated with the fact that exposures to miraculin through the presence of miracle fruit and miracle fruit in commercial products in the U.S. have not been associated with any reports on allergenic reactions.

Part 7. § 170.255 List of Supporting Data and Information

Aalberse RC (2000). Structural biology of allergens. J Allergy Clin Immunol 106(2):228-238. DOI:10.1067/mai.2000.108434.

Astwood J, Alibhai M, Lee T, Fuchs R, Sampson H (2000). Identification and characterization of IgE binding epitopes of patatin, a major food allergen of potato. J Allergy Clin Immunol 105(1, part 2):S184 [abstract 555]. DOI:10.1016/S0091-6749(00)90983-7.

Baur X, Pau M, Czuppon A, Fruhmann G (1996). Characterization of soybean allergens causing sensitization of occupationally exposed bakers. Allergy 51(5):326-330. DOI:10.1111/j.1398-9995.1996.tb04617.x.CDC (2020a). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015 [Page last reviewed: 2/21/2020].

CDC (2020b). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Page last reviewed: 2/21/2020].

Chen CC, Liu IM, Cheng JT (2006). Improvement of insulin resistance by miracle fruit (*Synsepalum dulcificum*) in fructose-rich chow-fed rats. Phytother Res 20(11):987-992. DOI:10.1002/ptr.1919.

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

Dioso MKM, Satsatin DCA, Ching J (2016). Hypoglycemic effects of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell (Miracle Berry) fruit and leaf extracts on the blood glucose level of albino rats. Pharm Lett 8(14):104-108.

EFSA (2017). Guidance on allergenicity assessment of genetically modified plants. (EFSA Panel on Genetically Modified Organisms/GMO) (Question no: EFSA-Q-2014-00547; adopted: 18 May 2017 by European Food Safety Authority). EFSA J 15(6):4862 [49pp]. DOI:10.2903/j.efsa.2017.4862. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/4862.

EFSA (2020). *Minutes of the 105th Plenary Meeting*, Aug. 31, 2020 [web conference] (Agreed on 04 September 2020). Parma, Italy: European Food Safety Authority (EFSA), Scientific Panel on Nutrition, Novel Foods and Food Allergens, Nutrition Unit. Available at: https://www.efsa.europa.eu/sites/default/files/event/2020/105th-plenary-meeting-nda-panel-minutes.pdf.

Ellis R, Morris ER, Philpot C (1977). Quantitative determination of phytate in the presence of high inorganic phosphate. Anal Biochem 77(2):536-539. 10.1016/0003-2697(77)90269-X.

European Commission (2018). *Summary of the [Novel Food] Dossier: Dried Miracle Berry (DMB)*. Applicant: Calle Marqués de Urquijo 47, Madrid, Spain: Baïa Food Co. (Medicinal Gardens S.L.). Submitted to Brussels, Belgium: European Commission. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/novel-food_sum_ongoing-app_2018-0709.pdf.

FAO/IZiNCG (2018). *FAO/INFOODS/IZiNCG Global Food Composition Database for Phytate – Version 1.0 (PhyFoodComp1.0)*. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / International Zinc Nutrition Consultative Group (FAO/IZiNCG). Available at: http://www.fao.org/infoods/infoods/tables-and-databases/faoinfoods-databases/en/.

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

FARRP (2021). Fruits. In: *Allergenic Foods and Their Allergens, with Links to Informall*. Lincoln (NE): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: https://farrp.unl.edu/informallfruit [Copyright 2021].

Hamerstrand GE, Black LT, Glover JD (1981). Trypsin inhibitors in soy products: modification of the standard analytical procedure. Cereal Chem 58(1):42-45.

Han H, Segal AM, Seifter JL, Dwyer JT (2015). Nutritional Management of kidney stones (nephrolithiasis). Clin Nutr Res 4(3):137-152. DOI:10.7762/cnr.2015.4.3.137.

Hileman RE, Silvanovich A, Goodman RE, Rice EA, Holleschak G, Astwood JD, et al. (2002). Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. Int Arch Allergy Immunol 128(4):280-291. DOI:10.1159/000063861.

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

Kattan JD, Sampson HA (2015). Clinical reactivity to soy is best identified by component testing to Gly m 8. J Allergy Clin Immunol Pract 3(6):970-972.e1. DOI:10.1016/j.jaip.2015.06.002.

Kurihara K, Beidler LM (1969). Mechanism of the action of taste-modifying protein. Nature 222(5199):1176-1179. DOI:10.1038/2221176a0.

Majamaa H, Seppolo U, Palosuo T, Turjanmaa K, Kalkkinen N, Reunala T (2001). Positive skin and oral challenge responses to potato and occurrence of immunoglobulin E antibodies to patatin (Sol t 1) in infants with atopic dermatitis. Pediatr Allergy Immunol 12(5):283-288. DOI:10.1034/j.1399-3038.2001.00096.x.

Moroz LA, Yang WH (1980). Kunitz soybean trypsin inhibitor: a specific allergen in food anaphylaxis. N Engl J Med 302(20):1126-1128. DOI:10.1056/NEJM198005153022005.

Morris JA (1976). Sweetening agents from natural sources. Lloydia 39(1):25-38.

Obafemi O, Akinmoladun A, Olaleye T, Adesanya TA, Onasanya A, Onikanni SA (2016). Amelioration of hematological and electrolyte imbalances in type 2 diabetic rats by methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum*. Int J Toxicol Pharmacol Res 8(5):326-331.

Obafemi TO, Akinmoladun AC, Olaleye MT, Agboade SO, Onasanya AA (2017). Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum* in type 2 diabetic rats. J Ayurveda Integr Med 8(4):238-246. DOI:10.1016/j.jaim.2017.01.008.

Obafemi TO, Olaleye MT, Akinmoladun AC (2019). Antidiabetic property of miracle fruit plant (*Synsepalum dulcificum* Shumach. & Thonn. Daniell) leaf extracts in fructose-fed streptozotocin-injected rats via anti-inflammatory activity and inhibition of carbohydrate metabolizing enzymes. J Ethnopharmacol 244:112124 [8pp]. DOI:10.1016/j.jep.2019.112124.

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

Quirce S, Fernández-Nieto M, Escudero C, Cuesta J, de Las Heras M, Sastre J (2006). Bronchial responsiveness to bakery-derived allergens is strongly dependent on specific skin sensitivity. Allergy 61(10):1202-1208. DOI:10.1111/j.1398-9995.2006.01189.x.

Roecklein JC, Leung PS, editors (1987). Sweet berry, miraculous-berry, miraculous fruit [*Synsepalum dulcificum*]. In: *A Profile of Economic Plants*. Oxford, UK: Transaction Publishers.

Saha S, Raghava GP (2006). AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. Nucleic Acids Res 34(Web Server issue):W202-W209. DOI:10.1093/nar/gkl343.

Seppälä U, Alenius H, Turjanmaa K, Reunala T, Palosuo T, Kalkkinen N (1999). Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. J Allergy Clin Immunol 103(1, Part 1):165-171. DOI:10.1016/s0091-6749(99)70541-5.

Sessa DJ, Ghantous PE (1987). Chemical inactivation of soybean trypsin inhibitors. J Am Oil Chem Soc 64(12):1682-1687. DOI:10.1007/BF02542503.

Shi YC, Lin KS, Jhai YF, Lee BH, Han Y, Cui Z, et al. (2016). Miracle fruit (*Synsepalum dulcificum*) exhibits as a novel anti-hyperuricaemia agent. Molecules 21(2):140 [11pp]. DOI:10.3390/molecules21020140.

Song P, Herman R, Kumpatla S (2015). 1:1 FASTA update: Using the power of *E*-values in FASTA to detect potential allergen cross-reactivity. Toxicol Rep 2:1145-1148. DOI:10.1016/j.toxrep.2015.08.005.

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, et al. (2019). Safety assessment of miraculin using *in silico* and *in vitro* digestibility analyses. Food Chem Toxicol 133:110762 [10pp]. DOI:10.1016/j.fct.2019.110762.

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, et al. (2020). Corrigendum to "Safety assessment of miraculin using in silico and in vitro digestibility analyses" [Food Chem Toxicol 133(2019 Nov):110762]. Food Chem Toxicol 137:111136. DOI:10.1016/j.fct.2020.111136.

Taylor SL, Remington BC, Panda R, Goodman RE, Baumert JL (2015). Detection and control of soybeans as a food allergen. In: Flanagan S, editor. *Handbook of Food Allergen Detection and Control*. (Woodhead Publishing Series in Food Science, Technology and Nutrition, No. 264). Cambridge, UK: Woodhead Publishing Ltd., pp. 341-366. DOI:10.1533/9781782420217.3.341.

