Tox Strategies

Innovative solutions
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July 17, 2023

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 for Use of Fava Bean Protein in Food

Dear Sir:

On behalf of Cooperative Koninkljike Cosun, U.A. (Cosun), 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. Previous email consultations with FDA regarding a potential GRAS Notification occurred on June 16, 2023 and June 30, 2023 (Chris Kampmeyer).

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,

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

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

JULY 17, 2023

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

SUBMITTED BY:

Cooperative Koninklijke Cosun U.A.
Kreekweg 1, 4671 VA Dinteloord
The Netherlands

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:

Donald F. Schmitt, MPH
ToxStrategies, Inc.
Naperville, IL 60540

July 17, 2023

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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
EFSA	European Food Safety Authority
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
4-HNE	4-hydroxynonenal
LCMS	liquid chromatography-mass spectrometry
IgE	immunoglobulin E
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kcal	kilocalories
kg	kilogram
LD_{50}	lethal dose in 50 percent of treated animals
LDL	low-density lipoprotein
mg	milligram
NaOH	sodium hydroxide
NOEL	no-observed-effect level
PDCAAS	Protein Digestibility Corrected Amino Acid Score
RBC	red blood cell
RDA	Recommended Dietary Allowance
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

Cosun, 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 certain specified foods for human consumption, in accordance with Subpart E of 21 CFR § 170.

(2) Name and Address

Cooperative Koninklijke Cosun U.A.
Kreekweg 1, 4671 VA Dinteloord
The Netherlands

(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

Cosun, 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

Cosun 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

Should this GRAS Notification be submitted to the FDA, 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. Cosun 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]

07/17/2023
Date

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

(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), its use limited only by GMP, as soy protein is also 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 ≥83% 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 Cosun Protein, Cooperative Koninklijke Cosun U.A. for this fava bean protein is Tendra™.

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 and convicine in three lots of the proposed fava bean protein ingredient. 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 liquid chromatography-mass spectrometry to be approximately 54-76 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 62.80 (Eurofins, 2021).

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

Protein	83 g
Moisture	6 g
Carbohydrates	3 g
Ash	4 g
Fat	0.5 g

Caloric value	370 kcal

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

Alkaloid	Lot 1000413751	Lot 1000413842	Lot 1000413891
Vicine	890	870	1540
Convicine	360	330	560

*by LC-MS method

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

Amino Acid	Fava Bean	Oat	Wheat	Rice	Soy	Whey
Alanine	3.90	4.37	4.0	5.67	NA	NA
Arginine	8.32	7.17	2.7	7.64	NA	NA
Aspartic Acid	10.1	7.48	3.2	9.33	NA	NA
Cysteine	0.97	2.45	1.3	2.09	NA	NA
Glutamic Acid	15.4	22.5	33.7	17.22	NA	NA
Glycine	3.76	4.13	5.1	4.33	NA	NA
Histidine	2.37	2.22	2.14	2.12	2.3	2.2
Isoleucine	4.13	4.38	3.39	4.4	4.7	5.8
Leucine	7.27	8.42	6.67	8.3	6.6	12
Lysine	6.16	3.38	2.5	3.4	5.4	10.8
Methionine	0.70	2.23	1.4	2.66	NA	NA
Methionine + Cysteine	1.67	4.68	4.33	5.61	2.9	4.2
Phenylalanine	4.06	5.95	4.2	5.38	NA	NA
Phenylalanine + Tyrosine	7.48	10	7.6	10.85	9.8	5.1
Proline	3.99	5.57	14.2	4.49	NA	NA
Serine	4.56	3.98	5.9	4.75	NA	NA
Threonine	3.29	3.5	2.96	3.7	4	7.2
Tryptophan	0.76	1.1	1.21	1.2	1.2	2.1
Tyrosine	3.42	4.05	2.7	4.84	NA	NA
Valine	4.39	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.

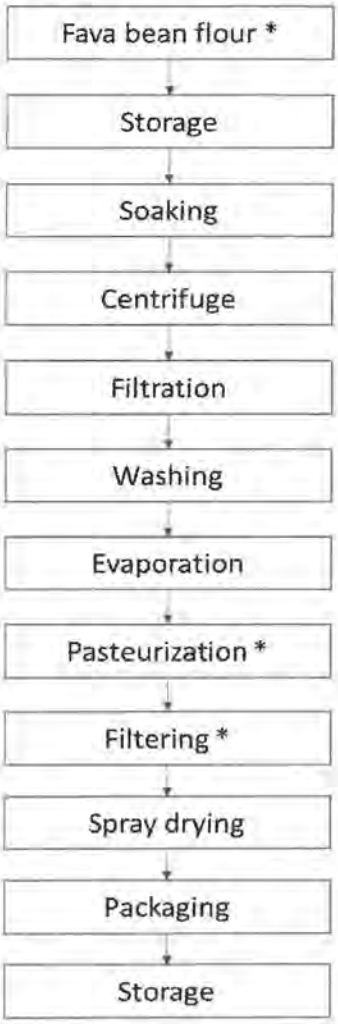
NA = not available

F. Manufacturing Process

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

The fava bean protein ingredient (≥83% protein) is produced by an extraction process from commercially available fava beans flour. 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. The fava bean flour is subsequently soaked (extraction), and sodium hydroxide (NaOH) is added to adjust the pH and sodium bisulfite is added as antioxidant. The slurry is separated by centrifugation and concentrated by filtration. The slurry is subsequently washed, where the NaOH and sodium bisulfite are removed. The product is subsequently evaporated, pasteurized and passed through a safety filter, to ensure all foreign particles are removed. The protein product is finally spray dried and packed.

Figure 1. Steps in the fava bean protein production process



*CCP

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)
Sodium bisulfite	7631-90-5	21 CFR §182.3739
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 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 due to its valuable content of both protein and energy (Crepon et al., 2010. It can be used throughout the year and is consumed in both raw and processed forms. In the human diet, the seed grain is primarily consumed. The fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to provide a benefit 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)	≥83	Kjeldahl
Carbohydrates (%, db)	≤10	Determined by difference
Ash (%)	≤6	Incineration
Fat (%, db)	≤2	Gravimetry
Moisture (%)	≤9	Gravimetry
pH	7–8	USP
Lead (mg/kg)	<0.1	ICP-MS
Arsenic (mg/kg)	<0.1	ICP-MS

Cadmium (mg/kg)	<0.1	ICP-MS
Mercury (µg/kg)	<100	ICP-MS
Total Plate Count (CFU/g)	≤100,000	ISO 4833-1
Yeast and mold (CFU/g)	≤200	ISO 21527-1
E. coli	Negative	ISO 16649-2
Salmonella	Negative	ISO 6579
Listeria monocytogenes	Negative	ISO 11290-1

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

Specification		**Lot No. 1000413562**	**Lot No. 1000413891**	**Lot No. 1000414223**
Protein (%)	≥83	87.5	87.5	88.6
Carbohydrates (%, db)	≤10	7.9	8.4	7.1
Ash (%)	≤6	4.2	3.8	4.0
Fat (%, db)	≤2	0.3	0.3	0.3
Moisture (%)	≤9	5.8	4.9	5.4
pH	7-8	7.7	7.8	7.7
Lead (mg/kg)	<0.1	<0.04	<0.04	<0.04
Arsenic (mg/kg)	<0.1	<0.04	<0.04	<0.04
Cadmium (mg/kg)	<0.1	<0.02	<0.02	<0.02
Mercury (µg/kg)	<100	<2	<2	<2
Total Plate Count (CFU/g)	≤100,000	14000	9000	2300
Yeast and mold (CFU/g)	≤200	<1	<1	<1
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus aureus	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 Cosun'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 (Lot no. 20210323-1) has been tested at 4°C under vacuum (Table 7) and under normal room temperature conditions (20°C; Table 8) for 12 months. Technical specifications for the product include shelf-life storage conditions of 12 months from the date of manufacture when stored in a closed container in a cool (below 20°C) and dry place away from strong light.

