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June 1, 2020

Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Campus Drive
College Park, MD 20740

Subject: GRAS Notification for Enzyme Treated Pea Protein

Dear Sir/Madam:

In accordance with 21 CFR part 170, subpart E, Yantai Oriental Protein Tech Co., Ltd. (Yantai), through Soni & Associates Inc. as its agent, hereby submits the enclosed notice of a claim that the food ingredient Enzyme Treated Pea Protein (>80%) described in the enclosed notification document 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.

Please note that in 2018, Yantai submitted a GRAS Notice (GRN 788) to FDA for pea protein prepared by employing base precipitation and acid neutralization to dissolve the protein. Based on marketing and commercialization experience with the pea protein (subject of GRN 788), Yantai proposes to make some changes to the production process and intends to use enzymes in the manufacturing of enzyme treated pea protein.

As regards submitting this GRAS notification, please also note that we also had email correspondence with Dr. Rachael Morissette and based on her email of December 5, 2019 (attached), we decided to submit a new GRAS notification instead of an amendment to GRN 788.

As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at sonim@bellsouth.net .

Sincerely,

Madhu G. Soni, Ph.D.

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ENZYME TREATED PEA PROTEIN

Submitted to:
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5001 Campus Drive
College Park, MD 20740-3835
USA

Submitted by::
Yantai Oriental Protein Tech Co., Ltd.
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Robert L. Martin., Ph.D.
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Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

May, 2020

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ENZYME TREATED PEA PROTEIN

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EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ENZYME TREATED PEA PROTEIN

1. Part I- SIGNED STATEMENT AND CERTIFICATION

1.1. Basis of Conclusion:

In accordance with 21 CFR § 170 Subpart E consisting of §170.203 through § 170.285, Yantai Oriental Protein Tech Co., Ltd. (Yantai) hereby informs the FDA that enzyme treated pea protein, as manufactured by Yantai, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Yantai's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. This GRAS conclusion for use of enzyme treated pea protein (>80%) as a food ingredient has been reached as per the requirements described in 21 CFR 170.220.

It should be noted that in 2018, Yantai submitted a GRAS Notice (GRN 788) to FDA for pea protein prepared by employing base precipitation and acid neutralization to dissolve the protein. Based on marketing and commercialization experience with the pea protein (subject of GRN 788), Yantai proposes to make some changes to the production process and intends to use enzymes in the manufacturing of enzyme treated pea protein. The proposed use of enzymes in the manufacturing primarily breaks the aggregation of the finished product. As discussed below, the changes to manufacturing does not significantly alter the final product specifications. The additionally processed product identity and specifications are substantially similar to the previous GRAS product (GRN 788).

1.2. Name and Address of Organization:

Yantai Oriental Protein Tech Co., Ltd.
#668 Jincheng Road,
Zhaoyuan City
CHINA 265400

1.3. Name of Substance:

The name of the substance of this GRAS assessment is enzyme-treated pea protein. The tradename is GINCORY.

1.4. Intended Conditions of use of Pea Protein:

Enzyme treated pea protein (containing >80% protein), derived from *Pisum sativum* L. seed-pods (peas), will be used as a food ingredient, formulation aid [21 CFR 170.3(o)(14)][1], nutrient supplements [21 CFR 170.3(o)(20)] [2], stabilizers and thickeners [21 CFR

[1]*Formulation aids*: Substances used to promote or produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.
[2]*Nutrient supplements*: Substances which are necessary for the body's nutritional and metabolic processes.

170.3(o)(28)][3] and texturizer [21 CFR 170.3(o)(32)][4] in conventional foods such as Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; Soups and Soup Mixes at levels ranging from 0.96 to 34.3%.

1.5. Statutory Basis for GRAS Conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6. Exemption from Premarket Approval Requirements:

Yantai has concluded that enzyme-treated pea protein (containing ≥80% protein) is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that pea protein (containing ≥80% protein), meeting the specifications cited herein, and when used as a formulation aid, nutrient supplements, stabilizers and thickeners, and texturizer is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that enzyme-treated pea protein (containing ≥80% protein), when used as described in this dossier, is GRAS based on scientific procedures.

1.7. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Ms. Dora Xu or Dr. Soni at the below addresses. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Ms. Dora Xu
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Or

[3]*Stabilizers and thickeners* : Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and bodying agents, setting agents, jellying agents, and bulking agents, etc.
[4]*Texturizers*: Substances which affect the appearance or feel of the food.

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1.8. Data exempt from Disclosure:

Parts 2 through Part 7 of this GRAS notification does not contain data or information that is exempt from disclosure under the Freedom of Information Act. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document. Therefore. All of the information contained in this dossier can be made publicly available.

1.9. Certification:

Yantai, certifies that to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Yantai, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of enzyme-treated pea protein. Yantai accepts responsibility for the GRAS conclusion that has been made for enzyme-treated pea protein as described in this dossier.

1.10. Name, position/title of responsible person who signs dossier and signature:

Ms. Dora Xu
Import and Export Manager
Yantai Oriental Protein Tech Co., Ltd.
#668 Jincheng Road,
Zhaoyuan City
CHINA 265400

Tel: +86-535-8072189
Mobile: +86-155-8959-1169
Email: doraxu@orientalprotein.com

Signature: 

1.11. FSIS/USDA – Use in Meat and/or Poultry:

Yantai does not intend to add pea protein to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

2. Part II-IDENTITY AND TECHNICAL INFORMATION

2.1. Description

The subject of this GRAS assessment, standardized enzyme treated pea protein, is derived from high quality non-GMO *Pisum sativum* L. seed-pods (peas) from US and Canada. The pea protein is prepared by water extraction followed by enzyme treatment to yield a protein rich (≥80% protein) fraction. Pea protein is a light cream colored powder with a bland and smooth taste. General descriptive characteristics and properties of pea protein manufactured by Yantai are presented in Table 1. These properties are not different from properties described for pea protein in GRAS notice GRN 788 submitted by Yantai.

Table 1. General Descriptive Characteristics of Enzyme Treated Pea Protein

Parameter	Description (Yantai, 2019)*
Botanical source	*Pisum sativum* L.
Source synonyms	*Pisum arvense* L., *Pisum humile* Boiss. & Noe,
Plant part used	Peas; seeds
Synonyms of part used	Golden pea; Yellow pea; Bush pea; False lupine
Product Appearance	Powder
Color	Light cream
Odor	Bland
Taste	Smooth
Storage	Store in a well closed, air tight container, protected from light and moisture, in a dry and cool place
Shelf life	Two years

*Based on information provided by Yantai (2019)

The taxonomic classification of the source material, *Pisum sativum* L., is provided in Table 2. As described in the USDA Plant Fact Sheet[5], the pea is a cool-season annual vine that is smooth and has a bluish-green waxy appearance. Vines can be up to nine feet long; the stem is hollow; and, the leaves are alternate, pinnately compound, and consist of two large leaf-like stipules, one to several pairs of oval leaflets, and terminal tendrils. Flowers have five green fused sepals and five white, purple or pink petals of different sizes. The fruit is a closed pod, 1 to 4 inches long that often has a rough inner membrane. Ripe seeds are round, smooth or wrinkled, and can be green, yellow, beige, brown, red-orange, blue-red, dark violet to almost black, or spotted (Pavek, 2012). A picture of split yellow peas is shown in Figure 1.

Table 2. Taxonomic Classification of *Pisum sativum* L.

Rank	Scientific Name – Common Name
Kingdom	Plantae- Plants
Subkingdom	Tracheobionta- Vascular plants
Superdivision	Spermatophyta- Seed plants
Division	Magnoliophyta- Flowering plants
Class	Dicotyledoneae
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Pisum*
Species	*Pisum sativum* L.

[5] Available at: https://plants.usda.gov/core/profile?symbol=pisa6

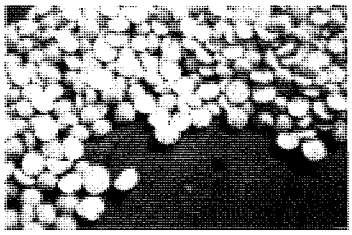

Figure 1. Typical Picture of Split Yellow Peas

2.2. Specifications and Identity

Yantai has established the food-grade specifications for enzyme treated pea protein. These specifications are presented in Table 3 and are substantially similar to our previous GRAS notice GRN 788. The protein content of the final product is ≥80%. The other components consist of fat, fiber and moisture. The product identity and quality is standardized by parameters such as the content of protein, crude fiber, moisture, ash, fat, and pH. The product specification also includes microorganism load and heavy metals levels. In order to demonstrate conformance with the food-grade specifications, Yantai has provided batch analysis data from five non-consecutive batches of enzyme treated pea protein. This data, presented in Appendix I, support the consistent manufacturing process. In order to further demonstrate the low and acceptable levels of aflatoxin and heavy metals, three additional batches of enzyme treated pea protein were analyzed at an independent laboratory and the reports are provided as Appendix II. Yantai recognizes that lot-to-lot variations occur, depending upon a variety of factors. However, the final product will comply with the standard specifications set forth in Table 3.

Table 3. Food Grade Specifications of Enzyme Treated Pea Protein and Comparison with GRN788*

Parameters	Current GRAS Values	Previous GRAS (GRN 788) Values	Assay method
Protein (n x 6.25)	≥ 80%	≥ 80%	AOAC 981.10
Crude fiber	≤ 0.5%	NA	AOAC991.43
Total carbohydrate	NA	≤ 3%	NLEA
Moisture	≤ 10%	≤ 10%	AOAC 925.09
Ash	≤ 8%	≤ 8%	AOAC 942.05
Crude Fats	≤ 10%	≤ 10%	AOAC 945.18
pH	6.5-8.5	6.5-8.5	Q/DFS0002S
Particle size	100 mesh	100 mesh	Sieve method
Heavy metals			
Lead	< 0.1 ppm	< 0.1 ppm	EN ISO17294-2
Arsenic	< 0.1 ppm	< 0.1 ppm	EN ISO17294-2
Cadmium	< 0.3 ppm	< 0.3 ppm	EN ISO17294-2
Mercury	< 0.02 ppm	< 0.02 ppm	EN ISO17294-2
Microbiological assays			
Total plate count	< 30000 cfu/g	< 30000 cfu/g	AOAC 990.12
Yeasts and Molds	< 50 cfu/g	< 100 cfu/g	AOAC 997.02
Salmonella	N.D./25g	Absent /10 g	AOAC 967.26
Escherichia coli	N.D./g	Absent /1 g	AOAC 991.14
Staphylococcus aureus	N.D./25g	Absent /1 g	AOAC975.55
Other Contaminants			
Aflatoxin B1 (G1+G2+B1+B2)	<5 μg/kg	<5 μg/kg	EN14123
Pesticides	Complies	Complies	BS EN 15662:2008

*Based on information provided by Yantai (2019); N.D.= none detected; NA= Not available

2.3. Manufacturing Process

The standardized enzyme treated pea protein is produced from yellow peas (*Pisum sativum* L.) according to current Good Manufacturing Practices (cGMP) at Yantai Oriental Protein Tech Co., Ltd. facility located in Shandong, China (Mainland). The production facility is ISO certified (9001:2015) and follows the HACCP protocols. Additionally, the facility has passed certifications such as CIQ registered, FDA registered under number 13488197390, KOSHER and HALAL, and has a QS Quality Safety Food Manufacturing Permit. The production process is provided in Figure 2.

In brief, the manufacturing process of pea protein involves selection of high quality dry peas from the suppliers. The peas obtained are cleaned and subjected to dehulling of the grains followed by grinding. The grinded pea powder is mixed with water resulting in the liquid mixture of protein and starch that is subjected to homogenization. Base precipitation and acid neutralization are used to dissolve the protein out from the mixture fluid. This mixture is processed to separate the starch liquid and protein liquid. The protein liquid is centrifuged and food grade enzymes (as described below and in Table 4) are added to break the aggregation. The protein is isolated and subjected to flash evaporation and drying by using spray dryers to obtain the final dry product. The heated spray drying also serves to inactivate the enzymes. The protein thus obtained is tested to ensure that it meets the specifications and packaged.

The preparation procedure assures a consistent and high-quality product. During manufacturing, no solvents other than water are used; the protein is an aqueous preparation derived from peas. The processing aids such as acid and base used are food grade and in compliance with the current regulations for such agents for food production. The food grade enzymes used is a mixture of four individual enzymes. All relevant details of the enzymes are provided in Table 4. The enzymes, used as processing aids in the treatment of pea protein, are used at levels not to exceed current good manufacturing practice (cGMP). The enzymes are added to achieve the desired specific functionality of the enzyme treated pea protein. The addition of the enzymes breaks the protein aggregation[6]. This variable is not considered significant to change the GRAS status of the pea protein product. All enzymes are provided by AB Enzymes GmbH, Germany. Information on the enzymes are described in Table 4. However, if required, Yantai will share additional information separately as confidential information to FDA.