Theerasilp S, Hitotsuya H, Nakajo S, Nakaya K, Nakamura Y, Kurihara Y (1989). Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. J Biol Chem 264(12):6655-6659.

Theerasilp S, Kurihara Y (1988). Complete purification and characterization of the taste-modifying protein, miraculin, from miracle fruit. J Biol Chem 263(23):11536-11539.

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, et al. (2004). A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regul Toxicol Pharmacol 39(2):87-98. DOI:10.1016/j.yrtph.2003.11.003.

U.S. FDA (1977). Miracle fruit berries, concentrates, and extracts; Denial of GRAS affirmation and determination of food additive status [Docket No. 76G-0412]. Fed Regist (US) 42(100):26467–26468.

U.S. FDA (2009). *Memorandum Re: 75 Day premarket notification for new dietary ingredient [miracle fruit from my M fruit LLC].* (NDI No. 574; Docket No. FDA-1995-S-0039). Washington (DC): U.S. Food and Drug administration (U.S. FDA), Office of Nutrition, Labeling and Dietary Supplements. https://www.regulations.gov/document?D=FDA-2009-S-0608-0049. [Apr. 30, 2009].

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

U.S. FDA (2020a). Part 170—Secondary direct food additives permitted in food for human consumption. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/ [current to 4-1-20, last updated 4-1-19].

U.S. FDA (2020b). Part 170—Secondary direct food additives permitted in food for human consumption. Section §170.30—Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/ [current to 4-1-20, last updated 4-1-19].

USDA (2019a). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2015-2016.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 10/17/2019].

USDA (2019b). 'Blueberries, raw'. In: *FoodData Central*. (FDC ID: 1102700). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102702/nutrients [FDC Published: 4/1/2019].

USDA (2019c). 'Sweet Cherries, raw'. In: *FoodData Central*. (FDC ID: 171719). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/171719/nutrients [FDC Published: 4/1/2019].

USDA (2019d). 'Sour cherries, raw'. In: *FoodData Central*. (FDC ID: 173954). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/173954/nutrients [FDC Published: 4/1/2019].

USDA (2020a). 'Blackberries, raw'. In: *FoodData Central*. (FDC ID: 1102700). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102700/nutrients [FDC Published: 10/30/2020].

USDA (2020b). 'Raspberries, raw'. In: *FoodData Central*. (FDC ID: 1102708). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102708/nutrients [FDC Published: 10/30/2020].

USDA (2020c). 'Grapes, raw'. In: *FoodData Central*. (FDC ID: 1102710). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102665/nutrients [FDC Published: 10/30/2020].

USDA (2020d). 'Strawberries, raw'. In: *FoodData Central*. (FDC ID: 1102665). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102710/nutrients [FDC Published: 10/30/2020].

Wold S, Jonsson J, Sjöström M, Sandberg M, Rännar S (1993). DNA and peptide sequences and chemical processes mutlivariately modelled by principal component analysis and partial least-squares projections to latent structures. Anal Chim Acta 277(2):239-253. DOI:10.1016/0003-2670(93)80437-P.

APPENDIX A – GRAS Panel Consensus Statement

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

19 October 2020

INTRODUCTION

At the request of Joywell Foods Inc. (Joywell Foods), 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 the data and information pertinent to the safety of miracle fruit pulp, miracle fruit powder, and miracle fruit protein to determine whether the intended uses of these ingredients in conventional food and beverage 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: Associate Professor Joseph Baumert (University of Nebraska-Lincoln), Professor Emeritus Robert J. Nicolosi (University of Massachusetts Lowell), and Professor Emeritus I. Glenn Sipes (University of Arizona).

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 Pulp, Miracle Fruit Powder and Miracle Fruit Protein*" (dated 11 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 pulp, powder, and protein. This dossier was prepared in part from a comprehensive search of the scientific literature through August 2020 and included information characterizing the identity and purity of the ingredients, the manufacture of the ingredients, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, and the safety of miracle fruit pulp, powder, and protein.

Following its independent and collective critical evaluation, and on the basis of scientific procedures, the GRAS Panel unanimously concluded that Joywell Foods' miracle fruit pulp, miracle fruit powder, and miracle fruit protein, meeting food-grade specifications and manufactured in accordance with current Good Manufacturing Practice (cGMP), are GRAS for their intended uses, 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 pulp, powder, and protein are obtained from the fruit of the miracle berry tree (*Synsepalum dulcificum* Daniell). Humans have been consuming fruit from this tree, since the early 1700s in Ghana. Cultivation of miracle berries in the United States (U.S.) began after its introduction in 1917 by the United States Department of Agriculture, and since then, has been in commercial use in the form of fresh berries, freeze-dried powder, or in tablet form available as dietary supplement products. The taste-modifying effect of the miracle berry is attributed to the glycoprotein, miraculin, which imparts its effect through interaction with the sweet receptors of the tongue, turning sour tastes into sweet (Morris, 1976). Miraculin is a single polypeptide of 220 amino acid residues, including a 29 amino acid N-terminal signal peptide that is removed through post-translational processing. The protein exists as a homodimer with a molecular weight of approximately 50 kDa, connected through a single inter-chain disulfide bond at Cys-138. The peptide sequence of a single chain of miraculin is publicly available under Accession No. P13087 in the UniProt database.

Miracle fruit pulp, powder, and protein are manufactured in accordance with cGMP and the principles of Hazard Analysis and Critical Control Points (HACCP). All processing aids and food contact materials used in the production process of these ingredients are food-grade or have previously been determined to be GRAS for their intended uses. Miracle berries are currently sourced within the U.S., though other international sources are also available through commercial production in Taiwan. The production process involves deseeding and maceration of washed berries and blending to produce the miracle fruit pulp. The pulp may then be freeze-dried and milled into a fine powder to yield the miracle fruit powder. Miracle fruit protein is obtained from the pulp. The miracle fruit pulp is mixed with an extraction buffer, pH-adjusted, homogenized, subject to a series of filtration steps (*e.g.,* sequential filtration, ultrafiltration), and then concentrated. The protein concentrate is then subject to diafiltration and washed with dialysis buffer, then passed through an ion exchange column. The concentrate is subject to a second diafiltration step, microfiltration, then frozen and freeze-dried. The miracle fruit protein is packaged and stored at room temperature and low humidity.

Specifications have been established for miracle fruit pulp, miracle fruit powder, and miracle fruit protein. These include parameters for proximate analysis, miraculin content (as a percentage of total protein), heavy metals, and microbiological contaminants. All methods of analysis are internationally recognized [*e.g.,* Association of Official Analytical Chemists (AOAC)] or equivalent. The miraculin content is measured using an internal method based on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and an internal method based on high-performance liquid chromatography (HPLC) that was developed and validated by Joywell Foods. The GRAS Panel reviewed the results of 3 non-consecutive batches of miracle fruit pulp, 4 non-consecutive batches of miracle fruit powder, and 4 non-consecutive batches of miracle fruit protein, and concluded that the manufacturing process produces a consistent product that meets the respective established specifications for each ingredient.

The proximate composition of miracle fruit pulp, powder, and protein are shown in Table 1 below. The average levels of miraculin varies from 0.2 to 1.2% of the total protein in miracle fruit pulp and powder to ~85.5% of the total protein in miracle fruit protein ingredient. The sugar profile of miracle fruit pulp and powder were analyzed and determined to be consistent across the production batches. The same production batches of miracle fruit pulp and powder were analyzed for antinutrients, including phytic acid, oxalic acid, and trypsin inhibitors. The GRAS Panel noted the absence of phytic acid and trypsin inhibitors, while oxalic acid content ranged from less than 400 to 1,170 ppm in miracle fruit pulp and 820 to 1,210 ppm in miracle fruit powder. The GRAS Panel reviewed the polyphenol content of 1 batch of miracle fruit pulp and 4 non-consecutive batches of miracle fruit powder and concluded the levels of polyphenols to be

consistent between miracle fruit pulp and miracle fruit powder. Miracle fruit protein is produced from miracle fruit pulp and considering that it undergoes several filtration and purification processes, it is expected that the levels of antinutrients and polyphenols in miracle fruit protein to be at minimum similar or less than those in the miracle fruit pulp or powder.

The GRAS Panel also reviewed the mineral profile of 4 non-consecutive batches of miracle fruit pulp, powder, and protein and concluded the levels to be consistent across all analyzed batches. Analysis of 4 non-consecutive batches of miracle fruit pulp also demonstrated the absence of pesticides. Considering that miracle fruit powder and protein are also produced from miracle fruit pulp, it is expected for the residual pesticides to also be absent in the miracle fruit powder and miracle fruit protein.