Table 7. Stability testing data (4°C)

	Time (Months)					
Specification	**Initial**	**1**	**3**	**6**	**9**	**12**
Aw value (25°C)		0.24	0.38	0.43	0.44	0.46
Insoluble matter (mg/kg)	6900	0.5	0.74	1.4	0.8	1.6
Mesophilic count (/10g)	2.7E+04	2.3E+04	3.7E+03	7.4E+03	4.2E+03	3.8E+03
Yeast (/10g)	<10	<10	<1	<1	<10	<10
Mold (/10g)	<10	<10	<1	<1	<10	<10

Table 8. Stability testing data (20°C)

	Time (Months)					
Specification	**Initial**	**1**	**3**	**6**	**9**	**12**
Aw value (25°C)		0.24	0.36	0.47	0.45	0.39
Insoluble matter (mg/kg)	6900	1	0.45	1.6	1.9	5.6
Mesophilic count (/10g)	2.7E+04	2.9E+04	5.4E+03	9.4E+02	3.1E+03	1.9E+03
Yeast (/10g)	<10	<10	<1	<1	<10	<10
Mold (/10g)	<10	<10	<1	<1	<10	<10

§ 170.235 Part 3, Dietary Exposure

Proposed Use

The focus of this GRAS determination of fava bean protein is for use in conventional foods at levels 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, 944; FDA 2016b, 2020b), 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), hemp seed (GRN 771; FDA 2018a) fava bean protein (GRN 879; FDA 2020a) and rice protein hydrolysate (GRN 944: 2020b). Cosun's fava bean–derived protein is intended for use as a source of protein for enrichment of processed foods in the same foods and use levels as the fava bean protein that previously received a "letter of no objection" (GRN 879; FDA, 2020a).

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 a safety based on upper limit of protein 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 protein intakes of twice the safe level are associated with any risk to healthy individuals.

As described in numerous GRAS Notifications including GRN Nos. 581 and 879 on pea protein and fava bean 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. Cosun proposes identical maximum use levels for its fava bean protein ingredient.

Table 10. 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 referenced GRNs on plant-derived proteins have demonstrated, the proposed use levels and the variety of food uses containing these plant-derived proteins, combined with the large average daily consumption of the described foods, results in a calculated daily intake of the added protein from these sources 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 can even exceed it at the 90th percentile consumption. This was true for GRN No. 327 (cruciferin-rich canola/rapeseed protein isolate and napin-rich protein canola/rapeseed protein isolate).

Cosun'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 including GRN 879 on fava bean protein, we do not realistically expect that the actual consumption of foods containing fava bean protein would result in daily consumption greater than the 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.

In summary, the proposed uses of the fava bean-derived protein ingredient will not result in an increase in the overall consumption of protein, but simply provide a well-characterized, alternative protein from fava beans for use in conventional foods.

§ 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 or flavor profile, either of which could affect consumer acceptability.

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

While there exists 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

GRN 879 for the use of fava bean protein in human foods in the United States received a "letter of no objection" from FDA in 2020. 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™, approximately 60% protein). In addition, 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 July 2023) present in them. These 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 PubMed, Embase, MedLine, and ToxLine.

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 animal feed. Fava beans contain approximately 250 g protein/kg seed and provides 320 kcal/100 g dry weight of energy. Fava bean, fava bean fractions, and its processing products (i.e., grains, hulls, and flours) also contain anti-nutritional factors, but these are substantially reduced in processing. The process of soaking and cooking in water is able to reduce the level of these anti-nutritional factors by up to 100%, thus limiting any safety concerns (Jamalian et al., 1999).

Fava bean is also a rich source of fiber and non-nutrient secondary metabolites that have been shown to be beneficial to human health (Aune et al. 2011). The protein content of fava beans is about twice that of other cereal crops and is several times that of root tubers (Wu Leung et al., 1968). 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, Beretta et al., 2023). The ingestion of untreated fava beans is associated with the hemolytic disease "favism" in certain glucose-6-phosphate dehydrogenase-deficient individuals (Kavehmansh et al., 2016; Skold et al., 2017; Luzzatto and Arese, 2018; Al-Dubai et al. 2021; Xin et al., 2022; Diegues et al., 2022). Favism is a condition causing red blood cells to break down in response to certain medications, infections, or other stressors. Typically, it is described as a condition when someone has a very severe reaction to fava beans and are at risk of

acute hemolysis, in which their red blood cells break down faster than the body can replace them. Typically, glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited condition usually occurring in males of African and Mediterranean descent.

Removal of these anti-nutrients is desired 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). These treatments can reduce the vicine and convicine content of fava beans by 94%–100% (Jamalian, 1999). 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.

Cosun soaks the fava beans as part of the manufacturing process for the fava bean protein product and this process results in a significant reduction in levels of vicine and convicine in the isolated protein. The toxicity of vicine, convicine, divicine, and isouramil are discussed in the safety section below, and the concentrations of the anti-nutrients vicine and convicine in three lots of the fava bean protein ingredient are presented in Table 11. If one were to consume the RDA for protein of 50–60 g all as fava bean protein only, that would represent approximately 130 mg of vicine plus convicine/day.

Table 11. Alkaloid concentration in three lots of fava bean protein (mg/kg)*

Alkaloid	Lot 1000413751	Lot 1000413842	Lot 1000413891
Vicine	890	870	1540
Convicine	360	330	560

*LC-MS 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 level decreases 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 54-76 mg/kg. If a person was to consume the RDA of 56 grams/day of protein, the maximum calculated exposure from this consumption is 4.25 mg of levodopa, which is 1.4% of the typical recommended daily dose (300 mg) and 0.28% 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 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 the historical consumption and general lack of toxicity. As 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. Given the publicly available information and data on the safety of fava beans and fava bean proteins, as well as the efforts to remove anti-nutritional compounds from fava beans and fava bean protein isolates intended for animal and/or human consumption, the conduct of toxicity studies was considered unnecessary and not an ethical use of animals. A summary of available published 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. Rats (three groups; 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. 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. Rats fed the *Vicia faba* diets had significantly lower body weights and energy intakes than did rats fed casein diets. The whole-seed diet induced a significant reduction in plasma triacylglycerol. Feeding rats diets containing fava bean seeds, or the protein isolate, produced 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* resulted from an increase in steroid fecal excretion. The authors concluded that the fava bean–protein isolate had a positive effect 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 (15/sex) were used per group. Gradually increasing 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 composition of the protein isolate was approximately 81% protein, 6% fat, and 3% carbohydrate. The low dose of 20 g/kg was identified as 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 (soaked and deep-fried dehulled faba beans) resulted in no toxicological effects at 50 g/kg doses (groups 3 and 4).

Anti-Nutritional Components

It has been long recognized that fava beans contain "anti-nutritional" compounds. 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), both of 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). As reviewed in GRN 879, 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 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.

For this reason, potential adverse reactions to fava bean ingestion are limited to a small, but significant, number of males with this X-linked genetic disease. There are many genetic variants of G6PD deficiency, with one common variant 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. G6PD deficiency in the U.S. is observed in approximately 10% of African-American males (Frank, 2005).

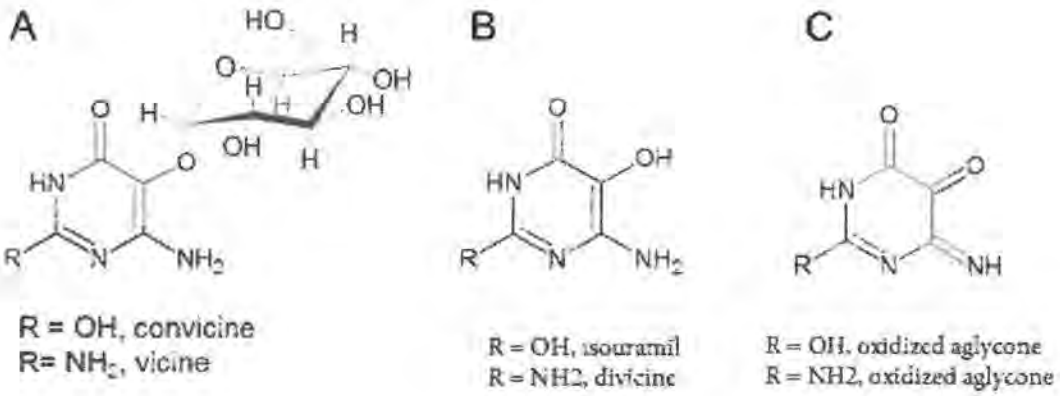
In the European, Middle Eastern, African, and Chinese areas where individuals prone to favism live, it is estimated that one meal can result in an intake of 2000 mg of vicine and 1000 mg of convicine (WHO, 1992). Numerous cases of favism related to consumption of raw, unprocessed fava beans by G6PD-deficient individuals have been reported (Alkhaaldi et al., 2014; Eghbal et al., 2012; Kavehmansh et al., 2016; Skold et al., 2017; Luzzatto and Arese, 2018; Al-Dubai et al. 2021; Xin et al., 2022; Diegues et al., 2022).

