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July 15, 2019

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Subject: GRAS Notification – Fava Bean Protein

Dear Sir:

On behalf of Yantai T.FULL Biotech Co., Ltd., ToxStrategies, Inc. (its agent) is submitting, for FDA review, a copy of the GRAS notification as required. The enclosed document provides notice of a claim that the food ingredient, fava bean protein, described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to food. The GRAS Notification was the subject of a pre-submission call/meeting with FDA on July 9, 2019.

In addition, the data and information in the GRAS notice can be shared with the Food Safety Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA).

If you have any questions or require additional information, please do not hesitate to contact me at 630-352-0303, or dschmitt@toxstrategies.com.

Sincerely,

[redacted]

Donald F. Schmitt, M.P.H.
Senior Managing Scientist

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FOOD ADDITIVE SAFETY

GRAS Determination of Fava Bean Protein for Use as an Ingredient in Human Food

JUNE 17, 2019

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GRAS Determination of Fava Bean Protein for Use as an Ingredient in Human Food

SUBMITTED BY:

Yantai T.FULL Biotech Co., Ltd.
Shiduitou, Zhangxing Town
Zhaoyuan City, Shandong Province
China

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
College Park MD 20740-3835

CONTACT FOR TECHNICAL OR OTHER INFORMATION

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June 17, 2019

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List of Acronyms

bw	body weight
cGMP	current Good Manufacturing Practice
C	centigrade
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CFU	colony-forming units
COA	Certificate of Analysis
db	dry basis
DRV	dietary reference value
FFDCA	Federal Food, Drug, and Cosmetic Act
FDA	U.S. Food and Drug Administration
g	gram
G6PD	glucose-6-phosphate dehydrogenase
GRAS	Generally Recognized as Safe
GRN	Generally Recognized as Safe Notification
GSH	glutathione
HCl	hydrochloric acid
4-HNE	4-hydroxynonenal
HMG	3-hydroxy-3-methylglutaryl
HPLC	high-pressure liquid chromatography
IgE	immunoglobulin E
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kcal	kilocalories
kg	kilogram
LD_{50}	lethal dose
LDL	low-density lipoprotein
mg	milligram
NaCl	sodium chloride
NaOH	sodium hydroxide
NOEL	no-observed-effect level
PDCAAS	Protein Digestibility Corrected Amino Acid Score
RBC	red blood cell
RDA	Recommended Dietary Allowance
SGF	simulated gastric fluid
US	United States
USC	United States Code
USDA	United States Department of Agriculture
V/C	vicine/convicine
VLDL	very low-density lipoprotein
WHO	World Health Organization

§ 170.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Yantai T.FULL Biotech Co., Ltd. (TFULL) , through its agent, ToxStrategies, Inc., hereby notifies the U.S. Food and Drug Administration (FDA) of the submission of a Generally Recognized as Safe (GRAS) notice for the use of fava bean protein in select foods for human consumption in accordance with Subpart E of 21 CFR § 170.

(2) Name and Address

Yantai T.FULL Biotech Co., Ltd.
Shiduitou, Zhangxing Town
Zhaoyuan City, Shandong Province
China

(3) Name of Notified Substance

The name of the substance that is the subject of this GRAS determination is a fava bean protein isolated from *Vicia faba* L., also referred to as broad bean, horse bean, field bean, and faba bean.

(4) Intended Use in Food

The fava bean protein is proposed for use only as an alternative to other plant-based protein ingredients in select foods for human consumption (except for infant formula), and the daily consumption of protein is not expected to increase as a result of its introduction.

(5) Statutory Basis for GRAS Determination

TFULL, through its agent, ToxStrategies, confirms that the fava bean protein ingredient, which meets the specifications described herein, has been determined to be GRAS through scientific procedures in accordance with 21 CFR § 570.30(a) and (b).

(6) Premarket Approval Statement

TFULL further asserts that the use of the fava bean protein ingredient, as described herein, is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act, based on a conclusion that the substance is GRAS under the conditions of its intended use.

(7) Availability of Information

The data and information that serve as the basis for this GRAS determination, as well any information that has become available since the GRAS determination, will be sent on request, or are available for the FDA's review and copying during customary business hours from ToxStrategies, Inc., Naperville, IL.

(8) Data and Information Confidentiality Statement

None of the data and information in the GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(9) GRAS Certification

To the best of our knowledge, the GRAS determination is a complete, representative, and balanced document. TFULL is not aware of any information that would be inconsistent with a finding that the proposed uses and use levels of the fava bean protein ingredient in food, meeting the appropriate specifications described herein, and used according to current Good Manufacturing Practice (cGMP), is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

[redacted]

July 15, 2019

Donald F. Schmitt, M.P.H.
Senior Managing Scientist
ToxStrategies, Inc.
Agent for TFULL

Date

(11) FSIS Statement

The fava bean protein ingredient will be used as an alternative meat extender and binder (similar to plant-based protein ingredients like pea protein), limited only by GMP, as soy protein is so limited by USDA.

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

A. Identity

The fava bean protein ingredient is an extract of fava beans consisting of ≥90% protein.

B. Common or Usual Name

Fava bean protein isolate. The ingredient will be referred to as fava bean protein throughout the document.

C. CAS Registry Number

Not applicable.

D. Trade Name

The trade name under Top Health Ingredients, Inc. for this fava bean protein is AdvantaFava™ (e.g., TFULL fava protein, AdvantaFava™ Nourish 90, AdvantaFava™ Resolve 90). TFULL's fava bean protein may be marketed by TFULL under a different trade name.

E. Fava Bean Protein Composition

Typical nutritional data for the fava bean protein is summarized in Table 1. Table 2 presents the alkaloid concentrations (anti-nutrients components vicine, convicine, divicine, and isouramil) in three lots of the proposed fava bean protein ingredient (see Appendix B for analytical method). Levodopa, also known as L-DOPA or L-3,4-dihydroxyphenylalanine, naturally occurs in fava beans, but its levels decrease significantly in heated/cooked beans (Carador-Martinez et al., 2012; Multari et al, 2015). The level of L-DOPA in the fava bean protein isolate was determined by high pressure liquid chromatography (see Appendix C) to be approximately 13.3 mg/kg .

Fava bean protein, due to its high protein content, is also rich in amino acids and Table 3 presents a comparison of its amino acid profile to a few other common food-derived proteins. The typical nutritional data and amino acid profile are also illustrated in the specification sheets in Appendix A. The % Protein Digestibility Corrected Amino Acid Score (PDCAAS) has been calculated to be 61.00 (PSL, 2018).

Table 1. Typical nutritional data for fava bean protein (per 100 g)

Protein	85 g
Moisture	7 g
Carbohydrates	3 g
Ash	3 g
Fat	2 g
Sodium	604 mg
Caloric value	370 kcal

Table 2. Alkaloid concentration in three lots of fava bean protein (mg/kg)*

Alkaloid	**Lot** [redacted]	**Lot** [redacted]	**Lot** [redacted]
Vicine	282	316	319
Convicine	66	91	86
Divicine	4	4	4
Isouramil	5	10	10
Sum	357	421	419

*by HPLC method (Appendix B)

Table 3. Typical amino acid profile (g dry basis per 100 g product)[1]

Amino Acid	Fava Bean	Oat	Wheat	Rice	Soy	Whey
Alanine	4.06	4.37	4.0	5.67	NA	NA
Arginine	8.58	7.17	2.7	7.64	NA	NA
Aspartic Acid	9.90	7.48	3.2	9.33	NA	NA
Cysteine	0.87	2.45	1.3	2.09	NA	NA
Glutamic Acid	16.8	22.5	33.7	17.22	NA	NA
Glycine	3.67	4.13	5.1	4.33	NA	NA
Histidine	2.37	2.22	2.14	2.12	2.3	2.2
Isoleucine	4.40	4.38	3.39	4.4	4.7	5.8
Leucine	8.05	8.42	6.67	8.3	6.6	12
Lysine	6.17	3.38	2.5	3.4	5.4	10.8
Methionine	0.79	2.23	1.4	2.66	NA	NA
Methionine + Cysteine	1.66	4.68	4.33	5.61	2.9	4.2
Phenylalanine	4.51	5.95	4.2	5.38	NA	NA
Phenylalanine + Tyrosine	7.90	10	7.6	10.85	9.8	5.1
Proline	4.27	5.57	14.2	4.49	NA	NA
Serine	4.81	3.98	5.9	4.75	NA	NA
Threonine	3.33	3.5	2.96	3.7	4	7.2
Tryptophan	1.03	1.1	1.21	1.2	1.2	2.1
Tyrosine	3.39	4.05	2.7	4.84	NA	NA
Valine	4.85	5.78	4.42	6	4.2	5.8

[1] Amino acid values for the other food proteins were excerpted from GRAS Notification (GRN) No. 575 for oat protein, GRN Nos. 26 and 575 for wheat protein, GRN Nos. 609 and 575 for rice protein, GRN No. 575 for soy protein, and GRN No. 575 for whey protein.

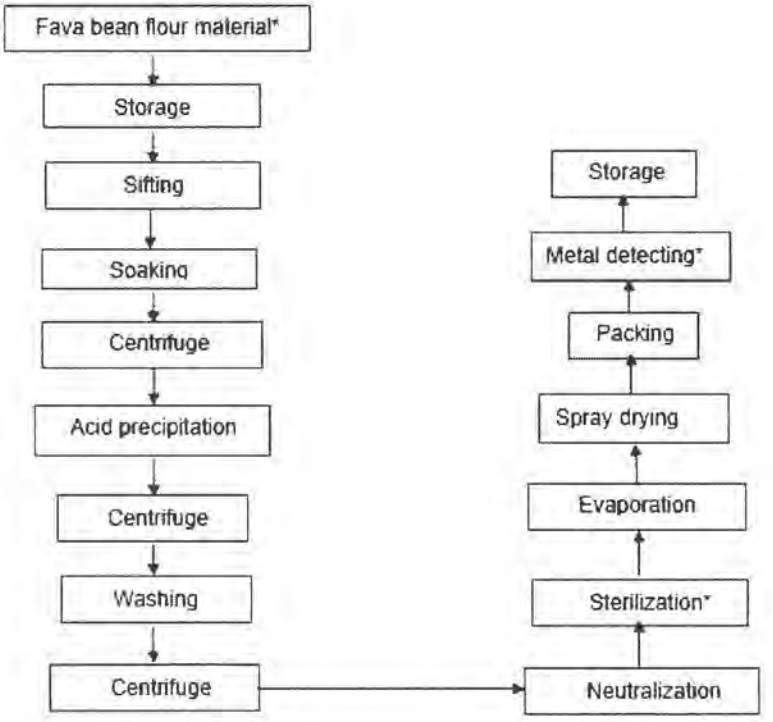
NA = not available

F. Manufacturing Process

A diagram of the fava bean protein product manufacturing process is shown below (Figure 1).

The fava bean protein ingredient (≥90% protein) is produced by an extraction process from commercially available fava beans. The starting material for the fava bean protein extraction process is fava bean flour which is produced from commercially available fava beans using a standard milling process. Following a soaking step (extraction), the slurry is separated by centrifugation. Hydrochloric acid (HCl) is then added to the slurry to precipitate the protein. The HCl is removed during the subsequent washing step. Sodium hydroxide (NaOH) is then added to neutralize the slurry as it reacts with remaining HCl to produce sodium chloride (NaCl) and water. The product is then sterilized, evaporated, and spray dried.

Figure 1. Steps in the fava bean protein production process



*CCPs

The only processing aids employed are safe and suitable for use in production of the fava bean protein ingredient. They are commonly used in food ingredient manufacturing processes, as described in Table 4.

Table 4. Processing aids

Processing Aid	CAS Number(s)	21 CFR/GRN Citation(s)
Hydrochloric acid (HCl)	7647-01-0	21 CFR §182.1057
Sodium hydroxide (NaOH)	1310-73-2	21 CFR §184.1763

G. Characterization of *Vicia faba* L.

Fava bean (*Vicia faba* L.), also referred to as broad bean, horse bean, faba bean, and field bean, is an early legume crop and belongs to the *Fabaceae* family. There is little evidence of the origins of its domestication, as its wild progenitor is still unknown. However, the oldest seeds of fava bean were found in the late 10th millennium, in north-west Syria (Willcox and Tanno, 2006). The fava bean has a long history of use as feed and food, likely because of its valuable content of both protein and energy (Crepon et al., 2010). Fava bean is a versatile crop and has the ability to grow in various climatic zones. It can be used throughout the year and is consumed in both raw and processed forms. In the human diet, it is mostly the seed grain that is consumed, while the pods are used as feed. The fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health. The protein content is about twice that of cereals and several times that of root tubers. Fava bean seeds are a rich source of proteins, carbohydrates, fiber, vitamins, and minerals (Aune et al. 2011).

H. Product Specifications

Specifications for the product are presented in Table 5. Analytical results from three non-consecutive lots are provided in Appendix A. A comparison of four non-consecutive lots of product to the specifications below can be found in Table 6.

Table 5. Specifications for fava bean protein

Parameter	Specification	Method
Protein (%, db)	≥90	Kjeldahl, AOAC 981.10 or equivalent
Ash (%)	≤5	AOAC 923.03 or equivalent
pH	6–7	USP or equivalent
Lead (mg/kg)	≤0.1	ICP-MS
Arsenic (mg/kg)	≤ 0.1	ICP-MS
Cadmium (mg/kg)	≤0.1	ICP-MS
Mercury (mg/kg)	≤0.1	ICP-MS
Total Plate Count (CFU/25g)	≤10,000	USP <2021/2022> or equivalent
Yeast and mold (CFU/25g)	≤100	USP <2021/2022> or equivalent
E. coli	Negative	USP <2021/2022> or equivalent

Salmonella	Negative	USP <2021/2022> or equivalent
Staphylococcus aureus	Negative	USP <2021/2022> or equivalent

Table 6. Analytical results for four non-consecutive lots of fava bean protein

Specification		Lot No. [redacted]	Lot No. [redacted]	Lot No. [redacted]	Lot No. [redacted]
Protein (%)	≥90	98.4	97.1	92.7	95.7
Ash (%)	≤5	4.5	2.4	1.2	4.1
pH	6–7	7.1	6.5	6.4	6.4
Lead (mg/kg)	≤0.1	≤0.02	≤0.02	≤0.02	≤0.02
Arsenic (mg/kg)	≤0.1	≤0.03	≤0.03	≤0.03	≤0.03
Cadmium (mg/kg)	≤0.1	≤0.02	≤0.02	≤0.02	≤0.02
Mercury (mg/kg)	≤0.1	≤0.02	≤0.02	≤0.02	≤0.02
Total Plate Count (CFU/25g)	≤10,000	160	730	≤10	≤10
Yeast and mold (CFU/25g)	≤100	80 yeast, <10 mold	≤10	≤10	≤10
E. coli	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative	Negative	Negative

The analytical results for the fava bean protein ingredient summarized in the above tables and included in Certificates of Analysis (COAs) in Appendix A confirm that the finished product meets the analytical specifications, demonstrates that TFULL's manufacturing process results in a consistently reproducible product, and confirms the lack of significant levels of impurities and/or contaminants (e.g., anti-nutritionals (Table 3), heavy metals, pesticides, aflatoxins, and microbiological contaminants).

I. Stability Data

The fava bean protein product has been tested under normal conditions (below 100°F) for 24 months. Technical specifications for the product include shelf-life storage conditions of 24 months from the date of manufacture when stored in a closed container in a cool (below 100°F) and dry place away from strong light. Stability testing data can be found in Table 7.

Table 7. Stability testing data

Lot [redacted]	Time (Months)				
Specification	**Initial**	**6**	**12**	**18**	**24**
Sensory	Regular	Regular	Regular	Regular	Regular
Moisture (%)	5.80	5.63	5.57	5.54	5.25
Protein (%)	86	85.81	85.98	86.02	89.94
Total Plate Count (CFU/25g)	8000	6900	5600	5000	1500
Coliform (CFU/25g)	<30	<30	<30	<30	<30

§ 170.235 Part 3, Dietary Exposure

Proposed Use

The focus of this GRAS determination is for food uses identical to what has been recognized in previous GRNs for current plant-based protein sources such as soy (GRN No. 134; FDA 2004), canola (GRN Nos. 327, 386, 683; FDA 2010, 2011, 2017a), pea (GRN Nos. 182, 581, 608, 788; FDA 2005, 2015b, 2016a, 2018b), wheat (GRN Nos. 26 and 182; FDA 1999, 2005), rice (GRN No. 609; FDA 2016b), whey (GRN Nos. 37, 633; FDA 2000, 2016c), potato (GRN No. 447; FDA 2013), oat (GRN 575; FDA 2015a), mung bean (GRN 684; FDA 2017b), and hemp seed (GRN 771; FDA 2018a). Similarly, fava bean–derived protein is intended for use as a source of protein for enrichment of processed foods.

Furthermore, as stated in GRN 575,

> "FDA has established a daily reference value (DRV) for protein of 50 g/day for adults and children four or more years of age. Furthermore, Dietary Guidelines for Americans (HHS/USDA, 2005) recommend that adults eat half their grains as whole grains, which include oats and wheat. The Institute of Medicine (IOM, 2005) recommends that adults consume 0.8 grams of protein per kilogram of body weight. IOM also set a wide range for acceptable protein intake, ranging from 10 - 35% of calories each day. In the U.S., the recommended daily allowance (RDA) of protein is 46 grams/day for women over 19 years of age, and 56 grams/day for men over 19 years of age."

However, the RDA does not represent an upper limit of consumption. Physically active persons on normal diets are known to exceed this level, and individuals involved in bodybuilding ingest much higher levels of protein (WHO, 2002). The accepted WHO safe level of intake is 0.83 g/kg per day, for proteins with a protein digestibility-corrected amino acid score value of 1.0. While WHO has stated that no safe upper limit has been identified, they also indicated that it is unlikely that intakes of twice the safe level are associated with any risk to healthy individuals.