Table 1 Proximate Composition of Miracle Fruit Pulp, Miracle Fruit Powder, and Miracle Fruit Protein

Parameter	Miracle Fruit Pulp[a]	Miracle Fruit Powder[a]	Miracle Fruit Protein[a,b]
Carbohydrate (%, as is)	*ca.* 12	*ca.* 78	*ca.* 5
Protein (%, as is)	*ca.* 1	*ca.* 6	*ca.* 32
Fat (%, as is)	*ca.* 1	*ca.* 10	-
Ash (%, as is)	*ca.* 0.4	*ca.* 4	-
Moisture (%)	*ca.* 85	*ca.* 1	*ca.* 5

[a] Average of 3 to 4 non-consecutive batches.
[b] Miracle fruit protein contains approximately 46% sodium chloride and 12% minerals.

The GRAS Panel reviewed the stability results of miracle fruit pulp, powder, and protein under various storage conditions (Table 2). The stability was considered *via* sensory testing by trained panelists consuming a standard sweet solution (0.24 M sucrose) or sour solution (0.023 M citric acid). The standard sweet solution was set at 100 on a scale of 0 to 200. The GRAS Panel concluded that miracle fruit pulp was stable when stored at -18°C for up to 188 days with some reported loss in color. The taste modification effect of miracle fruit pulp was significantly decreased when heated at 65°C for 30 minutes, while no significant changes were observed after storage at 37°C for 1 hour compared to storage at room temperature or after freeze-thaw cycles. Miracle fruit powder was demonstrated to be stable after storage at 40°C for 63 days, and no significant changes in the taste modification effect of miracle fruit powder were observed following storage in light or dark conditions. Miracle fruit protein was demonstrated to be stable over the 62-week duration in the real-time shelf-life study and under accelerated conditions (30°C for 56 days, 40°C for 28 days, or 50°C or 28 days). The taste modification effect of miracle fruit protein was demonstrated to last for over 35 minutes, with a sweetness score of greater than 50 over the duration of the shelf-life studies under accelerated conditions. The sweetness score of miracle fruit protein in powder form after storage at room temperature for 62 weeks was above 100, indicating no significant changes in the miraculin content. Miracle fruit protein was demonstrated to be tolerant of heat shock from 60 to 120°C. Miracle fruit protein in aqueous solution was demonstrated to be stable at lower temperatures (4°C) for up to 63 days, and 1 week at room temperature. The taste modification effect was not present after heating above 50°C for 1 hour, while retaining approximately 50% of its activity after heating at 30°C for 1 hour. A similar finding was observed after heating at 65°C for 30 minutes; these results indicate that miracle fruit protein is denatured at temperatures greater than 50°C in aqueous conditions.

The GRAS Panel concluded that the stability studies demonstrate that the miracle fruit pulp, powder, and protein are stable under controlled storage conditions with respect to humidity and temperature.

Table 2 Summary of Shelf-Life Stability Findings, Recommended Storage Conditions, and Use Considerations

Material	Storage Conditions	Shelf-Life (Days)	Heat-Shock Stability	Freeze/Thaw Stable	Light/Dark Stable	Manufacturing Use Considerations
Miracle Fruit Pulp	-18°C	188[a]	<37°C, <60 min	Yes	NT	Thaw and store at <10°C, use within 24 hrs
Miracle Fruit Powder	<25°C, <33% RH	210[a,b]	NT	Yes	Yes	Maintain at low relative humidity
Miracle Fruit Protein (dry powder)	<25°C, <56% RH	434[a]	<120°C, <60 min	Yes	NT	Maintain at low relative humidity
Miraculin Fruit Protein (aqueous solution)	<5°C	17	<25°C	Yes	NT	Handle at <25°C, store at <5°C, use within 24 hrs

hrs = hours; min = minutes; NT = not tested; RH = relative humidity.
[a] Based on real-time shelf-life stability testing.
[b] Based on accelerated shelf-life stability testing.

TECHNICAL EFFECT

Miracle fruit pulp, powder, and protein are intended for use in food and beverage products, including alcoholic and non-alcoholic beverages, chewing gum, coffee and tea, dairy products, grain products, fruit-based and vegetable-based beverages, and confectionary products. Each ingredient contains the active glycoprotein, miraculin, at varying amounts that is responsible for the sweetening and taste-modifying properties of these ingredients. The maximum sweetening effect of miraculin was achieved within 3 minutes of consumption and rapidly declined after 30 minutes (Kurihara and Beidler, 1969). This effect was reported to be concentration dependent. Similar findings were observed in the series of stability studies conducted with the miracle fruit pulp, powder, and protein reviewed by the GRAS Panel. Furthermore, the taste modification effect of miracle fruit was reported in a sensory panel test involving 6 trained panelists (Tafazoli *et al.,* 2019). Baseline sweetness was established through consumption of lemonade juice. Miracle fruit powder (0.08 g) was consumed by each panelist and held in the mouth for 1 minute then swallowed. Lemonade juice was consumed every 5 minutes for 30 minutes and the sweetness of each cup was recorded. A significant increase was observed in the perceived sweetness of lemonade juice, with the effect returning to baseline after 30 minutes. The GRAS Panel concluded that the available evidence indicates a clear taste modification effect of miracle fruit that is concentration-dependent with a duration of effect less than 30 minutes. Miraculin exerts its taste modifying effects by binding the taste receptors of the tongue to change taste from sour to sweet. There will be a limitation of this modification effect on taste due to the saturation of the receptors on the tongue, thus, limiting the levels of each ingredient that can be added in various food and beverage products.

INTENDED USE AND ESTIMATED EXPOSURE

Miracle fruit pulp, miracle berry powder, and miracle berry protein are intended for use as food ingredients in conventional food and beverages as outlined in Table A-1. The GRAS Panel reviewed the estimated intakes of these ingredients based on an assessment of dietary intakes under their intended conditions of use using the 2015-2016 cycle of National Health and Nutrition Examination Survey (NHANES). The GRAS Panel noted that the miracle fruit pulp, powder, and protein are not intended for use in food products consumed by infants and children up to 2 years of age.

For miracle fruit pulp, on a consumer-only basis, the resulting mean and 90th percentile intakes by the total U.S. population were estimated to be 12.24 g/person/day (0.19 g/kg body weight/day) and 24.27 g/person/day (0.39 g/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of miracle fruit pulp were determined to be 16.18 g/person/day (0.19 mg/kg body weight/day) and 30.92 g/person/day (0.35 mg/kg body weight/day), as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.45 and 13.48 g/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 0.52 and 1.11 g/kg body weight/day and the mean and 90th percentile intake.

For miracle fruit powder, on a consumer-only basis, the resulting mean and 90th percentile intakes by the total U.S. population from proposed food uses in the U.S. were estimated to be 1.70 g/person/day (27 mg/kg body weight/day) and 3.38 g/person/day (54 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 2.25 g/person/day (26 mg/kg body weight/day) and 4.31 g/person/day (50 mg/kg body weight/day), as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 0.90 and 1.88 g/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 73 and 155 mg/kg body weight/day and the mean and 90th percentile intake.

On a consumer-only basis, the resulting mean and 90th percentile intakes of miracle fruit protein by the total U.S. population from proposed food uses in the U.S. were estimated to be 11.61 mg/person/day (0.18 mg/kg body weight/day) and 23.35 mg/person/day (0.37 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of miracle fruit protein were determined to be 15.55 mg/person/day (0.18 mg/kg body weight/day) and 30.46 mg/person/day (0.35 mg/kg body weight/day), as identified among male adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.19 and 13.22 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily intakes, of 0.50 and 1.07 mg/kg body weight/day and the mean and 90thpercentile intake.

SAFETY NARRATIVE

The safety of miracle fruit berry, miracle fruit powder, and miracle fruit protein was evaluated using the following criteria:

1. There is a history of safe use of the ingredient in foods;

2. The ingredient is fully characterized with respect to exposure to natural toxins and anti-nutritional factors under the proposed conditions of use;

3. The protein is readily digested in validated *in vitro* digestive tests; and

4. There are no biological adverse effects associated with the ingredient with respect to allergenicity potential.

The GRAS Panel noted that miracle fruit has a long history of safe consumption both internationally and in the U.S. Various forms of miracle fruit are commercially available in the form of fresh berry, freeze-dried powder, or tablets. To date, no adverse events or serious side effects have been reported from consumption of these commercial forms of miracle fruit, supporting the general safety of the ingredient.

The miracle fruit pulp, miracle fruit powder, and miracle fruit protein are well characterized as shown in Table 1 above. The ingredients have been demonstrated analytically to be absent of antinutrients, microbiological contaminants, and chemical contaminants such as heavy metals and pesticides.