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 11) that is the subject of this GRAS dossier.

Arese et al. (2007) reported 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 11 for the proposed fava bean protein ingredient (e.g., approximately 1.2 mg/g for Lot No. 1000413842 and only slightly higher for the other three lots of fava bean protein). Arese et al. (2009) studied seven hemizygous males with G6PD deficiency using the FEVITA fava bean, which is low in vicine and convicine. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the maximum vicine and convicine level of 2.16 mg/g (Lot No. 1000413562) in the proposed ingredient, times 60 grams of protein/day (total replacement of RDA protein intake), results in an intake of approximately 130 mg vicine and convicine in the diet, an amount that is less than 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

The acute oral LD50 values for vicine and divicine in adult male albino rats are 2100 and 1950 mg/kg bw, respectively (Hussein, 2012). Oral administration of vicine to 10 rats/each dose/each group at doses of 700, 1400, 2100, 2800, 3500, and 4200 mg/kg bw resulted in 0, 2, 6, 8, 9, and 10 deaths, respectively. Similarly, oral administration of divicine to 10 rats/each dose/each group in doses of 500, 1000, 1500, 2000, 2500, and 3000 mg/kg bw resulted in 0, 1, 2, 5, 8, and 10 deaths, respectively. Possible causes of death included 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, respectively). The two treatment groups were given oral doses of convicine (20 mg/kg bw) for 15 or 30 days. Significant

convicine -related decreases ($p<0.05$) in red blood cell counts and hemoglobin content were produced. 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 study produced blood alterations similar to those observed in 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-defiicent male 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. No indication of hemolysis was observed as demonstrated 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 authors demonstrated that no 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 beans (low-tannin, low-V/C fava beans). This study provided important clinical evidence in hemizygous severely G6PD-defiicent male 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.

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.

Damiani et al. (2011) reported 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.

Jensen et al. (2008) 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 confirmed that cross-reactivity in peanut allergic individuals may ~~rarely~~ occur with fava bean protein just as it might with other legumes. 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).

A few reported cases of hemolytic anemia have been reported in breastfed infants of women who had consumed raw or cooked whole fava beans containing high levels of vicine and convicine. The concentration of these same alkaloids in the proposed protein product are very low. Therefore, it is unlikely that significant levels of vicine and convicine would be present in breast milk following consumption of a food containing the proposed fava bean protein ingredient that would represent a hazard to either the mother or infant.

The potential for fava bean proteins to cause an immune response is rare but consistent with similar, known allergic responses 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; Damiani et al., 2011; Jensen et al., 2008) clearly indicates that labeling of the presence of fava bean protein in any food product is warranted and recommended. More importantly, given the incidence of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. The Panel recommends that the ingredient labeling for the fava bean protein product clearly state

that it contains "fava bean protein" and that 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. Physically active persons on normal diets can 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). However, Martin et al. (2005) also 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 $\geq$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 from the intended uses in select foods proposed in this dossier will result in daily intake levels below those associated with effects on renal function. It is recommended by WHO (2002) that those who are at risk of kidney stones 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).

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 specified human foods 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 specified 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, rice, 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. 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 and GRN No. 879 for fava bean protein, 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.

Cosun's proposed fava bean protein is intended only to be an alternative source of protein for current uses of other proteins 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 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. 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 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. These 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). The removal of vicine and convicine ensures that the fava-causing aglycone compounds, divicine and isouramil, cannot be synthesized. Cosun soaks the fava beans as part of the

manufacturing process for the fava bean protein product and this results in a significant reduction in levels of vicine and convicine in the isolated protein.

In 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; Luzzatto and Arese, 2018; Al-Dubai et al. 2021; Xin et al., 2022).

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 and its intended uses in specified foods. 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 11 for the proposed fava bean protein ingredient (e.g., approximately 1.2 mg/g for Lot No. 1000413842 and only slightly higher for the other three lots of fava bean protein). 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. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the maximum vicine and convicine level of 2.16 mg/g (Lot No. 1000413562) in the proposed ingredient, times 60 grams of protein/day (total replacement of RDA protein intake), results in an intake of approximately 130 mg vicine and convicine in the diet, an amount that is less than 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 allergens found in 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; Damiani et al., 2011; Jensen et al., 2008) suggests that labeling of the presence of fava bean protein is both warranted and recommended. More importantly, given the incidence of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. The Panel recommends that the ingredient labeling for the fava bean protein product clearly state that it contains "fava bean protein" and that 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 83% 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). 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.

- 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 produced from metabolism of vicine and convicine). The ingestion of untreated fava beans is associated with an occurrence 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. 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. Cosun soaks the fava beans as part of the manufacturing process for the fava bean protein product and this results in a significant reduction in levels of vicine and convicine in the isolated protein.

- The proposed uses of the fava bean protein ingredient in specified foods 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.

- FDA has reviewed 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); GRNs No. 58, 608, and 788 (pea protein); GRN 879 (fava bean protein); and GRN 944 (rice protein hydrolysate).

- Given the long history of human consumption of fava beans as food (along with the protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by a history of 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 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 will 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 Cosun and composed of Paul Damian, Ph.D., M.P.H., DABT, ERT, Carol A. Knight, Ph.D., and Stanley M. Tarka, Jr., Ph.D., F.A.T.S. 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 Cosun's 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 concludes that the 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 Cosun's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Cosun 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.

Cosun 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

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APPENDIX A

COAs and Other Analytical Data

Cosun Innovation
Kreekweg 1
P.O. Box 20
4670 AA Dinteloord
The Netherlands

Certificate of Analysis of ***Cosun Fava Protein Isolate***, *Batch 1000413562*

Production date: 13 March 2022

Composition			*Method*
Moisture content	5.8	% as is	Gravimetry
Protein	87.5	% on dry matter	Kjeldahl
Ash	4.2	% on dry matter	Incineration
Fat	0.3	% on dry matter	Gravimetry

Microbial			*Method*
Total aerobic count	1.4E+4	CFU/g	ISO 4833-1
E. coli	Absent	in 25 g	ISO 16649-2
Salmonella spp.	Absent	in 25 g	ISO 6579
Listeria monocytogenes	Absent	in 25 g	ISO 11290-1
Yeasts	<1	CFU/g	ISO 21527-1
Moulds	<1	CFU/g	ISO 21527-1

Other			*Method*
Sulfite	0.0011	% as is	Monier-Williams method

Properties	
pH of a 1% solution	7.0 - 8.0
High solubility at neutral pH	
Mild/neutral taste at 1% in water	
2 year shelf life in un opened bag, stored dry at room temperature	

Cosun Innovation
Kreekweg 1
P.O. Box 20
4670 AA Dinteloord
The Netherlands

Certificate of Analysis of ***Cosun Fava Protein Isolate***, *Batch 1000413891*

Production date: 18 March 2022

Composition			*Method*
Moisture content	4.9	% as is	Gravimetry
Protein	87.5	% on dry matter	Kjeldahl
Ash	3.8	% on dry matter	Incineration
Fat	0.3	% on dry matter	Gravimetry

Microbial			*Method*
Total aerobic count	9.0E+3	CFU/g	ISO 4833-1
E. coli	Absent	in 25 g	ISO 16649-2
Salmonella spp.	Absent	in 25 g	ISO 6579
Listeria monocytogenes	Absent	in 25 g	ISO 11290-1
Yeasts	<1	CFU/g	ISO 21527-1
Moulds	<1	CFU/g	ISO 21527-1

Other			*Method*
Sulfite	0.0028	% as is	Monier-Williams method

Properties	
pH of a 1% solution	7.0 - 8.0
High solubility at neutral pH	
Mild/neutral taste at 1% in water	
2 year shelf life in un opened bag, stored dry at room temperature	