As described in numerous GRAS Notifications, including GRN No. 581 for un-hydrolyzed and hydrolyzed pea protein (see Table 8 below), the typical uses of protein for enrichment of foods include bakery products, snack foods, nutritional beverages such as high-protein drinks and milkshakes, instant powdered nutritional beverages, vegetarian food products and meat analogues, dairy products, and meal replacements/nutrition bars.

Table 8. Proposed maximum food use levels

Food Category	Maximum Use Level of Fava Bean Protein (%) as Consumed
Bakery products (e.g., breads, rolls, doughnuts, cookies, cakes, pies, batters, muffins, pasta, cereal bars, etc.)	10
Snack foods (e.g., crackers, cookies, breakfast/energy bars, snack chips, etc.)	10
Beverages, soups, nutritional beverages (e.g., protein-fortified smoothies, fruit juices, high protein drinks, vegetable-based soups, etc.)	50
Dairy products—imitation (e.g., cheese, spreads, creamers, desserts, dips, whipped topping, etc.)	10
Meal replacement/nutritional bars	20
Meat analogs (e.g., imitation meat products)	30
Processed meat products (where the addition of vegetable proteins is acceptable, such as unspecified products or those that are included in the Standard of Identity)	7
Dry-blend protein powders (e.g., protein shakes, instant protein powders)	90

As the previously submitted GRNs on plant-derived proteins have demonstrated, the proposed use concentrations and variety of food uses, combined with the large average daily consumption of the described foods, results in a calculated daily intake of the protein additives being a substantial fraction of the Recommended Dietary Allowance (RDA; 46 g/day for women over 19 years of age and 56 g/day for men over 19 years of age), and even exceed it at the 90th percentile consumption. This was the case for GRN No. 327 (cruciferin-rich canola/rapeseed protein isolate and napin-rich protein canola/rapeseed protein isolate). TFULL's proposed fava bean protein isolate is intended only to be an alternative source of protein for current uses in food. Therefore, a similar estimate of intake would be expected if fava bean protein were the only source of protein used in processed foods. As was concluded in the other GRAS notifications, we do not realistically expect that the actual consumption of foods containing fava bean protein would result in daily consumption greater than the DRV or RDA for protein. Most of the population's intake of protein is, and will remain, in the form of unprocessed foods, including meat, poultry, fish, and legumes. The proposed fava bean protein product is but one of many protein sources for use in processed foods, so only the known conservatism of intake calculations such as those described in the aforementioned GRNs suggest any possibility of exceeding the RDA at the 90th percentile (FDA, 2010, 2011).

In summary, the proposed uses of fava bean-derived protein will not result in an increase in the overall consumption of protein, but simply provide an alternative source of well-

characterized protein from fava beans for use in food. Therefore, an estimate of the cumulative daily intake is not considered necessary.

§ 170.240 Part 4, Self-Limiting Levels of Use

The use of fava bean protein in protein-enriched foods is considered to be self-limiting for technological reasons, such as product texture and/or flavor profile, either of which could affect consumer acceptability.

§ 170.245 Part 5, Experience Based on Common Use in Food

While there exists broad historical evidence of fava beans and the protein contained therein as food for human consumption, the statutory basis for our conclusion of its GRAS status in the notice is based on scientific procedures and not common use in food.

§ 170.250 Part 6, GRAS Narrative

History of Use and Regulatory Approval

There is no current formal approval for the use of fava bean protein in human foods in the United States. It should be noted that there are references in the public domain (e.g., general interest) to fava bean protein ingredients being self-determined as GRAS (e.g., Ingredion Vitessence™). However, fava beans and other legumes, and the protein from these sources, have been commonly consumed as food and feed around the world for decades (Crepon et al., 2010; Schmandke et al., 2010).

Safety

Introduction

Literature searches were performed to identify available safety data on fava beans, fava bean protein, and the associated anti-nutrients (through March 2019). This included searching sources of information such as publicly available assessments, databases, or reviews from organizations, including EFSA, Joint FAO/WHO Expert Committee on Food Additives (JECFA), U.S. FDA, and the World Health Organization (WHO), general internet searching, and searching databases such as Embase, MedLine, ToxLine, and PubMed.

As described previously, the fava bean can be used throughout the year and is consumed in both raw and processed forms. In the human diet, it is mostly the seed grain that is consumed, while the pods are used as feed. The pods provide macro-, micro- and non-nutrient phytochemicals and could be used as a source of functional compounds. Fava beans contain approximately 250 g protein/kg seed and provides 320 kcal/100 g dry weight of energy. Fava bean, its fractions, and its processing products (grains, hulls, and flours) also contain anti-nutritional factors, but soaking and cooking are able to reduce the amounts of the anti-nutritional factors up to 100%, thus limiting any safety concerns (Jamalian et al., 1999).

Fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health (Aune et al. 2011). The protein content is about twice that of cereals and several times that of root tubers (Wu Leung et al., 1968). However, the biological value of untreated fava beans is negatively affected by the presence of anti-nutritional factors such as the favism-inducing factors (aglycones: divicine and isouramil produced from metabolism of vicine and convicine; Jamalian et al., 1999; Frank, 2005; Luzzatto and Arese, 2018), and the ingestion of untreated fava beans is associated with precipitation of the hemolytic disease favism in certain glucose-6-phosphate dehydrogenase-deficient humans (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017; Frank, 2005; Luzzatto and Arese, 2018).

Removal of these anti-nutrients is the primary safety concern and is required for the effective utilization of fava bean components, including protein concentrates and isolates,

in human nutrition. Anti-nutrition factors are now removed routinely during commercial processing of fava beans (Cardador-Martinez et al., 2012; Jamalian et al., 1999). Some of the anti-nutrition factors are readily destroyed by heat processing (e.g., boiling, cooking, autoclaving, or extrusion cooking) during the production of fava bean flour, or can be eliminated by pretreatments of the beans, such as dehulling or soaking (Revilla 2015). Such treatments may reduce the vicine and convicine content of fava beans by 94%–100% (Jamalian, 1999). Later, Vioque et al. (2012) reported a reduction of vicine and convicine in fava protein "by more than 99%" by employing an extraction of the defatted seed flour, followed by precipitation at an isoelectric pH. The removal of vicine and convicine ensures that the favism-causing aglycone compounds, divicine and isouramil, cannot be synthesized. Soaking, acid hydrolysis, and sterilization are part of the manufacturing process for TFULL's fava protein and result in 1) significant (near total) reduction in levels of vicine and convicine in the isolated protein; and 2) inactivation of the beta-glucosidase activity normally inherent in fava beans. Beta-glucosidase activity is necessary for the conversion of vicine and convicine to their aglycone forms (divicine and isouramil, respectively). It is these aglycones that are the problematic compounds for those individuals with favism. The toxicity of vicine, convicine, divicine, and isouramil are discussed in the safety section below, and the concentrations of the anti-nutrients in three lots of the fava bean protein powder are presented in Table 9. If one were to consume the RDA for protein of 50–60 g all as fava bean protein only, that would represent approximately 25 mg of vicine plus convicine/day.

Table 9. Alkaloid concentration in three lots of fava bean protein (mg/kg)*

Alkaloid	**Lot** [redacted]	**Lot** [redacted]	**Lot** [redacted]
Vicine	282	316	319
Convicine	66	91	86
Divicine	4	4	4
Isouramil	5	10	10
Sum	357	421	419

*by HPLC method

Levodopa, also known as L-DOPA or L-3,4-dihydroxyphenylalanine, is a chemical precursor to dopamine and a common treatment for Parkinson's disease. L-DOPA naturally occurs in fava beans, but its levels decrease significantly in heated/cooked beans (Carador-Martinez et al., 2012; Multari et al, 2015). The level of L-DOPA in the fava bean protein isolate was determined to be approximately 13.3 mg/kg. If a person was to consume the RDA of 56 grams/day, the maximum calculated exposure is 0.74 mg of levodopa, which is 0.25% of the typical recommended daily dose (300 mg) and 0.05% of the maximum recommended daily dose (1500 mg) for the treatment of Parkinson's disease.

Safety Data

Fava Beans and/or Fava Bean Protein

Given the long history of global human consumption of fava beans as food (and the protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by their consumption and general lack of toxicity. As would be expected for a food that has been consumed by humans for centuries, fava beans and fava bean proteins have not been subjected to traditional toxicology studies. Furthermore, given the available information and data on the safety of fava beans and fava bean proteins, as well as the scientific efforts to remove anti-nutritional compounds from fava beans and fava bean protein isolates intended for animal and human consumption, conduct of toxicity studies was considered unnecessary and not an ethical use of animals. A summary of available safety-related information for fava beans and fava bean proteins is presented below.

Animal Studies

Macarulla et al. (2001) examined the effects of the whole fava bean seed versus a fava bean protein isolate on the cholesterol metabolism of hypercholesterolemic male Wistar rats. Three groups of rats (n=10) were fed *ad libitum* either high-fat diets rich in cholesterol-containing casein, whole seeds of *Vicia faba* (252 g protein/kg seed), or the protein isolate of fava beans (773 g protein/kg protein isolate) as a protein source for a period of 2 weeks. The fava bean protein isolate was prepared by isoelectric precipitation and spray drying. Analyses of serum, liver, and feces, as well as of the activity of hepatic 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, were assessed employing enzymatic methods. The rats fed on *Vicia faba* diets showed significantly lower body weights and energy intakes than did rats fed on casein diets. The whole-seed diet induced a significant reduction in plasma triacylglycerol. Feeding rats on diets containing fava bean seeds, or the protein isolate, induced a significant decrease in plasma (LDL+VLDL)-cholesterol but not in HDL-cholesterol. Hepatic cholesterol and triacylglycerol were also reduced. The authors reported that the hypocholesterolemic effects of *Vicia faba* were not the result of a reduction in cholesterol synthesis, as assessed by HMG-CoA reductase activity, but rather, resulted from an increase in steroid fecal excretion. The authors concluded that the fava bean–protein isolate was useful in improving the metabolic alterations induced by feeding with hypercholesterolemic diet compared to casein. No treatment-related adverse effects were noted.

Schmandke et al. (2000) conducted four-week feeding studies of several different processed dehulled fava bean materials in male and female Shoe:Wist/II rats. Treatment groups included (1) untreated beans, (2) beans steamed for 5 minutes at 120°C, (3) beans steamed for 15 min at 120°C, (4) beans soaked for 10 hours and heated for 2 minutes at 180°C by oil deep frying, and (5) a fava bean protein isolate. Thirty rats were used per group (15/sex). Graduated levels of the test materials were fed for 4 weeks, replacing the same amounts in the control diets. The fava bean protein isolate was fed at dose levels of 20, 40, and 80 g/kg. The protein isolate consisted of approximately 81% protein, 6% fat,

and 3% carbohydrate. The low dose of 20 g/kg was considered the no-observed-effect level (NOEL) for female rats consuming the protein isolate (group 5). An increase in adrenal weights was noted at the low and mid-dose levels for male rats and at the high dose of 80 g/kg for both sexes. In addition, an increase was noted in kidney weights and serum lymphocytes of female rats at the mid- and high-dose levels. No changes were noted in the weight of the spleen, liver or thymus. Heat treatment of the fava bean material resulted in no toxicological effects at 50 g/kg doses (groups 3 and 4).

Anti-Nutritional Components

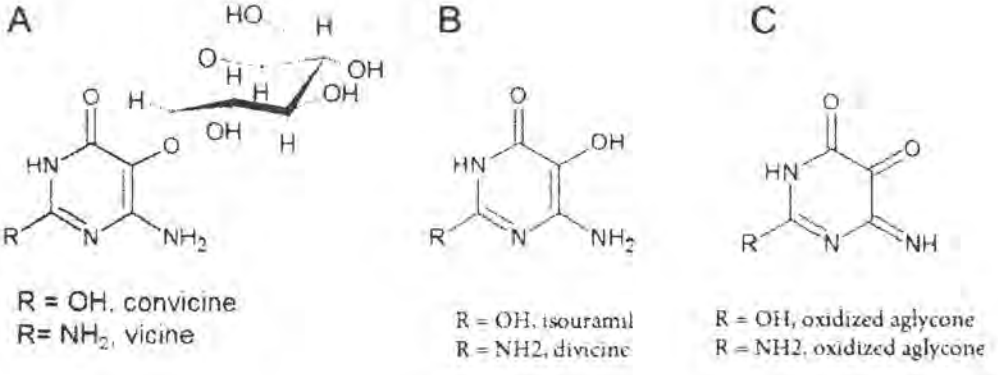
It has been recognized for decades that fava beans contain "anti-nutritional" compounds. As noted previously, those that are of most concern are vicine (2,6-diamino-4,5-dihydroxypyrimidine 5-(beta-D-glucopyranoside) and convicine (2,4,5-trihydroxy-6-aminopyrimidine 5-beta-D-glucopyranoside), which are glycosidic aminopyrimidine derivatives. The vicine and convicine content of raw fava beans have been reported to range from 0.02% to 1.46% (dry weight basis) (Khamassi et al., 2013). Vicine and convicine are hydrolyzed in the fresh fava bean by β-glucosidase to form the aglycones divicine and isouramil (Figure 2, below). These aglycones are the compounds responsible for "favism," by causing the oxidation of glutathione in red blood cells that cannot be sufficiently reversed in affected individuals who are mostly males. The erythrocytes of individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency cannot regenerate glutathione at a normal rate. Erythrocytes become rigid, experience aggregation of their proteins, produce methemoglobin and have their enzymes inactivated due to oxidative damage that cannot be controlled or reversed. The damaged red blood cells are then removed by macrophages in the spleen and liver, which could lead to a potentially fatal hemolytic anemia. Therefore, adverse reactions to fava bean ingestion is limited to a small, but significant, number of males with this X-linked genetic disease. There are many genetic variants of G6PD deficiency, one common variant is observed primarily in Africans and African-Americans and another in people with Mediterranean origins (e.g., Greeks, Italians, Armenians, several Semitic populations and others). Some Asian and middle eastern populations are also affected. In the US, G6PD deficiency is observed in approximately 10% of African-American males (Frank, 2005).

In the European, Middle Eastern, African, and Chinese areas where individuals prone to favism live, and where fava beans, and especially the immature pods, are consumed, it is estimated that there is an intake of 2000 mg of vicine and 1000 mg of convicine in one meal (WHO, 1992). Numerous cases of favism related to consumption of raw, unprocessed fava beans by G6PD-deficient individuals have been reported (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017; Luzzatto and Arese, 2018).

The total amount of convicine and vicine in regular fava bean varieties is 6–14 mg/g (Arese et al., 2007), an amount that is orders of magnitude greater than that found in the proposed fava bean protein ingredient (see Table 8). Arese et al. (2007) stated that the levels of vicine and convicine in FEVITA (a low vicine/convicine protein ingredient) were 10 – 20x lower than traditional cultivars (i.e., 6-14 mg/g). A 10-fold reduction

results in 0.6-1.4 mg/g and 0.3-0.7 mg/g for a 20-fold reduction. This is comparable to the levels shown in Table 8 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot [redacted]). In the Arese et al. (2009) study, seven hemizygous males with G6PD deficiency used the FEVITA fava bean, which is low in vicine and convicine due to reduced beta-glucosidase in the FEVITA cultivar which prevents conversion of vicine and convicine to divicine and isouramil. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the vicine and convicine level of 0.348 mg/g in the proposed ingredient, times 60 grams of protein/day (replacement of RDA protein intake), results in an intake of approximately 25 mg vicine and convicine in the diet, an amount that is 8 to18 % of the level employed in the Arese et al. (2009) study in which no hemolytic effects were reported.

Figure 2. Chemical structures of (A) vicine and convicine, (B) their aglycones, divicine and isouramil, and (C) suggested oxidized aglycones (Pulkkinen et al., 2016)



Animal Studies

Hussein (2012) reported acute oral LD50 values for vicine and divicine in adult male albino rats of 2100 and 1950 mg/kg bw, respectively. Oral administration of vicine at doses of 700, 1400, 2100, 2800, 3500, and 4200 mg/kg bw resulted in 0, 2, 6, 8, 9, and 10 deaths, respectively. Oral administration of divicine in doses of 500, 1000, 1500, 2000, 2500, and 3000 mg/kg bw resulted in 0, 1, 2, 5, 8, and 10 deaths, respectively. The authors considered the possible causes of death to include heart failure that can occur by malfunction, acute hypoglycemia, and/or hepato-damage.

Koreim et al. (2008) investigated the effects of oral administration of convicine in Sprague–Dawley rats. The study design included a control group and two treatment groups (five male and five female rats/group). The two treatment groups were given oral doses of convicine (20 mg/kg bw) for 15 or 30 days. Convicine produced significant decreases ($p<0.05$) in red blood cell counts and hemoglobin content. The serum

albumin/globulin ratio was decreased significantly, and there were significant increases in serum and liver total protein, serum bilirubin, globulin, and iron levels. The data indicated that convicine produced alterations in blood similar to those observed in the human metabolic disease known as favism.

Human Studies

Arese et al. (2009) conducted a clinical trial to examine the safety of fava beans with low levels of the anti-nutritionals vicine and convicine (V/C) in seven hemizygous severely G6PD-deficent male and female subjects (<5% normal enzyme activity). The subjects were fed 450 g/70 kg bw of fresh, raw, de-hulled beans, an amount known to exceed the normal quantity of fava bean consumption (i.e., 150 g/70 kg bw). This represents consumption of 135 – 630 mg of vicine plus convicine (i.e., 0.3 -1.4 mg vicine plus convicine/g times 450 g). Blood was drawn 24 hours before ingestion, and then 8–10, 24, and 48 hours after ingestion. Test parameters included 1) red blood cell (RBC) levels of reduced glutathione (GSH) (which is always decreased in favism); 2) markers indicating modification of RBC membranes (or oxidative stress), 3) formation of membrane adducts with 4 hydroxynonenal (4-HNE; a sensitive indicator of lipid peroxidation that accompanies oxidant insult, and 4) measurement of RBC filterability, a sensitive parameter of RBC deformability that is found during favism. In addition, RBC count, RBC volume, serum hemoglobin, hematocrit, and haptoglobin levels, as well as vitamin status, liver status, thyroid status, diabetes, and iron, were assessed to ensure that deficient subjects were without associated pathologies. Trial results gave no indication of hemolysis as shown by constant levels of RBC counts, hematocrit level, hemoglobin parameters, percentage of reticulocytes, and level of bilirubin; no deficiencies in clinical chemistry results; and no change in the steady-state level of GSH. All other study parameters were stable over time (e.g., 4-HNE adducts, autologous IgG, RBC deformability). The investigators concluded that none of the parameters indicative of hemolysis or oxidative RBC damage were changed in the short term (8–10 hours) or long term (48 hours) after ingestion of approximately 10-fold the normal amount of fava bean digestion of low-tannin, low-V/C fava beans, and provided evidence in hemizygous severely G6PD-deficent male and female subjects that low-tannin, low-V/C fava beans are safe for consumption.