Miraculin was demonstrated to be rapidly enzymatically digested in an *in vitro* simulated gastric fluid (SGF) model. The *in silico* analysis coupled with the rapid hydrolysis also 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 miracle fruit powder or pulp and were not considered appropriate for the safety assessment of miracle fruit pulp, powder, or protein.

Each of these safety considerations is discussed in detail below.

History of Consumption of Miracle Fruit

The source of miracle fruit powder, pulp, and protein, miracle fruit (*S. dulcificum*), has been consumed in West Africa since at least the 1700s, and in the 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 consumption of miracle fruit by U.S. consumers.

Compositional Analyses

Miracle fruit pulp, miracle fruit powder, and miracle fruit protein have been well characterized, as summarized in Table 1. Miracle fruit pulp and powder are produced by mechanical processing steps that do not involve the use of any chemical solvents or processing aids. Thus, these ingredients are considered to be minimally processed and their chemical composition are similar to that of miracle berry that they are sourced from. Analysis of several production batches of miracle fruit pulp and miracle fruit powder demonstrated the absence of environmental contaminants (*e.g.*, heavy metals and pesticides) and microbiological hazards that may have originated from the cultivation practices or manufacturing process. Analytical data for batches of miracle fruit pulp and powder also demonstrated that there is no safety

concern with the levels of antinutrients (*e.g.*, polyphenols, oxalic acid, phytic acid, trypsin inhibitors), which were demonstrated to be either absent or occurring as low levels. Considering that miracle fruit protein is obtained from miracle fruit pulp and undergoes several purification and filtration steps, similarly, no safety concerns are expected from the levels of antinutrients in miracle fruit powder.

Studies on Miracle Fruit from the Scientific Literature

A comprehensive search of the scientific literature was conducted to identify studies relevant to the safety of miracle berry pulp, miracle berry powder, and miracle berry protein through May 2020. A number of studies conducted with miracle fruit powder or the fruit or leaf extracts of miracle fruit were identified; however, these studies focused primarily on efficacy endpoints. These efficacy-focused studies included evaluation of 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). While these studies included limited toxicity-related endpoints, the GRAS Panel concluded that their relevance to the safety of miracle fruit pulp, miracle fruit powder, and miracle fruit protein is limited due to the following:

1. These studies were not conducted in accordance with international testing protocols or current Good Laboratory Practice (cGLP) and were non-standard toxicological studies as they focused on efficacy-related endpoints.

2. Detailed compositional analysis of the test articles and levels of miraculin were not reported.

3. The test articles used by Dioso *et al*. (2016), Obafemi *et al*. (2016, 2017, 2019), and Shi *et al*. (2016) were extracted using various solvents including methanol, ethanol, or butanol with compositional differences from miracle fruit pulp. As such, these test articles are not representative of Joywell Foods miracle fruit pulp, miracle fruit powder, and miracle fruit protein. Due to these compositional differences, any effects reported may be attributed to a concentrated component in the fruit/leaf or residual extraction solvents.

4. The findings of the study by Chen *et al.* (2006), which evaluated the effects of lyophilized miracle fruit powder on insulin resistance of male Wistar rats consuming a fructose-rich diet, appear to mainly reflect changes to those rats fed the fructose-rich diet and not the 'control' animals that were fed standard rat chow. In addition, no analysis on food/water intake or body weight was conducted in test animals in the fructose-rich experiments. Therefore, it is difficult to determine whether the test article that was administered 3 times daily was having an impact on food intake due to sensory/palatability issues, thereby reducing dietary consumption. This could have led to a decrease in the amount of fructose available for absorption from the fructose-rich diet, and its eventual impact on the blood glucose and consequently insulin levels.

Despite the limitations in the studies identified in the literature, the results of these studies do not suggest a safety concern associated with the use of miracle fruit powder, pulp, or protein as ingredients.

Safety Information Related to Miraculin, the Active Glycoprotein in Miracle Fruit Pulp, Powder, and Protein

The glycoprotein present in miracle fruit powder, pulp, and protein, miraculin, which is responsible for their taste-modifying effects, has been the subject of *in vitro* digestibility testing and a safety evaluation using *in silico* methods (Tafazoli *et al.,* 2019, 2020). The results of the *in vitro* digestibility study indicate the protein to be rapidly hydrolyzed by gastric fluid. Furthermore, the protein was concluded to not pose any allergenic or toxigenic risk to consumers. The GRAS Panel concluded the studies by Tafazoli *et al.* (2019, 2020) to be pivotal in the safety assessment of miracle fruit pulp, miracle fruit powder, and miracle fruit protein. These points are discussed further in the sections that follow.

In Vitro Digestibility of Miraculin

The potential digestibility of miraculin following oral consumption was investigated in an *in vitro* SGF model (Tafazoli *et al.,* 2019). The study was designed to mimic the conditions of the human stomach and followed methodology described by Thomas *et al.* (2004). Miraculin (0.1 mg/mL) was added to a preincubation mixture consisting of SGF (10 U/ µg pepsin) and incubated for up to 60 minutes. At various timepoints during incubation (0, 20, 40, and 60 minutes), the reaction was ceased with the addition of sodium bicarbonate, tricine buffer solution, and a reducing agent, and heated at 85°C for 10 minutes. The protein digestibility was evaluated by gel electrophoresis using Coomassie blue and silver stains. Miraculin was reported to be completely digested within 20 minutes. In addition, the effect of pepsin concentration on the digestibility of miraculin was evaluated in an SGF model containing different pepsin concentrations (5.45 or 10 U/µg), and incubated for up to 10 minutes. Miraculin was reported to be readily digested within 1 minute and the reaction to be pepsin dependent.

Proteolytic Fate of Miraculin

The proteolytic fate of miraculin following pepsin digestion was investigated using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Tafazoli *et al.,* 2019). Miraculin was added to SGF containing 5.45 U/µg pepsin and incubated for up to 10 minutes at 37°C, and digest samples were collected at various timepoints of 0, 0.5, 1, and 10 minutes. With longer digestion time, miraculin was increasingly digested with the number of unique peptides reported at 5, 33, 54, and 61 after 0, 0.5, 1, and 10 minutes of digestion, respectively (Tafazoli *et al.*, 2019). After 10 minutes of pepsin digestion, the number of unique peptides encompassed approximately 75% of the entire amino acid sequence. The only peptides that were not identified after 10 minutes of digestion were peptides with cysteine residues (*i.e.,* disulfide bonds) that were likely resistant to digestion (Tafazoli *et al.*, 2019). Based on the results of this study, the GRAS Panel concluded that miraculin would be rapidly digested by the gastric fluid into small peptides, and therefore would not be present as intact protein for absorption into the systemic circulation following ingestion.

Allergenicity of Miraculin

In the search for the full-length amino acid sequence, an identity cut-off value of 50% was used, considering that allergic cross-reactivity may occur at matches greater than 50% (Aalberse, 2000). However, cross-reactivity at 50% identity is rare, and generally allergic cross-reactivity requires 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, there were no hits greater than 50% identity, suggesting that the potential for cross-reactivity to putative allergens is very unlikely.

In the 80-amino acid sliding window alignment search, segments of 80-amino acids (1–80, 2–81, 3–82, *etc.*) derived from each full-length amino acid sequence were searched according to the methodology by the FAO/WHO (2001) and Codex Alimentarius (2003, 2009). Based upon these methodologies, significant homology was defined as an identity match of greater than 35% on the basis of the FAO/WHO (2001) and Codex Alimentarius (2003, 2009) criteria. At matches greater than 35% identity, immunoglobulin E (IgE) cross-reactivity to putative allergens may be considered a possibility. Using AllergenOnline, 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. The clinical significance of low identity matches (35 to 40% over 80 amino acid windows) is questionable and the recommended criterion of >35% identity over 80 amino acid windows is considered conservative. As such, other factors should be considered when the percent identity is low (Goodman, 2006).

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

Soybeans contain multiple allergenic proteins and are considered one of the most common allergenic foods in the world (Kattan and Sampson, 2015; Taylor *et al*., 2015). 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 contribute to the trypsin inhibitory effect and resistance to denaturation (Sessa and Ghantous, 1987). SKTI is considered an inhalation allergen associated with occupational exposure to flour dust in bakers, affecting bakers exposed to large amounts of inhaled soy flour (Baur *et al*., 1996; Quirce *et al.*, 2006). The incidence of allergic reactions related to inhaled SKTI is very low (Moroz and Yang, 1980). Instead, the major soy allergens have been reported to be 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), as well as Gly m 8 (a 2S albumin) (Kattan and Sampson, 2015; Taylor *et al*., 2015).