[6] Protein aggregation is a biological phenomenon in which mis-folded proteins aggregate (i.e., accumulate and clump together) either intra- or extra-cellularly. Aggregated proteins can be merely a nuisance factor or it can become a technical problem in the utility and production of products using proteins. By reducing the molecular weight of the protein, this problem can be mitigated.

Table 4. Details of Enzymes Used in Production of Enzyme Treated Pea Protein*

Name of Enzyme	Source organism	Function of Enzyme	Activity; Use Levels	Safety of Enzyme
Cellulase enzyme CAS No: 9012-54-8 IUB-No.: 3.2.1.4 ROHAMENT® CL	*Trichoderma reseei* classical strain	Hydrolyzing non-starch polysaccharides; viscosity reduction	Activity- 15,000 ECU/g; Use level- 50-100 g/t at 50°C for 1-2 hr	Meets purity specification of FCC; Source organism- Non-pathogenic, non-toxigenic. Microorganism not present in final product
Polygalacturonase enzyme preparation CAS No: 9032-75-1 IUB-No.: 3.2.1.15 ROHAMENT® MA	Genetically modified strain of *Trichoderma reesei* carrying a polygalacturonase gene from *Aspergillus tubingensis*	Pectinolytic enzyme- breaks down pectin; reduces viscosity	Up to 20 mg TOS/kg of final food	Complies with FCC specs; *T. reesei* - Non-pathogenic; non-toxigenic. Production strain is *T. reesei* RF6197 (GRAS: GRN 557)
Alkaline protease enzyme IUB-No.: 3.4.21.65 CAS No: 9014-01-1 COROLASE® APC	*Bacillus licheniformis* strain	Endopeptidase activities; Hydrolyzing proteins	Use levels- 0.05-1.0%	Fulfills purity specifications of FCC; Source organism- Non-pathogenic, non-toxigenic. Microorganism not present in final product
Protease enzyme- CAS No: 9001-92-7 IUB-No: 3.4.24.28 COROLASE® 7089	*Bacillus subtilis*	Hydrolyzing proteins at and around neutral pH	Use levels- 0.01-0.5%	Meets purity specification of FCC; Source organism-Non-pathogenic, non-toxigenic. Microorganism not present in final product

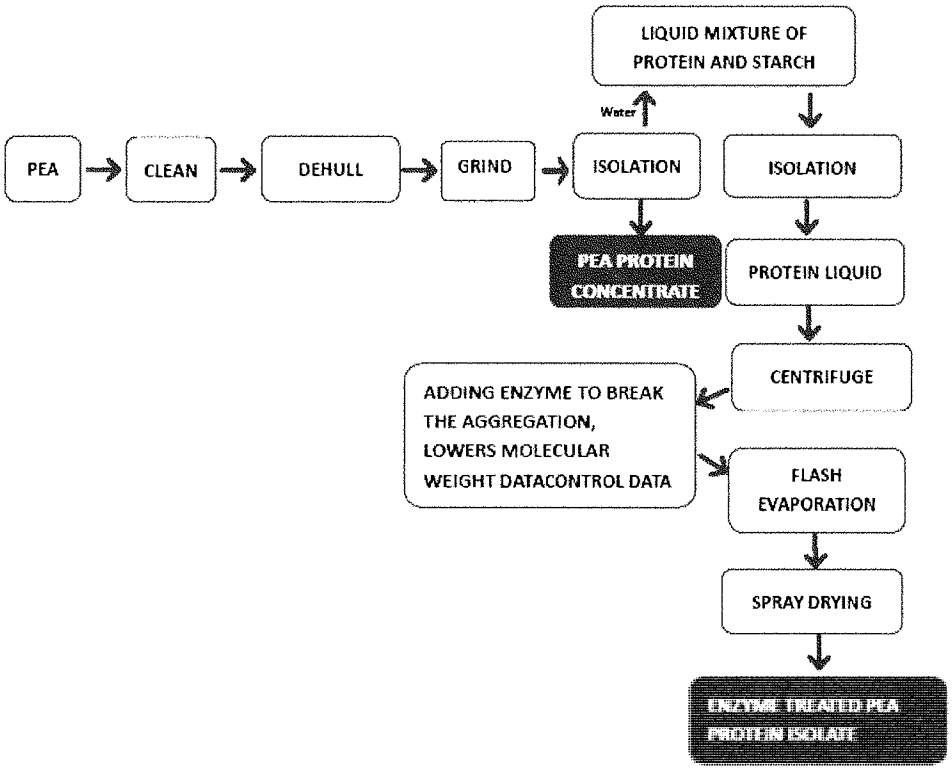

Figure 2. Manufacturing Process of Enzyme Treated Pea Protein

2.4. Compositional and Nutritional Analysis

Yellow peas offer many nutritional benefits, including being rich in dietary protein and fiber. The nutritional composition of enzyme treated pea protein (80% protein) is provided in Table 5. In Table 5, the nutritional composition of pea protein, the subject of this GRAS assessment, is also compared with unprocessed peas (green seeds, split, mature seeds, raw) and the subject of previous GRAS notice GRN 788 submitted by Yantai. The comparison with previous GRAS notice shows substantial similarity except for some minerals (sodium potassium, calcium) that are concentrated in the enzyme treated pea protein.

Table 5. Comparison of Nutritional Composition of Peas, Enzyme Treated Pea Protein and GRN 788

Nutrient	Content per 100 g		
	Pisum sativum[1]	**Enzyme Treated Pea Protein**	**Previous GRAS (GRN 788)**
Protein (g)	23.82	78.5 (as is); 84.12 (dry matter basis)	81.4
Total Fat (g)	1.16	9.20	9.6
Saturated Fat (g)	0.161	1.66	1.9
Cholesterol (mg)	0	N.D.	0
Total Carbohydrates	63.34	1.57	2.5
Total Dietary Fiber (g)	25.5	<0.5	1.6
Sugars (g)	8.00	0.59	0.6
Sodium (mg)	15	120	0.886
Potassium	0.823	346	0.0978
Calcium (mg)	37	46	17.9
Iron (mg)	4.82	6.35	32.6
Vitamin D3 (µg)	7	<0.25	0
Total Calories (Kcal)	352	403	422

Source: United States Department of Agriculture, 2013. National Nutrient Database for Standard Reference, Nutrient data for 16085, Peas, split, mature seeds, raw. Release 28. N.D.= Not detected

2.4.1. Amino Acid Profile

The amino acid profile of the subject of this GRAS, enzyme treated pea protein (≥80% protein), is presented in Table 6. The amino acid profile in Table 6 includes all amino acids such as essential, conditionally essential, and non-essential, and is compared with the source material (peas) amino acid. The information in Table 6 suggest that the amino acid profile of pea protein is similar to the amino acid composition of other peas as well as the pea protein subject of previous GRAS notice (GRN 788), except for some minor variations in a few amino acids. The comparative amino acid profile with unprocessed peas (source material) as well as pea protein concentrate (subject of GRN 788) suggest that the manufacturing process is unlikely to significantly affect the levels of the amino acids in the final product. It is recognized that pea protein is an incomplete protein. However, it is expected that pea protein will not be the sole source of protein in the diet. Therefore, other complementary proteins in the diet will compensate for the amino acids that are low in pea protein[7].

[7] Available at: https://www.accessdata.fda.gov/scripts/interactivenutritionfactslabel/factsheets/protein.pdf.

Table 6. Comparison of Amino Acid Profile of Peas and Pea Protein (enzyme treated and not treated)

Amino Acids	Peas		Pea Protein** (GRN 788)	Enzyme Treated Pea Protein
	g/100g*	% of Total Amino Acid*	% of Total Amino Acid	% of Total Amino Acid
Essential amino acids				
Phenylalanine	1.132	4.82	4.09	3.77
Valine	1.159	4.94	3.80	3.71
Threonine	0.872	3.72	3.30	3.16
Tryptophan	0.275	1.17	0.68	0.72
Methionine	0.251	1.07	0.79	0.93
Isoleucine	1.014	4.32	3.73	3.67
Leucine	1.760	7.50	6.59	6.98
Lysine	1.772	7.55	5.88	5.97
Histidine	0.597	2.54	1.97	2.00
Conditionally Essential amino acids				
Arginine	2.188	9.33	6.26	6.81
Cysteine	0.373	1.59	0.99	1.01
Glycine	1.092	4.65	3.09	3.44
Glutamic	4.196	17.88	12.33	12.55
Proline	1.014	4.32	3.95	3. 85
Serine	1.080	4.60	3.70	4.23
Tyrosine	0.711	3.03	3.11	3.03
Other amino acids				
Aspartic Acid	2.896	12.34	9.08	9.28
Alanine	1.080	4.60	3.32	3.66
Total Amino Acids	23.462	100	76.69	78.67

*Source: United States Department of Agriculture. National Nutrient Database for Standard Reference, Nutrient data for 16085, Peas, split, mature seeds, raw. Release 26. 2013; **Subject of GRN 788

3. Part III- DIETARY EXPOSURE

3.1. Intended Technical Effects and Food Categories

Yantai intends to use enzyme treated pea protein for the following technical effects as defined in 21 CFR 170.3(o) formulation aid (14), nutrient supplement (20), stabilizers and thickeners (28) and texturizers (31). Enzyme treated pea protein will be used as a substitute for, and/or in conjunction with, soy protein and whey protein in conventional food products. The targeted foods include snacks and cereals, high protein foods, gluten-free foods (pasta, baking), sports foods (mix, bars), and other conventional food products needing protein-source properties. The effects of enzyme treated pea protein in these foods include, promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, retention of oils and gelation, increase of water-solubility, and source of nutrients. The intended use levels of enzyme treated pea protein and food categories are presented Table 7 which are the same as what was discussed in GRN 608 and GRN 788.

It is recognized that there are Standard of Identity requirements for some of the foods and these foods will not be referred by their commonly recognized names such as milk, chocolate or yogurt. Foods that are intended for infants, such as infant formulas are excluded from the list of intended food uses of the subject enzyme treated pea protein.

3.1.1. Intended Uses and Estimated Intake

Enzyme treated pea protein by Yantai is intended for use in the same foods, and at identical use levels, mentioned in the GRN 608 and also in GRN 788. The substance mentioned in GRN 608 (Axiom Foods, 2015; FDA 2018) has been reported to contain ≥80% pea protein, which is the same as the subject of this present GRAS assessment. Enzyme treated pea protein will be added to Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; Soups and Soup Mixes at levels ranging from 0.96 to 34.3%. The use levels are based on the purity criteria of 80% protein.

As indicated above, enzyme treated pea protein is intended for use in the same foods, and at identical levels of addition, as notified by Axiom Foods in GRN 608 and by Yantai in GRN 788. The proposed uses and use levels of enzyme treated pea protein are provided in Table 7. The intended use of enzyme treated pea protein in the same foods and at the same levels as those in GRN 608 is not expected to noticeably affect the intake of enzyme treated pea protein in the overall diet of the public from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. In GRN 608 (Axiom Foods, 2015), estimates for the intake of pea protein were determined using the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2011-2012. The enzyme treated pea protein by Yantai is used as a substitute for, or in conjunction with, other proteins in conventional food products; and, that therefore, the enzyme treated pea protein will not contribute any additional exposure to protein for consumers.

Table 7. Summary of the Individual Proposed Food-Uses and Use-Levels of Enzyme Treated Pea Protein*

Food Category	Food-Uses	Proposed Use Level of Enzyme Treated Pea Protein (%)[1]
Baked Goods and Baking Mixes	Breads	4.8
	Rolls	4.8
	Bagels	4.4
	English Muffins	4.4
Beverages and Beverage Bases	Non-Milk Based Meal Replacements	1.04
Breakfast Cereals	Ready-to-Eat Breakfast Cereals	4.4 - 16
Dairy Product Analogs	Soy/Imitation Milks	1.04
Fats and Oils	Margarine[2]	17.12
	Salad Dressings	8
Grain Products and Pastas	Health Bars and Grain-Based Bars Containing Fruit and Vegetable[3]	20
Milk Products	Flavored Milk Drinks	1.04
	Milk-Based Meal Replacements	1.04
	Yogurt (Regular and Frozen)[2]	1.1 - 2.0
Plant Protein Products	Meat Alternatives	1 - 34.3
Processed Fruits and Fruit Juices	Fruit Juice[2]	1.04
	Fruit Nectars	1.04
	Fruit-Flavored Drinks	1.04
	Fruit Smoothies	20
Processed Vegetables and Vegetable Juices	Vegetable/Tomato Juice Including Vegetable Smoothies[4]	20
Soups and Soup Mixes	Prepared Soups, Dry Soup Mixes, and Condensed Soups	0.96

*Adapted from GRN 608 (Axiom Foods, 2015) and GRN 788 (Yantai, 2018); [1] Use levels are calculated based on the purity criteria of 80% protein; [2] These food-uses represent non-standardized food products; however, in order to obtain a conservative intake estimate, surrogate codes for the standardized food products were chosen; [3] It should be noted that there were no food codes identified for grain-based bars containing vegetable. However, for this assessment, it is assumed that the estimated consumption of grain-based bars containing fruit would also reflect the intake of grain-based bars containing vegetable; [4] There were no food codes identified for vegetable smoothies within the NHANES dataset; however, the intake estimate for vegetable-based juices is expected to be representative of the intake from both vegetable-based juices and vegetable smoothies. It was assumed that a consumer of vegetable-based juices would drink a vegetable smoothie in replacement of a vegetable-based juice.