Allergy

Mur Gimeno et al. (2007) reported a case of an allergic reaction to bread that included fava bean (*Vicia faba*) flour. A 25-year old woman presented with retrosternal and pharyngeal oppression and itching in the tongue and pharynx 5 minutes after eating the suspected bread. The subject had been allergic to legumes from the age of 8. A prick-by-prick test with the bread and a broad bean extract were positive. Additional immunoglobulin E (IgE)-related clinical testing supported the existence of allergens from broad beans in the bread that caused a type-I reaction in a legume-allergic patient. No other cases of allergy to fava beans was found in the publicly available literature.

Kumar et al. (2014) conducted studies to determine whether broad-bean proteins have the ability to elicit allergic responses due to the presence of clinically relevant allergenic proteins. A simulated gastric fluid (SGF) assay and IgE immunoblotting were employed

to identify pepsin-resistant and IgE-binding proteins. The allergenicity of broad beans was assessed in allergic patients, BALB/c mice, splenocytes, and RBL-2H3 cells. Eight broad-bean proteins of approximate molecular weight 70, 60, 48, 32, 23, 19, 15, and 10 kilodaltons that remained undigested in SGF, showed IgE-binding capacity. Of 127 allergic patients studied, broad-bean allergy was evident in 16 (12%). Mice sensitized with broad bean showed increased levels of histamine, total and specific IgE, and severe signs of systemic anaphylaxis compared with controls. Enhanced levels of histamine, prostaglandin D2, cysteinyl leukotriene, and beta-hexosaminidase release were observed in the primed RBL-2H3 cells following broad-bean exposure. The authors concluded that broad-bean proteins have the ability to elicit allergic responses.

The potential for fava bean proteins to cause an immune response is rare but consistent with similar, known allergies to other legumes. Although fava beans are not listed as one of eight major allergen groups by the FDA under the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)), the fact that the allergenicity of fava bean protein has been shown clinically (Mur Gimeno et al., 2007; Kumar et al., 2014) suggests that labeling of the presence of fava bean protein is warranted and recommended. More importantly, given the incidence in of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. Therefore, it is proposed that the ingredient labeling for the fava bean protein product clearly state that it contains "fava bean protein" and individuals who wish to avoid fava bean or fava bean protein consumption for any reason would be able to identify the presence of a fava bean–derived ingredient.

Other Protein-Related Safety Concerns

Protein Intake and Toxicity

IOM recommends that adults not consume more than twice the 0.8 g/kg protein RDA per day. However, physically active persons on normal diets easily exceed this level, and individuals involved in bodybuilding ingest much higher levels of protein (WHO, 2002). WHO (2002) recommends body-weight-based protein consumption rates for both genders. For example, the safe protein consumption level for a 40-kg adult is 33 g/day, and that for an 80-kg adult is 66 g/day.

Kidney Function

Dietary protein is known to alter renal function. Increased protein intakes lead to increased excretion of urea and creatinine, due to increased renal blood flow causing a higher glomerular filtration rate. Excess protein intake has been found to advance chronic kidney disease due to increased glomerular pressure and hyperfiltration (Martin et al., 2005; WHO, 2002). Martin et al. (2005) state that, although protein restriction may be indicated in treatment of existing kidney disease, the existing evidence does not indicate an adverse effect of high protein consumption on renal function in healthy individuals who for centuries have consumed high-protein Western diets. In addition, several studies indicate that hyperfiltration, the reported mechanism for kidney effects, is a normal adaptive response to increased demands for renal clearance due to higher nitrogen load.

Martin et al. (2005) defined a "high protein diet" as a daily intake of ≥1.5 g/kg-day, nearly double the current recommended intake level set by IOM. In persons with decreased renal function, a high-protein diet that leads to a renal solute load in excess of the kidneys' excretory functions can contribute to progressive kidney failure (Martin et al., 2005). The intake of the fava bean protein ingredient will result in daily intake levels below those associated with effects on renal function. As to the issue of kidney stone formation, it is recommended that those who are at risk should consume the recommended safe level of protein (0.83 g/kg-day), preferably from vegetable sources, but not high levels (>1.4 g/kg/day) (WHO, 2002).

Calcium Balance/Bone Health

Excess protein intake can adversely affect the body's calcium balance and calcium concentration in bone. Persons consuming high-protein diets are known to excrete increased amounts of urinary calcium, and doubling protein intake can increase urinary calcium excretion by 50%. WHO (2002) states that the existing scientific evidence indicates that dietary protein, when consumed as part of a well-balanced diet, is likely beneficial for bone, potentially even at dietary levels exceeding the recommended consumption rates.

Summary

The proposed uses and consumption of fava bean protein in the foods specified in Part 3 do not raise concerns regarding the noted protein safety-related outcomes.

Basis for the GRAS Determination

Introduction

The regulatory framework for determining whether a substance can be considered GRAS in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act") is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety

through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of the fava bean protein ingredient in human food that is the subject of this GRAS determination is GRAS based on scientific procedures. All data relied upon in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the Federal Food, Drug, and Cosmetic Act (FFDCA).

Safety Determination

The subject of this GRAS determination is the use of fava bean protein as an alternative source of dietary protein for addition to processed foods. Humans have consumed fava beans and other legumes, as well as proteins from these sources, for centuries, along with proteins from many food sources such as meats, dairy, fruits, vegetables, nuts, and seeds. Other natural, plant-based sources of protein concentrates that have been safely consumed for years include soy, canola, potato, wheat, whey, and mung bean.

IOM (2005) recommends that adults consume a minimum of 0.8 g protein/kg and has set a range for acceptable protein intake of 10%–35% of daily calories. In the US, the recommended daily allowance of protein is 56 g/day and 46 g/day for adult men and women (>19 years of age), respectively. FDA (2015) has established a protein daily reference value (DRV) of 50 g/day for adults and children four years of age or older. However, it should be noted that the RDA does not represent an upper limit of consumption.

As described in numerous GRAS Notifications, including GRN No. 386 for canola protein isolate and hydrolyzed canola protein isolate, the typical uses of protein for enrichment of foods include bakery products, snack foods, ready-to-drink beverages, soups and nutritional beverages, high-protein drinks and milkshakes, powdered nutritional/protein beverages, nutrition bars, vegetarian food products, meat analogues, processed meat products, dairy and imitation dairy products, and meal replacements/nutrition bars.

TFULL's proposed fava bean protein is intended only to be an alternative source of protein for current uses of protein in food. Therefore, a similar estimate of intake would be expected if fava bean protein was the only source of protein used in processed foods. As was concluded in numerous other GRAS notifications for plant-based dietary proteins for which FDA issued "no objection" letters, it is not realistic to expect that the actual consumption of foods containing fava bean protein would result in daily protein consumption greater than the existing DRV or RDA for protein. It is reasonable to expect that most of the population's daily intake of protein will remain in the form of unprocessed foods, including meat, poultry, fish, and legumes (FDA, 2010, 2011).

Fava beans are a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health. The protein content is about twice that of cereals and several times that of root tubers. Fava bean seeds are a rich source of proteins, carbohydrates, fiber, vitamins, and minerals (Aune et al., 2011). However, the biological value of untreated fava beans is negatively affected by the presence of anti-nutritional factors such as the favism-inducing factors (the aglycones: divicine and isouramil, from vicine and convicine), and the ingestion of untreated fava beans is associated with precipitation of the hemolytic disease favism in certain glucose-6-phosphate dehydrogenase-deficient humans.

Therefore, removal of these anti-nutrients is the primary safety concern and is required for the effective utilization of fava bean components, including protein concentrates and isolates, in human nutrition. Anti-nutrition factors are now removed routinely during commercial processing of fava beans (Jamalian, 1999). Some of the anti-nutrition factors are readily destroyed by heat processing (boiling, cooking, autoclaving, or extrusion cooking) during the production of fava bean flour, or can be eliminated by pretreatment of the beans, such as dehulling or soaking (Revilla 2015). An example of such treatments of the fava bean, wherein the vicine content was reduced by 94%–100% and the convicine content was reduced by 100%, was described by Jamalian (1999). Later, Vioque et al. (2012) reported a reduction of vicine and convicine in fava protein "by more than 99%" by employing an extraction of the defatted seed flour, followed by precipitation at an isoelectric pH. The removal of vicine and convicine ensures that the fava-causing aglycones compounds, divicine and isouramil, cannot be synthesized. Soaking, acid hydrolysis, and sterilization are part of the manufacturing process of T-FULL's fava protein and results in vicine and convicine being significantly reduced; an inactivation of β-glucosidase, which is necessary for conversion of vicine and convicine to the aglycones; and the elimination of the favism-causing compounds divicine and isouramil.

In the European, Middle Eastern, and Chinese areas where individuals prone to favism live, and where fava beans, and especially the immature pods, are consumed, it is estimated that there is an intake of 2000 mg of vicine and 1000 mg of convicine in one meal (WHO, 1992). Numerous cases of favism related to consumption of raw, unprocessed fava beans by G6PD-deficient individuals have been reported (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017).

The total amount of convicine and vicine in a regular fava bean variety is 6–14 mg/g, an amount that is orders of magnitude greater than that found in the proposed fava bean protein ingredient. Arese et al. (2007) stated that the levels of vicine and convicine in FEVITA (a low vicine/convicine protein ingredient) were 10 – 20x lower than traditional cultivars (i.e., 6-14 mg/g). A 10-fold reduction results in 0.6-1.4 mg/g and 0.3-0.7 mg/g for a 20-fold reduction. This is comparable to the levels shown in Table 8 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot No. [redacted]). In the Arese et al. (2009) study, seven hemizygous males and females with G6PD deficiency used the FEVITA fava bean, which is low in vicine and convicine due to reduced beta-glucosidase in the FEVITA cultivar which prevents conversion of vicine

and convicine to divicine and isouramil. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the vicine and convicine level of 0.348 mg/g in the proposed ingredient, times 60 grams of protein/day (replacement of RDA protein intake), results in an intake of approximately 25 mg vicine and convicine in the diet, an amount that is 8 to18 % of the level employed in the Arese et al. (2009) study in which no hemolytic effects were reported.

The potential for fava bean proteins to cause an immune response is rare but consistent with similar, known allergies to other legumes. Although fava beans are not listed as one of eight major allergen groups by the FDA under the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)), the fact that the allergenicity of fava bean protein has been shown clinically (Mur Gimeno et al., 2007; Kumar et al., 2014) suggests that labeling of the presence of fava bean protein is warranted and recommended. More importantly, given the incidence in of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. Therefore, it is proposed that the ingredient labeling for the fava bean protein product clearly state that it contains "fava bean protein" and individuals who wish to avoid fava bean or fava bean protein consumption for any reason would be able to identify the presence of a fava bean–derived ingredient.

General Recognition of the Safety of Fava Bean Protein Ingredient

The intended use of the fava bean protein ingredient in human food has been determined to be safe through scientific procedures set forth in 21 CFR§170.3(b), thus satisfying the so-called "technical" element of the GRAS determination, based on the following:

- Fava bean protein is manufactured from commercially available fava beans, following current cGMP for food (21 CFR § Part 110). The raw materials and processing aids used in the manufacturing process are food grade and/or approved for use in food. The fava bean protein product has been characterized appropriately, contains a minimum of 90% protein, and meets appropriate food-grade specifications.

- Fava beans have been consumed as food (and the protein contained therein) for centuries, along with many other food sources of protein (e.g., meats, dairy, fruits, vegetables, nuts).

- The biological value of untreated fava beans is negatively affected by the presence of anti-nutritional factors such as the favism-inducing factors (aglycones: divicine and isouramil produced from metabolism of vicine and convicine). The ingestion of untreated fava beans is associated with precipitation of the hemolytic disease favism in certain glucose-6-phosphate dehydrogenase-deficient humans.

- Removal of these anti-nutrients is the primary safety concern and is required for the effective utilization of fava bean components, including protein concentrates

and isolates, in human nutrition. Anti-nutrition factors are now removed routinely during commercial processing of fava beans via boiling, cooking, autoclaving, or extrusion cooking during the production of fava bean flour, or by pretreatments of the beans, such as dehulling or soaking. Such treatments reduce the vicine and convicine content of fava beans as is the case in the proposed fava bean protein ingredient.

- The proposed uses of the fava bean protein ingredient will provide an alternative to other dietary sources of protein and will not increase the overall consumption of protein but will simply provide another source of well-characterized protein for use in food products.

- Fava beans are a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health. The protein content is about twice that of cereals and several times that of root tubers.

- FDA has reviewed extensive published information and data on many protein products as part of GRAS notifications for animal and plant-based protein isolates and concentrates and subsequently issued "no objection letters." Examples include GRN No. 26 (isolated wheat protein); GRN No. 37 (whey protein isolate and dairy product solids); GRN No. 168 (poultry protein); GRN No. 182 (hydrolyzed wheat gluten isolate; pea protein isolate); GRN No. 313 (beef protein); GRN No. 314 (pork protein); GRN 386 (canola protein isolate and hydrolyzed canola protein isolate); GRN No. 447 (potato protein isolates); GRN No. 575 (oat protein); and GRNs No. 58, 608, and 788 (pea protein).

- Given the long history of global human consumption of fava beans as food (and the protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by their consumption and general lack of toxicity. As would be expected for a food that has been consumed by humans for centuries, fava beans and fava bean proteins have not been subjected to traditional toxicology studies. However, the available summarized preclinical and clinical study data support its safe use as proposed.

- Concerns related to the allergenicity of fava bean protein as well as the effect of fava beans consumption by G6PD deficient individuals can be addressed through appropriate labeling of food products as containing fava bean protein and individuals who wish to avoid fava bean protein consumption would be able to identify the presence of a fava bean–derived ingredient.

- The body of publicly available scientific literature on the consumption and safety of fava beans and fava bean protein is sufficient to support the safety and GRAS status of the proposed fava bean protein ingredient.

Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status of the fava bean protein ingredient that is the subject of this self-determination has been made through the deliberations of a GRAS Panel of qualified experts convened by TFULL and composed of Michael Carakostas, DVM, Ph.D., Paul Damian, Ph.D., M.P.H., DABT, ERT, and Carol A. Knight, Ph.D . These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to food. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that the fava bean protein ingredient, produced in a manner consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concluded that use of the fava bean protein ingredient in human food is GRAS based on scientific procedures, and that other experts qualified to assess the safety of food and food ingredients for human consumption would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit 1 to this document.

It is also TFULL's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. TFULL has concluded that the fava bean protein ingredient is GRAS under the intended conditions of use on the basis of scientific procedures; and therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

TFULL is not aware of any information that would be inconsistent with a finding that the use of the fava bean protein ingredient in food for human consumption, meeting appropriate specifications, and used according to GMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

§ 170.250 Part 7, Supporting Data and Information

References

Alkhaaldi A, Al Tenejii M, Faisal Khanani M, Baroudi M, Jamil A, Trad O, Al Reyami L, Ahmed Awan N. 2014. Two cases of glucose-6-phosphate dehydrogenase deficiency presenting as symptomatic methemoglobinemia with persistently low saturations after fava bean ingestion in United Arab Emirates. Applied Clinical research, Clinical Trials, and Regulatory Affairs 1(3):176-179.

Arese P, Gerard D, lessire M, Marget P. 2007. Low vicine-convicine and zero tannin FEVITA faba beans. Grain Legumes 48:16-17.

Arese P, Schwarzer E, Gallo V, Simula L, Duc G. 2009. Risk assessment for subjects with G6PD-deficiency of new faba bean cultivars with low content of vicine and convicine (FEVITA beans). Report: PRO0818.
http://proof.saskpulse.com/files/general/PRO0818.pdf

Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, Norat T. 2011. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ 343:1-20.

Cardador-Martinez A, Maya-Ocana K, Ortiz-Moreno A, Herrera-Cabrera BE, Davila-Ortiz G Muzquiz M, Martin-Pedrosa M, Burbano C, Cuadrado C, Jimenez-Martinez C. 2012. Effect of roasting and boiling on the content of vicine, convicine and L-3,4-dihydroxyphenylalanine in *Vicia faba* L. Journal of Food Quality 35:419-428.

Crepon K, Marget P, Peyronnet C, Carrou'ee B, Arese P, Duc G. 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Res 115(3):329–339.

Eghbal F, Fakaoorziba MR, Eghbal MH, Latifnia S. 2012. Survey on causes of hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient pediatric patients.Pak J Med Sci 28(4):666-669.

Food and Drug Administration (US FDA). 1999. GRN No. 26. GRAS Assessment of isolated wheat protein.

Food and Drug Administration (US FDA). 2000. GRN No. 37. GRAS Notification for whey protein isolate and dairy product solids.

Food and Drug Administration (US FDA). 2004. GRN No. 134. GRAS Notification for soy protein hydrolysate with enzyme-modified lecithin.

Food and Drug Administration (US FDA). 2005. GRN 182. GRAS Assessment of plant proteins in winemaking (hydrolyzed wheat gluten isolate; pea protein isolate).