Cosun Innovation
Kreekweg 1
P.O. Box 20
4670 AA Dinteloord
The Netherlands

Certificate of Analysis of ***Cosun Fava Protein Isolate***, *Batch 1000414223*

Production date: 23 March 2022

Composition			*Method*
Moisture content	5.4	% as is	Gravimetry
Protein	88.6	% on dry matter	Kjeldahl
Ash	4.0	% on dry matter	Incineration
Fat	0.3	% on dry matter	Gravimetry

Microbial			*Method*
Total aerobic count	2.3E+3	CFU/g	ISO 4833-1
E. coli	Absent	in 25 g	ISO 16649-2
Salmonella spp.	Absent	in 25 g	ISO 6579
Listeria monocytogenes	Absent	in 25 g	ISO 11290-1
Yeasts	<1	CFU/g	ISO 21527-1
Moulds	<1	CFU/g	ISO 21527-1

Other			*Method*
Sulfite	0.0011	% as is	Monier-Williams method

Properties	
pH of a 1% solution	7.0 - 8.0
High solubility at neutral pH	
Mild/neutral taste at 1% in water	
2 year shelf life in un opened bag, stored dry at room temperature	

eurofins

Certificate of analysis

Analytical Service Manager	Gerda De Vries
Report electronically validated by:	Wouter Hagedoorn
Client id	HE0000060
Your project name	*Fava protein*
Your project number	*G12220*
Your order number	*G12220*
Quotationcode	LTI&BX20006058
Batchcode	EUNLHE-00669069
Number of samples	1
Enclosure	
Report date	25 May 2022
Date sampling	*Unknown*
Time sampling	*Unknown*
Samples received on	20 May 2022
Sample taking Eurofins	No
Sample taker	*Marlies Geerts*

Cosun Research & Development
Attention Renate Bremen
Kreekweg 1
4671 VA DINTELOORD

Certificate number:	**AR-22-HE-141396-01**
Sample number:	**888-2022-05190970**
Start date	20 May 2022
Your sample number:	*Batch 1000413562*
Sample description:	*batch 1000413562*
Reception	*20-05-2022*
Your project name	*Fava protein*
Your order number	G12220
Your project number	G12220
Reception condition	Uncooled
Sampler	Marlies Geerts

	Lab	TC	Analysis	Unit	Result
Q#	EUNLBA	FF1S1	Arsenic (As) Method: Internal Method, ICP-MS		
			Arsenic (As)	mg/kg	< 0.040
Q#	EUNLBA	FF1S5	Cadmium (Cd) Method: Internal Method, ICP-MS		
			Cadmium (Cd)	mg/kg	< 0.020
Q#	EUNLBA	FF1SB	Lead (Pb) Method: Internal Method, ICP-MS		
			Lead (Pb)	mg/kg	< 0.040
Q#	EUNLBA	FF1SE	Mercury (Hg) Method: Internal Method, ICP-MS		
			Mercury (Hg)	µg/kg	< 2.0
Q#	EUDE11	ZP914	Pesticide Screening LC-GHT Method: DIN EN 15662:2018-07 mod., LC-MS/MS		
			Piperonyl butoxide	mg/kg	0.081
			Other screened pesticides		Not Detected

The results are only valid for the sample as received. The uncertainty of measurement for the applied methods of analysis are retrievable from the ASM department Opinions and interpretations in this certificate are outside the scope of accreditation. The samples will be stored until 21 days after date of reception. The samples are not stored for microbiological analyzes, unless otherwise agreed with the customer.

Eurofins Food Testing Netherlands B.V.

Icarus 12

8448 CJ Heerenveen NL

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Fax : +31 88 83 10 100
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https://www.eurofinsfoodtesting.nl

D. Weijens
National Division Leader

eurofins

Legend

Lab	Laboratory		Accreditation
EUDE11	Eurofins Dr. Specht Laboratorien GmbH	Q#	DIN EN ISO/IEC 17025:2018 DAkkS D-PL-14198-01-00
EUNLBA	Eurofins Analytico B.V	Q#	NEN EN ISO/IEC 17025: 2017, RvA L010

Q# = Accreditation for the lab performing the analyses but not issuing the certificate

The data in italics is provided by the customer and may affect the validity of the results.

The results are only valid for the sample as received. The uncertainty of measurement for the applied methods of analysis are retrievable from the ASM department Opinions and interpretations in this certificate are outside the scope of accreditation. The samples will be stored until 21 days after date of reception. The samples are not stored for microbiological analyzes, unless otherwise agreed with the customer.

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D. Wetjens
National Division Leader

eurofins

Certificate of analysis

Analytical Service Manager	Gerda De Vries
Report electronically validated by:	Wouter Hagedoorn
Client id	HE0000060
Your project name	*Fava protein*
Your project number	*G12220*
Your order number	*G12220*
Quotationcode	LTI&BX20006058
Batchcode	EUNLHE-00669069
Number of samples	1
Enclosure	
Report date	25 May 2022
Date sampling	*Unknown*
Time sampling	*Unknown*
Samples received on	20 May 2022
Sample taking Eurofins	No
Sample taker	*Marlies Geerts*

Cosun Research & Development
Attention Renate Bremen
Kreekweg 1
4671 VA DINTELOORD

Certificate number:	**AR-22-HE-141394-01**
Sample number:	**888-2022-05190968**
Start date	20 May 2022
Your sample number:	*Batch 1000413891*
Sample description:	*batch 1000413891*
Reception	*20-05-2022*
Your project name	*Fava protein*
Your order number	G12220
Your project number	G12220
Reception condition	Uncooled
Sampler	Marlies Geerts

	Lab	TC	Analysis	Unit	Result
Q#	EUNLBA	FF1S1	Arsenic (As) Method: Internal Method, ICP-MS		
			Arsenic (As)	mg/kg	< 0.040
Q#	EUNLBA	FF1S5	Cadmium (Cd) Method: Internal Method, ICP-MS		
			Cadmium (Cd)	mg/kg	< 0.020
Q#	EUNLBA	FF1S8	Lead (Pb) Method: Internal Method, ICP-MS		
			Lead (Pb)	mg/kg	< 0.040
Q#	EUNLBA	FF1SE	Mercury (Hg) Method: Internal Method, ICP-MS		
			Mercury (Hg)	µg/kg	< 2.0
Q#	EUDE11	ZP914	Pesticide Screening LC-GHT Method: DIN EN 15662:2018-07 mod., LC-MS/MS		
			Piperonyl butoxide	mg/kg	0.12
			Tebuconazole	mg/kg	0.019
			Other screened pesticides		Not Detected

The results are only valid for the sample as received. The uncertainty of measurement for the applied methods of analysis are retrievable from the ASM department Opinions and interpretations in this certificate are outside the scope of accreditation. The samples will be stored until 21 days after date of reception. The samples are not stored for microbiological analyzes, unless otherwise agreed with the customer.

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National Division Leader

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Legend

Lab	Laboratory		Accreditation
EUDE11	Eurofins Dr. Specht Laboratorien GmbH	Q#	DIN EN ISO/IEC 17025:2018 DAkkS D-PL-14198-01-00
EUNLBA	Eurofins Analytico B.V	Q#	NEN EN ISO/IEC 17025: 2017, RvA L010

Q# = Accreditation for the lab performing the analyses but not issuing the certificate

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Certificate of analysis

Analytical Service Manager	Gerda De Vries
Report electronically validated by:	Wouter Hagedoorn
Client id	HE0000060
Your project name	*Fava protein*
Your project number	*G12220*
Your order number	*G12220*
Quotationcode	LTI&BX20006058
Batchcode	EUNLHE-00669069
Number of samples	1
Enclosure	
Report date	25 May 2022
Date sampling	*Unknown*
Time sampling	*Unknown*
Samples received on	20 May 2022
Sample taking Eurofins	No
Sample taker	*Marlies Geerts*

Cosun Research & Development
Attention Renate Bremen
Kreekweg 1
4671 VA DINTELOORD