The intake analysis (Table 8) revealed that approximately 98% of the total U.S. population was identified as potential consumers of pea protein from the proposed food uses (Axiom Foods, 2015). The estimated mean and 90th percentile all-user intakes of pea protein was determined as 10.3 g/person/day (181 mg/kg bw/day) and 17.3 g/person/day (388 mg/kg bw/day), respectively. As described in GRN 608, among the individual population groups, male adults were determined to have the greatest mean and 90th percentile all-user intakes of pea protein on

an absolute basis, at 11.2 and 20.5 g/person/day, respectively. A summary of dietary intake calculations from the intended food categories is presented in Table 8. For safety assessment purposes the highest 90th percentile intake of 20.5 g/person/day, noted in male adults, was considered. Similar to that as described in GRN 608, Yantai also intends to market enzyme treated pea protein as a directly consumed supplemental protein at levels ranging from 5 to 15 g/serving when used as a protein supplement in sports nutrition or meal replacement applications, where consumers prepare their own beverages. These products can be used by consumers two times per day for lower protein use levels and one time per day for higher use levels. Thus, the maximum intake of enzyme treated pea protein from its proposed uses in sports nutrition will be 30 g/person/day.

Table 8. Summary of the Estimated Daily Intake of Enzyme Treated Pea Protein from Proposed Food-Uses*

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 3	5.9	12.4	83.2	683	7.1	13.4
Children	4 to 11	9.4	14.8	99.9	1,347	9.4	14.8
Female Teenagers	12 to 19	10.5	16.5	98.8	526	10.6	16.5
Male Teenagers	12 to 19	11.8	18.7	98.5	508	12.0	19.7
Female Adults	20 and up	9.7	16.1	99.8	2,204	9.7	16.1
Male Adults	20 and up	11.1	20.3	98.8	2,067	11.2	20.5
Total Population	**All Ages**	**10.1**	**17.2**	**98.4**	**7,335**	**10.3**	**17.3**

*Adapted from GRN 608 (Axiom Foods, 2015)

In addition to the above described two GRAS notices (GRN 608 and 788), in three additional more recent GRAS notices (GRN 851, GRN 803 and 804), use of pea protein in foods has been proposed (FDA, 2020; FDA, 2019a; FDA, 2019b). In GRN 803, the use of pea protein is proposed in various foods, including meat and poultry products, at levels that will not increase the consumer's overall exposure to protein (Ingredion, 2018). In GRN 804, the use of pea protein was proposed in foods such as such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, coffee and tea, confections and frostings, dairy product analogs, egg products, fats and oils, fish products, frozen dairy desserts, fruit and water ice, gelatins, puddings, and fillings, grain products and pastas, gravies and sauces, meat products, milk products, nut and nut products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes at use levels ranging from 1 to 35 g pea protein per 100 g of food. In this GRAS notice, the notifier, Burcon NutraScience Corporation (Burcon, 2018), provided detailed intake estimates, on consumer-only basis; the resulting mean and 90th percentile intakes of protein by the total U.S. population from all proposed food-uses, were estimated to be 28.42 g/person/day (0.47 g/kg bw/day) and 51.62 g/person/day (0.97 g/kg bw/day), respectively. These GRAS notices received a "no question" letter from FDA for the proposed uses of pea protein.

Yantai does not intend to add enzyme treated pea protein in meat and poultry products; however, for other products, Yantai may change the use levels of enzyme treated pea protein

similar to those described in GRN 804 (except meat and poultry). The resulting mean and 90th percentile intake of 28.42 and 51.62 g/person/day, respectively, from GRN 804 is also applicable to enzyme treated pea protein and is expected to be lower than these values as enzyme treated pea protein will not be added to meat and poultry products.

3.2. Background Exposure to Peas and its Protein

The available information indicate that peas are a commonly consumed food in the US. As per USDA nutritional database, peas is a general food product category under legumes. The USDA National Nutrient Database categorized peas under a general food group that includes several products such as Legumes and Legume Products (6), Soups, Sauces, and Gravies (15), Vegetables and Vegetable Products (31), and Baby Foods (3). The USDA database has listed 55 food products that contain peas (USDA, 2013). The Reference Amount Customarily Consumed (RACC) for peas is 85 g/serving (FDA, 2013). This figure was promulgated by the FDA based on data on consumption of peas reported in the 1987-88 Nationwide Food Consumption Survey and the 1989-90 and 1990-91 Continuing Surveys of Food Intakes by Individuals, and it represents an average intake of peas by Americans at a single serving. Multiple servings during the day or larger-than-average servings result in a daily intake of peas well in excess of the 85 g average single serving. The FDA recommends that the 90th percentile of intake can normally be approximated by doubling the mean (FDA, 2006). This suggests that a reasonable estimate of the 90th percentile daily intake of peas is 170 g.

Based on the above information, and as peas have been reported to contain approximately 24.55% protein, the intake of protein from the consumption of peas at the 90th percentile in the U.S. is estimated to be 41.7 g/person/day. The 90th percentile intake of enzyme treated pea protein from the intended uses of enzyme treated pea protein in different food categories of less than 51.62 g/person/day is higher than the 90th percentile daily intake of pea protein resulting from the dietary consumption of peas.

In addition to the above information, Smiciklas-Wright et al. (2002) reported the mean and 90th percentile total for dried beans plus peas consumption in the U.S. for all individuals to be 96 and 197 g/person/day, respectively. This data does not separate beans from peas and it is likely that, for some individuals, the entire consumption of this food category may come from peas. As peas contain approximately 24.55% protein, the intake of protein from consumption of peas at the mean and 90th percentile in the U.S. is estimated to be 23.57 and 48.36 g/person/day, respectively. The 90th percentile intake of enzyme treated pea protein from the proposed uses of the enzyme treated pea protein in different food categories of 51.62 g/person/day is similar to the daily intake of pea protein resulting from the consumption of peas (dried bean and peas). As enzyme treated pea protein will not be added to meat and poultry products, the likely intake will be less than 51.62 g/person/day. The intended use of enzyme treated pea protein is unlikely to add significantly to the existing intake of protein from consumption of peas or from other sources, as the use of enzyme treated pea protein as a macro-ingredient will likely replace the intake of other similar foods and not in addition to the other food products.

4. Part IV- SELF LIMITING LEVELS OF USE

Excessive amounts of enzyme treated pea protein is unlikely to be added to food products, given the water binding properties of protein ingredients. Excessive use levels can make the food product dry, gummy and difficult to prepare. The projected use levels are supported by the current protein levels in marketed products.

5. Part V- EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of enzyme treated pea protein in this document is not based on common use in foods before 1958. The GRAS assessment is based on scientific procedures. As described below, the source material for enzyme treated pea protein, peas have been commonly used in foods prior to 1958. Notwithstanding this, it is reasonable to conclude that, humans are exposed to pea protein from consumption of peas, suggesting that it was present in foods prior to 1958.

6. Part VI- NARRATIVE

6.1. Safe Uses of Peas

The available information suggest that peas are consumed safely as staple for centuries by human beings around the world. Peas is one of the earliest food crops. Peas are part of the legume family, which consists of plants that produce pods with seeds inside. The history of food uses of legumes is intertwined with that of human civilization. Archaeologists exploring the "Spirit Cave" on the border between Burma and Thailand, revealed the evidence of consumption of wild peas by humans that dates back to 9750 BC. Peas are one of the oldest cultivated crops in the world. In Neolithic sites in China dating as far back as 7000 to 6000 BC domesticated peas were found (Simoons, 1991). Peas cultivation brought stability to once nomadic tribes, and made it possible for peas to be brought by travelers and explorers into the countries of the Mediterranean as well as to the Far East. Because of their content of protein, starch and other nutrients, pulses, including peas, have long been important part of the human diet. Amon the crops cultivated by human beings, the field pea (*P. sativum,* L.) was the first crops. It is believed that main center of pea developments was middle Asia, including northwest India and Afghanistan, as pea cultivation requires cool weather.

In some counties such as Afghanistan, Iran, and Ethiopia, wild field peas of related species can still be found. Yellow or green cotyledon varieties of peas known as dry, smooth or field peas are grown around the world for human and animal consumption. Peas (*P. sativum*), or field peas, originated in southwest Asia are now cultivated in temperate areas (Aykroyd and Doughty, 1982). Peas that are cultivated have been classified into garden peas (*P. sativum* sp. hortense) and filed peas (*P. sativum* sp. arvense). Garden peas are identified by the wrinkled nature of their seed and cotyledon, while field peas commonly known as dry peas. Among the different varieties of dry peas grown throughout the world, two main varieties are the dry green cotyledon and the dry yellow cotyledon. Split peas are simply dry peas (green, yellow, or red) that have been split.

Legumes became an important staple by providing essential supplementing protein, as well as key vitamins and minerals, in times when meat was not available. Given the high content of protein, pulses was the major reason for the development of alternative source of protein, especially in Europe. Pulses, including peas, remains an important dietary component of many millions of people around the world, often combined with a cereal crop to provide energy. In developing countries, pulses are considered to be a very important group of plant food stuffs as a cheap source of protein when animal protein is scarce. A significant part of the human population relies on legumes, including peas, as staple food for subsistence, particularly in combination with cereals. The USDA's My Plate Guidelines for food intake recommends consuming at least three cups of dry beans and peas per week.

Currently, pulses crops, including peas, provide over 12% of the plant protein consumed by humans globally, more than either potatoes or vegetables (FAO, 2009). Generally, pulses, including peas, are considered as a low cost source of dietary protein, fiber and starch. The leading pea-producing countries around the world include Canada, Russia, China, USA and India (Dahl et al., 2012). The annual production of peas worldwide is reported to be over 10 million tons. Europe accounts for 50-75% of world pea production. Lazanyi (2005) reported yearly rise of pea production during the 1980's by 6-10% in developed countries of the European Union. In the 1990's, the European Union produced 4-5 million tons of dry peas, of which 3-4 million tons

were used for feed and 1 million tons for export. Consumed fresh or dry, peas are a major staple diet throughout the world. Dry peas are a valued source of vegetable protein. Peas provide a cost-effective and convenient source of protein, complex carbohydrates, vitamins and minerals (Dahl et al., 2012). In keeping with the increasingly popular use of vegetable proteins as functional ingredients in the food industry, dry peas have proven especially sought after due to their wide acceptance as part of the human diet. Pulses, including peas, are cultivated on about 3 million acres in the USA, with an annual production value in excess of $1 billion. The high nutrient density of peas makes them a valuable food commodity, capable of meeting the dietary needs of the estimated 800-900 million undernourished individuals around the world (Dahl et al., 2012). Consumption of dry peas as a food is primarily concentrated in developing countries, where grain legumes represent a useful complement to cereal-based diets as a relatively inexpensive source of high quality protein (Lazanyi, 2005). In developing countries, shortage of grain legumes has adverse effects on the nutritional standard of poor people.

In summary, the available information suggest that there is common knowledge that human beings have regularly consumed peas and thus the protein present in it, without any safety concerns.

6.2. Nutritional Properties of Pea Protein and Safety

Similar to other legumes, peas provide protein, complex carbohydrates, vitamins and minerals to millions of people and animals worldwide. Approximately half of the protein in the human diet is derived from cereals. As cereals are a poor source of the amino acid lysine, other protein sources are required to enrich the human diet (Coyne et al., 2005). Lysine-rich legumes (including peas) make for an excellent complimentary protein source to cereals. The available information suggest that the rate of digestive utilization of protein in peas is high, similar to that found for fava beans and much higher than that of lentils, chickpeas, and beans (Urbano et al., 2003).

6.2.1. Comparison of Enzyme Treated Pea Proteins with other Protein

For human beings, dietary sources of protein include both animals and plants. The animal protein sources include meats, dairy products, fish and eggs, while the plant proteins are grains, legumes and nuts. It is well recognized that quality of protein depends on the source. Given the differences in protein sources, in Table 9, the nutrient profile of pea protein, the subject of present GRAS, is compared with other proteins such as soy, whey and casein. This comparison reveals that nutritional profile of enzyme treated pea protein is substantially similar to other commonly available proteins. FDA has recognized several sources of protein such as whey (21 CFR 184.1979), reduced lactose whey (21 CFR 184.1979a), reduced minerals whey 21 CFR 184.1979b, and whey protein concentrate (21 CFR 184.1979c) as direct food substances affirmed as GRAS. Similarly, peptones, a variable mixture of polypeptides, oligopeptides, and amino acids produced by partial hydrolysis of casein as well as from soy protein isolate is GRAS (21 CFR 184.1553). Additionally, whey protein isolate and dairy product solids that has been subject of GRN 37 received no question letter from the FDA. The available information suggest that enzyme treated pea protein, the subject of present GRAS assessment, is similar to other common proteins that are used in several marketed products as per FDA regulation.