Food and Drug Administration (US FDA). 2010. GRN No. 327. GRAS Notification for cruciferin-rich canola/rapeseed and napin-rich canola/rapeseed protein isolate.

Food and Drug Administration (US FDA). 2011. GRN No. 386. GRAS Assessment of canola protein isolate and hydrolyzed canola protein isolate.

Food and Drug Administration (US FDA). 2013. GRN 447. GRAS Assessment of potato protein isolates.

Food and Drug Administration (US FDA). 2015a GRN 575. GRAS Assessment of oat protein.

Food and Drug Administration (US FDA). 2015b. GRN 581. GRAS Assessment of pea protein.

Food and Drug Administration (US FDA). 2016a. GRN 608. GRAS Assessment of pea protein concentrate.

Food and Drug Administration (US FDA). 2016b. GRN 609. GRAS Assessment of rice protein

Food and Drug Administration (US FDA). 2016c. GRN 633. GRAS Assessment of whey protein

Food and Drug Administration (US FDA). 2017a. GRN 683. GRAS Assessment of canola protein isolate.

Food and Drug Administration (US FDA). 2017b. GRN 684. GRAS Assessment of mung bean protein isolate.

Food and Drug Administration (US FDA). 2018a. GRN 771. GRAS Assessment of hemp seed protein.

Food and Drug Administration (US FDA). 2018b. GRN 788. GRAS Assessment of pea protein concentrate.

Frank JE. 2005. Diagnosis and management of G6PD deficiency. American Family Physician 72(&):1277-1282.

Hussein, MA. 2012. Anti-inflammatory effect of natural heterocycle glucoside vicine obtained from *Vicia faba* L. its aglucone (divicine) their effect on some oxidative stress biomarkers in Albino rats. Free Radicals and Antioxidants 2(2):44-54.

Institute of Medicine (IOM) of the National Academies. 2005. Dietary, Functional and Total Fiber. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids Washington DC. National Academies Press

Jamalian J. 1999. Removal of favism-inducing factors vicine and convicine and the associated effects on the protein content and digestibility of faba beans (*Vicia faba* L). Journal of the Science of Food and Agriculture. 79(13):1909–1914.

Kavehmansch Z, Arab A, Abolghasemi H, Torabi SM. 2016. Fava bean ingestion: the most important risk factor of hemolysis in G6PD deficiency in Iran. IJBC 8(2):38-42.

Khamassi K, Stoddard FL, O'Sullivan D, Jones H. 2013. A baseline study of vicine-convicine levels in faba bean (Vicia faba L.) germplasm. Plant Genetic Resources 1-8.

Kumar D, Kumar S, Verma AK, Sharma A, Tripathi A, Chaudhari BP, Kant S, Das M, Jain SK, Dwivedi PD. 2014. Hypersensitivity linked to exposure to broad bean protein(s) in allergic patients and BALB/c mice. Nutrition 30(7-8): 903-914.

Luzzatto L, Arese P. 2018. Favism and glucose-6-phosphate dehydrogenase deficiency. N Engl J Med 378(1):60-71.

Macarulla MT, Medina C, De Diego MA, Chávarri M, Zulet MA, Martínez JA, Nöel-Suberville C, Higueret P, Portillo MP. 2001. Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolaemic rats. Br J Nutr. 85(5):607-14.

Martin WF, Armstrong LE, Rodriguez NR. 2005. Dietary protein intake and renal function. Nutr. Metab. (Lond). 2(25). Doi:10.1186/1743-7075-2-25.

Multari S, Stewart D, Russell WR. 2015. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety 14:512-522.

Mur Gimeno P, Brito FF, Iglesias AM, Vega ML, Martinez PB. 2007. Allergic reaction caused by a new hidden food, broad bean flour. Eur J Allergy Clin Immun 62(11):1340-1341.

Product Safety Labs (PSL). 2018. Protein Digestibility Corrected Amino Acid Score (PDCAAS). Study Number 48060. May 4.

Pulkkinen M, Shou X, Lampi A-M, Piironen V. 2016. Determination and stability of divicine and isouramil produced by enzymatic hydrolysis of vicine and convicine of faba bean. Food Chemistry 212:10-19.

Revilla I. 2015. Processing and Impact on Active Components in Food. Chapter 40 – Impact of Thermal Processing on Faba Bean (*Vicia faba*) Composition. pp 337–343.

Schmandke H, Plass R, Lewerenz H-J, Bleyl DWR. 2000. Four week feeding studies with differently processes dehulled faba bean (*Vicia faba* L. minor) products in rats. Molecular Nutrition and Food Research 44(2):133-135.

Skold MB, Svendsen RP, Pedersen EB. 2017. Favism after ingestion of fava beans in a three-year old child with glucose-6-phosphate dehydrogenase deficiency. Ugeskr Laeger 15:179.

Vioque J, Alaiz M, Girón-Calle J. 2012. Nutritional and functional properties of *Vicia faba* protein isolates and related fractions. Food Chem 132(1):67-72.

Willcox G, Tanno K-I. 2006. How fast was wild wheat domesticated? Science 313:296-297.

World Health Organization.1992. Final report of the steering group on naturally occurring toxins of plant origin, Carshalton, UK, 20-22 May. http://apps.who.int/iris/bitstream/10665/67268/1/WHO_PCS_92.69.pdf

World Health Organization (WHO). 2002. Technical Report Series 935: Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation.

Wu Leung W, Busson F, Jardin C. 1968. Food composition table for use in Africa. FAO-Nutrition Information Documents Series (3).

APPENDIX A

COAs and Other Analytical Data

Advanta™ Resolve 90
TECHNICAL SPECIFICATION

Document number: TS-042
Effective date: 28 Feb 2019
Version number: 1.1

Product Name: *AdvantaFava™* Resolve 90
Description: 90% Fava Bean Protein Powder

Items Analyzed	Specification	Test Method
Chemical Composition		
Protein Content	≥ 90% (db)	Kjeldahl, AOAC 981.10 or equivalent, Conversion factor 6.25
Loss on Drying	≤ 10%	Oven Drying at 105ºC or equivalent
Sulphated Ash	≤ 5%	AOAC 923.03, or equivalent
Physical Properties:		
Appearance	Fine powder	Visual
Color	Light yellow	Visual
pH	6-7	USP or equivalent
Heavy Metal Analysis:		
Lead (Pb)	≤ 0.4 ppm	ICP-MS or equivalent
Arsenic (As)	≤ 0.2 ppm	ICP-MS or equivalent
Cadmium (Cd)	≤ 0.4 ppm	ICP-MS or equivalent
Mercury (Hg)	≤ 0.2 ppm	ICP-MS or equivalent
Microbiological Analysis:		
Total plate count	≤ 10,000 CFU/25g	USP <2021/2022> or equivalent
Yeast and Mold	≤ 100 CFU/25g	USP <2021/2022> or equivalent
Escherichia coli	Negative	USP <2021/2022> or equivalent
Salmonella	Negative	USP <2021/2022> or equivalent
S. aureus	Negative	USP <2021/2022> or equivalent

Shelf-Life: 24 months from the date of manufacturing when properly stored in a closed container in a cool (below 100°F) and dry place away from strong light.

Packaging: 20 Kg poly-lined kraft bags

Safety: Safe, non-toxic

Label Declaration: Fava bean protein isolate

Certifications: Kosher and Halal certified

Source: Non-GMO, Canadian fava beans, further processed in US and China

HEALTH INGREDIENTS

10045 81 Ave., Edmonton, AB T6E 1W7 Ph: 780-439-1424, www.tophealthingredients.com

AdvantaFava™ Resolve 90
TECHNICAL SPECIFICATION

Document number: TS-042
Effective date: 28 Feb 2019
Version number: 1.1

Allergen Statement: ***AdvantaFava™*** does not contain eggs, milk, mustard, peanuts, seafood (fish, crustaceans, shellfish), sesame, soy, sulphites, tree nuts and wheat.

Typical Nutritional Data per 100 g

Proteins	86 g
Moisture	5 g
Carbohydrates	3 g
Dietary Fiber	1 g
Total Sugars	0 g
Ash	3 g
Total Fat	3 g
Cholesterol	0 g
Calcium	33 mg
Iron	33 mg
Sodium	604 mg
Potassium	46 mg
Vitamin A	< 50 IU or < 15 µg
Vitamin C	< 0.5 mg
Calorie Value	370 Kcal

Typical Amino Acid Profile (g, db per 100 g product):

Alanine	4.06
Arginine	8.58
Aspartic Acid	9.90
Cysteine	0.87
Glutamic Acid	16.8
Glycine	3.67
Histidine	2.37
Isoleucine	4.40
Leucine	8.05
Lysine	6.17
Methionine	0.79
Phenylalanine	4.51
Proline	4.27
Serine	4.81
Threonine	3.33
Tryptophan	1.03
Tyrosine	3.39
Valine	4.85

Other Properties:

Particle size	98% through 100 mesh
Taste	Bland

Applications:

- Baked Goods
- Breakfast Cereals
- Beverages and Juices
- Confectionary
- Meat Products
- Dairy Products
- Jams and Jellies
- Meal Replacements
- Nutritional bars
- Sauces, Puddings & Dressings
- Snack Foods
- Powdered Shakes

Disclaimer: *Ingredient specifications are based on anticipated composition, yield and performance. Prospective purchasers should conduct their own tests, studies and regulatory review to determine the product's fitness for their particular purposes, product claims and specifications.*

TOPHEALTH INGREDIENTS

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HEALTH INGREDIENTS

Top Health Ingredients Inc.

10045-81 Ave.
Edmonton, Alberta CANADA T6E 1W7
Telephone: 780-439-1425

CERTIFICATE OF ANALYSIS

C of A #: [redacted]

Brand Name	***AdvantaFava™ 90B***	Product No.	8710-01
Commodity	Fava Bean Isolate	Batch Number	[redacted]
Source	Canadian Fava Bean	Quantity	300 Kg
Country of Origin	China	Test Date	6 Apr 2018
Manufacturer	Yantai TFULL	Manufacturing Date	6 Dec 2017
		Expiry Date	5 Dec 2019

Item	Standard	Result
Protein Content, Kjeldahl, AOAC 981.10, %	≥ 90 (db)	98.4
Loss on Drying, %	≤ 10	8.8
Sulphated Ash, AOAC 923.03, %	≤ 5	4.5
Appearance, visual	Fine powder	Complies
Color, visual	Light Yellow	Complies
pH, USP	7 - 8	7.1
Lead (Pb); ICP-MS, mg/kg (ppm)	≤ 0.4	< 0.02
Arsenic (As); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.03
Cadmium (Cd); ICP-MS, mg/kg (ppm)	≤ 0.4	< 0.02
Mercury (Hg); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.02
Total Plate Count, USP <2021/2022 or equivalent>, CFU/25g	≤ 10,000	160
Yeast and mold, USP<2021/2022>, CFU/25g	≤ 100	80 yeast, < 10 mold
Escherichia coli, USP<2021/2022>	Negative	Negative
Salmonella, USP<2021/2022>	Negative	Negative
Staphylococcus aureus, USP<2021/2022>	Negative	Negative
Approved	[redacted]	Date: 6 Apr 2018

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CERTIFICATE OF ANALYSIS

C of A #: [redacted]

Brand Name	***AdvantaFava™ 90B***	Product No.	8710-01
Commodity	Fava Bean Isolate	Batch Number	[redacted]
Source	Canadian Fava Bean	Quantity	2000 Kg
Country of Origin	China	Test Date	17 Aug 2018
Manufacturer	Yantai TFULL	Manufacturing Date	7 Jul 2018
		Expiry Date	6 Jul 2020

Item	Standard	Result
Protein Content, Kjeldahl, AOAC 981.10, %	≥ 90 (db)	91.3
Loss on Drying, %	≤ 10	4.3
Sulphated Ash, AOAC 923.03, %	≤ 5	4.2
Appearance, visual	Fine powder	Complies
Color, visual	Light Yellow	Complies
pH, USP	7 - 8	7.5
Lead (Pb); ICP-MS, mg/kg (ppm)	≤ 0.4	< 0.02
Arsenic (As); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.03
Cadmium (Cd); ICP-MS, mg/kg (ppm)	≤ 0.4	< 0.02
Mercury (Hg); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.02
Total Plate Count, USP <2021/2022 or equivalent>, CFU/25g	≤ 10,000	80
Yeast and mold, USP<2021/2022>, CFU/25g	≤ 100	< 10
Escherichia coli, USP<2021/2022>	Negative	< 10
Salmonella, USP<2021/2022>	Negative	Negative
Staphylococcus aureus, USP<2021/2022>	Negative	Negative
Approved	[redacted]	Date: 17 Aug 2018

HEALTH INGREDIENTS

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CERTIFICATE OF ANALYSIS

C of A #: [redacted]

Brand Name	***AdvantaFava™ 90***	Product No.	8720-01
Commodity	Fava Bean Isolate	Batch Number	[redacted]
Source	Canadian Fava Bean	Quantity	8000 kg
Country of Origin	China	Test Date	9 Jan 2019
Manufacturer	Yantai TFULL	Manufacturing Date	6 Jul 2018
		Expiry Date	5 Jul 2020

Item	Standard	Result
Protein Content, Kjeldahl, AOAC 981.10, %	≥ 90 (db)	92.7
Loss on Drying, %	≤ 10	7.7
Sulphated Ash, AOAC 923.03, %	≤ 5	1.2
Appearance, visual	Fine powder	Complies
Color, visual	Light Yellow	Complies
pH, USP	6 - 7	6.4
Lead (Pb); ICP-MS, mg/kg (ppm)	≤ 0.4	<0.02
Arsenic (As); ICP-MS, mg/kg (ppm)	≤ 0.2	<0.03
Cadmium (Cd); ICP-MS, mg/kg (ppm)	≤ 0.4	<0.02
Mercury (Hg); ICP-MS, mg/kg (ppm)	≤ 0.2	<0.02
Total Plate Count, USP <2021/2022 or equivalent>, CFU/25g	≤ 10,000	<10
Yeast and mold, USP<2021/2022>, CFU/25g	≤ 100	<10
Escherichia coli, USP<2021/2022>	Negative	Negative
Salmonella, USP<2021/2022>	Negative	Negative
Staphylococcus aureus, USP<2021/2022>	Negative	Negative
Approved	[redacted]	Date: 17 Jan 2019

Top Health Ingredients Inc.

10045-81 Ave.
Edmonton, Alberta CANADA T6E 1W7
Telephone: 780-439-1425

CERTIFICATE OF ANALYSIS

C of A #: [redacted]

Brand Name	***AdvantaFava™ 90***	Product No.	8720-01
Commodity	Fava Bean Isolate	Batch Number	[redacted]
Source	Canadian Fava Bean	Quantity	5000 kg
Country of Origin	China	Test Date	7 Nov 2018
Manufacturer	Yantai TFULL	Manufacturing Date	30 Jul 2018
		Expiry Date	29 Jul 2020

Item	Standard	Result
Protein Content, Kjeldahl, AOAC 981.10, %	≥ 90 (db)	95.7
Loss on Drying, %	≤ 10	5.9
Sulphated Ash, AOAC 923.03, %	≤ 5	4.1
Appearance, visual	Fine powder	Complies
Color, visual	Light Yellow	Complies
pH, USP	6 - 7	6.4
Lead (Pb); ICP-MS, mg/kg (ppm)	≤ 0.4	0.0
Arsenic (As); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.03
Cadmium (Cd); ICP-MS, mg/kg (ppm)	≤ 0.4	< 0.02
Mercury (Hg); ICP-MS, mg/kg (ppm)	≤ 0.2	< 0.02
Total Plate Count, USP <2021/2022 or equivalent>, CFU/25g	≤ 10,000	< 10
Yeast and mold, USP<2021/2022>, CFU/25g	≤ 100	< 10
Escherichia coli, USP<2021/2022>	Negative	Negative
Salmonella, USP<2021/2022>	Negative	Negative
Staphylococcus aureus, USP<2021/2022>	Negative	Negative
Approved	[redacted]	Date: 7 Nov 2018

SGS

Test Report **QDF18-026082-12** **Date: Sep 05 2018**

Client name:	YanTai T.Full Biotech CO.,LTD
Client address:	SHIDUITOU,ZHANGXING TOWN,ZHAOYUAN, SHANDONG,CHINA
Sample name:	FAVA BEAN PROTEIN 90C
Sample Batch No.:	/
Production Date:	/
Manufacturer:	/

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS reference No.:	TAOFD1803971101
Date of sample received:	Aug 23 2018
Test Period:	Aug 23 2018 ~ Sep 04 2018
Test Requested:	Selected test(s) as requested by client.
Test Method:	Please refer to next page(s).
Test Result(s):	Please refer to next page(s).

Chinese shall prevail in this report
Unless otherwise stated the results shown in this test report refer only to the sample(s) tested, and for clients internal use only, not to the society has the proof function. This document cannot be used for publicity, without prior approval of the SGS.

.....
SGS Authorized Signature

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SGS

Test Report **QDF18-026082-12** **Date: Sep 05 2018**

Sample Description:

Specimen No.	SGS Sample ID	Description
1	QDF18-026082.001	Sample in bag

Chemical test

Test Result(s):

Test item(s)	Unit(s)	Test method(s)	Test result(s)	LOQ(s)
Aflatoxin G_2	μg/kg	GB 5009.22-2016 III	Not detected	0.2
Aflatoxin G_1	μg/kg	GB 5009.22-2016 III	Not detected	0.3
Aflatoxin B_2	μg/kg	GB 5009.22-2016 III	Not detected	0.2
Aflatoxin B_1	μg/kg	GB 5009.22-2016 III	Not detected	0.3
Aflatoxin (B_1 +B_2 +G_1, G_2)	μg/kg	GB 5009.22-2016 III	Not detected	1

Remark:LOQ= Limit of Quantitation

*** End of Report***

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SGS

Test Report

QDF19-008084-02 Date: 29 Mar 2019

Client Name: YanTai T.Full Biotech CO.,LTD
Client Address: SHIDUITOU, ZHANGXING TOWN, ZHAOYUAN CITY, SHANDONG PROVINCE, CHINA

Sample Name:	FAVA BEAN PROTEIN
Manufacturer:	/
Sample Batch No.:	/
Production Date:	/

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

Date of Sample Received :	25 Mar 2019
Testing Period :	25 Mar 2019 - 29 Mar 2019
Test Requested :	Selected test(s) as requested by client.
Test Method :	Please refer to next page(s).
Test Result(s) :	Please refer to next page(s).