Certificate number:	**AR-22-HE-141395-01**
Sample number:	**888-2022-05190969**
Start date	20 May 2022
Your sample number:	*Batch 1000414223*
Sample description:	*batch 1000414223*
Reception	*20-05-2022*
Your project name	*Fava protein*
Your order number	G12220
Your project number	G12220
Reception condition	Uncooled
Sampler	Marlies Geerts

	Lab	TC	Analysis	Unit	Result
Q#	EUNLBA	FF1S1	Arsenic (As) Method: Internal Method, ICP-MS		
			Arsenic (As)	mg/kg	< 0.040
Q#	EUNLBA	FF1S5	Cadmium (Cd) Method: Internal Method, ICP-MS		
			Cadmium (Cd)	mg/kg	< 0.020
Q#	EUNLBA	FF1S8	Lead (Pb) Method: Internal Method, ICP-MS		
			Lead (Pb)	mg/kg	< 0.040
Q#	EUNLBA	FF1SE	Mercury (Hg) Method: Internal Method, ICP-MS		
			Mercury (Hg)	µg/kg	< 2.0
Q#	EUDE11	ZP914	Pesticide Screening LC-GHT Method: DIN EN 15662:2018-07 mod., LC-MS/MS		
			Piperonyl butoxide	mg/kg	0.075
			Other screened pesticides		Not Detected

The results are only valid for the sample as received. The uncertainty of measurement for the applied methods of analysis are retrievable from the ASM department Opinions and interpretations in this certificate are outside the scope of accreditation. The samples will be stored until 21 days after date of reception. The samples are not stored for microbiological analyzes, unless otherwise agreed with the customer.

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Legend

Lab	Laboratory		Accreditation
EUDE11	Eurofins Dr. Specht Laboratorien GmbH	Q#	DIN EN ISO/IEC 17025:2018 DAkkS D-PL-14198-01-00
EUNLBA	Eurofins Analytico B.V	Q#	NEN EN ISO/IEC 17025: 2017, RvA L010

Q# = Accreditation for the lab performing the analyses but not issuing the certificate
The data in italics is provided by the customer and may affect the validity of the results.

The results are only valid for the sample as received. The uncertainty of measurement for the applied methods of analysis are retrievable from the ASM department Opinions and interpretations in this certificate are outside the scope of accreditation. The samples will be stored until 21 days after date of reception. The samples are not stored for microbiological analyzes, unless otherwise agreed with the customer.

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Legend

(*)	The test is accredited
CAS	The CAS Registry Number is a unique identifier assigned by the Chemical Abstracts Service to every chemical substance
LOQ	Limit of quantification

ZP914-1 (*) Pesticide Screening LC-GHT

Aim

To provide a quick and cheap method to screen residues of more than 150 currently used pesticides and metabolites in plant material that are not analysable applying SP911.

Method

The procedure involves initial single-phase extraction of 10g sample with 10 mL acetonitrile, followed by liquid-liquid partitioning formed by addition of Magnesium Sulfate plus Sodium Chloride. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid phase extraction (dispersive SPE), in which Magnesium Sulfate and primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile extract. The dispersive SPE with PSA removes many polar matrix components such as organic acids, certain polar pigments and sugars to some extent from the food extracts. Liquid Chromatogarphy applying tandem MS detection is then used for qualitative and quantitative determination of LC-amenable pesticides.

Reference method

DIN EN 15662:2009-02

Applied on

Coffee beans (green), Coffee products (exc. beans & liquids), Plant extract, Sauces and condiments, Soup and broth

Laboratory

Eurofins Dr. Specht Laboratorien GmbH — DIN EN ISO/IEC 17025:2005 D-PL-14198-01-00

Parameters

Parameter	CAS	LOQ
2,4-D	94-75-7	0.02 mg/kg
3-Hydroxycarbofuran	16655-82-6	0.01 mg/kg
Abamectin	71751-41-2	0.02 mg/kg
Acetamiprid	135410-20-7	0.01 mg/kg
Acrinathrin	101007-06-1	0.01 mg/kg
Aldicarb	116-06-3	0.01 mg/kg
Aldicarb (sum)		
Aldicarb-sulfone	1646-88-4	0.01 mg/kg
Aldicarb-sulfoxide	1646-87-3	0.01 mg/kg
Aminocarb	2032-59-9	0.01 mg/kg
Amitraz	33089-61-1	0.01 mg/kg
Atrazine	1912-24-9	0.01 mg/kg
Azadirachtin	11141-17-6	0.02 mg/kg
Azinphos-methyl	86-50-0	0.03 mg/kg
Azoxystrobin	131860-33-8	0.01 mg/kg
Benalaxyl	71626-11-4	0.01 mg/kg
Bendiocarb	22781-23-3	0.01 mg/kg
Benfuracarb	82560-54-1	0.02 mg/kg
Bensulide	741-58-2	0.05 mg/kg
Bentazone	25057-89-0	0.01 mg/kg
Benthiavalicarb, isopropyl-	177406-68-7	0.01 mg/kg
Benzalkonium chloride (total) (BAC)		
Benzethonium Chloride	121-54-0	0.1 mg/kg
Benzyldimethyldodecylammonium chloride (BAC-C12)	139-07-1	0.1 mg/kg
Bitertanol	55179-31-2	0.01 mg/kg
Boscalid	188425-85-6	0.01 mg/kg
Bromoxynil	1689-84-5	0.01 mg/kg

27-2-2019

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BNP Paribas S.A. 227 9248 27
VAT/BTW No. NL 8037.24.123.B01
KvK No. 01070347
IBAN:NL65BNPA0227924827 BIC:BNPANL2A
BNP Paribas S.A., Amsterdam, The Netherlands

Bromuconazole (Sum)		
Bromuconazole, cis-		0.01 mg/kg
Bromuconazole, trans-	116255-48-2	0.01 mg/kg
Bupirimate	41483-43-6	0.01 mg/kg
Buprofezin	69327-76-0	0.01 mg/kg
Butocarboxim	34681-10-2	0.02 mg/kg
Butocarboxim (sum)		
Butocarboxim-sulfoxide	34681-24-8	0.02 mg/kg
Butoxycarboxim	34681-23-7	0.01 mg/kg
Carbaryl	63-25-2	0.01 mg/kg
Carbendazim	10605-21-7	0.01 mg/kg
Carbendazim/Benomyl (sum)		0.01 mg/kg
Carbofuran	1563-66-2	0.01 mg/kg
Carbofuran (Sum)		
Carbosulfan	55285-14-8	0.01 mg/kg
Carboxin	5234-68-4	0.01 mg/kg
Carfentrazone-ethyl	128639-02-1	0.5 mg/kg
Cetalkonium chloride (BAC-C16)	122-18-9	0.1 mg/kg
Chlorantraniliprole	500008-45-7	0.01 mg/kg
Chlorbromuron	13360-45-7	0.01 mg/kg
Chlorbufam	1967-16-4	0.02 mg/kg
Chlorfluazuron	71422-67-8	0.01 mg/kg
Chloridazone	1698-60-8	0.02 mg/kg
Chlorotoluron	15545-48-9	0.01 mg/kg
Chloroxuron	1982-47-4	0.01 mg/kg
Chlorpropham	101-21-3	0.03 mg/kg
Chlorpyrifos (-ethyl)	2921-88-2	0.02 mg/kg
Chlorpyrifos-methyl	5598-13-0	0.03 mg/kg
Cinidon-ethyl	142891-20-1	0.01 mg/kg
Clefoxydim	139001-49-3	0.01 mg/kg
Clethodim	99129-21-2	0.01 mg/kg
Clofentezine	74115-24-5	0.01 mg/kg
Clomazone	81777-89-1	0.01 mg/kg
Clopyralid	1702-17-6	0.2 mg/kg
Clothianidin	210880-92-5	0.01 mg/kg
Cyazofamid	120116-88-3	0.01 mg/kg
Cycloxydim	101205-02-1	0.01 mg/kg
Cymoxanil	57966-95-7	0.01 mg/kg
Cyproconazole	94361-06-5	0.01 mg/kg
Cyprodinil	121552-61-2	0.01 mg/kg
Cyromazine	66215-27-8	0.05 mg/kg
DDAC C10 - Didecyldimethylammoniumchloride	7173-51-5	0.1 mg/kg
Demeton-S-methyl	919-86-8	0.01 mg/kg
Demeton-S-methyl-sulfone	17040-19-6	0.01 mg/kg
Desmedipham	13684-56-5	0.01 mg/kg
Diafenthiuron	80060-09-9	0.01 mg/kg
Diazinon	333-41-5	0.01 mg/kg
Diethofencarb	87130-20-9	0.01 mg/kg
Diethyltoluamide	134-62-3	0.01 mg/kg
Difenoconazole	119446-68-3	0.01 mg/kg
Difenoxuron	14214-32-5	0.01 mg/kg
Diflubenzuron	35367-38-5	0.01 mg/kg
Dimefuron	34205-21-5	0.02 mg/kg
Dimethenamid	87674-68-8	0.01 mg/kg
Dimethoate	60-51-5	0.01 mg/kg
Dimethomorph	110488-70-5	0.01 mg/kg
Diniconazole	83657-24-3	0.01 mg/kg