Allergic reactions following the ingestion of potatoes have been infrequently described. Several allergenic proteins have been identified in potato with the major allergen in potatoes being reported, as Sol t 1, a 43 kD protein known as patatin, the main storage protein of the potato tuber (Seppälä *et al*., 1999; Astwood *et al*., 2000; Majamaa *et al*., 2001). Therefore, the importance of these proteinase inhibitors from potato, as allergens remains questionable. Considering the low identity of these proteins with miraculin, the likelihood of cross-reactivity between miraculin and potato proteins is very low.

In a recent publication by Tafazoli *et al.* (2019, 2020), the allergenic potential of peptide digests of miraculin was investigated. 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, which 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. 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 raise any allergenic risk (Aalberse, 2000). The 80-amino acid sliding window searches with each peptide digest did not identify 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).

Miraculin has been demonstrated to be rapidly and fully digested in an *in vitro* digestibility model with SGF, suggesting that the glycoprotein will be readily digested into small peptides that are considered transient in nature and, ultimately, will be broken down into individual amino acids, as such, reducing the potential for absorption to elicit an allergenic response. The totality of the available evidence, 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 through the presence of miracle fruit and miracle fruit in commercial products in the U.S., have not been associated with any reports on allergenic reactions.

Toxigenicity of Miraculin

The miraculin amino acid sequence was compared against downloaded protein sequences obtained from a curated database of animal venom proteins and toxins maintained in the UniProtKB/Swiss-Prot Tox-Prot[1] database using the Basic Local Alignment Search Tool (BLAST) maintained by the National Center for Biotechnology Information. The results of the BLAST search demonstrated several matches to animal toxins/venoms, with sequence identities ranging from 25 to 54% and corresponding E-values of 0.61 to 9.3 with generally low query coverage (<25%). Currently, there are no formal guidelines established for what constitutes a significant sequence similarity between an introduced protein and protein toxins (Hammond *et al.,* 2013). Taking into account 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, virulence factors or harbors any toxic potential.

Summary and Basis for GRAS

Joywell Foods intends to market miracle fruit pulp, miracle fruit powder, and miracle fruit protein, derived from the miracle berry (*Synsepalum dulcificum*) fruit, as ingredients for use in conventional food and beverage products in the U.S. Miracle fruit has a long history of safe use both internationally and in the U.S. Various forms of miracle fruit are commercially available in the form of fresh berry, freeze-dried powder, or tablets. To date, no adverse events or serious side effects have been reported from consumption of these commercial forms of miracle fruit, supporting the general safety of the ingredient. Joywell Foods' ingredients are well characterized. Miracle fruit pulp is mainly comprised of moisture (on average ~85%) with the remaining components being carbohydrates (on average ~12%, as is), protein (on average ~1%, as is), fat (on average ~1%, as is), and ash (on average ~0.4%, as is). Miracle fruit powder mainly consists of carbohydrates (on average ~ 78%, as is), fat (on average ~10%, as is), protein (on average ~6%, as is), ash (on average ~4%, as is) and moisture (on average ~1%). Miracle fruit protein ingredient comprises protein (on average ~32%, as is), salt (on average ~46% NaCl), carbohydrates (on average ~5%, as is), and moisture (on average ~5%). The results of the analysis for several representative batches of miracle fruit pulp, miracle fruit powder, and miracle fruit protein demonstrate that the levels of microbiological contaminants, heavy metals (lead, arsenic, mercury, and cadmium), and pesticides remain within specified and/or acceptable levels. The low levels of antinutrients (trypsin inhibitors, oxalic acid, phytic acid and

[1] 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.

polyphenols) are not expected to negatively affect the availability of other nutrients in foods to which the ingredient is added and are of no safety concern.

The mean and 90th percentile consumer-only intakes of miracle fruit powder, among the total population, were determined to be 1.70 and 3.38 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 2.25 and 4.31 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 0.90 and 1.88 g/person/day, respectively. Among the total population (all ages), the mean and 90th percentile consumer-only intakes of miracle fruit pulp were determined to be 12.24 and 24.27 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 pulp on an absolute basis, at 16.18 and 30.92 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.45 and 13.48 g/person/day, respectively. The mean and 90th percentile consumer-only intakes of miracle fruit protein were determined to be 11.61 and 23.35 mg/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 protein on an absolute basis, at 15.55 and 30.46 mg/person/day, respectively.

Miracle fruit protein was demonstrated to be rapidly enzymatically digested in an *in vitro* SGF model. The *in silico* analysis coupled with the rapid hydrolysis also indicates a lack of cross-reactivity with any known allergen. Published studies that reported on the potential health benefits of the miracle berry did not use test articles that were considered to be representative of Joywell Foods products and were, therefore, considered inappropriate for the safety assessment of miracle fruit powder, pulp, or miracle fruit protein.

The data and information summarized in this dossier demonstrate that the proposed uses of miracle fruit powder, miracle fruit pulp and miracle fruit protein manufactured by Joywell Foods in accordance with cGMP and meeting appropriate food-grade specifications, are GRAS based on scientific procedures.

CONCLUSION

We, the members of the GRAS Panel, have, independently and collectively, critically evaluated the data and information summarized above, and unanimously conclude that Joywell Foods' miracle fruit pulp, miracle fruit powder, and miracle fruit protein do not pose a safety concern under the proposed conditions of use.

We further unanimously conclude that the proposed uses of Joywell Food's miracle fruit pulp, miracle fruit powder, and miracle fruit protein 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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REFERENCES

Aalberse RC (2000). Structural biology of allergens. J Allergy Clin Immunol 106(2):228–238. DOI:10.1067/mai.2000.108434.

Astwood J, Alibhai M, Lee T, Fuchs R, Sampson H (2000). Identification and characterization of IgE binding epitopes of patatin, a major food allergen of potato. J Allergy Clin Immunol 105(1, part 2):S184 [abstract 555]. DOI:10.1016/S0091-6749(00)90983-7.

Baur X, Pau M, Czuppon A, Fruhmann G (1996). Characterization of soybean allergens causing sensitization of occupationally exposed bakers. Allergy 51(5):326-330. DOI:10.1111/j.1398-9995.1996.tb04617.x.

Bushey DF, Bannon GA, Delaney B, Graser G, Hefford M, Jiang X, et al. (2014). Characteristics and safety assessment of intractable proteins in genetically modified crops. Regul Toxicol Pharmacol 69(2):154-170. DOI:10.1016/j.yrtph.2014.03.003.

Chen CC, Liu IM, Cheng JT (2006). Improvement of insulin resistance by miracle fruit (*Synsepalum dulcificum*) in fructose-rich chow-fed rats. Phytother Res 20(11):987-992. DOI:10.1002/ptr.1919.

Codex Alimentarius (2003). *Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms*. (CAC/GL 46-2003; Adopted in 2003, Annexes II and III adopted in 2008). Rome Italy: Codex Alimentarius. Available at: http://www.fao.org/fileadmin/user_upload/gmfp/resources/CXG_046e.pdf.

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

Dioso MKM, Satsatin DCA, Ching J (2016). Hypoglycemic effects of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell (Miracle Berry) fruit and leaf extracts on the blood glucose level of albino rats. Pharm Lett 8(14):104-108.

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

Goodman RE (2006). Practical and predictive bioinformatics methods for the identification of potentially cross-reactive protein matches. Mol Nutr Food Res 50(7):655-660. DOI:10.1002/mnfr.200500277.

Hammond B, Kough J, Herouet-Guicheney C, Jez JM (2013). Toxicological evaluation of proteins introduced into food crops. Crit Rev Toxicol 43(Suppl. 2):25-42. DOI:10.3109/10408444.2013.842956.

Kattan JD, Sampson HA (2015). Clinical reactivity to soy is best identified by component testing to Gly m 8. J Allergy Clin Immunol Pract 3(6):970-972.e1. DOI:10.1016/j.jaip.2015.06.002.

Kurihara K, Beidler LM (1969). Mechanism of the action of taste-modifying protein. Nature 222(5199):1176-1179. DOI:10.1038/2221176a0.

Majamaa H, Seppolo U, Palosuo T, Turjanmaa K, Kalkkinen N, Reunala T (2001). Positive skin and oral challenge responses to potato and occurrence of immunoglobulin E antibodies to patatin (Sol t 1) in infants with atopic dermatitis. Pediatr Allergy Immunol 12(5):283-288. DOI:10.1034/j.1399-3038.2001.00096.x.

Moroz LA, Yang WH (1980). Kunitz soybean trypsin inhibitor: a specific allergen in food anaphylaxis. N Engl J Med 302(20):1126-1128. DOI:10.1056/NEJM198005153022005.