Chinese shall prevail in this report.

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SGS Authorized signature

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Test Report

QDF19-008084-02

Date: 29 Mar 2019

Sample Description :

Specimen No.	SGS Sample ID	Description
1	QDF19-008084.001	sample in bag

Pesticide residues

Test Method(s) : BS EN 15662:2018

Test Result(s) :

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
1	2-Phenylphenol 邻苯基苯酚	mg/kg	90-43-7	ND	0.01
2	Acephate 乙酰甲胺磷	mg/kg	30560-19-1	ND	0.02
3	Acetamiprid 啶虫脒	mg/kg	135410-20-7	ND	0.01
4	Acetochlor 乙草胺	mg/kg	34256-82-1	ND	0.01
5	Acrinathrin 氟丙菊酯	mg/kg	101007-06-1	ND	0.05
6	Aldicarb (sum of Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide) 涕灭威(涕灭威、涕灭威砜和涕灭威亚砜之和)	mg/kg	-	ND	-
7	Aldicarb sulfone 涕灭威砜	mg/kg	1646-88-4	ND	0.1
8	Aldicarb 涕灭威	mg/kg	116-06-3	ND	0.01
9	Aldicarb sulfoxide 涕灭威亚砜	mg/kg	1646-87-3	ND	0.02
10	Aldrin 艾氏剂	mg/kg	309-00-2	ND	0.01
11	Amidosulfuron 酰嘧磺隆	mg/kg	120923-37-7	ND	0.05
12	Amisulbrom 唑菌磺胺	mg/kg	348635-87-0	ND	0.05
13	Amitraz 双甲脒	mg/kg	33089-61-1	ND	0.05
14	Anthraquinone 蒽醌	mg/kg	84-65-1	ND	0.01
15	Aramite 杀螨特	mg/kg	140-57-8	ND	0.05
16	Atrazine 莠去津	mg/kg	1912-24-9	ND	0.02
17	Azinphos-ethyl 益棉磷	mg/kg	2642-71-9	ND	0.01
18	Azinphos-methyl 保棉磷	mg/kg	86-50-0	ND	0.01
19	Azoxystrobin 嘧菌酯	mg/kg	131860-33-8	ND	0.01
20	Benalaxyl 苯霜灵	mg/kg	71626-11-4	ND	0.05
21	Bendiocarb 恶虫威	mg/kg	22781-23-3	ND	0.01
22	Benfluralin 乙丁氟灵	mg/kg	1861-40-1	ND	0.01
23	Benoxacor 解草噁	mg/kg	98730-04-2	ND	0.05
24	Bensulfuron-methyl 苄嘧磺隆	mg/kg	83055-99-6	ND	0.02

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Test Report QDF19-008084-02 Date: 29 Mar 2019

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
25	BHC (Sum of alpha-BHC, beta-BHC, gamma -BHC, delta-BHC and epsilon-BHC)六六六(α-六六六、β-六六六、γ-六六六和δ-六六六之和ε-六六六)	mg/kg	-	ND	-
26	BHC delta isomer δ-六六六	mg/kg	319-86-8	ND	0.05
27	Bifenthrin 联苯菊酯	mg/kg	82657-04-3	ND	0.01
28	Boscalid 啶酰菌胺	mg/kg	188425-85-6	ND	0.1
29	Bromophos 溴硫磷	mg/kg	2104-96-3	ND	0.01
30	Bromophos-ethyl 乙基溴硫磷	mg/kg	4824-78-6	ND	0.01
31	Bromopropylate 溴螨酯	mg/kg	18181-80-1	ND	0.01
32	Bupirimate 乙嘧酚磺酸酯	mg/kg	41483-43-6	ND	0.05
33	Buprofezin 噻嗪酮	mg/kg	69327-76-0	ND	0.01
34	Butachlor 丁草胺	mg/kg	23184-66-9	ND	0.01
35	Butocarboxim 丁酮威	mg/kg	34681-10-2	ND	0.02
36	Cadusafos 硫线磷	mg/kg	95465-99-9	ND	0.01
37	Carbaryl 甲萘威	mg/kg	63-25-2	ND	0.01
38	Carbendazim and Benomyl 多菌灵&苯菌灵	mg/kg	10605-21-7&17804-35-2	ND	0.02
39	Carbofuran(sum of Carbofuran,Carbofuran-3-Hydroxy) 克百威(克百威和3-羟基克百威之和)	mg/kg	-	ND	-
40	Carbofuran 克百威	mg/kg	1563-66-2	ND	0.01
41	Carbofuran-3-hydroxy 3-羟基克百威	mg/kg	16655-82-6	ND	0.05
42	Carfentrazone-ethyl 氯酮唑草	mg/kg	128639-02-1	ND	0.01
43	Chlorantraniliprole 氯虫苯甲酰胺	mg/kg	500008-45-7	ND	0.05
44	Chlorbenzuron 灭幼脲	mg/kg	57160-47-1	ND	0.05
45	trans-Chlordane 反式氯丹	mg/kg	5103-74-2	ND	0.01
46	Chlorfenapyr 虫螨腈	mg/kg	122453-73-0	ND	0.01
47	Chlorfenvinphos 毒虫畏	mg/kg	470-90-6	ND	0.01
48	Chlorfluazuron 氟啶脲	mg/kg	71422-67-8	ND	0.02
49	Chlorpropham 氯苯胺灵	mg/kg	101-21-3	ND	0.01
50	Chlorpyrifos Methyl 甲基毒死蜱	mg/kg	5598-13-0	ND	0.01
51	Chlorpyrifos 毒死蜱	mg/kg	2921-88-2	ND	0.01
52	Chlorthal-dimethyl 氯酞酸甲酯	mg/kg	1861-32-1	ND	0.01
53	cis-Chlordane 顺式氯丹	mg/kg	5103-71-9	ND	0.01

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Test Report

QDF19-008084-02 Date: 29 Mar 2019

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
54	Clethodim 烯草酮	mg/kg	99129-21-2	ND	0.1
55	Clofentezine 四螨嗪	mg/kg	74115-24-5	ND	0.05
56	Clothianidin 噻虫胺	mg/kg	210880-92-5	ND	0.02
57	Cyanazine 氰草津	mg/kg	21725-46-2	ND	0.02
58	Cyflufenamid 环氟菌胺	mg/kg	180409-60-3	ND	0.02
59	Cyfluthrin Beta-cyfluthrin 氟氯氰菊酯 高效氟氯氰菊酯	mg/kg	68359-37-5	ND	0.02
60	Cyhalofop-butyl 氰氟草酯	mg/kg	122008-85-9	ND	0.01
61	Cyhalothrin& λ-Cyhalothrin 氯氟氰菊酯&高效氯氟氰菊酯	mg/kg	68085-85-8 & 91465-08-6	ND	0.01
62	Cymoxanil 霜脲氰	mg/kg	57966-95-7	ND	0.02
63	Cypermethrin Zeta-Cypermethrin & Beta-Cypermethrin 氯氰菊酯 氯氰菊酯 (Zeta)& 高效氯氰菊酯	mg/kg	52315-07-8 & 65731-84-2	ND	0.05
64	Cyproconazole 环丙唑醇	mg/kg	94361-06-5	ND	0.01
65	Cyprodinil 嘧菌环胺	mg/kg	121552-61-2	ND	0.02
66	Total DDT (Sum of o,p'-DDT, p,p'-DDD, o,p'-DDD,p,p'-DDE, o,p'-DDE,p,p'-DDT) 滴滴涕 (o,p'-滴滴涕, p,p'-滴滴滴, o,p'-滴滴滴,p,p'-滴滴伊,o,p'-滴滴伊, p,p'-滴滴涕之和)	mg/kg	-	ND	-
67	p,p'-DDT p,p'-滴滴涕	mg/kg	50-29-3	ND	0.05
68	o,p'-DDT o,p'-滴滴涕	mg/kg	789-02-6	ND	0.01
69	p,p'-DDE p,p'-滴滴伊	mg/kg	72-55-9	ND	0.01
70	p,p'-DDD p,p'-滴滴滴	mg/kg	72-54-8	ND	0.01
71	Diafenthiuron 丁醚脲	mg/kg	80060-09-9	ND	0.05
72	Diazinon 二嗪磷	mg/kg	333-41-5	ND	0.01
73	Dichlorvos 敌敌畏	mg/kg	62-73-7	ND	0.05
74	Dicloran 氯硝胺	mg/kg	99-30-9	ND	0.01
75	Dicofol 三氯杀螨醇	mg/kg	115-32-2	ND	0.01
76	Dieldrin 狄氏剂	mg/kg	60-57-1	ND	0.05
77	Diethofencarb 乙霉威	mg/kg	87130-20-9	ND	0.05
78	Difenoconazole 苯醚甲环唑	mg/kg	119446-68-3	ND	0.05
79	Diflufenican 吡氟酰草胺	mg/kg	83164-33-4	ND	0.02
80	Dimethoate 乐果	mg/kg	60-51-5	ND	0.02
81	Dimethomorph 烯酰吗啉	mg/kg	110488-70-5	ND	0.02
82	Diniconazole 烯唑醇	mg/kg	83657-24-3	ND	0.02

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Test Report QDF19-008084-02 Date: 29 Mar 2019

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
83	Dinotefuran 呋虫胺	mg/kg	165252-70-0	ND	0.05
84	Diuron 敌草隆	mg/kg	330-54-1	ND	0.01
85	Edifenphos 敌瘟磷	mg/kg	17109-49-8	ND	0.05
86	Emamectin benzoate 甲氨基阿维菌素苯甲酸盐	mg/kg	155569-91-8	ND	0.01
87	Endosulfan (Sum of α-endosulfan,β-endosulfan,endosulfan sulfate) 硫丹(α-硫丹、β-硫丹、硫丹硫酸酯之和)	mg/kg	-	ND	-
88	Endosulfan alpha α-硫丹	mg/kg	959-98-8	ND	0.01
89	Endosulfan beta β-硫丹	mg/kg	33213-65-9	ND	0.01
90	Endosulfan sulfate 硫丹硫酸酯	mg/kg	1031-07-8	ND	0.05
91	Endrin 异狄氏剂	mg/kg	72-20-8	ND	0.01
92	EPN 苯硫磷	mg/kg	2104-64-5	ND	0.01
93	Epoxiconazole 氟环唑	mg/kg	106325-08-0	ND	0.02
94	Ethalfluralin 乙丁烯氟灵	mg/kg	55283-68-6	ND	0.01
95	Ethiofencarb 乙硫苯威	mg/kg	29973-13-5	ND	0.01
96	Ethion 乙硫磷	mg/kg	563-12-2	ND	0.01
97	Ethoprophos 灭线磷	mg/kg	13194-48-4	ND	0.01
98	Etofenprox 醚菊酯	mg/kg	80844-07-1	ND	0.01
99	Etoxazole 乙螨唑	mg/kg	153233-91-1	ND	0.05
100	Etrimfos 乙嘧硫磷	mg/kg	38260-54-7	ND	0.01
101	Famoxadone 恶唑菌酮	mg/kg	131807-57-3	ND	0.01
102	Fenamiphos 苯线磷	mg/kg	22224-92-6	ND	0.01
103	Fenarimol 氯苯嘧啶醇	mg/kg	60168-88-9	ND	0.01
104	Fenazaquin 喹螨醚	mg/kg	120928-09-8	ND	0.01
105	Fenbuconazole 腈苯唑	mg/kg	114369-43-6	ND	0.02
106	Fenhexamid 环酰菌胺	mg/kg	126833-17-8	ND	0.02
107	Fenitrothion 杀螟硫磷	mg/kg	122-14-5	ND	0.01
108	Fenobucarb 仲丁威	mg/kg	3766-81-2	ND	0.01
109	Fenoxycarb 苯氧威	mg/kg	79127-80-3	ND	0.05
110	Fenpropathrin 甲氰菊酯	mg/kg	64257-84-7	ND	0.01
111	Fenpropimorph 丁苯吗啉	mg/kg	67564-91-4	ND	0.05
112	Fenpyroximate 唑螨酯	mg/kg	111812-58-9	ND	0.02
113	Fenthion 倍硫磷	mg/kg	55-38-9	ND	0.01
114	Fenvalerate & Esfenvalerate 氰戊菊酯& S-氰戊菊酯	mg/kg	51630-58-1& 66230-04-4	ND	0.01

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QDF19-008084-02 Date: 29 Mar 2019

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
115	Fipronil (sum of Fipronil,Fipronil sulfoxide,Fipronil sulfone) 氟虫腈（氟虫腈&氟虫腈亚砜&氟虫腈砜之和）	mg/kg	-	ND	-
116	Fipronil sulfide氟虫腈亚砜	mg/kg	120067-83-6	ND	0.02
117	Fipronil sulfone氟虫腈砜	mg/kg	120068-36-2	ND	0.02
118	Fipronil 氟虫腈	mg/kg	120068-37-3	ND	0.005
119	Fluazifop-Butyl&Fluazifop-P-Butyl 吡氟禾草灵&精吡氟禾草灵	mg/kg	69806-50-4&79241-46-6	ND	0.05
120	Flucythrinate 氟氰戊菊酯	mg/kg	70124-77-5	ND	0.01
121	Flufenoxuron 氟虫脲	mg/kg	101463-69-8	ND	0.05
122	Flusilazole 氟硅唑	mg/kg	85509-19-9	ND	0.01
123	Fosthiazate 噻唑磷	mg/kg	98886-44-3	ND	0.01
124	Haloxyfop 吡氟氯禾灵	mg/kg	69806-34-4	ND	0.01
125	Haloxyfop-methyl 氟吡甲禾灵	mg/kg	69806-40-2	ND	0.05
126	BHC alpha isomer α-六六六	mg/kg	319-84-6	ND	0.01
127	BHC beta isomer β-六六六	mg/kg	319-85-7	ND	0.01
128	BHC epsilon isomer ε-六六六	mg/kg	6108-10-7	ND	0.01
129	Heptenophos 庚烯磷	mg/kg	23560-59-0	ND	0.05
130	Hexachlorobenzene 六氯苯	mg/kg	118-74-1	ND	0.05
131	Hexaconazole 己唑醇	mg/kg	79983-71-4	ND	0.1
132	Hexaflumuron 氟铃脲	mg/kg	86479-06-3	ND	0.05
133	Hexythiazox 噻螨酮	mg/kg	78587-05-0	ND	0.02
134	Imazalil 抑霉唑	mg/kg	35554-44-0	ND	0.01
135	Imidacloprid 吡虫啉	mg/kg	138261-41-3	ND	0.05
136	Indoxacarb 茚虫威	mg/kg	173584-44-6	ND	0.02
137	Iprobenfos 异稻瘟净	mg/kg	26087-47-8	ND	0.01
138	Iprodione 异菌脲	mg/kg	36734-19-7	ND	0.01
139	Iprovalicarb 缬霉威	mg/kg	140923-17-7	ND	0.02
140	Isocarbophos 水胺硫磷	mg/kg	24353-61-5	ND	0.01
141	Isofenphos 异柳磷	mg/kg	25311-71-1	ND	0.01
142	Isofenphos-methyl 甲基异柳磷	mg/kg	99675-03-3	ND	0.01
143	Isoprocarb 异丙威	mg/kg	2631-40-5	ND	0.02
144	Isoprothiolane 稻瘟灵	mg/kg	50512-35-1	ND	0.01
145	Isoproturon 异丙隆	mg/kg	34123-59-6	ND	0.01
146	Kresoxim-methyl 醚菌酯	mg/kg	143390-89-0	ND	0.01
147	Lactofen 乳氟禾草灵	mg/kg	77501-63-4	ND	0.02
148	Lenacil 环草啶	mg/kg	2164-08-1	ND	0.02

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QDF19-008084-02 Date: 29 Mar 2019

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
149	Lindane/BHC gamma isomer γ-六六六/林丹	mg/kg	58-89-9	ND	0.01
150	Linuron 利谷隆	mg/kg	330-55-2	ND	0.02
151	Lufenuron 虱螨脲	mg/kg	103055-07-8	ND	0.05
152	Malathion 马拉硫磷	mg/kg	121-75-5	ND	0.01
153	Metalaxyl&metalaxyl-M 甲霜灵&精甲霜灵	mg/kg	57837-19-1&70630-17-0	ND	0.01
154	Metamitron 苯嗪草酮	mg/kg	41394-05-2	ND	0.1
155	Methacrifos 虫螨畏	mg/kg	62610-77-9	ND	0.01
156	Methamidophos 甲胺磷	mg/kg	10265-92-6	ND	0.01
157	Methidathion 杀扑磷	mg/kg	950-37-8	ND	0.02
158	Methiocarb 甲硫威	mg/kg	2032-65-7	ND	0.01
159	Methomyl 灭多威	mg/kg	16752-77-5	ND	0.02
160	Methoxyfenozide 甲氧虫酰肼	mg/kg	161050-58-4	ND	0.01
161	Metolachlor 异丙甲草胺	mg/kg	51218-45-2	ND	0.05
162	Mevinphos 速灭磷	mg/kg	7786-34-7	ND	0.01
163	Monocrotophos 久效磷	mg/kg	6923-22-4	ND	0.01
164	Myclobutanil 腈菌唑	mg/kg	88671-89-0	ND	0.01
165	Nicosulfuron 烟嘧磺隆	mg/kg	111991-09-4	ND	0.02
166	Nitrothal-isopropyl 酞菌酯	mg/kg	10552-74-6	ND	0.01
167	o,p'-DDD o,p'-滴滴滴	mg/kg	53-19-0	ND	0.01
168	o,p'-DDE o,p'-滴滴伊	mg/kg	3424-82-6	ND	0.01
169	Omethoate 氧化乐果	mg/kg	1113-02-6	ND	0.01
170	Oxadiazon 恶草酮	mg/kg	19666-30-9	ND	0.05
171	Oxadixyl 恶霜灵	mg/kg	77732-09-3	ND	0.01
172	Oxamyl 杀线威	mg/kg	23135-22-0	ND	0.01
173	Oxycarboxin 氧化萎锈灵	mg/kg	5259-88-1	ND	0.01
174	Oxydemeton-methyl 亚砜磷	mg/kg	301-12-2	ND	0.01
175	Oxyfluorfen 乙氧氟草醚	mg/kg	42874-03-3	ND	0.1
176	Paclobutrazol 多效唑	mg/kg	76738-62-0	ND	0.01
177	Parathion 对硫磷	mg/kg	56-38-2	ND	0.01
178	Parathion-Methyl 甲基对硫磷	mg/kg	298-00-0	ND	0.01
179	Penconazole 戊菌唑	mg/kg	66246-88-6	ND	0.01
180	Pendimethalin 二甲戊灵	mg/kg	40487-42-1	ND	0.01
181	Pentachloroaniline 五氯苯胺	mg/kg	527-20-8	ND	0.01
182	Permethrin 氯菊酯	mg/kg	52645-53-1	ND	0.05
183	Phenthoate 稻丰散	mg/kg	2597-03-7	ND	0.01