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Dinotefuran	165252-70-0	0.01 mg/kg
Dioxacarb	6988-21-2	0.02 mg/kg
Diuron	330-54-1	0.01 mg/kg
Dodine	2439-10-3	0.5 mg/kg
Emamectin	119791-41-2	0.02 mg/kg
Epoxiconazole	133855-98-8	0.01 mg/kg
Ethiofencarb	29973-13-5	0.01 mg/kg
Ethiofencarb (sum)		
Ethiofencarb-sulfone	53380-23-7	0.01 mg/kg
Ethiofencarb-sulfoxide	53380-22-6	0.01 mg/kg
Ethofumesate	26225-79-6	0.02 mg/kg
Ethoxyquin	91-53-2	0.02 mg/kg
Etofenprox	80844-07-1	0.02 mg/kg
Fenarimol	60168-88-9	0.01 mg/kg
Fenazaquin	120928-09-8	0.01 mg/kg
Fenbuconazole	114369-43-6	0.01 mg/kg
Fenhexamid	126833-17-8	0.01 mg/kg
Fenobucarb	3766-81-2	0.01 mg/kg
Fenoxycarb	72490-01-8	0.01 mg/kg
Fenpropimorph	67564-91-4	0.01 mg/kg
Fenpyroximate	134098-61-6	0.01 mg/kg
Fipronil	120068-37-3	0.01 mg/kg
Fipronil (sum)		
Fipronil, desulfinyl-	111246-15-2	0.01 mg/kg
Fipronil-sulfide	120067-83-6	0.01 mg/kg
Fipronil-sulfone	120068-36-2	0.01 mg/kg
Flazasulfuron	104040-78-0	0.01 mg/kg
Flonicamid	158062-67-0	0.02 mg/kg
Fluazifop (Sum)		
Fluazifop-P	83066-88-0	0.02 mg/kg
Fluazifop-P-butyl	79241-46-6	0.02 mg/kg
Fluazinam	79622-59-6	0.01 mg/kg
Flubendiamide	272451-65-7	0.01 mg/kg
Fludioxonil	131341-86-1	0.01 mg/kg
Flufenacet	142459-58-3	0.01 mg/kg
Flufenoxuron	101463-69-8	0.01 mg/kg
Fluopicolid	239110-15-7	0.01 mg/kg
Fluopyram	658066-35-4	0.01 mg/kg
Fluoxastrobin	361377-29-9	0.1 mg/kg
Flusilazole	85509-19-9	0.01 mg/kg
Forchlorfenuron	68157-60-8	0.01 mg/kg
Formetanate	22259-30-9	0.05 mg/kg
Fosthiazate	98886-44-3	0.01 mg/kg
Fuberidazole	3878-19-1	0.01 mg/kg
Furalaxyl	57646-30-7	0.01 mg/kg
Furathiocarb	65907-30-4	0.01 mg/kg
Haloxyfop	69806-34-4	0.02 mg/kg
Hexaconazole	79983-71-4	0.01 mg/kg
Hexaflumuron	86479-06-3	0.01 mg/kg
Hexythiazox	78587-05-0	0.01 mg/kg
Imazalil	35554-44-0	0.01 mg/kg
Imibenconazole	86598-92-7	0.01 mg/kg
Imidacloprid	138261-41-3	0.01 mg/kg
Indoxacarb	144171-61-9	0.01 mg/kg
Iprodione	36734-19-7	0.01 mg/kg
Iprovalicarb	140923-17-7	0.01 mg/kg
Isoprocarb	2631-40-5	0.01 mg/kg
Isoproturon	34123-59-6	0.02 mg/kg
Isoxaflutole	141112-29-0	0.01 mg/kg

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Kresoxim-methyl	143390-89-0	0.02 mg/kg
Linuron	330-55-2	0.01 mg/kg
Lufenuron	103055-07-8	0.01 mg/kg
Malathion	121-75-5	0.01 mg/kg
Malathion/Malaoxon (sum)		
Mandipropamid	374726-62-2	0.02 mg/kg
MCPA	94-74-6	0.05 mg/kg
MCPA/MCPB (sum)		
MCPB	94-81-5	0.05 mg/kg
Mepanipyrim	110235-47-7	0.01 mg/kg
Metaflumizone	139968-49-3	0.01 mg/kg
Metalaxyl	57837-19-1	0.01 mg/kg
Metalaxyl/Metalaxyl-M (sum)		
Metamitron	41394-05-2	0.01 mg/kg
Methabenzthiazuron	18691-97-9	0.01 mg/kg
Methamidophos	10265-92-6	0.05 mg/kg
Methidathion	950-37-8	0.01 mg/kg
Methiocarb	2032-65-7	0.01 mg/kg
Methiocarb (sum)		
Methiocarb-sulfone	2179-25-1	0.01 mg/kg
Methiocarb-sulfoxide	2635-10-1	0.01 mg/kg
Methomyl	16752-77-5	0.01 mg/kg
Methomyl/Thiodicarb (sum)		
Methoxyfenozide	161050-58-4	0.01 mg/kg
Metolachlor	51218-45-2	0.01 mg/kg
Metolcarb	1129-41-5	0.01 mg/kg
Metoxuron	19937-59-8	0.01 mg/kg
Metribuzin	21087-64-9	0.01 mg/kg
Miristalkonium chloride (BAC-C14)	139-08-2	0.1 mg/kg
Monolinuron	1746-81-2	0.01 mg/kg
Monuron	150-68-5	0.01 mg/kg
Myclobutanil	88671-89-0	0.01 mg/kg
Neburon	555-37-3	0.01 mg/kg
Nitenpyram	120738-89-8	0.05 mg/kg
Novaluron	116714-46-6	0.01 mg/kg
Nuarimol	63284-71-9	0.01 mg/kg
Omethoate	1113-02-6	0.02 mg/kg
Oxadixyl	77732-09-3	0.02 mg/kg
Oxamyl	23135-22-0	0.01 mg/kg
Oxydemeton-methyl	301-12-2	0.02 mg/kg
Oxydemeton-methyl (sum)		
Penconazole	66246-88-6	0.01 mg/kg
Pencycuron	66063-05-6	0.01 mg/kg
Pendimethalin	40487-42-1	0.1 mg/kg
Penflufen	494793-67-8	0.05 mg/kg
Phenmedipham	13684-63-4	0.01 mg/kg
Phosalone	2310-17-0	0.03 mg/kg
Phosmet	732-11-6	0.01 mg/kg
Phoxim	14816-18-3	0.01 mg/kg
Piperonyl butoxide	51-03-6	0.01 mg/kg
Pirimicarb	23103-98-2	0.01 mg/kg
Pirimicarb (Sum)		
Pirimicarb, desmethyl-	30614-22-3	0.01 mg/kg
Pirimicarb, desmethyl-formamido-	27218-04-8	0.01 mg/kg
Pirimiphos-methyl	29232-93-7	0.01 mg/kg
Prochloraz	67747-09-5	0.01 mg/kg
Procymidone	32809-16-8	0.05 mg/kg
Promecarb	2631-37-0	0.01 mg/kg