Table 9. Comparison of Nutritional Profile of Enzyme Treated Pea Protein with Other Common Proteins

Parameters	Enzyme Treated Pea Protein*	Optimum Soy	Optimum Whey	Cellucor Whey	Optimum Casein	Muscle Pharm Whey	Weight Loss Lab. Whey
Serving size (g)	35	31.5	39	33	34	38	40
Protein (g)	29.44	25	30	25	24	38	20
Total Fat (g)	3.22	1.5	1	1.5	1	1.5	2
Saturated Fat (g)	0.58	0	0.5	0.5	0.5	1	1
Cholesterol (mg)	0	0	5	35	15	75	55
Total Carbohydrates (g)	0.55	2	2	3	3	7	15
Dietary Fiber (g)	0.17	NA	NA	1	1	1	8
Sugars (g)	0.21	0	1	1	1	3	5
Sodium (mg)	42	330	190	130	280	159	95
Total Calories	141	120	140	120	120	150	160
Calories from Fat	29	15	10	10	10	20	15

*Based on data from Table 5. Adapted from GRN 608 and GRN 788

6.2.2. Comparison of Amino Acid Profile of Pea and other Proteins

In an attempt to understand the similarity and differences between enzyme treated pea protein (subject of present GRAS) and other commonly marketed protein, the amino acid profile of enzyme treated pea protein is compared with amino acid profile of peas and with other currently marketed proteins such as whey, soy products, pea protein from GRN 608 and pea protein from GRN 788 (Table 10). This comparison shows that the amino acid profile of enzyme treated pea protein is substantially similar to peas and other commonly marketed high-protein concentration products. As pea and soybeans are legumes, there are some similarities in the amino acid profile of their proteins. Similar to soy protein, pea protein has a high content of arginine and low content of methionine, as compared to whey protein. Additionally, in pea and soybean proteins the ratio of arginine: lysine is higher as compared to casein.

In an extensive chemical analysis of pea protein isolate and concentrate, Tomoskozi et al. (2001) studied the gross chemical composition, amino acid content, and functional properties (solubility profile, emulsifying--and foaming properties, water--and oil absorption) of pea protein concentrate and isolate. These investigators also compared the findings from pea protein analysis with soy and lupin protein product parameters. The findings from these investigations revealed that the solubility of pea protein isolates is similar to other legume proteins, such as soy, and that pea protein isolate provided an advantageous amino acid composition and acceptable functional properties over soy protein. These investigators concluded that pea protein concentrate and isolate can be successfully used in bakery products for enrichment in protein and improvement of biological value.

Table 10. Comparison of Amino Acid Profile of Enzyme Treated Pea Protein with Other Proteins

Amino Acids	*Pisum sativum* % of total amino acids (AA)	Present GRAS % of total AA*	GRN 788 pea protein % of total AA[1]	GRN 608 pea protein % of total AA[2]	Whey[2] % of total AA	Soy[2] % of total AA
Alanine	4.60	3.66	3.32	3.60	4.82	4.07
Arginine	9.33	6.81	6.26	7.07	3.16	7.57
Aspartic Acid	12.34	9.28	9.08	9.79	12.26	11.58
Cysteine	1.59	1.01	0.99	0.84	2.28	1.25
Glutamic Acid	17.88	12.55	12.33	14.01	15.41	19.80
Glycine	4.65	3.44	3.09	3.44	2.00	4.09
Histidine	2.54	2.00	1.97	2.06	2.41	2.61
Isoleucine[3,4]	4.32	3.67	3.76	4.06	6.41	4.83
Leucine[3,4]	7.50	6.98	6.59	7.08	11.60	7.70
Lysine[3]	7.55	5.97	5.88	6.15	9.83	6.04
Methionine[3]	1.07	0.93	0.79	0.90	2.35	1.28
Phenylalanine[3]	4.82	3.77	4.09	4.54	3.56	5.21
Proline	4.32	3.85	3.95	3.62	6.28	5.63
Serine	4.60	4.23	3.70	4.32	6.24	5.21
Threonine[3]	3.72	3.16	3.30	3.11	8.44	3.56
Tryptophan[3]	1.17	0.72	0.68	0.87	1.80	1.27
Tyrosine	3.03	3.03	3.11	3.20	3.26	3.66
Valine[3,4]	4.94	3.71	3.80	4.39	6.09	4.65

*Provided by Yantai; [1]Adapted from GRN 608 and [2]GRN 788; [3]Essential Amino Acid; [4]Branched Chain Amino Acid

6.2.3. Human Protein Requirements

Along with fats and carbohydrate, protein is an important macronutrient that is required in the daily diet. It is essential to maintain and build body tissues and muscle. Thus, protein is an important building block of bones, muscles, cartilage, skin, and blood. The Recommended Dietary Allowance (RDA) for protein is a modest 0.8 g protein/kg bw/day. For an individual weighing 60 kg, this will be 48 g/person/day which is sufficient to meet basic nutritional requirements. The Institute of Medicine (IOM, 2005) estimated the background dietary intakes of protein for the U.S. population using USDA Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998. The mean and 90th percentile protein intake for adults ranged from 56 to 104 g/day and from 76 to 142 g/day, respectively.

Adequate consumption of high-quality proteins from animal products (*e.g.*, lean meat and milk) is essential for optimal growth, development, and health of humans. Lack of sufficient dietary intake of protein has been associated with adverse effects in human health and development. For infants, aged 0 to 6 months, adequate intake (AI) of protein was set at 1.52 g/kg bw/day. In the absence of sufficient information, the IOM concluded that the Tolerable Upper Intake Levels (UL) for total protein or individual amino acids cannot be established. In a review article, Bilsborough (2006) suggested that the maximum daily protein intake of approximately 176 g for an 80 kg individual on a 2867 kcal/day diet is safe.

6.3. FDA Evaluation of GRAS Notices on Pea Protein

Since 2006, FDA has received and reviewed five GRAS notices for use of pea protein in foods. All these GRAS notices received 'no question' letter from FDA. A summary of all these GRAS notices reviewed by FDA is provided in Table 11.

Table 11. Comparison of the Subject of Present GRAS, Enzyme Treated Pea Protein with other FDA Accepted GRAS Notices

Parameters	Current GRAS*	GRN 851	GRN 804	GRN 803	GRN 788	GRN 608	GRN 581	GRN 182
Pea protein	Enzyme treated	Isolate Enzyme treated	Concentrate	Concentrate	Concentrate	Concentrate	Unhydrolyzed/hydrolyzed	Isolate
Pea Protein (%)	≥80	>84%	85 and 65	≥80	≥80	≥80	80	90
Basis	Scientific procedures	Scientific procedures	Scientific procedures	Scientific procedures	Scientific Procedure	Scientific procedures	Scientific procedures	Scientific procedures
Intended uses	Multiple food	Multiple food including meat poultry	Multiple food including meat poultry	Multiple food including meat poultry	Multiple food	Multiple food	Multiple food including meat poultry	Fining agents in wine making
Use levels	Up to 35%	1-90%	Up to 35%	Up to 34.3%	Up to 34.3%	Up to 34.3%	Up to 90%	0.5 g/L
EDI	51.62 g/day (90th %tile)	<DRV and <DRI of protein	51.62 g/day (90th %tile)	Substitute	17.3 g/day (90th %tile)	17.3 g/day (90th %tile)	10.76 g/ day (90th %tile)	No dietary exposure
ADI	At proposed use levels	At proposed use levels	At proposed use levels	At proposed use levels	At proposed use levels	At proposed use levels	At proposed use levels	Not determined
Safety determination	Totality of evidence	Totality of evidence	Totality of evidence	Totality of evidence	Totality of evidence	Totality of evidence	Totality of evidence	Totality of evidence

*Based on information provided by Yantai; ADI = Acceptable Daily Intake; DRI = Dietary Reference Intake; DRV = Daily Reference Value

Among the above described GRAS notices (Table 11), GRN 804 submitted by Burcon (2018) is further described here. In this recent GRAS notice, Burcon proposed use of pea protein as a source of protein in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, coffee and tea, confections and frostings, dairy product analogs, egg products, fats and oils, fish products, frozen dairy desserts, fruit and water ices, gelatins, puddings, and fillings, grain products and pastas, gravies and sauces, meat products, milk products, nut and nut products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes at use levels ranging from 1 to 35 g pea protein per 100 g of food.

In the GRAS notice (GRN 804) by Burcon (2018), pea protein products are described as two powders that are formulated to contain either ≥85% protein or ≥65% protein. The product containing ≥85% protein is an off-white to slightly yellow powder that is bland and has a very low or no pea flavor, while the product containing ≥65% protein is a yellow-beige powder that

has a mild pea flavor. Both formulations may also contain small amounts of moisture, fat, fiber, and ash. The typical composition and specifications for pea protein were described. In the manufacturing of pea protein, Burcon (2018) also stated that food grade enzymes may be used in the process either prior to the pasteurization steps in each product line or prior to the acidification step to reduce viscosity and increase solubility of the proteins according to customer requirements. These optional enzymes used in the manufacturing are in accordance with a regulation or are GRAS for this use.

In the GRAS notice (GRN 804) by Burcon (2018), the resulting dietary exposure to protein from the intended use of pea protein from all intended food uses was estimated as 28.42 g/person/day (0.47 g/kg body weight/day) at the mean and 51.62 g/person/day (0.97 g/kg body weight/day) at the 90th percentile. In the GRAS notice, Burcon (2018) incorporated the safety information from GRN 000608 and GRN 000581 and stated that it concurs with the safety conclusions of both GRN 000608 and GRN 000581 and that the intended use of the pea protein is GRAS. For the GRAS assessment (GRN 804), literature search through June 2018 was conducted with regard to any new information. The literature search did not reveal any new information was found that would contradict its GRAS conclusion. Based on the information presented in the notice (GRM 804), Burcon (2018) concluded that pea protein is GRAS for its intended use in food. In a response letter to the Burcon on November 8, 2019, the FDA stated that the agency has 'no questions" regarding the conclusion that pea protein concentrate is GRAS under the intended conditions of use (FDA, 2019b).

6.4. Safety Studies of Pea and its Protein

6.4.1. Metabolism

Gausseres et al. (1997) evaluated postprandial absorption of pea protein as well as exogenous nitrogen retention in seven adult volunteers (4 males and 3 females with mean body weight 64 kg, ranging from 46 to 77 kg). The gastrointestinal absorption of pea protein following ingestion of 21.45 g (195 mMol N) of [^{15}N]-labeled pea protein [each meal contained 75 g pea flour (195 mMol N)] was studied. Total absorption was estimated at 89.4±1.1%, resulting in 19.2 g being absorbed in the 8-hour postprandial period at a rate of 2.4 g/hour. Following pea ingestion, the absorption correlated with a significant increase in [^{15}N]-enrichment in the plasma amino acids and in the nitrogen incorporation into the body urea pool for 1 hour. At 24 hour after pea ingestion, the enrichment remained significantly higher as compared to the basal values in these pools. The recovery of total urinary exogenous nitrogen after 22 hour was 31.1±9.3 mmol N. The kinetics of [^{15}N]-labeled pea amino acids deamination reached a plateau of 39 mmol. Under these conditions, pea nitrogen retention represented 78% of the absorbed dietary nitrogen in healthy humans. The results of this study suggest good true nitrogen digestibility and retention of pea protein in humans.

In another human study, Mariotti et al. (2001) studied the bioavailability and metabolic utilization of pea albumins and globulins in healthy human subjects. In this study, volunteers ingested a mixed meal of 30 g of raw purified pea protein either as [^{15}N]-globulins (G, n=9; 6 men and 3 women) or as a mix of [^{15}N]-globulins and [^{15}N]-albumins (GA, n=7; 4 men and 3 women) in their natural proportions (22:8). Following the ingestion of protein meal, postprandial sampling was done hourly for eight hours. The pea albumin fraction significantly lowered the real ileal digestibility of pea protein, did not promote acute intestinal losses of endogenous nitrogen and did not significantly improve the postprandial biological value of pea protein,

despite the fact that it corrected the globulin deficiency in sulfur amino acids. The ileal digestibility was 94.0±2.5% and 89.9±4.0% for the globulins, and globulins plus albumins meals respectively yielding amino acid absorption rates of approximately 3.5 and 3.4 g/hour. The investigators concluded that both globulins as well as mixture of globulin and albumin are of good nutritional value for humans and show that cysteine-rich albumins have a far more modest effect on the efficiency of postprandial dietary protein utilization than would be expected from the amino acid scores. It was also noted that, when given selectively to healthy humans, pea proteins exhibit a good nutritional value, similar to that of soy protein.