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
184	Phorate (sum of Phorate,Phorate sulphone&Phorate sulphoxide) 甲拌磷(甲拌磷、甲拌磷砜和甲拌磷亚砜之和)	mg/kg	-	ND	-
185	Phorate sulphone 甲拌磷砜	mg/kg	2588-04-7	ND	0.01
186	Phorate sulphoxide 甲拌磷亚砜	mg/kg	2588-03-6	ND	0.01
187	Phorate 甲拌磷	mg/kg	298-02-2	ND	0.01
188	Phosalone 伏杀硫磷	mg/kg	2310-17-0	ND	0.02
189	Phosmet 亚胺硫磷	mg/kg	732-11-6	ND	0.02
190	Phoxim 辛硫磷	mg/kg	14816-18-3	ND	0.05
191	Picoxystrobin 啶氧菌酯	mg/kg	117428-22-5	ND	0.05
192	Piperonyl butoxide 增效醚	mg/kg	51-03-6	ND	0.01
193	Pirimicarb 抗蚜威	mg/kg	23103-98-2	ND	0.02
194	Pirimiphos-ethyl 嘧啶磷	mg/kg	23505-41-1	ND	0.01
195	Pirimiphos-methyl 甲基嘧啶磷	mg/kg	29232-93-7	ND	0.01
196	Prochloraz 咪鲜胺	mg/kg	67747-09-5	ND	0.05
197	Procymidone 腐霉利	mg/kg	32809-16-8	ND	0.01
198	Profenofos 丙溴磷	mg/kg	41198-08-7	ND	0.02
199	Promecarb 猛杀威	mg/kg	2631-37-0	ND	0.02
200	Propachlor 毒草胺	mg/kg	1918-16-7	ND	0.01
201	Propamocarb 霜霉威	mg/kg	24579-73-5	ND	0.01
202	Propanil 敌稗	mg/kg	709-98-8	ND	0.05
203	Propargite 炔螨特	mg/kg	2312-35-8	ND	0.02
204	Propham 苯胺灵	mg/kg	122-42-9	ND	0.01
205	Propiconazole 丙环唑	mg/kg	60207-90-1	ND	0.01
206	Propoxur 残杀威	mg/kg	114-26-1	ND	0.01
207	Propyzamide 炔苯酰草胺	mg/kg	23950-58-5	ND	0.01
208	Pymetrozine 吡蚜酮	mg/kg	123312-89-0	ND	0.01
209	Pyraclostrobin 百克敏	mg/kg	175013-18-0	ND	0.02
210	Pyraoxystrobin 唑菌酯	mg/kg	862588-11-2	ND	0.05
211	Pyrazophos 吡菌磷	mg/kg	13457-18-6	ND	0.01
212	Pyridaben 哒螨灵	mg/kg	96489-71-3	ND	0.05
213	Pyridaphenthion 哒嗪硫磷	mg/kg	119-12-0	ND	0.01
214	Pyrimethanil 嘧霉胺	mg/kg	53112-28-0	ND	0.02
215	Pyrimidifen 嘧螨醚	mg/kg	105779-78-0	ND	0.05
216	Quinalphos 喹硫磷	mg/kg	13593-03-8	ND	0.01
217	Quinoxyfen 喹氧灵	mg/kg	124495-18-7	ND	0.02

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
218	Quintozene 五氯硝基苯	mg/kg	82-68-8	ND	0.01
219	Quizalofop-ethyl & Quizalofop-P-ethyl 喹禾灵 &精喹禾灵	mg/kg	76578-14-8 & 100646-51-3	ND	0.1
220	Rimsulfuron 砜嘧磺隆	mg/kg	122931-48-0	ND	0.02
221	Rotenone 鱼藤酮	mg/kg	83-79-4	ND	0.02
222	S-421 八氯二丙醚	mg/kg	127-90-2	ND	0.01
223	Simazine 西玛津	mg/kg	122-34-9	ND	0.02
224	Spinosad 多杀霉素	mg/kg	168316-95-8	ND	0.02
225	Spirodiclofen 螺螨酯	mg/kg	148477-71-8	ND	0.05
226	Spiroxamine 螺噁茂胺/螺环菌胺	mg/kg	118134-30-8	ND	0.05
227	Tebuconazole 戊唑醇	mg/kg	107534-96-3	ND	0.02
228	Tebufenozide 虫酰肼	mg/kg	112410-23-8	ND	0.05
229	Tebufenpyrad 吡螨胺	mg/kg	119168-77-3	ND	0.1
230	Tecnazene 四氯硝基苯	mg/kg	117-18-0	ND	0.01
231	Tefluthrin 七氟菊酯	mg/kg	79538-32-2	ND	0.01
232	Terbacil 特草定	mg/kg	5902-51-2	ND	0.02
233	Terbufos 特丁硫磷	mg/kg	13071-79-9	ND	0.01
234	Tetrachlorvinphos 杀虫畏	mg/kg	22248-79-9	ND	0.01
235	Tetraconazole 氟醚唑	mg/kg	112281-77-3	ND	0.01
236	Tetradifon 三氯杀螨砜	mg/kg	116-29-0	ND	0.01
237	Thiabendazole 噻菌灵	mg/kg	148-79-8	ND	0.01
238	Thiacloprid 噻虫啉	mg/kg	111988-49-9	ND	0.02
239	Thiamethoxam 噻虫嗪	mg/kg	153719-23-4	ND	0.05
240	Thifensulfuron-methyl 噻吩磺隆	mg/kg	79277-27-3	ND	0.01
241	Thiofanox sulfone 久效威砜	mg/kg	39184-59-3	ND	0.01
242	Thiofanox-sulfoxide 久效威亚砜	mg/kg	39184-27-5	ND	0.01
243	Thiophanate methyl 甲基硫菌灵	mg/kg	23564-05-8	ND	0.05
244	Tolclofos-methyl 甲基立枯磷	mg/kg	57018-04-9	ND	0.01
245	Tolfenpyrad 唑虫酰胺	mg/kg	129558-76-5	ND	0.05
246	Triadimefon and Triadimenol 三唑酮和三唑醇之和	mg/kg	-	ND	-
247	Triadimefon 三唑酮	mg/kg	43121-43-3	ND	0.02
248	Triadimenol 三唑醇	mg/kg	55219-65-3	ND	0.01
249	Triasulfuron 醚苯磺隆	mg/kg	82097-50-5	ND	0.05
250	Triazophos 三唑磷	mg/kg	24017-47-8	ND	0.01
251	Trichlorphon 敌百虫	mg/kg	52-68-6	ND	0.05

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
252	Tricyclazole 三环唑	mg/kg	41814-78-2	ND	0.02
253	Tridemorph 十三吗啉	mg/kg	81412-43-3	ND	0.02
254	Trifloxystrobin 肟菌酯	mg/kg	141517-21-7	ND	0.1
255	Triflumizole 氟菌唑	mg/kg	68694-11-1	ND	0.02
256	Trifluralin 氟乐灵	mg/kg	1582-09-8	ND	0.01
257	Triflusulfuron-methyl 氟胺磺隆	mg/kg	126535-15-7	ND	0.01
258	Triticonazole 灭菌唑	mg/kg	131983-72-7	ND	0.05
259	Vamidothion 蚜灭磷	mg/kg	2275-23-2	ND	0.01
260	Vinclozolin 乙烯菌核利	mg/kg	50471-44-8	ND	0.01

Remark:
1.ND = Not Detected
2.LOQ = Limit of Quantitation

*** End ***

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APPENDIX B

Analytical Method for Vicine, Convicine, Divicine, and Isouramil

HPLC determination of alkaloids in fava bean protein

Preparation of fava bean protein samples

1. Weight 0.1 – 0.2 g of fava bean protein into a 20-mL glass liquid scintillation vial
2. Add 20.0 mL of Milli-Q Plus water, and a stir bar
3. Cap vial, mix well, and stir vigorously for one hour at room temperature
4. Centrifuge an aliquot at 15,000 x g for 5 minutes
5. Analyze via HPLC (per method below)

HPLC analysis

- Column: YMC ODS-AQ, 4.6 x 250 mm, 5 μm
- Mobile Phase A: 0.05 M KH_2PO_4, pH 2.9
- Mobile Phase B: 200 mL Milli-Q Plus water, 800 mL acetonitrile
- Flow rate: 0.5 mL/minute
- Temperature: 25 °C
- Injection volume: 10 μL
- Elution Program: 0% B from 0-20 minutes, 100% B from 20-25 minutes, 0% B from 25-43 minutes
- Detection: UV at 276 nm, 214 nm

References

1) Vioque J, et al., Nutritional and functional properties of Vicia faba protein isolates and related fractions, *Food Chem*, 132 (2012) 67-72
2) Pulkkinen M, et al., Determination of vicine and convicine from faba bean with an optimized high-performance liquid chromatographic method, *Food Res Intl*, 76 (2015) 168-177
3) Multari S, et al., Potential of fava bean as future protein supply to partially replace meat intake in the human diet, *Comp Rev Food Sci Food Safety*, 14 (2015) 511
4) Pulkkinen M, et al., Determination and stability of divicine and isouramil produced by enzymatic hydrolysis of vicine and convicine of faba bean, *Food Chem*, 212 (2016) 10-19
5) Purves PW, et al., Quantification of vicine and convicine in faba bean seeds using hydrophilic interaction liquid chromatography, *Food Chem*, 240 (2018) 1137-1145
6) Valente IM, et al., Unravelling the phytonutrients and antioxidant properties of European *Vicia faba* L. seeds, *Food Res Intl*, 116 (2019) 888-896

APPENDIX C

Analytical Method for L-DOPA

HPLC determination of Levodopa in fava bean protein

Preparation of standard solutions

1. Dissolve 10-11 mg of USP levodopa reference standard (lot [redacted]) in 25 ml of Milli-Q Plus water. This is the Stock Standard Solution.
2. Dilute 0.200 ml of Stock Standard Solution to 10 ml with HPLC Mobile Phase A. This is the High Standard Solution.
3. Dilute 0.100 ml of Stock Standard Solution to 10 ml with HPLC Mobile Phase A. This is the Middle Standard Solution.
4. Dilute 0.0200 ml of Stock Standard Solution to 10 ml with HPLC Mobile Phase A. This is the Low Standard solution.

Preparation of protein ingredients

1. Weigh 0.9 - 1.1 g of protein ingredient into a tared 20-ml glass LSC vial.
2. Add 20.0 ml of HPLC Mobile Phase A.
3. Add a stir bar, tightly cap vial, and stir suspension vigorously for 30 minutes at RT.
4. Filter suspension through Whatman No. 41 paper.
5. Syringe filter filtrate (from step 4) through a 0.2 µm PTFE membrane into an HPLC autosampler vial.

HPLC analysis

- Column: Zorbax Eclipse Plus C18, 4.6 x 250 mm, 5 µm
- Mobile Phase A: 1000 ml 0.05 M KH_2P0_4, pH 2.9; 25 ml acetonitrile
- Mobile Phase B: 200 ml Milli-Q Plus water, 800 ml acetonitrile
- Flow rate: 0.4 mL/minute
- Temperature: 20 °C
- Injection volume: 5 µL
- Elution Program: 0% B from 0-25 minutes, 100% B from 25-30 minutes, 0% B from 30-50 minutes
- Detection: FLD, Ex = 280 nm, Em = 310 nm

References

7) Remenar et al., Pharmaceutical compositions and methods of using levodopa and carbidopa, *EP167045081*, 2011-01-19
8) Stennett et al., *Am J Hosp Pharm, 4317* [1986] 1726-1728

EXHIBIT 1

Report of the Expert Panel

OPINION OF AN EXPERT PANEL ON THE SAFETY AND GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF FAVA BEAN PROTEIN FOR USE IN FOOD

Introduction

An independent panel of experts (Expert Panel), qualified by scientific training and experience to evaluate the safety of food and food ingredients, was requested by TFULL to determine the safety and Generally Recognized as Safe (GRAS) status of the use of fava bean protein for use in food for human consumption. Fava bean protein is intended for use as a source of protein for enrichment of processed foods. The fava bean protein ingredient is manufactured in accordance with current Good Manufacturing Practice (cGMP) and meets the proposed specifications.

A detailed review based on the existing scientific literature (through March 2019) on the safety of fava bean protein was conducted by ToxStrategies, Inc. (ToxStrategies) and is summarized in the attached dossier. The Expert Panel members reviewed the dossier prepared by ToxStrategies and other pertinent information and convened on June 25, 2019 via teleconference. Based on an independent, critical evaluation of all of the available information and discussions during the June 25, 2019 teleconference, the Expert Panel unanimously concluded that the intended uses described herein for TFULL's fava bean protein ingredient, meeting appropriate food-grade specifications as described in the supporting dossier (**GRAS Determination of Fava Bean Protein for Use as an Ingredient in Human Food**) and manufactured according to cGMP, are safe, suitable, and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

Summary and Basis for GRAS Determination

Description

Fava bean protein is a protein ingredient derived from fava bean (*Vicia faba* L.) that is ≥90% protein (dry matter basis). The remainder of the ingredient is ash, fat, carbohydrates, and water. The fava bean protein ingredient is also rich in amino acids.

Manufacturing Process

The fava bean protein ingredient (≥90% protein) is produced by an extraction process from commercially available fava beans. The starting material for the fava bean protein extraction process is fava bean flour which is produced from commercially available fava beans using a standard milling process. Following a soaking step (extraction), the slurry is separated by centrifugation. Hydrochloric acid (HCl) is then added to the slurry to precipitate the protein. The HCl is removed during the subsequent washing step. Sodium hydroxide (NaOH) is then added to neutralize the slurry as it reacts with remaining HCl to produce sodium chloride (NaCl) and water. The product is then sterilized, homogenized, and spray dried.

Analytical (chemical and microbiological) results for the fava bean protein product confirm that the finished product meets the proposed specifications as demonstrated by the consistency of production, the lack of impurities and contaminants (e.g., heavy metals, pesticides, microorganisms (*Salmonella* and *E. coli*), and mycotoxins), and is stable for 24 months when stored under recommended storage conditions: in a closed container in a cool (below 100°F) and dry place away from strong light.

History of Use

Fava bean (*Vicia faba* L.), also referred to as broad bean, horse bean, faba bean, and field bean, is an early legume crop and belongs to the *Fabaceae* family. There is little evidence of the origins of its domestication, as its wild progenitor is still unknown. However, the oldest seeds of fava bean were found in the late 10th millennium, in north-west Syria (Willcox and Tanno, 2006). The fava bean has a long history of use as feed and food, likely because of its valuable content of both protein and energy (Crepon et al., 2010). Fava bean is a versatile crop and has the ability to grow in various climatic zones. It can be used throughout the year and is consumed in both raw and processed forms. In the human diet, it is mostly the seed grain that is consumed, while the pods are used as feed. The fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health. The protein content is about twice that of cereals and several times that of root tubers. Fava bean seeds are a rich source of proteins, carbohydrates, fiber, vitamins, and minerals (Aune et al. 2011).

Protein is found throughout the body - in bone, muscle, hair, skin, and virtually every tissue and body part. At least 10,000 different proteins exist in the human body. Proteins are made up of amino acid building blocks, some of which must be provided by the diet (essential amino acids) as they cannot be made by the body. In some regions of the world outside of the US, many people experience protein malnutrition due to an inadequate dietary protein consumption, resulting in a condition known as kwashiorkor. While animal sources of protein tend to deliver all the essential amino acids, plant protein sources also deliver the majority of the required amino acids and have become an important option for added protein in processed foods. Plant and cereal grain sources of added protein currently used in food include peas, soy, lentils, chickpeas, beans, canola, rice, potatoes, and wheat.

Humans have consumed fava beans, as well as proteins from these sources for centuries, along with proteins from many food sources such as meats, dairy, fruits, vegetables, nuts, and seeds. The USDA recommends that half of a person's meal consist of grains and protein foods (equal amounts), and the other half should contain fruits and vegetables, with a serving of dairy. The Institute of Medicine (IOM, 2005) recommends that adults consume a minimum of 0.8 g protein/kg and has set a range for acceptable protein intake of 10 - 35% of daily calories. In the US, the recommended daily allowance (RDA) of protein is 56 and 46 grams/day for adult men and women (>19 years of age), respectively. FDA has established a protein daily reference value (DRV) of 50 g/day for adults and children four years of age or older (21 CFR § 101.9). Physically active

persons on normal diets may easily exceed this level, and individuals involved in bodybuilding ingest much higher levels of protein (WHO, 2002). WHO (2002) recommends body weight-based protein consumption rates for both genders. For example, the safe protein consumption level for a 40-kg adult is 33 g/day, and that for an 80-kg adult is 66 g/day. For adults, the protein requirement per kg body weight is considered to be the same for both sexes, at all ages, and for all body weights within the acceptable range. The value accepted as a safe level of intake is 0.83 g/kg per day, for proteins with a protein digestibility-corrected amino acid score value of 1.0 (WHO, 2002). While WHO states that no safe upper limit has been identified, they also indicate that it is unlikely that intakes of twice the safe level are associated with any risk.