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Propamocarb	24579-73-5	0.01 mg/kg
Propaquizafop	111479-05-1	0.01 mg/kg
Propargite	2312-35-8	0.01 mg/kg
Propham	122-42-9	0.05 mg/kg
Propiconazole	60207-90-1	0.01 mg/kg
Propoxur	114-26-1	0.01 mg/kg
Propoxycarbazone	145026-81-9	0.01 mg/kg
Prosulfocarb	52888-80-9	0.01 mg/kg
Prothioconazole	178928-70-6	0.01 mg/kg
Pymetrozine	123312-89-0	0.02 mg/kg
Pyraclostrobin	175013-18-0	0.01 mg/kg
Pyrethrins	8003-34-7	0.02 mg/kg
Pyridaben	96489-71-3	0.01 mg/kg
Pyridate	55512-33-9	0.1 mg/kg
Pyrimethanil	53112-28-0	0.01 mg/kg
Pyriproxyfen	95737-68-1	0.01 mg/kg
Quinoxyfen	124495-18-7	0.01 mg/kg
Quizalofop	76578-12-6	0.05 mg/kg
Rimsulfuron	122931-48-0	0.01 mg/kg
Rotenone	83-79-4	0.01 mg/kg
Sethoxydim	74051-80-2	0.01 mg/kg
Simazine	122-34-9	0.01 mg/kg
Spinetoram	935545-74-7	0.1 mg/kg
Spinosad	168316-95-8	0.01 mg/kg
Spirodiclofen	148477-71-8	0.01 mg/kg
Spiromesifen	283594-90-1	0.01 mg/kg
Spirotetramat	203313-25-1	0.01 mg/kg
Spirotetramate (Sum)		
Spirotetramat-enol	203312-38-3	0.1 mg/kg
Spirotetramat-enolglucoside	1172614-86-6	0.1 mg/kg
Spirotetramat-ketohydroxy	1172134-11-0	0.1 mg/kg
Spirotetramat-monohydroxy	1172134-12-1	0.1 mg/kg
Spiroxamine	118134-30-8	0.01 mg/kg
Sulfentrazone	122836-35-5	0.01 mg/kg
Tebuconazole	107534-96-3	0.01 mg/kg
Tebufenozide	112410-23-8	0.01 mg/kg
Tebufenpyrad	119168-77-3	0.01 mg/kg
Teflubenzuron	83121-18-0	0.01 mg/kg
Tepraloxydim	149979-41-9	0.01 mg/kg
Terbacil	5902-51-2	0.01 mg/kg
Terbuthylazine	5915-41-3	0.01 mg/kg
Tetraconazole	112281-77-3	0.01 mg/kg
Thiabendazole	148-79-8	0.01 mg/kg
Thiacloprid	111988-49-9	0.01 mg/kg
Thiamethoxam	153719-23-4	0.01 mg/kg
Thiodicarb	59669-26-0	0.01 mg/kg
Thiofanox	39196-18-4	0.05 mg/kg
Thiofanox (total)		
Thiofanox-sulfone	39184-59-3	0.01 mg/kg
Thiofanox-sulfoxide	39184-27-5	0.02 mg/kg
Thiophanate-methyl	23564-05-8	0.02 mg/kg
Tolclofos-methyl	57018-04-9	0.05 mg/kg
Tralkoxydim	87820-88-0	0.01 mg/kg
Triadimefon	43121-43-3	0.01 mg/kg
Triadimenol	55219-65-3	0.02 mg/kg
Tricyclazole	41814-78-2	0.01 mg/kg
Tridemorph	81412-43-3	0.01 mg/kg
Trifloxystrobin	141517-21-7	0.01 mg/kg
Triflumizol/FM-6-1 (Sum)		

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	Triflumizole	99387-89-0			0.01 mg/kg	
	Triflumuron	64628-44-0			0.01 mg/kg	
	Triforine	26644-46-2			0.01 mg/kg	
	Trimethacarb, 3,4,5-	2686-99-9			0.01 mg/kg	
	XMC	2655-14-3			0.01 mg/kg	
	Zoxamide	156052-68-5			0.01 mg/kg	
	Screened pesticides					
	Other screened pesticides					
Sampling / shipping requirements	**Optimal quantity**	100 g	**Minimal quantity**	50 g	**Container**	Original packaging
Related / preparation tests (see appendix)	Extraction	ZPP11-1 (*)	Extraction "QuEChERS"		Preparation	

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Appendix

ZPP11-1 (*)	Extraction "QuEChERS"		
Aim	Created as a necessary extraction module in addition to QuEChERs tests for fresh and dried food.		
Method	Extraction of sample with Acetonitrile, solid phase cleanup		
Reference method	DIN EN 15662:2009-02		
Applied on	Food / Feed		
Laboratory	Eurofins Dr. Specht Laboratorien GmbH		DIN EN ISO/IEC 17025:2005 D-PL-14198-01-00
Parameters	**Parameter** Weighted sample	**CAS**	**LOQ**

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EXHIBIT 1

Report of the Expert Panel

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

Introduction

An independent panel of experts (GRAS Panel), qualified by scientific training and experience to evaluate the safety of food and food ingredients, was requested by Cosun to determine the safety and Generally Recognized as Safe (GRAS) status of the use of fava bean protein as an ingredient in human food. 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 July 2022) on the safety of fava bean protein was conducted by ToxStrategies, Inc. (ToxStrategies) and is summarized in the attached dossier. The GRAS Panel members reviewed the dossier prepared by ToxStrategies and other pertinent information and convened on October 27, 2022 via teleconference. Based on an independent, critical evaluation of all of the available information and discussions during the October 27, 2022 teleconference, the GRAS Panel unanimously concluded that the intended uses and use levels described herein for Cosun'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 GRAS 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 ≥83% 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 (≥85 % protein) is produced by an extraction process from commercially available fava bean flour. 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. The fava bean flour is subsequently soaked (extraction), and sodium hydroxide (NaOH) is added to adjust the pH and sodium bisulfite is added as antioxidant. The slurry is separated by centrifugation and concentrated by filtration. The slurry is subsequently washed, where the NaOH and sodium bisulfite are removed. The product is subsequently evaporated, pasteurized and

passed through a safety filter, to ensure all foreign particles are removed. The protein product is finally spray-dried and packed.

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., anti-nutritionals, heavy metals, pesticides, aflatoxins, and microbiological contaminants), and is stable for 12 months when stored under recommended storage conditions: in a closed container in a cool (below 20°C) 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. The fava bean has a long history of use as fboth animal feed and food, likely because of its valuable content of both protein and energy (Crepon et al., 2010). 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 that have been shown to be beneficial to human health. The protein content of the fava bean 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. 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. 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 of fava bean protein is for its use in conventional foods at levels 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, 944; FDA 2016b, 2020b), 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), hemp seed (GRN 771; FDA 2018a) fava bean protein (GRN 879; FDA 2020a) and rice protein hydrolysate (GRN 944: 2020b). 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, 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 the variety of food uses containing these plant-derived proteins, combined with the large average daily consumption of the described foods, results in a calculated daily intake of the added protein from these sources 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 can 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).

Cosun'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 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.

In summary, the proposed uses of the fava bean-derived protein ingredient will not result in an increase in the overall consumption of protein, but simply provide a relatively

minor proportion of well-characterized, alternative protein from fava beans for use in conventional foods.

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. 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 animal 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 also a rich source of fiber and non-nutrient secondary metabolites that have been shown to be beneficial to human health (Aune et al. 2011). The protein content of fava beans is about twice that of other cereal crops and is 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). The ingestion of untreated fava beans is associated with the hemolytic disease "favism" in certain glucose-6-phosphate dehydrogenase-deficient individuals (Kavehmansh et al., 2016; Skold et al., 2017; Luzzatto and Arese, 2018; Al-Dubai et al. 2021; Xin et al., 2022; Diegues et al., 2022).

Removal of these anti-nutrients is desired 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). These treatments can reduce the vicine and convicine content of fava beans by 94%–100% (Jamalian, 1999). 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.

Cosun soaks the fava beans as part of the manufacturing process for the fava bean protein product and this process results in a significant reduction in levels of vicine and convicine in the isolated protein. The toxicity of vicine, convicine, divicine, and isouramil are discussed in the safety section below, and the concentrations of the anti-nutrients vicine and convicine in three lots of the fava bean protein ingredient are presented in Table 11. If one were to consume the RDA for protein of 50–60 g all as fava bean protein only, that would represent approximately 130 mg of vicine plus convicine/day.