6.4.2. Clinical Studies in Humans

In an open-label, randomized, exploratory study in 44 healthy overweight subjects with cardio-metabolic syndrome (CMS) risk factors, Dahlberg et al. (2017) investigated the safety and tolerability of a proprietary lifestyle modification program without (DIET) and with (PROG) targeted dietary supplementation, including phytosterols, antioxidants, probiotics, fish oil, bebeerine, and soy, pea, and whey proteins over a period of 13 weeks. In this study, the subjects in the PROG diet received protein shake containing soy, pea or whey protein. The subjects within the study were allowed to choose between commercially available soy, whey, or pea proteins with scoop size normalized to deliver 20 g of protein. The daily intake of pea protein was reported as 12 g/day. Estimates of the relative soy, pea, and whey protein consumption during the study were made from returned product canisters. Soy protein shakes were most popular and represented 50% of the consumed snakes followed by pea protein at 30% and whey protein at 20%. Key metrics were recorded at baseline and weeks 9 and 13. For the DIET and PROG groups, compliance was 85% and 86%, respectively, with no adverse events related to the diet or supplements. Although data were not reported, the investigators stated that complete blood counts and metabolic profiles covering baseline, week 9, and week 13 were normal. Twelve subjects discontinued participation before week 9 for reasons unrelated to the study. The results of this study show that intake of pea protein at a daily dose of 12 g/day was well tolerated.

In a randomized, double-blind, placebo controlled, three-way, cross-over meal test study, Kristensen et al. (2016) compared the acute meal-induced appetite sensations of meals based on vegetable protein sources (beans/peas) with animal protein sources (veal/pork). In this study, 43 healthy, normal-weight, young men participated. The meals [all 3.5 MJ, 28 energy-% (E%) fat] were either high protein (39 g/100 g) based on veal and pork meat, HP-Meat (19 E% protein, 53 E% carbohydrate, 6 g fiber/100 g); high protein (38 g/100 g) based on legumes (beans and peas), HP-Legume (19 E% protein, 53 E% carbohydrate, 25 g fiber/100 g); or low-protein (18 g/100 g) based on legumes, LP-Legume (9 E% protein, 62 E% carbohydrate, 10 g fiber/100 g). Subjective appetite sensations were recorded at baseline and every half hour using visual analog scales until the *ad libitum* meal three hours after the test meal. HP-Legume induced lower composite appetite score, hunger, prospective food consumption, and higher fullness compared to HP-Meat and LP-Legume. Furthermore, satiety was higher after HP-Legume compared to HP-Meat. The investigators concluded that vegetable-based meals (beans/peas) influenced appetite sensations favorably compared to animal based meals (pork/veal) with similar energy and protein content, but lower fiber content. Vegetable-based meal with low protein content was as satiating and palatable as an animal-based meal with high protein content.

In a double-blind, randomized, placebo-controlled trial, Babault et al. (2015) investigated the effects of oral supplementation with pea protein vs. whey protein and placebo on biceps brachii muscle thickness and strength following a 12-week resistance training program. In this

study, 161 male volunteers (age 18-35 years) were divided into three groups: pea protein (n=53), whey protein (n=54) or placebo (n=54) group. The subjects underwent 12 weeks of resistance training on upper limb muscles. During the 12-week training period all subjects received 25 g of the proteins or placebo twice a day (50 g/day). Tests were performed on biceps muscles three times, and supplementation compliance or adverse effects were recorded. A significant time effect for biceps brachii muscle thickness was noted that was significantly greater in the pea protein group as compared to placebo whereas there was no difference between whey and the two other conditions. Muscle strength also increased with time but without any statistical difference between groups. Of the 161 subjects who took protein products, three presented an adverse event in the whey group (7.4%), four in the placebo group (7.4%) and one in the pea group (1.9%). Except for two digestive disorders (diarrhea) in the placebo group, the adverse effects were all musculotendinous or back pains related to their usual daily activity throughout the study. All symptoms disappeared spontaneously except for an elbow tendinopathy in the whey group which persisted at the end of the trial but any association with the product intake was ruled out. Given the lack of adverse effects of pea protein at levels of 50 g/day for 12 weeks, the findings from this study support safety of pea protein concentrate. The findings from this study support the safety of proposed uses of pea protein, the subject of the present GRAS.

Teunissen-Beekman et al. (2012) studied the effects of increased protein intake at two levels (about 25% compared with about 15% of energy intake that isoenergetically replaces carbohydrate intake) for four weeks to lower blood pressure of male and female subjects during office and daytime as compared with increased carbohydrate intake. In this randomized, double-blind, parallel clinical trial, consumption of 3×20 g protein/day (20% pea, 20% soy, 30% egg, and 30% milk-protein isolate) with 3×20 g maltodextrin/day was compared. In this study, protein or maltodextrin were isoenergetically substituted for a sugar-sweetened drink. For this study, a total of 99 male and female subjects (20-70 years old; BMI 25-35 kg/m^2) with untreated elevated BP (BP ≥130/85 and <160/100 mm Hg) were randomized. Ninety-four subjects, 51 subjects in the maltodextrin group and 43 subjects in the protein group, completed the study and were included in the analyses. In the protein group, the office systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 4.9±1.7 mm Hg and 2.7±1.3 mm Hg lower, respectively. Daytime SBP was 4.6±1.7 mm Hg lower in the protein group, whereas daytime DBP did not differ between groups. Urinary sodium excretion was higher in the maltodextrin group. During the study, five participants in the protein group dropped out; two participants stopped because of immediate adverse effects after consumption of the supplement (one subjects experienced nausea, and one subject experienced a lightly swollen face, abdomen, and thighs); two participants were excluded after randomization because they started with antihypertensive medication; and one subject stopped for personal reasons that were not related to the intervention. The investigators concluded that increased protein intake, at the expense of maltodextrin, lowers blood pressure in overweight adults with upper-range pre-hypertension and grade 1 hypertension.

In two separate additional studies, Teunissen-Beekman et al. (2015) investigated the effects of dietary proteins and carbohydrates on markers of endothelial dysfunction (ED) and low-grade inflammation (LGI) in overweight/obese individuals with untreated elevated blood pressure. In the first study, 52 subjects consumed a protein mix or maltodextrin (3×20 g/day) for four weeks. The protein mix consisted of 20% pea protein, 20% soy protein, 30% egg-white protein, 30% milk protein isolates. Fasting levels and 12 hour postprandial responses of markers of ED (soluble intercellular adhesion molecule 1 (sICAM), soluble vascular cell adhesion molecule 1 (sVCAM), soluble endothelial selectin and von Willebrand factor) and markers of

LGI (serum amyloid A, C-reactive protein and sICAM) were evaluated before and after intervention. In the second study, 4 hour postprandial responses of ED and LGI markers in 42 participants was compared after ingestion of pea protein, milk protein and egg-white protein at a dose level of 0.6 g/kg. In addition, postprandial responses after maltodextrin intake were compared with a protein mix and sucrose.

In the first study, significantly lower fasting ED Z-scores and sICAM after four weeks on the high-protein diet were noted (Teunissen-Beekman et al., 2015). The postprandial studies found no clear differences of ED and LGI between test meals. However, postprandial sVCAM decreased more after the protein mix compared with maltodextrin in both studies. The investigators concluded that dietary protein is beneficial for fasting ED, but not for fasting LGI, after four weeks of supplementation. On the basis of Z-scores, postprandial ED and LGI were not differentially affected by protein sources or carbohydrates. In this publication, no safety related parameters or adverse effects were reported by the investigators.

In two separate randomized single-blind cross-over studies, Abou-Samra et al. (2011) investigated the effect of different proteins, including pea protein, on satiation and short-term satiety. In the first study, the effects of a preload containing 20 g of casein, whey, pea protein, egg albumin or maltodextrin vs. water control on food intake 30 min later in 32 male volunteers (25±4 years, BMI 24±0.4 kg/m^2) was investigated. The results of this study revealed that food intake was significantly lower only after casein and pea protein compared to water control. Caloric compensation was 110, 103, 62, 56 and 51% after casein, pea protein, whey, albumin and maltodextrin, respectively. Feelings of satiety were significantly higher after casein and pea protein compared to other preloads. Blood glucose response to the meal was significantly lower when whey protein was consumed as a preload compared to other groups. In the second study, the effect of 20 g of casein, pea protein or whey vs. water control on satiation in 32 male volunteers (25±0.6 years, BMI 24±0.5 kg/m^2) was investigated. No difference between preloads on *ad libitum* intake was noted. No adverse effects were reported.

In a 3-week randomized, double blind, placebo-controlled, crossover human intervention trial in hypertensive human subjects, Li et al. (2011) studied the effect of a pea protein hydrolysate that contained <3 kDa peptides, isolated by membrane ultrafiltration from the thermolysin (a metallopeptidase used to cleave peptide bonds at specific junctions) digest of pea protein isolate. The focus of the study was to investigate the blood pressure lowering effect of a pea protein hydrolysate. The study was carried out in 7 volunteers (4 females and 3 males, ages 30-55 years, 145-185 lb) with systolic blood pressure ranging from 125 to 170 mm Hg. There were 3 treatments: placebo (50 mL of orange juice), 1.5 and 3.0 g of pea protein hydrolysate per day divided into 3 doses of 0.5 or 1 g each and taken at breakfast, lunch and dinner. Results from the human intervention study demonstrated that 3 g/day of pea protein hydrolysate, containing <3 kDa peptides, compared to placebo resulted in reductions in SBP of 5 and 6 mmHg, respectively, in human subjects at weeks 2 and 3 but not in the first week. In contrast, at a dosage of 1.5 g/day there was no significant effect on blood pressure when compared with the placebo group. The findings from this study suggest that pea protein hydrolysate reduces blood pressure in hypertensive human subjects. No safety related clinical chemistry or hematological parameters were investigated. The investigators did mention that during the three-week duration of this experiment, none of the participants reported any adverse side effects. These investigators also investigated similar effects in rats.

In countries where soybean is not a native crop, or when soybean protein cannot be used due to allergic reactions or intolerances, the use of pea protein in infant formula has been suggested as an alternative to soybean formula. In a study in healthy non-anemic women (n=20; 10/study; mean age 22 years; weight 55 kg), Davidsson et al. (2001) investigated the absorption iron (Fe) from experimental infant formulas based on pea protein isolate. The effects of phytic acid and ascorbic acid on iron absorption were investigated. Fe absorption from experimental infant formulas based on pea-protein isolate was measured in women. Phytic acid has negative effects on Fe absorption while ascorbic acid has a positive effect on Fe absorption. The stable-isotope technique was used to analyze the effects, and the results indicated that pea protein had improved Fe absorption effects compared to the soy protein.

6.4.3. Pre-Clinical Studies of Pea Protein

6.4.3.1. Repeat-Dose Animal Toxicity Study

In a repeat-dose 90-day (subchronic) toxicity study, Aouatif et al. (2013a) investigated the effects of pea protein isolate in Wistar rats. The pea protein isolate (Nutralys) used in this study was manufactured and supplied by Roquette Freres, France. The isolate is a high quality white powder source food grade with 85% pea protein content, extracted in water. In this dose-response study designed and conducted as per OECD Guidelines, rats were maintained on diet containing pea protein isolate at levels of 0 ppm, 25,000 ppm (low), 50,000 ppm (intermediate) and 100,000 ppm (high) for 90 days. For this study, six groups of rats (10/sex/group): G1 (control), G2 (Low dose- 2.5%), G3 (Intermediate dose- 5%), G4 (High dose- 10%), G5 (Satellite control) and G6 (Satellite high dose- 10%) group. At end of 90 days, the satellites groups were given only diet without the test item for an additional 28 days to evaluate any possible reversal effects.

No treatment-related adverse effects on clinical signs, body weights, feed consumption, water consumption, hematological, blood biochemical and urinalysis parameters following exposure to pea protein were noted as compared with concurrent control animals. Additionally, organ weights, gross and histological examinations did not reveal any systemic toxicity induced by pea protein exposure. Statistical changes in some hematological and clinical chemistry parameters such as eosinophil in male rats and prothrombin in females of low dose rats; significant decrease in platelets and neutrophils and increase in lymphocyte counts observed in female rats of the high dose group; triglyceride levels in all the three treated groups of female rats; absolute weight of the testes of male rats in the low dose group; and, absolute weight of the spleen of female rats in the high dose group, were observed. The absolute magnitude of these effects were minimal, no corresponding histological changes were reported, and the effect did not occur in both sexes. Hence these effects could be regarded as random and without toxicological relevance. Overall, pea protein isolate exposure in diet did not alter liver or kidney function or have an adverse effect on the hemopoietic system. Further, histological and gross examinations of organs did not reveal abnormal findings. The changes noted in the satellite group were not considered as treatment related (Aouatif et al., 2013a).