Many protein products are currently available in the marketplace. To date, FDA has reviewed extensive published information and data as part of GRAS notifications for animal and plant-based protein isolates and concentrates and subsequently issued "no questions letters".

Intended Use and Intake Assessment

The focus of this GRAS determination is for food uses identical to what has been recognized in previous GRNs for plant-based protein sources such as soy (GRN No. 134; FDA 2004), canola (GRN Nos. 327, 386, 683; FDA 2010, 2011, 2017a), pea (GRN Nos. 182, 581, 608, 788; FDA 2005, 2015b, 2016a, 2018b), wheat (GRN Nos. 26 and 182; FDA 1999, 2005), rice (GRN No. 609; FDA 2016b), whey (GRN Nos. 37, 633; FDA 2000, 2016c), potato (GRN No. 447; FDA 2013), oat (GRN 575; FDA 2015a), mung bean (GRN 684; FDA 2017b), and hemp seed (GRN 771; FDA 2018a). Similarly, fava bean–derived protein is intended for use as a source of protein for enrichment of processed foods.

As described in numerous GRAS Notifications, including GRN No. 581 for un-hydrolyzed and hydrolyzed pea protein, the typical uses of protein for enrichment of foods include bakery products, snack foods, nutritional beverages such as high-protein drinks and milkshakes, instant powdered nutritional beverages, vegetarian food products and meat analogues, dairy products, and meal replacements/nutrition bars.

As the previously submitted GRNs on plant-derived proteins have demonstrated, the proposed use concentrations and variety of food uses, combined with the large average daily consumption of the described foods, results in a calculated daily intake of the protein additives being a substantial fraction of the Recommended Dietary Allowance (RDA; 46 g/day for women over 19 years of age and 56 g/day for men over 19 years of age), and even exceed it at the 90th percentile consumption. This was the case for GRN No. 327 (cruciferin-rich canola/rapeseed protein isolate and napin-rich protein canola/rapeseed protein isolate). TFULL's proposed fava bean protein isolate is intended only to be an alternative source of protein for current uses in food. Therefore, a similar estimate of intake would be expected if fava bean protein were the only source of protein used in processed foods. As was concluded in the other GRAS notifications, we do not realistically expect that the actual consumption of foods containing fava bean protein

would result in daily consumption greater than the DRV or RDA for protein. Most of the population's intake of protein is, and will remain, in the form of unprocessed foods, including meat, poultry, fish, and legumes. The proposed fava bean protein product is but one of many protein sources for use in processed foods, so only the known conservatism of intake calculations such as those described in the aforementioned GRNs suggest any possibility of exceeding the RDA at the 90th percentile (FDA, 2010, 2011).

In summary, the proposed uses of fava bean-derived protein will not result in an increase in the overall consumption of protein, but simply provide an alternative source of well-characterized protein from fava beans for use in food. Therefore, an estimate of the cumulative daily intake is not considered necessary.

Safety Data

Fava beans and other legumes, and the protein from these sources, have been commonly consumed as food and feed around the world for decades (Crepon et al., 2010; Schmandke et al., 2010). Humans have consumed fava beans for centuries, along with proteins from many food sources such as meats, dairy, fruits, vegetables, nuts and seeds. The IOM (2005) recommends that adults consume a minimum of 0.8 g protein/kg and has set a range for acceptable protein intake of 10 - 35% of daily calories.

Many protein products are currently available in the marketplace. To date, FDA has reviewed extensive published information and data as part of GRAS notifications for animal and plant-based protein isolates and concentrates and subsequently issued "no questions letters"

The fava bean is consumed in both raw and processed forms. In the human diet, it is mostly the seed grain that is consumed, while the pods are used as feed. The pods provide macro-, micro- and non-nutrient phytochemicals and could be used as a source of functional compounds. Fava beans contain approximately 250 g protein/kg seed and provides 320 kcal/100 g dry weight of energy. Fava bean, its fractions, and its processing products (grains, hulls, and flours) also contain anti-nutritional factors, but soaking and cooking are able to reduce the amounts of the anti-nutritional factors up to 100%, thus limiting any safety concerns (Jamalian et al., 1999).

Fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health (Aune et al. 2011). The protein content is about twice that of cereals and several times that of root tubers (Wu Leung et al., 1968). However, the biological value of untreated fava beans is negatively affected by the presence of anti-nutritional factors such as the favism-inducing factors (aglycones: divicine and isouramil produced from metabolism of vicine and convicine; Jamalian et al., 1999; Frank, 2005; Luzzatto and Arese, 2018), and the ingestion of untreated fava beans is associated with precipitation of the hemolytic disease favism in certain glucose-6-phosphate dehydrogenase-deficient humans (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017; Frank, 2005; Luzzatto and Arese, 2018).

Removal of these anti-nutrients is the primary safety concern and is required for the effective utilization of fava bean components, including protein concentrates and isolates, in human nutrition. Anti-nutrition factors are now removed routinely during commercial processing of fava beans (Cardador-Martinez et al., 2012; Jamalian et al., 1999). Some of the anti-nutrition factors are readily destroyed by heat processing (e.g., boiling, cooking, autoclaving, or extrusion cooking) during the production of fava bean flour, or can be eliminated by pretreatments of the beans, such as dehulling or soaking (Revilla 2015). Such treatments may reduce the vicine and convicine content of fava beans by 94%–100% (Jamalian, 1999). Later, Vioque et al. (2012) reported a reduction of vicine and convicine in fava protein "by more than 99%" by employing an extraction of the defatted seed flour, followed by precipitation at an isoelectric pH. The removal of vicine and convicine ensures that the favism-causing aglycone compounds, divicine and isouramil, cannot be synthesized. Soaking, acid hydrolysis, and sterilization are part of the manufacturing process for T-FULL's fava protein and result in 1) significant (near total) reduction in levels of vicine and convicine in the isolated protein; and 2) inactivation of the beta-glucosidase activity normally inherent in fava beans. Beta-glucosidase activity is necessary for the conversion of vicine and convicine to their aglycone forms (divicine and isouramil, respectively). It is these aglycones that are the problematic compounds for those individuals with favism. The toxicity of vicine, convicine, divicine, and isouramil are discussed below, and the concentrations of the anti-nutrients in three lots of the fava bean protein powder are presented in the following Table. If one were to consume the RDA for protein of 50–60 g all as fava bean protein only, that would represent approximately 25 mg of vicine plus convicine/day.

Alkaloid concentration in three lots of fava bean protein (mg/kg)

Alkaloid	**Lot** [redacted]	**Lot** [redacted]	**Lot** [redacted]
Vicine	282	316	319
Convicine	66	91	86
Divicine	4	4	4
Isouramil	5	10	10
Sum	357	421	419

Levodopa, also known as L-DOPA or L-3,4-dihydroxyphenylalanine, is a chemical precursor to dopamine and a common treatment for Parkinson's disease. L-DOPA naturally occurs in fava beans, but its levels decrease significantly in heated/cooked beans (Carador-Martinez et al., 2012; Multari et al, 2015). The level of L-DOPA in the fava bean protein isolate was determined to be approximately 13.3 mg/kg. If a person was to consume the RDA of 56 grams/day, the maximum calculated exposure is 0.74 mg of levodopa, which is 0.25% of the typical recommended daily dose (300 mg) and 0.05% of the maximum recommended daily dose (1500 mg) for the treatment of Parkinson's disease.

Given the long history of global human consumption of fava beans as food (and the

protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by their consumption and general lack of toxicity. As would be expected for a food that has been consumed by humans for centuries, fava beans and fava bean proteins have not been subjected to traditional toxicology studies. Furthermore, given the available information and data on the safety of fava beans and fava bean proteins, as well as the scientific efforts to remove anti-nutritional compounds from fava beans and fava bean protein isolates intended for animal and human consumption, conduct of toxicity studies was considered unnecessary and not an ethical use of animals.

It has been recognized for decades that fava beans contain "anti-nutritional" compounds. As noted previously, those that are of most concern are vicine (2,6-diamino-4,5-dihydroxypyrimidine 5-(beta-D-glucopyranoside) and convicine (2,4,5-trihydroxy-6-aminopyrimidine 5-beta-D-glucopyranoside), which are glycosidic aminopyrimidine derivatives. The vicine and convicine content of raw fava beans have been reported to range from 0.02% to 1.46% (dry weight basis) (Khamassi et al., 2013). Vicine and convicine are hydrolyzed in the fresh fava bean by β-glucosidase to form the aglycones divicine and isouramil. These aglycones are the compounds responsible for "favism," by causing the oxidation of glutathione in red blood cells that cannot be sufficiently reversed in affected individuals who are mostly males. The erythrocytes of individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency cannot regenerate glutathione at a normal rate. Erythrocytes become rigid, experience aggregation of their proteins, produce methemoglobin and have their enzymes inactivated due to oxidative damage that cannot be controlled or reversed. The damaged red blood cells are then removed by macrophages in the spleen and liver, which could lead to a potentially fatal hemolytic anemia. Therefore, adverse reactions to fava bean ingestion is limited to a small, but significant, number of males with this X-linked genetic disease. There are many genetic variants of G6PD deficiency, one common variant is observed primarily in Africans and African-Americans and another in people with Mediterranean origins (e.g., Greeks, Italians, Armenians, several Semitic populations and others). Some Asian and middle eastern populations are also affected. In the US, G6PD deficiency is observed in approximately 10% of African-American males (Frank, 2005).

In the European, Middle Eastern, African, and Chinese areas where individuals prone to favism live, and where fava beans, and especially the immature pods, are consumed, it is estimated that there is an intake of 2000 mg of vicine and 1000 mg of convicine in one meal (WHO, 1992). Numerous cases of favism related to consumption of raw, unprocessed fava beans by G6PD-deficient individuals have been reported (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017; Luzzatto and Arese, 2018).

The total amount of convicine and vicine in regular fava bean varieties is 6–14 mg/g (Arese et al., 2007), an amount that is orders of magnitude greater than that found in the proposed fava bean protein ingredient. Arese et al. (2007) stated that the levels of vicine and convicine in FEVITA (a low vicine/convicine protein ingredient) were 10 – 20x lower than traditional cultivars (i.e., 6-14 mg/g). A 10-fold reduction results in 0.6-1.4 mg/g and 0.3-0.7 mg/g for a 20-fold reduction. This is comparable to the levels shown in

Table 8 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot No.). In the Arese et al. (2009) study, seven hemizygous males and females with G6PD deficiency used the FEVITA fava bean, which is low in vicine and convicine due to reduced beta-glucosidase in the FEVITA cultivar which prevents conversion of vicine and convicine to divicine and isouramil. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the vicine and convicine level of 0.348 mg/g in the proposed ingredient, times 60 grams of protein/day (replacement of RDA protein intake), results in an intake of approximately 25 mg vicine and convicine in the diet, an amount that is 8 to18 % of the level employed in the Arese et al. (2009) study in which no hemolytic effects were reported.

The potential for fava bean proteins to cause an immune response is rare but consistent with similar, known allergies to other legumes. Although fava beans are not listed as one of eight major allergen groups by the FDA under the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)), the fact that the allergenicity of fava bean protein has been shown clinically (Mur Gimeno et al., 2007; Kumar et al., 2014) suggests that labeling of the presence of fava bean protein is warranted and recommended. More importantly, given the incidence in of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. Therefore, it is proposed that the ingredient labeling for the fava bean protein product clearly state that it contains "fava bean protein" and individuals who wish to avoid fava bean or fava bean protein consumption for any reason would be able to identify the presence of a fava bean–derived ingredient.

Taken together, the available published safety data demonstrate that the fava bean protein isolate from *Vicia faba* has little potential for toxicity when used in foods for human consumption.

General Recognition of the Safety of Fava Bean Protein

The intended use of fava bean protein has been determined to be safe through scientific procedures as set forth in 21 CFR§170.3(b), thus satisfying the so-called "technical" element of the GRAS determination and is based on the following:

- Fava bean protein is manufactured from commercially available fava beans, following current cGMP for food (21 CFR § Part 110). The raw materials and processing aids used in the manufacturing process are food grade and/or approved for use in food. The fava bean protein product has been characterized appropriately, contains a minimum of 90% protein, and meets appropriate food-grade specifications.

- Fava beans have been consumed as food (and the protein contained therein) for centuries, along with many other food sources of protein (e.g., meats, dairy, fruits, vegetables, nuts).

- The biological value of untreated fava beans is negatively affected by the presence of anti-nutritional factors such as the favism-inducing factors (aglycones: divicine and isouramil produced from metabolism of vicine and convicine). The ingestion of untreated fava beans is associated with precipitation of the hemolytic disease favism in certain glucose-6-phosphate dehydrogenase-deficient humans

- Removal of these anti-nutrients is the primary safety concern and is required for the effective utilization of fava bean components, including protein concentrates and isolates, in human nutrition. Anti-nutrition factors are now removed routinely during commercial processing of fava beans via boiling, cooking, autoclaving, or extrusion cooking during the production of fava bean flour, or by pretreatments of the beans, such as dehulling or soaking. Such treatments reduce the vicine and convicine content of fava beans as is the case in the proposed fava bean protein ingredient.

- The proposed uses of the fava bean protein ingredient will provide an alternative to other dietary sources of protein and will not increase the overall consumption of protein but will simply provide another source of well-characterized protein for use in food products.

- Fava beans are a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health. The protein content is about twice that of cereals and several times that of root tubers.

- FDA has reviewed extensive published information and data on many protein products as part of GRAS notifications for animal and plant-based protein isolates and concentrates and subsequently issued "no objection letters." Examples include GRN No. 26 (isolated wheat protein); GRN No. 37 (whey protein isolate and dairy product solids); GRN No. 168 (poultry protein); GRN No. 182 (hydrolyzed wheat gluten isolate; pea protein isolate); GRN No. 313 (beef protein); GRN No. 314 (pork protein); GRN 386 (canola protein isolate and hydrolyzed canola protein isolate); GRN No. 447 (potato protein isolates); GRN No. 575 (oat protein); and GRNs No. 58, 608, and 788 (pea protein).

- Given the long history of global human consumption of fava beans as food (and the protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by their consumption and general lack of toxicity. As would be expected for a food that has been consumed by humans for centuries, fava beans and fava bean proteins have not been subjected to traditional toxicology studies. However, the available summarized preclinical and clinical study data support its safe use as proposed.

- Concerns related to the allergenicity of fava bean protein as well as the effect of fava beans consumption by G6PD deficient individuals can be addressed through appropriate labeling of food products as containing fava bean protein and

individuals who wish to avoid fava bean protein consumption would be able to identify the presence of a fava bean–derived ingredient.

- The body of publicly available scientific literature on the consumption and safety of fava beans and fava bean protein is sufficient to support the safety and GRAS status of the proposed fava bean protein ingredient.

Conclusions of the Expert Panel

We, the undersigned independent qualified members of the GRAS Panel, have individually and collectively, critically reviewed the published and ancillary information pertinent to the identification, use, and safety of TFULL's fava bean protein product. We unanimously conclude that the intended use of the TFULL fava bean protein ingredient produced consistent with good manufacturing practice (cGMP) and meeting appropriate food-grade specifications as presented in the supporting dossier ["**GRAS Determination of Fava Bean Protein for Use as an Ingredient in Human Food**"] is safe.

We the members of the GRAS Panel, further unanimously conclude that the intended use of the TFULL fava bean protein product, produced consistent with good manufacturing practice (cGMP) and meeting appropriate food-grade specifications as presented in the supporting dossier is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in conventional foods specified herein.

It is our professional opinion that other qualified experts critically evaluating the same information, would concur with this conclusion.

_____ _____

Michael Carakostas, DVM, PhD
Consultant
MC Scientific Consulting LLC

Date

_____ _____

Paul Damian, PhD, MPH, DABT
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Carol A. Knight, PhD
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[redacted]

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8 July 2019
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Paul Damian, PhD, MPH, DABT
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Date

References

Alkhaaldi A, Al Tenejii M, Faisal Khanani M, Baroudi M, Jamil A, Trad O, Al Reyami L, Ahmed Awan N. 2014. Two cases of glucose-6-phosphate dehydrogenase deficiency presenting as symptomatic methemoglobinemia with persistently low saturations after fava bean ingestion in United Arab Emirates. Applied Clinical research, Clinical Trials, and Regulatory Affairs 1(3):176-179.

Arese P, Gerard D, Iessire M, Marget P. 2007. Low vicine-convicine and zero tannin FEVITA faba beans. Grain Legumes 48:16-17.

Arese P, Schwarzer E, Gallo V, Simula L, Duc G. 2009. Risk assessment for subjects with G6PD-deficiency of new faba bean cultivars with low content of vicine and convicine (FEVITA beans). Report: PRO0818. http://proof.saskpulse.com/files/general/PRO0818.pdf

Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, Norat T. 2011. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ 343:1-20.

Cardador-Martinez A, Maya-Ocana K, Ortiz-Moreno A, Herrera-Cabrera BE, Davila-Ortiz G Muzquiz M, Martin-Pedrosa M, Burbano C, Cuadrado C, Jimenez-Martinez C. 2012. Effect of roasting and boiling on the content of vicine, convicine and L-3,4-dihydroxyphenylalanine in *Vicia faba* L. Journal of Food Quality 35:419-428.

Crepon K, Marget P, Peyronnet C, Carrou´ee B, Arese P, Duc G. 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Res 115(3):329–339.