Morris JA (1976). Sweetening agents from natural sources. Lloydia 39(1):25-38.

Obafemi O, Akinmoladun A, Olaleye T, Adesanya TA, Onasanya A, Onikanni SA (2016). Amelioration of hematological and electrolyte imbalances in type 2 diabetic rats by methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum*. Int J Toxicol Pharmacol Res 8(5):326-331.

Obafemi TO, Akinmoladun AC, Olaleye MT, Agboade SO, Onasanya AA (2017). Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum* in type 2 diabetic rats. J Ayurveda Integr Med 8(4):238-246. DOI:10.1016/j.jaim.2017.01.008.

Obafemi TO, Olaleye MT, Akinmoladun AC (2019). Antidiabetic property of miracle fruit plant (*Synsepalum dulcificum* Shumach. & Thonn. Daniell) leaf extracts in fructose-fed streptozotocin-injected rats via anti-inflammatory activity and inhibition of carbohydrate metabolizing enzymes. J Ethnopharmacol 244:Article 112124. DOI:10.1016/j.jep.2019.112124.

Pearson WR (2000). Flexible sequence similarity searching with the FASTA3 program package. In: Misener S, Krawetz SA, editors. *Bioinformatics Methods and Protocols*. (Methods in Molecular Biology, vol. 132). Totowa (NJ): Humana Press Inc., pp. 185-219.

Quirce S, Fernández-Nieto M, Escudero C, Cuesta J, de Las Heras M, Sastre J (2006). Bronchial responsiveness to bakery-derived allergens is strongly dependent on specific skin sensitivity. Allergy 61(10):1202-1208. DOI:10.1111/j.1398-9995.2006.01189.x.

Seppälä U, Alenius H, Turjanmaa K, Reunala T, Palosuo T, Kalkkinen N (1999). Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. J Allergy Clin Immunol 103(1, Part 1):165-171. DOI:10.1016/s0091-6749(99)70541-5.

Sessa DJ, Ghantous PE (1987). Chemical inactivation of soybean trypsin inhibitors. J Am Oil Chem Soc 64(12):1682-1687. DOI:10.1007/BF02542503.

Shi YC, Lin KS, Jhai YF, Lee BH, Han Y, Cui Z, et al. (2016). Miracle fruit (*Synsepalum dulcificum*) exhibits as a novel anti-hyperuricaemia agent. Molecules 21(2):140 [11pp]. DOI:10.3390/molecules21020140.

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, et al. (2019). Safety assessment of miraculin using *in silico* and *in vitro* digestibility analyses. Food Chem Toxicol 133:Article 110762 [10pp]. DOI:10.1016/j.fct.2019.110762.

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, et al. (2020). Corrigendum to "Safety assessment of miraculin using in silico and in vitro digestibility analyses" [Food Chem Toxicol 133(2019 Nov) 110762]. Food Chem Toxicol 137:Article 111136. DOI:10.1016/j.fct.2020.111136.

Taylor SL, Remington BC, Panda R, Goodman RE, Baumert JL (2015). Detection and control of soybeans as a food allergen. In: Flanagan S, editor. *Handbook of Food Allergen Detection and Control*. (Woodhead Publishing Series in Food Science, Technology and Nutrition). Cambridge, UK: Woodhead Publishing Ltd., pp. 341-366. DOI:10.1533/9781782420217.3.341.

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, et al. (2004). A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regul Toxicol Pharmacol 39(2):87-98. DOI:10.1016/j.yrtph.2003.11.003.

U.S. FDA (2020a). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs* (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/ [current to 4-1-20, last updated 4-1-19].

U.S. FDA (2020b). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

ATTACHMENT A
Individual Proposed Food Uses and Use Levels for Miracle Fruit Protein, Miracle Fruit Powder, and Miracle Fruit Pulp in the U.S.

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

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses[a]	RACC[b] (g or mL)	Miracle Fruit Protein Level (mg/serving)	Miracle Fruit Protein Use Levels (mg/100 g)	Miracle Fruit Powder Level (g/serving)	Miracle Fruit Powder Use Level (g/100 g)	Miracle Fruit Pulp Level (g/serving)	Miracle Fruit Pulp Use Level (g/100 g)
Baked Goods and Baking Mixes	Cheesecake	125	5.0	4.00	0.70	0.56	5.0	4.00
Beverages, alcoholic	Cocktail drinks (pre-packaged)	360	5.0	1.39	0.70	0.19	5.0	1.39
	Malt beverages	355	5.0	1.41	0.70	0.20	5.0	1.41
	Distilled liquors	44	5.0	11.36	0.70	1.59	5.0	11.36
	Wine	148	5.0	3.38	0.70	0.47	5.0	3.38
Beverages and Beverages Bases, non-alcoholic	Packaged water-based beverages	360	5.0	1.39	0.70	0.19	5.0	1.39
	Non-milk-based meal replacement beverages and protein drinks	240	5.0	2.08	0.70	0.29	5.0	2.08
Chewing Gum	Chewing gum	3	2.0	66.67	0.20	6.67	NA	N/A
Coffee and Tea	Ready-to-drink coffee beverages	360	5.0	1.39	0.70	0.19	5.0	1.39
	Ready-to-drink tea beverages	360	5.0	1.39	0.70	0.19	5.0	1.39
Dairy Product Analogs	Milk analogs	240	5.0	2.08	0.70	0.29	5.0	2.08
	Non-dairy yogurts	170	5.0	2.94	0.70	0.41	5.0	2.94
Frozen Dairy Desserts and Mixes	Ice cream	130	2.0	1.54	0.70	0.54	5.0	3.85
	Frozen yogurt	90	2.0	2.22	0.70	0.78	5.0	5.56
	Frozen milk desserts and bars	129	2.0	1.55	0.70	0.54	5.0	3.88
Fruit and Water Ices	Edible Ices	157	2.0	1.27	0.70	0.45	5.0	3.18
	Sherbet	100	2.0	2.00	0.70	0.70	5.0	5.00
	Sorbet	133	2.0	1.50	0.70	0.53	5.0	3.76
Grain Products and Pastas	Cereal bars, granola bars, energy, protein, and meal replacement bars	40	5.0	12.50	0.70	1.75	5.0	12.50
	Granola	40	3.0	7.50	0.70	1.75	5.0	12.50
Milk Products	Packaged milk-based beverages	240	5.0	2.08	0.70	0.29	5.0	2.08
	Yogurt	170	5.0	2.94	0.70	0.41	5.0	2.94
	Yogurt drinks	93 to 207	5.0	2.42	0.70	0.34	5.0	2.42

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

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses[a]	RACC[b] (g or mL)	Miracle Fruit Protein Level (mg/serving)	Miracle Fruit Protein Use Levels (mg/100 g)	Miracle Fruit Powder Level (g/serving)	Miracle Fruit Powder Use Level (g/100 g)	Miracle Fruit Pulp Level (g/serving)	Miracle Fruit Pulp Use Level (g/100 g)
Processed Fruits and Fruit Juices	Packaged fruit juices, nectar, fruit drinks and ades, and fruit-based smoothies	240	5.0	2.08	0.70	0.29	5.0	2.08
Processed Vegetables and Vegetable Juices	Packaged vegetable juices and blends	240	5.0	2.08	0.70	0.29	5.0	2.08
Snack Foods	Fruit-based bars (without granola)	30	5.0	16.67	0.70	2.33	5.0	16.67
Soft Candy	Confectionery and chewy candy coatings and fillings	30	4.0	13.33	0.70	2.33	5.0	16.67
	Gummy candy	30	2.0	6.67	0.70	2.33	5.0	16.67

CFR = Code of Federal Regulations; NA = not applicable; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

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

[b] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2020b). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

APPENDIX B - Results of Allergenicity Assessment with Pepsin Digests

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
DSAPNPVLDIDGEKLRT	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.21	50	100	12
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.23	58.3	91.7	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.28	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.29	50	100	12
DSAPNPVLDIDGEKLRTGTNY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.018	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.25	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.33	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.34	50	100	12
	Proteinase inhibitor	*Solanum tuberosum*	221	0.53	40	70	20
DSAPNPVLDIDGEKLRTGTNYY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.037	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.4	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.58	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.6	50	100	12
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.62	50	75	16
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.62	50	75	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.63	43.8	81.3	16
	Proteinase inhibitor	*Solanum tuberosum*	221	0.86	40	70	20
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.85	50	81.3	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.85	50	75	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.85	50	81.3	16