Based on the findings of this 90-day feeding toxicity study in Wistar rats, Aouatif et al. (2013a) considered the highest dose tested of 10% of pea protein in diet, equivalent to 8726 for male and 9965 for female mg/kg bw/day as the no-observed-adverse-effect-level (NOAEL). The pea protein isolate (85%) used in the Aouatif et al. (2013a) study is similar to the pea protein concentrate (80% minimum), the subject of present GRAS assessment. The highest safe dose

noted in the Aouatif et al. (2013a) study is 17 to 20 fold higher as compared to the highest dose of 30 g/person/day of pea protein. The results of this study support the safety of pea protein. The available information indicate that similar to the subject of present GRAS, the pea protein used in the Aouatif et al. (2013a) study is hydrolyzed using enzymes. The findings from this study support the safety of proposed uses of enzyme treated pea protein.

6.4.3.2. Mutagenicity and Genotoxicity Studies

In addition to repeat dose toxicity study, Aouatif et al. (2013b) also investigated the potential mutagenic and genotoxic effects of pea protein isolate (85%) as evaluated by Ames assay, *in vitro* chromosomal aberration test, and *in vivo* micronucleus test. All these assays were conducted as per OECD guidelines. For the Ames reverse mutation assay, five tester strains of *Salmonella typhimurium* (TA100, TA102, TA1535, TA98, and TA1537) were used. The effects of pea protein isolate was tested at concentrations of 312.5, 625, 1250, 2500, and 5000 µg/plate in the presence and absence of metabolic activation (S9). Under the experimental conditions employed, pea protein was non-mutagenic. The *in vitro* chromosomal aberration test was conducted in cultured human peripheral blood lymphocytes. In this test, pea protein isolate was tested at concentrations of 125, 250, and 500 µg/mL for potentials to induce structural and numerical aberrations. The findings from this study suggest that pea protein isolate did not induce genotoxic responses in human lymphocytes. For the *in vivo* micronucleus assay, a limit test was performed in mice. In the limit test male and female CD1 mice received a single and two-day treatments (24 hours apart) of pea protein isolate at the highest dose of 2000 mg/kg bw. No evidence of increase in the frequencies of micro-nucleated polychromatic erythrocytes (MN-PCE) was observed in the treated group as compared to that of the concurrent vehicle control groups at all time points of euthanasia. The findings from this study suggest that pea protein isolate was non-genotoxic in single- and two-day treatments.

In summary, pea protein isolate is non-mutagenic and non-genotoxic, as per the Ames assay, *in vitro* chromosomal aberration test, and the *in vivo* bone marrow micronucleus test in mice (Aouatif et al., 2013b). The findings from this study with pea protein isolate (85%) are applicable to the subject of present GRAS assessment.

6.4.3.3. Additional Safety-Related Studies

Li et al. (2011) also studied the blood pressure lowering effects of pea protein isolate in hypertensive rats, in addition to the above described human studies. For these investigations, pea protein hydrolysate (PPH) that contained <3 kDa peptides, isolated by membrane ultrafiltration from the thermolysin (a metallopeptidase used to cleave peptide bonds at specific junctions) digest of pea protein isolate, was administered orally to spontaneously hypertensive rats (SHR) at doses of 100 and 200 mg/kg bw. Pea protein hydrolysate administration to rats resulted in lowering of hourly systolic blood pressure (SBP), with a maximum reduction of 19 mm Hg at four hours after the treatment. However, oral administration of pea protein isolate (unhydrolyzed) had no blood pressure reducing effect in spontaneously hypertensive rats, indicating that thermolysin hydrolysis may have been responsible for releasing bioactive peptides from the native protein.

In the experiment with model of chronic kidney disease related hypertension, Han:SPRD-cy rat were fed one of the following diets for 8 weeks: casein (20%), 0.5% PPH (19.5% casein + 0.5% PPH), or 1.0% PPH (19% casein + 1% PPH). Oral administration of the pea protein hydrolysate (PPH) to the Han:SPRD-cy rat over an 8-week period led to 29 and 25 mmHg

reductions in SBP and diastolic blood pressure, respectively. The pea protein hydrolysate-fed rats had lower plasma levels of angiotensin II, the major vasopressor involved in development of hypertension, but there was no effect on plasma activity or renal mRNA levels of ACE. However, renal expression of renin mRNA levels was reduced by approximately 50% in the pea protein hydrolysate-fed rats, suggesting that reduced renin may be responsible for the reduced levels of angiotensin II. The investigators also mentioned that during the 8-week period, there were no differences in feed consumption (average 25-30 g/day) and growth rate (325-340 g at week 8) of rats in the control and pea protein hydrolysate-fed groups. The information on rat feed consumption was not provided in the publication. No other safety related parameters were mentioned.

In an *in vitro* study, Li and Aluko (2010) investigated the inhibitory activities of multifunctional peptides from pea protein isolate against Calmodulin-dependent phosphodiesterase (CaMPDE), renin, and angiotensin I-converting enzyme (ACE). Results showed that pea protein isolate peptides do exhibit inhibitory activities against ACE, renin, and CaMPDE, indicating an improved health response, and suggesting the peptides "may be used as potential ingredients to formulate multifunctional food products and nutraceuticals".

Gawalko et al. (2009) compared the levels of toxic trace elements in field peas from Canada with the international (CODEX) maximum limits for these trace element. In this study, a total of 295 field pea samples from 35 regional varieties from the years 2004-2006 were analyzed. The results revealed mean total cadmium content of 0.023 mg/kg, arsenic and lead mean values of 0.050 mg/kg and total mean mercury level of <0.002 mg/kg. All measured values were below the maximum residue levels (MRLs) established by the Food and Agriculture Association (FAO) and the World Health Organization (WHO). The results of this study suggest that Canadian field peas are in compliance with CODEX standards (Gawalko et al., 2009).

Ndiaye et al. (2012) investigated the anti-oxidant, anti-inflammatory and immune-modulating characteristics of enzymatic pea protein hydrolysate (derived from yellow field pea seeds). In the *in vitro* study with macrophages, the pea protein hydrolysate, after a 12 hours pre-treatment showed inhibition of nitric oxide production by activated macrophages up to 20%, TNF-α up to 35% and IL-6 up to 80%. Oral administration to pea protein hydrolysate to mice, enhanced phagocytic activity of their peritoneal macrophages and stimulated the gut mucosa immune response.

6.4.3.4. Sensitization and Allergy

Peas are part of a family of plants called legumes that also include alfalfa, clover, beans, lentils, mesquite, carob, soybeans, peanuts, tamarind, and wisteria. As a cereal grain, pea also contains proteins that are similar to those found in other cereal grains. The available information shows that individuals allergic to cereal grain products are allergic to some of the specific proteins found in some cereals. Allergenic reaction to legumes may range from mild skin reactions to life-threatening anaphylactic reactions. In a comprehensive review of legume allergy, Verma et al. (2013) reported that overall, allergic reaction due to consumption of legumes, in decreasing order, may be peanut, soybean, lentil, chickpea, pea, mung bean, and red gram. The most common foods causing immunologically-mediated reactions include milk, eggs, fish, crustaceans, nuts, wheat, soy, peanuts, peas and other legumes. Thus far, several allergens from different legumes have been identified and characterized. Most of the identified allergens belong to the storage protein family, profilins, or the pathogenesis-related proteins. Legumes also

exhibit the property of immunological cross-reactivity among themselves and from other sources that also increases the severity of allergenic response to a particular legume.

In a recent study, Smits et al. (2018) analyzed legume sensitization data (peanut, soybean, lupin, lentil, and pea) from studies in relation to consumption data obtained from national food consumption surveys using the European Food Safety Authority (EFSA), Global Environment Monitoring System (GEMS), and What We Eat in America-Food Commodity Intake Database (WWEIA-FCID) databases. Forty-two articles met the inclusion criteria and were subsequently included in the analysis. Of the selected articles, 41 articles investigated peanut sensitization, 17 soybean sensitization, 4 lupin sensitization, 2 lentil sensitization, and 1 pea sensitization in the general population. The investigators noted that vicilin and convicilin from pea were identified as major allergens, and cross-reactivity with the major allergen from lentil (Len c 1) occurred in all 18 pea allergic patients in Spain. Additionally, in peanut-allergic patients, co-sensitization to lupine (82%), pea (55%), and soybean (87%) is often seen. Consumption and sensitization data were available for soybean (n=17), lupin (n=4), lentil (n=2), and pea (n=1). Given the low number of data points for pea, weighted least squares linear regression was not calculated for pea. The findings from this study show that sensitization to pea low.

Among the food allergies, legumes, especially peanut, have been reported to be a cause of allergy. In the US, approximately 0.6% to 1.3% of the population is affected by peanut allergies (FARE, 2014). Sanchez-Monge et al. (2004) reported that peanut and soybeans are the major legume allergies known in the United States, United Kingdom, and Japan, while lentils, chickpeas and pea allergies are more common in the Mediterranean area and India. Pea proteins are mainly storage protein comprised of albumins and globulins. Albumins and globulins separate into two major fractions; the 7S vicilin and convicilin fraction, and an 11S fraction made up mostly of legumin (Casey et al., 1985). Legume allergies are most often caused by these storage proteins (albumins, globulins, prolamins) (University of Nebraska - Lincoln, 2014). Food allergies can be identified scientifically by determining the effect on IgE antibodies. IgE antibody synthesis is stimulated by cytokines such as Interleukin 4 (IL-4), IL-5, and IL-13, which are produced by Type II T-Helper Cell (T_H2).

In Spanish children, legume allergy, mainly to lentils and chickpeas, is the fifth most common cause of food allergy. In these children, Ibanez et al. (2003) reported a great degree of cross-reactivity among lentil, chick-pea, pea and peanut by ELISA inhibition (>50% max inhibition). The majority of patients showed symptoms with more than one legume (median 3 legumes). These investigators challenged (open or simple blind) 39 patients with two or more legumes and 32 (82%) reacted to two or more legumes: 43.5% to 3, 25.6% to 2, 13% to 4 legumes. Among these patients, 73% challenged with lentil and pea had positive reactions to both, 69.4% to lentil and chick-pea, 60% to chick-pea and 64.3% to lentil, chick-pea and pea simultaneously. In this study, 82% of the children allergic to legumes had a sensitization to pollen. Ibanez et al. (2003) suggested that the decision to eliminate one legume from the diet should be based on a positive oral food challenge.

Dziuba et al. (2014) reported that selected legume proteins (soybean, lentil, pea, bean) have shown IgE mediated cross-reactivity, which could be caused by the inability of IgE specific antibodies to distinguish between the proteins of different sources, which have very similar tertiary structure and amino acid sequences. Sanchez-Monge et al. (2009) attempted to identify the main IgE binding components from pea seeds and to study their potential cross-reactivity with lentil vicilin. For this assessment, serum pool or individual sera from 18 patients with pea

allergy were used to detect IgE binding proteins from pea seeds by immune-detection and immunoblot inhibition assays. IgE immune-detection of crude pea extracts revealed that convicilin, as well as vicilin and one of its proteolytic fragments (32 kDa), reacted with more than 50% of the individual sera tested. The findings from this study show that vicilin and convicilin are potential major allergens found in pea seeds. Additionally, proteolytic fragments from vicilin are also relevant IgE binding pea components.

Wensing et al. (2003) reported that patients with anaphylaxis to pea can have peanut allergy caused by cross-reactive IgE to vicilin. These investigators described three patients with a history of anaphylaxis to pea who subsequently had symptoms after ingestion of peanut. In this study, peanut-related symptoms were documented according to case history or double-blind, placebo-controlled food challenge results. Skin prick tests were performed, and specific IgE levels were determined for pea and peanut. All patients had a positive skin prick test response and an increased IgE level to pea and peanut. These investigators concluded that clinically relevant cross-reactivity between pea and peanut does occur. The molecular basis for cross reactivity was determined to be vicilin homologues in pea and peanut (Ara h 1).