Eghbal F, Fakaoorziba MR, Eghbal MH, Latifnia S. 2012. Survey on causes of hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient pediatric patients.Pak J Med Sci 28(4):666-669.

Food and Drug Administration (US FDA). 1999. GRN No. 26. GRAS Assessment of isolated wheat protein.

Food and Drug Administration (US FDA). 2000. GRN No. 37. GRAS Notification for whey protein isolate and dairy product solids.

Food and Drug Administration (US FDA). 2004. GRN No. 134. GRAS Notification for soy protein hydrolysate with enzyme-modified lecithin.

Food and Drug Administration (US FDA). 2005. GRN 182. GRAS Assessment of plant proteins in winemaking (hydrolyzed wheat gluten isolate; pea protein isolate).

Food and Drug Administration (US FDA). 2010. GRN No. 327. GRAS Notification for cruciferin-rich canola/rapeseed and napin-rich canola/rapeseed protein isolate.

Food and Drug Administration (US FDA). 2011. GRN No. 386. GRAS Assessment of canola protein isolate and hydrolyzed canola protein isolate.

Food and Drug Administration (US FDA). 2013. GRN 447. GRAS Assessment of potato protein isolates.

Food and Drug Administration (US FDA). 2015a GRN 575. GRAS Assessment of oat protein.

Food and Drug Administration (US FDA). 2015b. GRN 581. GRAS Assessment of pea protein.

Food and Drug Administration (US FDA). 2016a. GRN 608. GRAS Assessment of pea protein concentrate.

Food and Drug Administration (US FDA). 2016b. GRN 609. GRAS Assessment of rice protein

Food and Drug Administration (US FDA). 2016c. GRN 633. GRAS Assessment of whey protein

Food and Drug Administration (US FDA). 2017a. GRN 683. GRAS Assessment of canola protein isolate.

Food and Drug Administration (US FDA). 2017b. GRN 684. GRAS Assessment of mung bean protein isolate.

Food and Drug Administration (US FDA). 2018a. GRN 771. GRAS Assessment of hemp seed protein.

Food and Drug Administration (US FDA). 2018b. GRN 788. GRAS Assessment of pea protein concentrate.

Frank JE. 2005. Diagnosis and management of G6PD deficiency. American Family Physician 72(&):1277-1282.

Institute of Medicine (IOM) of the National Academies. 2005. Dietary, Functional and Total Fiber. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids Washington DC. National Academies Press

Jamalian J. 1999. Removal of favism-inducing factors vicine and convicine and the associated effects on the protein content and digestibility of faba beans (*Vicia faba* L). Journal of the Science of Food and Agriculture. 79(13):1909–1914.

Kavehmansch Z, Arab A, Abolghasemi H, Torabi SM. 2016. Fava bean ingestion: the most important risk factor of hemolysis in G6PD deficiency in Iran. IJBC 8(2):38-42.

Khamassi K, Stoddard FL, O'Sullivan D, Jones H. 2013. A baseline study of vicine-convicine levels in faba bean (Vicia faba L.) germplasm. Plant Genetic Resources 1-8.

Kumar D, Kumar S, Verma AK, Sharma A, Tripathi A, Chaudhari BP, Kant S, Das M, Jain SK, Dwivedi PD. 2014. Hypersensitivity linked to exposure to broad bean protein(s) in allergic patients and BALB/c mice. Nutrition 30(7-8): 903-914.

Luzzatto L, Arese P. 2018. Favism and glucose-6-phosphate dehydrogenase deficiency. N Engl J Med 378(1):60-71.

Multari S, Stewart D, Russell WR. 2015. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety 14:512-522.

Mur Gimeno P, Brito FF, Iglesias AM, Vega ML, Martínez PB. 2007. Allergic reaction caused by a new hidden food, broad bean flour. Eur J Allergy Clin Immun 62(11):1340-1341.

Revilla I. 2015. Processing and Impact on Active Components in Food. Chapter 40 – Impact of Thermal Processing on Faba Bean (*Vicia faba*) Composition. pp 337–343.

Schmandke H, Plass R, Lewerenz H-J, Bleyl DWR. 2000. Four week feeding studies with differently processes dehulled faba bean (*Vicia faba* L. minor) products in rats. Molecular Nutrition and Food Research 44(2):133-135.

Skold MB, Svendsen RP, Pedersen EB. 2017. Favism after ingestion of fava beans in a three-year old child with glucose-6-phosphate dehydrogenase deficiency. Ugeskr Laeger 15:179.

Vioque J, Alaiz M, Girón-Calle J. 2012. Nutritional and functional properties of *Vicia faba* protein isolates and related fractions. Food Chem 132(1):67-72.

Willcox G, Tanno K-I. 2006. How fast was wild wheat domesticated? Science 313:296-297.

World Health Organization.1992. Final report of the steering group on naturally occurring toxins of plant origin, Carshalton, UK, 20-22 May. http://apps.who.int/iris/bitstream/10665/67268/1/WHO_PCS_92.69.pdf

World Health Organization (WHO). 2002. Technical Report Series 935: Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation.

Wu Leung W, Busson F, Jardin C. 1968. Food composition table for use in Africa. FAO-Nutrition Information Documents Series (3).

Bonnette, Richard

From: Don Schmitt <dschmitt@toxstrategies.com>
Sent: Wednesday, August 28, 2019 11:03 AM
To: Bonnette, Richard
Subject: Re: Fava bean GRAS submission - FSIS info

Hi Richard,

I have spoken with all parties to the GRN submission and they wish to exclude the meat and poultry uses from the submission for fava bean protein.

Let me know if there is anything else you require regarding this matter.

Don

Donald F. Schmitt, M.P.H.
Senior Managing Scientist

ToxStrategies, Inc.
739 Thornapple Drive
Naperville, IL 60540
phone: 630.352.0303
email: dschmitt@toxstrategies.com

From: "Donald Schmitt, MPH" <dschmitt@toxstrategies.com>
Date: Friday, August 16, 2019 at 9:22 AM
To: "Bonnette, Richard" <Richard.Bonnette@fda.hhs.gov>
Subject: Re: Fava bean GRAS submission - FSIS info

Hi Richard,

Thank you for the note. I have contacted the client and hope to have an answer for you today.

Don

Donald F. Schmitt, M.P.H.

From:	Don Schmitt
To:	Downey, Jason
Cc:	Judy Yeung; Kimmo Lucas; Hanlon, Paul R; Weeks, Kelly J
Subject:	Re: GRN 879 - Fava bean protein isolate - Follow-Up Questions
Date:	Thursday, November 21, 2019 12:15:33 PM
Attachments:	image003.png FDA fava bean GRN questions and answers 112119.pdf

Dear Dr. Downey,

We have attached responses to all of the follow up questions raised by FDA in your email dated November 14, 2019. Please let me know if you have any further needs.

Best regards,

Don

Donald F. Schmitt, M.P.H.
Senior Managing Scientist

ToxStrategies, Inc.
739 Thornapple Drive
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From: "Donald Schmitt, MPH" <dschmitt@toxstrategies.com>
Date: Thursday, November 14, 2019 at 10:56 AM
To: "Downey, Jason" <Jason.Downey@fda.hhs.gov>
Subject: Re: GRN 879 - Fava bean protein isolate - Follow-Up Questions

Thank you, Jason. We will have responses back in 10 business days (before the Thanksgiving holiday if possible).

Don

Donald F. Schmitt, M.P.H.
Senior Managing Scientist

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From: "Downey, Jason" <Jason.Downey@fda.hhs.gov>
Date: Thursday, November 14, 2019 at 10:49 AM
To: "Donald Schmitt, MPH" <dschmitt@toxstrategies.com>
Subject: GRN 879 - Fava bean protein isolate - Follow-Up Questions

Dear Mr. Schmitt,

During our review of GRN 879, which you submitted on behalf of Yantai T. FULL Biotech Co., Ltd. for use of fava bean protein isolate in select conventional food categories, we noted six clarifying comments and questions that are attached to this email. Please provide responses to the attached comments and questions within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. If you have questions or need further clarification, please feel free to contact me. Thank you in advance for you attention to our comments.

Regards,

Jason

Jason Downey, PhD

Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
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1. In Part 1 of your Notice on page 6, you cite the statutory basis for your GRAS conclusion. We note that 21 CFR Section 570 deals with animal drugs, feed, and related products. Please clarify the statutory basis for your GRAS conclusion as required by 21 CFR 170.225(c)(5).

   Answer: The statement should read as follows: TFULL, through its agent, ToxStrategies, confirms that the fava bean protein ingredient, which meets the specifications described herein, has been determined to be GRAS through scientific procedures in accordance with 21 CFR § 170.30(a) and (b).

2. Table 2 on page 9 of your Notice is titled "Alkaloid concentration in three lots of fava bean protein (mg/kg)" and Table 3 on page 10 is titled "Typical amino acid profile (g dry basis per 100 g product)". On page 13 you appear to refer to Table 3 as showing levels of anti-nutrients in your fava bean protein isolate ("anti-nutritionals (Table 3)"). Please indicate whether the reference on page 13 should read "anti-nutritionals (Table 2)".

   Answer: The reference on page 13 should read "anti-nutritionals (Table 2)".

3. Table 6 on page 13 of your Notice provides analytical results for four non-consecutive lots of fava bean protein isolate. We note that the results for "Lot No. [redacted]" in Table 6 differ from those reported in the Certificate of Analysis for that lot in Appendix A of your Notice. Please clarify these discrepancies or provide a corrected Table 6.

   Answer: See corrected Table 6 below.

Table 6. Analytical results for four non-consecutive lots of fava bean protein

Specification		Lot No. [redacted]	Lot No. [redacted]	Lot No. [redacted]	Lot No. [redacted]
Protein (%)	≥90	98.4	91.3	92.7	95.7
Ash (%)	≤5	4.5	4.2	1.2	4.1
pH	6–7	7.1	7.5	6.4	6.4
Lead (mg/kg)	≤0.1	<0.02	<0.02	<0.02	<0.02
Arsenic (mg/kg)	≤0.1	<0.03	<0.03	<0.03	<0.03
Cadmium (mg/kg)	≤0.1	<0.02	<0.02	<0.02	<0.02
Mercury (mg/kg)	≤0.1	<0.02	<0.02	<0.02	<0.02
Total Plate Count (CFU/25g)	≤10,000	160	80	<10	<10
Yeast and mold (CFU/25g)	≤100	80 yeast, <10 mold	<10	<10	<10
E. coli	Negative	Negative	<10	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative	Negative	Negative

4. In your Notice, Table 8 is titled "Proposed maximum food use levels" and Table 9 is titled "Alkaloid concentration in three lots of fava bean protein (mg/kg)". On several pages you refer to "Table 8" as showing the levels of anti-nutrients in three lots of your fava bean protein isolate. Thus, it appears that some references to Table 8 in the Notice should have been to "Table 9". In addition, on page 24 you reference a "Lot No. " in "Table 8" (which we assume meant Table 9). However, this lot is not included in Table 9. Please rectify these inconsistencies with Table 8 and 9 in your Notice, either by specifying/clarifying each instance in a response or make available the corrected pages/sections of the Notice.

   Answer: There are three references to "Table 8" that should be corrected to read "Table 9". On page 23, last paragraph, the sentence should read "The total amount of convicine and vicine in regular fava bean varieties is 6–14 mg/g (Arese et al., 2007), an amount that is orders of magnitude greater than that found in the proposed fava bean protein ingredient (see Table 9)." On page 24, the first sentence should read "This is comparable to the levels shown in Table 9 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot No.)." On page 29, last paragraph, 4th sentence should read "This is comparable to the levels shown in Table 9 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot No.)." In addition, page 7, line 1 of the Expert Panel Report (Exhibit 1) should be corrected to read "Table 9" and not "Table 8". On page 24, the reference to "Lot No. in "Table 8" should read "This is comparable to the levels shown in Table 9 for the proposed fava bean protein ingredient (e.g., approximately 0.348 mg/g for Lot No.)."

5. In your section on the allergenicity of your product (pages 25 and 26) you state that you were only able to find one publication in the public record that presented a case report on the allergenicity of the fava bean. FDA has found two other case reports of allergenicity related to fava beans (broad beans). Reference 1 discusses fried broad bean allergenicity in a 5-year-old male and reference 2 discusses allergenicity in a farmer. Furthermore, we found an additional reference that showed that the IgE from patients with peanut allergy produced some cross-reactivity with protein extracts from fava beans. Please discuss whether these additional citations impact your safety assessment. In addition, give assurance that all relevant publicly available information has been extracted and reviewed during your GRAS review.

   Answer: We thank FDA for the additional references and discuss each paper below. They were identified in our literature search but not included in the GRN. We can assure FDA that our literature search was comprehensive and included more than 460 hits which were reviewed for relevance.

   It is our opinion that the additional references do not change any safety conclusions in the GRN or expert panel report. The GRAS dossier states that allergic reactions occur rarely and the additional references do not dispute that conclusion. They report either very special situations (Rodriguez-Mazariego et al., 2016; Damiani et al., 2011) or very weak skin prick test results in a small minority of ten patients selected for their significant peanut allergy status.

   The Rodriguez-Mazariego et al. (2016) paper reports allergy to oral exposure to broad beans only in one very specific situation - when they are fried - but not when they are cooked. Skin Prick Test results were negative in this patient when exposed to raw, cooked and fried broad beans - only oral challenge to fried beans caused a problem. The paper did not explore what changes frying induced in broad beans that caused the allergic reaction, but the proposed fava bean

protein product is not produced using frying so this paper identifies a situation that is most likely not applicable.

The Damiani et al. (2011) paper reports a prolonged, and likely very high, occupational exposure to fava beans that resulted in hypersensitivity. There are many known situations where very high occupational exposures to substances can induce hypersensitivity that is absent in typical consumer exposure to much lower levels with intermittent exposure. Consumer exposure via food is very unlikely to replicate this farmer's situation.

The Jensen et al. (2008) paper performed allergy cross-reactivity testing on ten children with moderate to severe peanut allergy. Broad beans produced positive Skin Prick Test results in only two of the 10 children. One had a 3.5 mm wheal that barely exceeded the minimal positive level (3mm). The other child showed a significant positive result with broad bean and every other food protein tested indicating a high propensity to show a cross-reactivity response. The result simply confirms what the dossier already stated - that cross-reactivity in peanut allergic individuals may rarely occur with fava bean protein just as it might with other legumes. Labeling was strongly recommended by the expert panel as this protein ingredient is not substantially different from any other legume or legume-derived protein. In addition, although fava beans did show cross-reactivity in some peanut-allergy patients, additional tests showed that fava bean proteins were about 1000-fold less potent than peanut proteins in inducing histamine release (Figure 1, Jensen et al., 2008).

6. On page 26, you state that "it is proposed that the ingredient labeling for the fava bean protein product clearly state that it contains 'fava bean protein' and individuals who wish to avoid fava bean or fava bean protein consumption for any reason would be able to identify the presence of a fava bean-derived ingredient." However, given that: a) many consumers who have not previously consumed fava beans would not necessarily know that they have G6PD deficiency or that they are sensitized to fava beans, b) a number of cases of hemolytic anemia in infants have been reported due to breastfeeding mothers who consumed fava beans and, c) your intended uses include products expected to be consumed by pregnant and/or breastfeeding women as well as children, please provide a narrative to ensure safe consumption of fava bean protein isolate for both children and adults under the existing regulation.

   Answer: It is true that individuals who are G6PD deficient sometimes find out by consuming a food that creates a clinical problem, as diagnostic testing is not necessarily conducted on every infant or individual. However, the proposed ingredient is a refined protein product derived from fava beans, with a documented low level of vicine and convicine as indicated in Table 9. The GRAS determination indicates that these levels are not only low in the proposed product - but so low that they are unlikely to cause a problem as evidenced in a challenge study in G6PD deficient individuals (Arese et al., 2007).

   Regarding breastfeeding, fava bean flour is used as the starting material in the production of the fava bean protein product and the proposed product contains very low levels of the vicine and convicine that cause a problem in G6PD deficient individuals. Table 9 demonstrates that the alkaloid concentrations in three lots of the proposed protein product are indeed quite low - especially in comparison to the information reported by Arese et al. (2007) in which G6PD individuals were challenged with fava beans. The total amount of convicine and vicine in regular fava bean varieties is 6–14 mg/g (Arese et al., 2007), an amount that is orders of magnitude greater than that found in the proposed fava bean protein ingredient. Anderson (2018) is

not specific about what foods containing fava beans might have caused the cited problem following breastfeeding but is most likely raw or cooked fava beans and not a low vicine/convicine fava bean-derived protein ingredient.

In summary, given the fact that reported cases of hemolytic anemia in breastfed infants occurred in women consuming raw or cooked whole fava beans containing high levels of vicine and convicine, and the concentration of the same alkaloids in the proposed protein product are very low, it is unlikely that significant levels of vicine and convicine would be present in breast milk following consumption of a food containing the proposed protein ingredient that would represent a hazard to either the mother or infant.

From:	Don Schmitt
To:	Downey, Jason
Cc:	Judy Yeung
Subject:	Re: GRN 879 - Fava bean protein isolate - Requests for Clarification
Date:	Tuesday, February 11, 2020 2:48:24 PM
Attachments:	image003.png

Hi Jason,

The following provides answers to the two questions raised in your email of February 10, 2020.

1. On page 11 of the notice, the notifier indicates that the fava bean protein extraction begins by soaking fava bean flour. Please state the solvent that is used for this soaking and extraction. Please state whether the solvent is food-grade.

   Answer: The solvent used is water purified through a reverse osmosis process and is food-grade.

2. On page 11 of the notice, the notifier states that sodium hydroxide is added to the slurry to neutralize it. However, the process diagram in figure 1 indicates neutralization occurs after the slurry is separated and the protein is precipitated and washed. Please clarify to which material (e.g. slurry or protein precipitate) during which processing step the sodium hydroxide is added.

   Answer: The sodium hydroxide is added during the protein precipitate step.

Regards,

Don

Donald F. Schmitt, M.P.H.
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