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

Alkaloid	Lot 1000413751	Lot 1000413842	Lot 1000413891
Vicine	890	870	1540
Convicine	360	330	560

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 level decreases 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 54-76 mg/kg. If a person was to consume the RDA of 56 grams/day of protein, the maximum calculated exposure from this consumption is 4.25 mg of levodopa, which is 1.4% of the typical recommended daily dose (300 mg) and 0.28% 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 the historical 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. Given the publicly available information and data on the safety of fava beans and fava bean proteins, as well as the efforts to remove anti-nutritional compounds from fava beans and fava bean protein isolates intended for animal and/or human consumption, the conduct of toxicity studies was considered unnecessary and not an ethical use of animals.

It has been long recognized that fava beans contain "anti-nutritional" compounds. 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), both of 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). As reviewed in GRN 879, 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 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.

For this reason, potential adverse reactions to fava bean ingestion are limited to a small, but significant, number of males with this X-linked genetic disease. There are many genetic variants of G6PD deficiency, with one common variant 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. G6PD deficiency in the U.S. 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, it is estimated that one meal can result in an intake of 2000 mg of vicine and 1000 mg of convicine (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; Al-Dubai et al. 2021; Xin et al., 2022).

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 11) that is the subject of this GRAS dossier.

Arese et al. (2007) reported 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 11 for the proposed fava bean protein ingredient (e.g., approximately 1.2 mg/g for Lot No. 1000413842 and only slightly higher for the other three lots of fava bean protein). Arese et al. (2009) studied seven hemizygous males with G6PD deficiency using the FEVITA fava bean, which is low in vicine and convicine. The subjects were fed 450 g of the FEVITA fava bean which contained 135 - 315 mg of vicine and convicine. Given the maximum vicine and convicine level of 2.16 mg/g (Lot No. 1000413562) in the proposed ingredient, times 60 grams of protein/day (total replacement of RDA protein intake), results in an intake of approximately 130 mg vicine and convicine in the diet, an amount that is less than 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 allergic responses 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; Damiani et al., 2011; Jensen et al., 2008) clearly indicates that labeling of the presence of fava bean protein in any food product is warranted and recommended. More importantly, given the incidence of G6PD deficiency in the US, consumers may not expect fava bean protein to be present in their food. The Panel recommends that the ingredient labeling for the fava bean protein product clearly state that it contains "fava bean protein" and that 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 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 83% 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). 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.

- 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 produced from metabolism of vicine and convicine). The ingestion of untreated fava beans is associated with an occurrence 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. 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. Cosun soaks the fava beans as part of the manufacturing process for the fava bean protein

product and this results in a significant reduction in levels of vicine and convicine in the isolated protein.

- The proposed uses of the fava bean protein ingredient in specified foods 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.

- FDA has reviewed 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); GRNs No. 58, 608, and 788 (pea protein); GRN 879 (fava bean protein); and GRN 944 (rice protein hydrolysate).

- Given the long history of human consumption of fava beans as food (along with the protein contained therein), the safety of the fava bean protein ingredient derived from them is supported by a history of 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 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 will 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 Cosun's fava bean protein ingredient for use an alternative source of protein in specified foods. We unanimously conclude that the intended use of Cosun's fava bean protein ingredient, produced consistent with current good manufacturing practice (cGMP) and meeting the 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 uses and use levels of Cosun's fava bean protein ingredient in specified foods, produced consistent with current good manufacturing practice (cGMP) and meeting the 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 foods as described herein.

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

_____ _____

Paul Damian, PhD, MPH, DABT Date
Consultant
Damian Applied Toxicology, LLC

_____ _____

Carol A. Knight, PhD Date
Consultant
Knight International

_____ _____

Stanley M. Tarka, Jr., Ph.D., Fellow, ATS Date
The Tarka Group, Inc.
The Pennsylvania State University, College of Medicine

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

[redacted]

Paul Damian, PhD, MPH, DABT
Consultant
Damian Applied Toxicology, LLC

10/27/2022
Date

Carol A. Knight, PhD
Consultant
Knight International

Date

Stanley M. Tarka, Jr., Ph.D., Fellow, ATS
The Tarka Group, Inc.
The Pennsylvania State University, College of Medicine

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Paul Damian, PhD, MPH, DABT — Date
Consultant
Damian Applied Toxicology, LLC

[redacted]

10/27/22

Carol A. Knight, PhD — Date
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Knight International

Stanley M. Tarka, Jr., Ph.D., Fellow, ATS — Date
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It is our professional opinion that other qualified experts critically evaluating the same information would concur with this conclusion.

Paul Damian, PhD, MPH, DABT
Consultant
Damian Applied Toxicology, LLC

Date

Carol A. Knight, PhD
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27 October 2022

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Date

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GRN 1151 Items for Clarification

1. Regarding our Q2, we consider that your response is incomplete. According to the information on page 10 of GRN 001151, "The fava bean flour is subsequently soaked (extraction), and sodium hydroxide (NaOH) is added to adjust the pH. The slurry is separated by centrifugation and concentrated by filtration." Please clarify if the protein is extracted from fava bean flour by water or by aqueous alkaline, and if the pH of the slurry was adjusted to alkaline by NaOH. Additionally, please clarify if the pH of the resulting alkaline slurry was subsequently adjusted to neutral or acidic pH to induce protein precipitation before centrifugation and concentration.

   Response: Fava bean flour is suspended in water and the pH is adjusted from approximately pH 6.5 to pH 7.5. The fava bean protein is mainly extracted in the water phase. Nevertheless, the slight increase in pH, from pH 6.5 to pH 7.5, has a positive effect on the protein solubility and subsequently the protein yield (approximately 5% increase). Yet, the increase of pH is especially important for the filtration step, where better performance is observed at pH 7.5 compared to pH 6.5. The whole process is operated at neutral pH (pH 7.5), subsequently, the pH is not adjusted to neutral or acidic pH. In addition, the protein is not precipitated during the process.

2. Regarding our Q3(b), you responded that there is a typographical error in the Table 6 (page 12) and provided corrected sample sizes for microbiological parameters. For the administrative record, please provide a revised Table 6, which includes *Listeria monocytogenes* and sample sizes for *E. coli, Salmonella*, and *Listeria monocytogenes*.

   Response: See the following revised Table 6.

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

Specification		**Lot No. 1000413562**	**Lot No. 1000413891**	**Lot No. 1000414223**
Protein (%)	≥83	87.5	87.5	88.6
Carbohydrates (%, db)	≤10	7.9	8.4	7.1
Ash (%)	≤6	4.2	3.8	4.0
Fat (%, db)	≤2	0.3	0.3	0.3
Moisture (%)	≤9	5.8	4.9	5.4
pH	7-8	7.7	7.8	7.7
Lead (mg/kg)	<0.1	<0.04	<0.04	<0.04
Arsenic (mg/kg)	<0.1	<0.04	<0.04	<0.04
Cadmium (mg/kg)	<0.1	<0.02	<0.02	<0.02
Mercury (μg/kg)	<100	<2	<2	<2
Total Plate Count (CFU/g)	≤100,000	14000	9000	2300
Yeast and mold (CFU/g)	≤200	<1	<1	<1
E. coli (CFU/25g)	Negative	Negative	Negative	Negative
Salmonella (CFU/25g)	Negative	Negative	Negative	Negative
Listeria monocytogenes (CFU/25 g)	Negative	Negative	Negative	Negative

3. You also informed us that you wish to remove the "Processed meat products" category from the intended uses of fava bean protein. Please address the impact of this change on the dietary exposure to fava bean protein from the intended uses and provide a revised Table 10 (page 15), excluding "Processed meat products".

Response: See revised Table 10 below.

Table 10. 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
Dry-blend protein powders (e.g., protein shakes, instant protein powders)	90

Removal of the processed meat products category from the proposed uses of the fava bean protein ingredient would lower the potential exposure to the proposed fava bean protein. Cosun'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 including GRN 879 on fava bean protein, we do not realistically expect that the actual consumption of foods containing fava bean protein would result in daily consumption greater than the 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.

In summary, the proposed uses of the fava bean-derived protein ingredient will not result in an increase in the overall consumption of protein, but simply provide a well-characterized, alternative protein from fava beans for use in conventional foods.