As several cases of severe anaphylaxis to dun pea have been registered by the French Allergy Vigilance network, Richard et al. (2015) evaluated the rate of sensitization to dun pea in legume-allergic patients and in peanut-allergic patients, and to search for modification of allergenicity induced by food technologies. A series of 36 patients with legume and/or peanut allergy was studied. The findings from this study showed that subjects with isolated legume allergy had positive prick tests to dun pea, whereas patients with isolated peanut allergy had negative prick tests. Cross-reactivity between Specific IgE (sIgE) to peanut and dun pea was observed. Further investigation revealed that protein epitopes were presented differently in dun pea seeds, isolate, and flour. Immunoblots of serum from a patient (male 18 years) with allergy to all legumes since infancy with the same amount of protein for each extract illustrate the difference of IgE reactivity with seed, flour and isolate extracts. Hence, allergenic profiles were different between flour and isolate; IgE recognized the 9 kDa proteins in dun pea seed but not in flour or isolate; the absence of inhibition of the 9 kDa proteins of seed by flour confirmed that the 9 kDa proteins present in flour and isolate were no longer able to bind the sIgE; and finally seed and flour differentially inhibited IgE binding to 28 kDa and 50 kDa proteins in seed. Based on these observations, the investigators hypothesized that manufacturing processes may be different for the two types of ingredients, thus modifying the allergenicity of native proteins. The investigators concluded that this study identifies, for the first time, a risk of dun pea allergy in legume-allergic patients and in a subset of peanut-allergic patients.

In summary, the available information indicates that allergy to pea has been reported and the frequency to pea allergy varies among different populations. Cross-reactivity among lentil, chick-pea, pea and peanut has been reported. Some of the specific proteins in pea are responsible for the allergic reaction. Although people with peanut allergies may also be sensitive to peas, allergy to peas is actually quite rare and the frequency to pea allergy varies among different populations. Yantai acknowledges that pea protein does not contain any of the eight foods (milk, egg, fish, crustacean shellfish, tree nuts, peanuts, soybeans, wheat) considered to be major food allergens under the U.S. Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA).

6.4.3.5. Mycotoxins and Safety

Mycotoxins are secondary metabolites synthesized by a variety of fungal species such as Aspergillus, Penicillium, Fusarium, and Alternaria. These secondary metabolites, even at small amounts, are toxic and have a significant impact if they enter the production and food chain. Mycotoxins are capable of causing disease and death in humans and other animals (Bennett and Klich, 2003). Several countries have enforced different thresholds to limit the passage of mycotoxins along the food chain. Aflatoxins are considered unavoidable contaminants in the US food supply, especially in corn and peanuts, and levels are regulated by the FDA. The FDA has established action levels for poisonous or deleterious substances in human food and animal feed[8]. The action levels and tolerances are established based on the unavoidability of the poisonous or deleterious substances and do not represent permissible levels of contamination where it is avoidable. The FDA established action levels for aflatoxins present in human food that include 0.5 ppb (aflatoxin M1) for milk and 20 ppb for foods such as peanuts and peanut products, brazil and pistachio nuts.

In an article on mycotoxin monitoring for commercial foodstuffs, Chen et al. (2016) summarized the limits of aflatoxins, ochratoxin A, and citrinin in different food commodities set by the CODEX Alimentarius Commission, European Commission, USA and Japan. These investigators reported the levels of mycotoxins from 712 food samples in Taiwan. This analysis revealed that approximately 97% of samples were found in compliance with Taiwan government regulation of 15 ppb for aflatoxin and 10 ppb for other food products. The National Grain and Feed Association described FDA Regulatory Guidance for Mycotoxins[9]. This document stated that FDA has not established regulatory limits for mycotoxins in specific food or feed, although it has stated its intent to eventually establish such limits for aflatoxin. The document summarizes the above described aflatoxin limits. An FDA advisory level for vomitoxin in finished wheat products for human consumption is 1 ppm. FDA guidance levels for fumonisin (FB1, FB2, FB3) for corn and corn products intended for human food range from 2 to 4 ppm.

In enzyme treated pea protein, the subject of present GRAS assessment, the aflatoxins (B1+B2+G1+G2 sum of the four) levels from five lots were measured. The aflatoxin profile as analyzed by European Committee for Standardization EN 14123 method was non-detectable for the individual and sum of Aflatoxins B1, B2, G1, G2 at a detection limit of < 5 µg/kg. The low (undetectable) levels of aflatoxins in enzyme treated pea protein is below the FDA established action levels of 20 ppb for foods in general and thus is considered as safe.

6.5. GRAS Panel Review, Summary and Discussion

In 2018, Yantai Oriental Protein Tech Co., Ltd. (Yantai) submitted a GRAS notification (GRN 788) to FDA for use of pea protein in conventional foods. Following FDA review of the GRAS notice, on October 12, 2018 Yantai received a 'no question' letter from FDA. The subject of GRN 788, pea protein is prepared by base precipitation and acid neutralization to dissolve the protein. Given the marketing and commercialization experiences with the pea protein and to meet market demand, Yantai proposes to make some changes to the manufacturing, particularly use enzymes in the production of protein. The enzymes used as processing aids in the hydrolysis

[8] Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm

[9] Available at: www.ngfa.org

of pea protein are used at levels not to exceed current good manufacturing practice (cGMP). The proposed use of enzymes primarily breaks the aggregation of the finished product. Given this change, Yantai has undertaken the GRAS assessment for its new product, enzyme treated pea protein.

For the new product, enzyme treated pea protein, Yantai convened an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[10], qualified by their scientific training and relevant national and international experience with safety of food and food ingredients, to evaluate the Generally Recognized As Safe (GRAS) status of enzyme treated pea protein (≥80% protein) derived from *Pisum sativum* L. seed-pods (peas) as a food ingredient, formulation aid and texturizer, in conventional foods such as Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; and, Soups and Soup Mixes at levels ranging from 0.96 to 34.3%. A comprehensive updated search of the scientific literature for safety and toxicity information on pea and its protein was conducted through March 2020 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Yantai and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on May 22, 2020 and unanimously agreed to the decision described herein.

Yantai ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. Efforts were also placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel were not contingent upon the outcome of their deliberations.

The available information suggest that peas are one of the oldest cultivated crops in the world and an important source of protein for humans and animals. It is a hardy winter legume grain that has been consumed since ancient times as a food around the world. Peas are considered as an excellent source of the amino acid lysine and protein. Peas contain approximately 25% protein. In addition to nutritional properties, enzyme treated pea protein has several functional effects in foods, such as promotion of ease of dry flow, ability to mask off-flavors, improves texture, increases water-solubility, etc. The available information demonstrates common knowledge of the human consumption of peas and thus its protein. The USDA Nutrient Database list includes peas and its preparations as foods. Based on USDA data on food consumption, the mean and 90th percentile estimated daily intake of peas is 96 and 197 g/person/day, respectively. As peas contains about 25% protein, the protein intake from pea consumption will be 23.57 and 48.36 g pea protein/person/day, respectively. As regards daily protein intake from all sources, the Institute of Medicine (IOM, 2005) has established the recommended daily intake of protein of 0.8 g/kg bw for an adult. The IOM has also reported that the mean adult protein intake ranges from 56 - 104 g/day, depending on age group. At the 90th percentile, adult protein intakes ranged from 76 g/day to 142 g/day.

[10] Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

Yantai intends to use enzyme treated pea protein (80%) as a multifunctional food ingredient. The processes by which enzyme treated pea protein is derived from raw field peas are mechanical such as sifting, centrifugation, drying, and sieving. Additionally, in the manufacturing enzymes are used to improve the acceptability of final product. The enzyme treated pea protein is manufactured as per current GMP from yellow peas by base precipitation, acid neutralization, enzyme treatment and isolation of protein. The function of the enzymes used in the manufacturing is to improve functionality of the protein. The addition of enzymes break the aggregation. As such, use of enzymes in the manufacturing does not affect the safety profile of the protein.

The enzyme treated pea protein has been well characterized for its nutritional composition and characteristics. The nutritional components and amino acid profile comparison of enzyme treated pea protein with other protein concentrates such as whey, casein and soy, revealed substantial similarity. Whey protein concentrate has been recognized as GRAS by the FDA. Additionally, peptones produced by partial hydrolysis of casein as well as from soy protein isolate are also recognized as GRAS. Additionally, whey protein isolate and dairy product solids has been the subject of GRAS (GRN 37) that received no question letter from the FDA. Furthermore, pea protein preparations has been the subject of seven separate GRAS notices (GRN 851; GRN 804; GRN 803; GRN 788; GRN 608; GRN 581; GRN 182) to FDA, all of which have received 'no question' letter from the FDA.

The proposed uses of enzyme treated pea protein by Yantai includes Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; Soups and Soup Mixes at levels ranging 0.96 to 34.3%. The use of enzyme treated pea protein in these foods will result in estimated total maximum intake of 51.62 g/person/day. This intake of enzyme treated pea protein is similar as compared to the 90th percentile background intake of protein (48.36 g/person/day, respectively) from the consumption of peas as a staple. As compared to the IOM established daily protein intake that ranges from 76 g/day to 142 g/day, the resulting intake of enzyme treated pea protein from its uses in different food categories, including in sports nutrition is 1.5-2.8 fold lower.

There is sufficient qualitative and quantitative scientific, as well as history of use evidence to determine the safety-in-use of the enzyme treated pea protein in the above mentioned food applications. Similar to other dietary protein, enzyme treated pea protein is digested in the human gastrointestinal tract. The comparison of enzyme treated pea protein proximate as well as amino acid profile with other commonly consumed proteins such as whey, soy, casein, other pea protein, suggest that enzyme treated pea protein, the subject of this GRAS assessment determination, is similar to other commonly used proteins. In human clinical studies, supplementation with pea protein at levels up to 50 g/day for 12 weeks did not reveal any adverse effects. In a subchronic toxicity study conducted as per OECD guidelines, the NOAEL of pea protein isolate (80% protein) in male and female rats was determined as 8,726 and 9,965 mg/kg bw/day. This safe dose is similar to the highest dose of 51.62 g/person/day of enzyme treated pea protein. The results of available animal and human studies did not indicate any potential for adverse effects of pea protein. The enzyme treated pea protein is unlikely to cause allergic reaction.

In response to seven GRAS notices (GRN 851; GRN 804; GRN 803; GRN 788; GRN 608; GRN 581; GRN 182), the FDA did not question the safety of pea protein concentrate for the specified food uses. The subject of this present GRAS assessment is substantially equivalent to the pea protein concentrate that have been the subjects of the FDA GRAS notified substances. The use of a similar pea protein that is the subject of this GRAS assessment and the ones that have been the subject of seven FDA notifications suggests that the differences between pea protein products would be limited to minor variations in the amino acid profile, and to differences in the residual levels of other components. These observations also suggest that the safety information on pea protein products can be interchangeable. The use of enzymes will partially hydrolyze the pea protein and the peptides have not been filtered, in order to obtain a mixture with smaller peptides and larger protein. Given this, as well as similar product specification and compositional analysis, including amino acid profile, of enzyme treated pea protein to the subject of previous GRAS notice GRN 788, the safety assessment from previous GRAS and other GRAS notices is applicable to present GRAS.

The totality of the available evidence from dietary consumption of peas for centuries, the current intake of peas as a staple, the substantial equivalence of enzyme treated pea protein produced by Yantai with other similarly marketed GRAS protein concentrates or isolates, and available safety studies in animals and humans described in this document, suggest that consumption of enzyme treated pea protein from the intended uses at use levels ranging 0.96 to 34.3% in specified foods is safe. On the basis of scientific procedures corroborated by exposure from natural dietary sources, consumption of enzyme treated pea protein, as an added food ingredient to the food supply, or its use as a nutritional supplement, is safe at daily consumption levels up to 30 g/person/day. The proposed uses are compatible with current regulations, *i.e.*, the enzyme treated pea protein is used as a food ingredient in Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; and, Soups and Soup Mixes, when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

6.6. Conclusion

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below have individually and collectively concluded that consumption of enzyme treated pea protein as a food ingredient in selected food products such as Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Dairy Product Analogs; Fats and Oils; Grain Products and Pastas; Milk Products; Plant Protein Products; Processed Fruits and Fruit Juices; Processed Vegetables and Vegetable Juices; and, Soups and Soup Mixes at levels ranging 0.96 to 34.3%, when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the maximum daily intake of up to 51.62 g/person is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information, further corroborated by history of safe use, would reach the same conclusion. Therefore, the Panelists also concluded that enzyme treated pea protein, when used as described, is GRAS based on scientific procedures.