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
DSAPNPVLDIDGEKLRTGTNYYIVPVLRD	Kunitz trypsin inhibitor	*Glycine max*	208	0.00078	45.5	86.4	22
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00099	50	81.8	22
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.001	50	81.8	22
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.0026	40	76	25
	Proteinase inhibitor	*Solanum tuberosum*	221	0.29	39.1	69.6	23
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.62	50	83.3	18
	Trypsin inhibitor subtype B	*Glycine max*	217	0.63	50	77.8	18
	Trypsin inhibitor subtype A	*Glycine max*	217	0.63	50	83.3	18
DSAPNPVLDIDGEKLRT	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.21	50	100	12
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.23	58.3	91.7	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.28	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.29	50	100	12
DSAPNPVLDIDGEKLRTGTNY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.018	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.25	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.33	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.34	50	100	12
	Proteinase inhibitor	*Solanum tuberosum*	221	0.53	40	70	20

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
DSAPNPVLDIDGEKLRTGTNYY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.022	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.30	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.41	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.42	50	100	12
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.45	50	75	16
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.45	50	75	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.46	43.8	81.3	16
	Proteinase inhibitor	*Solanum tuberosum*	221	0.64	40	70	20
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.85	50	81.3	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.85	50	75	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.85	50	81.3	16
DSAPNPVLDIDGEKLRTGTNYYIVPVLRD	Kunitz trypsin inhibitor	*Glycine max*	208	0.00078	45.5	86.4	22
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00099	50	81.8	22
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.001	50	81.8	22
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.0026	40	76	25
	Proteinase inhibitor	*Solanum tuberosum*	221	0.29	39.1	69.6	23
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.62	50	83.3	18
	Trypsin inhibitor subtype B	*Glycine max*	217	0.63	50	77.8	18
	Trypsin inhibitor subtype A	*Glycine max*	217	0.63	50	83.3	18

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
SAPNPVLDIDGEKLRTGTNY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.016	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.23	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.29	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.30	50	100	12
	Proteinase inhibitor	*Solanum tuberosum*	221		40	70	20
SAPNPVLDIDGEKLRTGTNYY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.025	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.32	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.41	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.42	50	100	12
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.48	50	75	16
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.48	50	75	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.48	43.8	81.3	16
	Proteinase inhibitor	*Solanum tuberosum*	221	0.66	40	70	20
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.87	50	81.3	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.88	50	81.3	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.88	50	75	16
APNPVLDIDGEKLRT	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.14	50	100	12
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.15	58.3	91.7	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.19	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.2	50	100	12

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
APNPVLDIDGEKLRTGTNY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.018	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.24	50	100	12
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.31	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.32	50	100	12
	Proteinase inhibitor	*Solanum tuberosum*	221	0.9	43.8	75	16
APNPVLDIDGEKLRTGTNYY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.025	44.4	83.3	18
	Ani s 11-like protein precursor	*Anisakis simplex*	160	0.32	50	100	12
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.47	50	75	16
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.47	50	75	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.47	43.8	81.3	16
	Ani s 11-like protein 2 precursor	*Anisakis simplex*	287	0.40	50	100	12
	Ani s 11 allergen precursor	*Anisakis simplex*	307	0.41	50	100	12
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.85	50	81.3	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.85	50	75	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.85	50	81.3	16
NPVLDIDGEKLRTGTNYY	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.32	43.8	81.3	16
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.33	50	75	16
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.33	50	75	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.34	43.8	81.3	16
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.62	50	81.3	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.62	50	75	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.62	50	81.3	16
	Proteinase inhibitor	*Solanum tuberosum*	221	0.84	43.8	75	16
LDIDGEKLRT	No sequences with E(< 1.000000)						

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
LDIDGEKLRTGTNY	No sequences with E(< 1.000000)						
LDIDGEKLRTGTNYY	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.73	46.7	73.3	15
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.73	46.7	73.3	15
	Kunitz trypsin inhibitor	*Glycine max*	208	0.71	40	80	15
LDIDGEKLRTGTNYYIVPVLRD	Kunitz trypsin inhibitor	*Glycine max*	208	0.00036	42.9	85.7	21
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00047	47.6	81	21
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.00047	47.6	81	21
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.57	33.3	71.4	21
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.63	47.1	82.4	17
	Trypsin inhibitor subtype B	*Glycine max*	217	0.63	47.1	76.5	17
	Trypsin inhibitor subtype A	*Glycine max*	217	0.63	47.1	82.4	17
DIDGEKLRT	No sequences with E(< 1.000000)						
DIDGEKLRTGTNYY	Kunitz trypsin inhibitor	*Glycine max*	208	0.84	42.9	78.6	14
IDGEKLRTGTNY	No sequences with E(< 1.000000)						
IDGEKLRTGTNYY	No sequences with E(< 1.000000)						
NYYIVPVLRD	Kunitz trypsin inhibitor	*Glycine max*	208	0.17	62.5	100	8
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.24	62.5	100	8
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.24	62.5	100	8
	11S globulin subunit beta	*Cucurbita maxima*	480	0.84	55.6	88.9	9
NYYIVPVLRDHGGGLT	Kunitz trypsin inhibitor	*Glycine max*	208	0.0012	57.1	92.9	14
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0016	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.018	50	92.9	14
	Vacuolar serine protease	*Penicillium citrinum*	358	0.85	57.1	78.6	14
	Vacuolar serine protease, partial	*Fusarium proliferatum*	386	0.95	57.1	78.6	14

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
NYYIVPVLRDHGGGLTV	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0008	56.3	87.5	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.002	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.028	50	92.9	14
NYYIVPVLRDHGGGLTVSA	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00046	50	88.9	18
	Kunitz trypsin inhibitor	*Glycine max*	208	0.0016	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.024	50	92.9	14
	Vacuolar serine protease	*Penicillium citrinum*	358	0.84	57.1	78.6	14
	Vacuolar serine protease, partial	*Fusarium proliferatum*	386	0.90	57.1	78.6	14
YYIVPVLRD	Kunitz trypsin inhibitor	*Glycine max*	208	0.073	62.5	100	8
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.11	62.5	100	8
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.11	62.5	100	8
YYIVPVLRDHGGGLT	Kunitz trypsin inhibitor	*Glycine max*	208	0.00094	57.1	92.9	14
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0012	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.014	50	92.9	14
YYIVPVLRDHGGGLTV	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00082	56.3	87.5	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.002	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.028	50	92.9	14
YYIVPVLRDHGGGLTVS	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00091	56.3	87.5	16
	Kunitz trypsin inhibitor	*Glycine max*	208	0.0022	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.031	50	92.9	14
YYIVPVLRDHGGGLTVSA	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00063	50	88.9	18
	Kunitz trypsin inhibitor	*Glycine max*	208	0.0021	57.1	92.9	14
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.029	50	92.9	14
YYIVPVLRDHGGGLTVSAT	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.00034	52.6	89.5	19
	Kunitz trypsin inhibitor	*Glycine max*	208	0.0019	47.4	78.9	19
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.032	42.1	84.2	19

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
IVPVLRDHGGGLT	Kunitz trypsin inhibitor	*Glycine max*	208	0.14	50	91.7	12
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.18	50	91.7	12
VVQTRKEVDHDRPLAFF	No sequences with E(< 1.000000)						
QTRKEVDHDRPLAFF	No sequences with E(< 1.000000)						
QTRKEVDHDRPLAFFPENPKED	No sequences with E(< 1.000000)						
QTRKEVDHDRPLAFFPENPKEDVVRVSTDLN	No sequences with E(< 1.000000)						
TRKEVDHDRPLAFF	No sequences with E(< 1.000000)						
LAFFPENPKE	No sequences with E(< 1.000000)						
LAFFPENPKEDVVRVSTDLN	Fra e 2.01 allergen	*Fraxinus excelsior*	134	0.43	36.8	68.4	19
FFPENPKED	No sequences with E(< 1.000000)						
FFPENPKEDVV	No sequences with E(< 1.000000)						
FFPENPKEDVVRVS	No sequences with E(< 1.000000)						
FFPENPKEDVVRVST	No sequences with E(< 1.000000)						
FFPENPKEDVVRVSTDL	No sequences with E(< 1.000000)						
FFPENPKEDVVRVSTDLN	Fra e 2.01 allergen	*Fraxinus excelsior*	134	0.80	35.3	70.6	17
FFPENPKEDVVRVSTDLNI	Fra e 2.01 allergen	*Fraxinus excelsior*	134	0.78	35.3	70.6	17
FFPENPKEDVVRVSTDLNINFS	No sequences with E(< 1.000000)						
FPENPKEDVVRVSTDL	No sequences with E(< 1.000000)						
FPENPKEDVVRVSTDLN	Fra e 2.01 allergen	*Fraxinus excelsior*	134	0.67	35.3	70.6	17
FPENPKEDVVRVSTDLNINFS	No sequences with E(< 1.000000)						
EDVVRVSTDL	No sequences with E(< 1.000000)						
EDVVRVSTDLN	No sequences with E(< 1.000000)						
EDVVRVSTDLNI	No sequences with E(< 1.000000)						
EDVVRVSTDLNINFS	No sequences with E(< 1.000000)						
VVRVSTDLNINFS	No sequences with E(< 1.000000)						

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
RWTSSTVWRLDKYDESTGQYF	Pollen allergen	*Lolium perenne*	263	0.21	36	60	25
	Group I pollen allergen	*Poa pratensis*	263	0.21	42.1	63.2	19
	Proteinase inhibitor	*Solanum tuberosum*	221	0.26	46.7	66.7	15
	Pollen allergen	*Lolium perenne*	252	0.28	42.1	68.4	19
	Pollen allergen	*Lolium perenne*	263	0.28	42.1	68.4	19
	Pollen allergen Lol p 1	*Lolium perenne*	263	0.28	42.1	68.4	19
	Crystal Structure of Phl p 1, a Major Timothy Grass Pollen Allergen	*Phleum pratense*	241	0.36	36.8	68.4	19
	Protein with incomplete signal sequence	*Holcus lanatus*	248	0.37	42.1	68.4	19
	Phl p I allergen	*Phleum pratense*	263	0.38	36.8	68.4	19
	Major group I allergen Hol l 1	*Holcus lanatus*	263	0.38	42.1	68.4	19
	Allergen Hol-II	*Holcus lanatus*	265	0.38	42.1	68.4	19
	Major pollen allergen Pha a 1	*Phalaris aquatica*	269	0.39	36.8	68.4	19
	Group 1 allergen Dac g 1.01 precursor	*Dactylis glomerata*	240	0.49	36.8	68.4	19
	Group 1 allergen-like	*Dactylis glomerata*	264	0.51	36.8	68.4	19

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
STVWRLDKYDESTGQYF	Pollen allergen	*Lolium perenne*	252	0.38	50	71.4	14
	Pollen allergen	*Lolium perenne*	263	0.40	50	71.4	14
	Pollen allergen	*Lolium perenne*	263	0.40	50	71.4	14
	Pollen allergen Lol p 1	*Lolium perenne*	263	0.40	50	71.4	14
	group I pollen allergen	*Poa pratensis*	263	0.40	40	73.3	15
	Crystal Structure of Phl p 1, a Major Timothy Grass Pollen Allergen	*Phleum pratense*	241	0.47	42.9	71.4	14
	Protein with incomplete signal sequence	*Holcus lanatus*	248	0.49	50	71.4	14
	Phl p I allergen	*Phleum pratense*	263	0.53	42.9	71.4	14
	major group I allergen Hol l 1	*Holcus lanatus*	263	0.53	50	71.4	14
	Allergen Hol-II	*Holcus lanatus*	265	0.536	50	71.4	14
	Major pollen allergen Pha a 1	*Phalaris aquatica*	269	0.54	42.9	71.4	14
	Proteinase inhibitor	*Solanum tuberosum*	221	0.56	46.2	69.2	13
	Group 1 allergen Dac g 1.01 precursor	*Dactylis glomerata*	240	0.62	42.9	71.4	14
	Group 1 allergen-like	*Dactylis glomerata*	264	0.70	42.9	71.4	14

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
TVWRLDKYDESTGQYF	Pollen allergen	*Lolium perenne*	252	0.59	50	71.4	14
	Pollen allergen	*Lolium perenne*	263	0.61	50	71.4	14
	Pollen allergen	*Lolium perenne*	263	0.61	50	71.4	14
	Pollen allergen Lol p 1	*Lolium perenne*	263	0.61	50	71.4	14
	group I pollen allergen	*Poa pratensis*	263	0.61	40	73.3	15
	Crystal Structure of Phl p 1, a Major Timothy Grass Pollen Allergen	*Phleum pratense*	241	0.72	42.9	71.4	14
	Protein with incomplete signal sequence	*Holcus lanatus*	248	0.75	50	71.4	14
	Phl p I allergen	*Phleum pratense*	263	0.80	42.9	71.4	14
	major group I allergen Hol l 1	*Holcus lanatus*	263	0.80	50	71.4	14
	Allergen Hol-II	*Holcus lanatus*	265	0.81	50	71.4	14
	Major pollen allergen Pha a 1	*Phalaris aquatica*	269	0.82	42.9	71.4	14
	Proteinase inhibitor	*Solanum tuberosum*	221	0.85	46.2	69.2	13
	Group 1 allergen Dac g 1.01 precursor	*Dactylis glomerata*	240	0.93	42.9	71.4	14
WRLDKYDESTGQYF	No sequences with E(< 1.000000)						
LDKYDESTGQYFV	No sequences with E(< 1.000000)						
FVTIGGVKGNPGPET	No sequences with E(< 1.000000)						
FVTIGGVKGNPGPETISSW	No sequences with E(< 1.000000)						
FVTIGGVKGNPGPETISSWF	No sequences with E(< 1.000000)						
TIGGVKGNPGPETISSWF	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPET	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPETI	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPETISS	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPETISSW	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPETISSWF	No sequences with E(< 1.000000)						
YFVTIGGVKGNPGPETISSWFK	No sequences with E(< 1.000000)						

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
YFVTIGGVKGNPGPETISSWFKIEEF	No sequences with E(< 1.000000)						
SWFKIEEF	Proteinase inhibitor	*Solanum tuberosum*	221	0.39	100	100	5
	Major pollen allergen Lol p 11	*Lolium perenne*	134	0.88	80	100	5
	Trypsin inhibitor subtype B	*Glycine max*	217	0.92	80	100	5
	Pollen allergen Phl p 11	*Phleum pratense*	143	0.94	80	100	5
SWFKIEEFC	No sequences with E(< 1.000000)						
GIYIDQKGRRRLALS	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0024	66.7	80	15
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.0024	66.7	80	15
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.021	64.3	85.7	14
	Proteinase inhibitor	*Solanum tuberosum*	221	0.039	63.6	90.9	11
	Allergen Pen m 2	*Fenneropenaeus chinensis*	53	0.14	46.7	86.7	15
	Allergen Pen m 2	*Fenneropenaeus chinensis*	53	0.14	46.7	86.7	15
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.38	56.3	75	16
	Trypsin inhibitor subtype A	*Glycine max*	217	0.38	56.3	75	16
	Trypsin inhibitor subtype B	*Glycine max*	217	0.38	56.3	75	16

Table B-1 Search Results of AllergenOnline Database Version 21 with Peptides Following Pepsin Digest Mapping

Peptide Sequence	Description	Source	Full Length				
			Length	E-value	% Identity	% Similarity	Amino acid overlap
GIYIDQKGRRRLALSDKPFAF	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0043	60	80	20
	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.0044	60	80	20
	Proteinase inhibitor	*Solanum tuberosum*	221	0.045	52.9	88.2	17
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.14	50	85	20
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.30	52.4	76.2	21
	Trypsin inhibitor subtype A	*Glycine max*	217	0.30	52.4	76.2	21
	Trypsin inhibitor subtype B	*Glycine max*	217	0.30	52.4	76.2	21
	Allergen Pen m 2	*Fenneropenaeus chinensis*	53	0.83	43.8	87.5	16
	Allergen Pen m 2	*Fenneropenaeus chinensis*	53	0.83	43.8	87.5	16
GIYIDQKGRRRLALSDKPFAFE	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.0013	56.5	78.3	23
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.0046	52.2	78.3	23
	Proteinase inhibitor	*Solanum tuberosum*	221	0.061	52.9	88.2	17
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.18	50	85	20
	Kunitz trypsin inhibitor; KTi	*Glycine max*	216	0.29	45.8	75	24
	Trypsin inhibitor subtype A	*Glycine max*	217	0.29	45.8	75	24
	Trypsin inhibitor subtype B	*Glycine max*	217	0.29	45.8	75	24
YIDQKGRRRLALSDKPFAFE	Kunitz trypsin inhibitor KTi2	*Glycine max*	204	0.037	55	80	20
	Proteinase inhibitor	*Solanum tuberosum*	221	0.041	52.9	88.2	17
	Kunitz trypsin inhibitor KTi1	*Glycine max*	203	0.13	50	80	20
	Aspartic protease inhibitor 11 (Cathepsin D inhibitor)	*Solanum tuberosum*	188	0.43	53.3	93.3	15
LSDKPFAFE	No sequences with E(< 1.000000)						
EFNKTVYF	No sequences with E(< 1.000000)						