Signatures

[redacted]

Robert L. Martin, Ph.D. — May 28, 2020 (Date)

[redacted]

John A. Thomas, Ph.D., F.A.T.S., F.A.C.T. — May 28 2020 (Date)

[redacted]

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — May 29, 2020 (Date)

Advisor to Expert Panel

7. Part VII- SUPPORTING DATA AND INFORMATION

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8. APPENDIX I

Analytical data from five non-consecutive manufacturing lots of Enzyme Treated Pea Protein

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CERTIFICATE OF ANALYSIS

NO: DFPG1213.1-II

Sample Analyzed	Enzyme Treated Pea Protein Isolate 80%	Produce Date	March 05, 2019
Date of Analysis	March 06, 2019	Date of Expiration	March 04, 2021
BATCH NO	20190305-1		
Packaging	2kg	Model	HPI80

Item	Testing Method	Standard	Result
Appearance	Visual	Powder	Powder
Color	Visual	Yellowish	Yellowish
Taste and Odor	Sensory Analysis	Bland	Bland
Protein(dry basis) (NX6.25),%	AOAC 981.10	≥80	82.7
Moisture,%	AOAC 925.09	≤8	7.3
Ash,%	AOAC 942.05	≤8	4.1
TPC, cfu/g	AOAC 990.12	≤ 30000	$6.1*10^3$
Staphylococcus aureus, /25g	AOAC 975.55	N.D.	N.D.
Salmonella, /25g	AOAC 2003.09	N.D.	N.D.
Gluten, mg/kg	R7001	<20	<5
PH	Q/DFS0001S-2017	6.5~8.5	7.1
Lead (Pb), mg/kg	GB5009.12-2017	≤0.1	<0.1
Arsenic (As), mg/kg	GB5009.11-2014	≤0.1	<0.1
Cadmium (Cd), mg/kg	GB5009.15-2014	≤0.3	<0.3
Mercury(Hg), mg/kg	GB5009.17-2014	≤0.02	<0.02
Aflatoxin, μg/kg	EN14123	<5	<5

Assesor signature: WU MEIYAN Inspector signature: CHENG GANG

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CERTIFICATE OF ANALYSIS

NO: DFPG1213.2-II

Sample Analyzed	Enzyme Treated Pea Protein Isolate 80%	Produce Date	February 05, 2019
Date of Analysis	February 06, 2019	Date of Expiration	February 04, 2021
BATCH NO	20190205-3		
Packaging	2kg	Model	HPI80

Item	Testing Method	Standard	Result
Appearance	Visual	Powder	Powder
Color	Visual	Yellowish	Yellowish
Taste and Odor	Sensory Analysis	Bland	Bland
Protein(dry basis) (NX6.25), %	AOAC 981.10	≥80	82.9
Moisture, %	AOAC 925.09	≤8	7.1
Ash, %	AOAC 942.05	≤8	4.5
TPC, cfu/g	AOAC 990.12	≤ 30000	$8.7*10^3$
Total Coliform, cfu/g	AOAC 991.14	≤10	<10
Gluten, mg/kg	R7001		<5
PH	Q/DFS0001S-2017	6.5-8.5	7.1
Lead (Pb), mg/kg	GB5009.12-2017	≤0.1	<0.1
Arsenic (As), mg/kg	GB5009.11-2014	≤0.1	<0.1
Cadmium (Cd), mg/kg	GB5009.15-2014	≤0.3	<0.3
Mercury(Hg), mg/kg	GB5009.17-2014	≤0.02	<0.02
Aflatoxin, μg/kg	EN14123	<5	<5

Assesor signature: WU MEIYAN **Inspector signature:** CHENG GANG

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CERTIFICATE OF ANALYSIS

NO: DFPG1213.3-II

Sample Analyzed	Enzyme Treated Pea Protein Isolate 80%	Produce Date	August 04, 2019
Date of Analysis	August 05, 2019	Date of Expiration	August 03, 2021
BATCH NO	20190804-2		
Packaging	1kg	Model	HPI80

Item	Testing Method	Standard	Result
Appearance	Visual	Powder	Powder
Color	Visual	Yellowish	Yellowish
Taste and Odor	Sensory Analysis	Bland	Bland
Protein(dry basis) (NX6.25),%	AOAC 981.10	≥80	84.8
Moisture,%	AOAC 925.09	≤10	7.1
Ash,%	AOAC 942.05	≤8	4.8
TPC, cfu/g	AOAC 990.12	≤ 30000	$6.9*10^3$
Total Coliform, cfu/g	AOAC 991.14	≤10	<10
Gluten, mg/kg	R7001	<20	<5
PH	Q/DFS0001S-2017	6.5~8.5	7.2
Lead (Pb), mg/kg	GB5009.12-2017	≤0.1	<0.1
Arsenic (As), mg/kg	GB5009.11-2014	≤0.1	<0.1
Cadmium (Cd), mg/kg	GB5009.15-2014	≤0.3	<0.3
Mercury(Hg), mg/kg	GB5009.17-2014	≤0.02	<0.02
Aflatoxin, μg/kg	EN14123	<5	<5

Assesor signature: WU MEIYAN **Inspector signature:** CHENG GANG

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CERTIFICATE OF ANALYSIS

NO: DFPG1213.4-II

Sample Analyzed	Enzyme Treated Pea Protein Isolate 80%	Produce Date	August 02, 2019
Date of Analysis	August 03, 2019	Date of Expiration	August 01, 201
BATCH NO	20190802-1		
Packaging	1kg	Model	HPI80

Item	Testing Method	Standard	Result
Appearance	Visual	Powder	Powder
Color	Visual	Yellowish	Yellowish
Taste and Odor	Sensory Analysis	Bland	Bland
Protein(dry basis) (NX6.25), %	AOAC 981.10	≥80	84.1
Moisture, %	AOAC 925.09	≤10	7.0
Ash, %	AOAC 942.05	≤8	4.2
TPC, cfu/g	AOAC 990.12	≤ 30000	$7.3*10^3$
Total Coliform, cfu/g	AOAC 991.14	≤10	<10
Gluten, mg/kg	R7001	<20	<5
PH	Q/DFS0001S-2017	6.5~8	
Lead (Pb), mg/kg	GB5009.12-2017	≤0.	<0.
Arsenic (As), mg/kg	GB5009.11-2014	≤0.	<0.
Cadmium (Cd), mg/kg	GB5009.15-2014	≤0.3	<0.3
Mercury(Hg), mg/kg	GB5009.17-2014	≤0.02	<0.02
Aflatoxin, μg/kg	EN14123	<5	<5

Assesor signature: WU MEIYAN **Inspector signature:** CHENG GANG

YAN TAI ORIENTAL PROTEIN TECH CO., LTD

东方科技
ORIENTAL TECH

Yantai Oriental Protein Tech Co., Ltd.

Tel: +86-535-8072188 www.pea-protein.com.cn
Fax: +86-535-8072199 info@orientalprotein.com
668 Jincheng Rd., Zhaoyuan City, Shandong Province, China.

CERTIFICATE OF ANALYSIS

NO: DFPG11213.5-II

Sample Analyzed	Enzyme Treated Pea Protein Isolate 80%	Produce Date	August 05, 2019
Date of Analysis	August 06, 2019	Date of Expiration	August 04, 2021
BATCH NO	20190805-2		
Packaging	1kg	Model	HPI80

Item	Testing Method	Standard	Result
Appearance	Visual	Powder	Powder
Color	Visual	Yellowish	Yellowish
Taste and Odor	Sensory Analysis	Bland	Bland
Protein(dry basis) (NX6.25), %	AOAC 981.10	≥80	84.4
Moisture, %	AOAC 925.09	≤10	7.1
Ash, %	AOAC 942.05	≤8	4.4
TPC, cfu/g	AOAC 990.12	≤ 30000	$9.4*10^3$
Total Coliform, cfu/g	AOAC 991.14	≤10	<10
Gluten, mg/kg	R7001	<20	<5
PH	Q/DFS0001S-2017	6.5~8.5	7.1
Lead (Pb), mg/kg	GB5009.12-2017	≤0.1	<0.1
Arsenic (As), mg/kg	GB5009.11-2014	≤0.1	<0.1
Cadmium (Cd), mg/kg	GB5009.15-2014	≤0.3	<0.3
Mercury(Hg), mg/kg	GB5009.17-2014	≤0.02	<0.02
Aflatoxin, μg/kg	EN14123	<5	<5

Assesor signature: WU MEIYAN Inspector signature: CHENG GANG

YAN TAI ORIENTAL PROTEIN TECH CO., LTD

9. APPENDIX II

Additional Data on Afflatoxin and Heavy Metal Analysis from three batches of Enzyme Treated Pea Protein

Analytical Report

Sample Code	128-2020-00000101	Report date	06-Jan-2020
Certificate No.	AR-20-VV-000461-01		

Yantai Oriental Protein Tech. Co., Ltd.
Juyi Cao
North Jincheng Road, Zhaoyuan City
Yantai, Shandong Province, P.R.China

Our reference:	128-2020-00000101/ AR-20-VV-000461-01
Client Sample Code:	201912272
Sample described as:	Enzyme Treated Pea Protein (80%)
Sample Packaging:	Sealed plastic bag
Sample reception date:	02-Jan-2020
Analysis starting date:	02-Jan-2020
Analysis ending date:	06-Jan-2020
Arrival Temperature (°C)	15.4
Sample Weight	130g
Sample Type	Powder

		Results	Unit	LOQ	LOD
VV10H	Aflatoxins B1, B2, G1, G2 Method: GB 5009.22-2016 First method				
	Aflatoxin B1	<0.1	µg/kg	0.1	
	Aflatoxin B2	<0.2	µg/kg	0.2	
	Aflatoxin G1	<0.1	µg/kg	0.1	
	Aflatoxin G2	<0.2	µg/kg	0.2	
	Sum of Aflatoxins B1,B2,G1,G2	N/A	µg/kg		

		Results	Unit	LOQ	LOD
☆ SU05E	Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Arsenic (As)	0.017	mg/kg	0.005	
☆ SU05D	Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Lead (Pb)	<0.05	mg/kg	0.05	
☆ SU05G	Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Cadmium (Cd)	0.089	mg/kg	0.005	
☆ SU007	Mercury (AAS) Method: BS EN 13806:2002				
	Mercury (Hg)	<0.005	mg/kg	0.005	

		Results	Unit	LOQ	LOD
☆ SU0SX	Heavy metals as Pb Method: GB 5009.74-2014				
	Heavy metals as Pb	<10	mg/kg	10	

SIGNATURE

Kevin Fu
Authorized Signatory

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
☆ means the test is subcontracted within Eurofins group
⊙ means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
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For and on behalf of Eurofins Technology Service (Qingdao) Co., Ltd.

END OF REPORT

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Analytical Report

Sample Code	128-2020-00000102	Report date	06-Jan-2020
Certificate No.	AR-20-VV-000462-01		

Yantai Oriental Protein Tech. Co., Ltd.
Juyi Cao
North Jincheng Road, Zhaoyuan City
Yantai, Shandong Province, P.R.China

Our reference:	128-2020-00000102/ AR-20-VV-000462-01
Client Sample Code:	20191126
Sample described as:	Enzyme Treated Pea Protein (80%)
Sample Packaging:	Sealed plastic bag
Sample reception date:	02-Jan-2020
Analysis starting date:	02-Jan-2020
Analysis ending date:	06-Jan-2020
Arrival Temperature (°C)	15.4
Sample Weight	150g
Sample Type	Powder

	Results	Unit	LOQ	LOD
VV10H Aflatoxins B1, B2, G1, G2 Method: GB 5009.22-2016 First method				
Aflatoxin B1	<0.1	µg/kg	0.1	
Aflatoxin B2	<0.2	µg/kg	0.2	
Aflatoxin G1	<0.1	µg/kg	0.1	
Aflatoxin G2	<0.2	µg/kg	0.2	
Sum of Aflatoxins B1,B2,G1,G2	N/A	µg/kg		

	Results	Unit	LOQ	LOD
☆ SU05E Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
Arsenic (As)	0.011	mg/kg	0.005	
☆ SU05D Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
Lead (Pb)	<0.05	mg/kg	0.05	
☆ SU05G Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
Cadmium (Cd)	0.031	mg/kg	0.005	
☆ SU007 Mercury (AAS) Method: BS EN 13806:2002				
Mercury (Hg)	<0.005	mg/kg	0.005	

	Results	Unit	LOQ	LOD
☆ SU0SX Heavy metals as Pb Method: GB 5009.74-2014				
Heavy metals as Pb	<10	mg/kg	10	

SIGNATURE

Kevin Fu
Authorized Signatory

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EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
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Analytical Report

Sample Code	128-2020-00000103	Report date	06-Jan-2020
Certificate No.	AR-20-VV-000463-01		

Yantai Oriental Protein Tech. Co., Ltd.
Juyi Cao
North Jincheng Road, Zhaoyuan City
Yantai, Shandong Province, P.R.China

Our reference:	128-2020-00000103/ AR-20-VV-000463-01
Client Sample Code:	20190805
Sample described as:	Enzyme Treated Pea Protein (80%)
Sample Packaging:	Sealed plastic bag
Sample reception date:	02-Jan-2020
Analysis starting date:	02-Jan-2020
Analysis ending date:	06-Jan-2020
Arrival Temperature (°C)	15.4
Sample Weight	160g
Sample Type	Powder

		Results	Unit	LOQ	LOD
VV10H	Aflatoxins B1, B2, G1, G2 Method: GB 5009.22-2016 First method				
	Aflatoxin B1	<0.1	µg/kg	0.1	
	Aflatoxin B2	<0.2	µg/kg	0.2	
	Aflatoxin G1	<0.1	µg/kg	0.1	
	Aflatoxin G2	<0.2	µg/kg	0.2	
	Sum of Aflatoxins B1,B2,G1,G2	N/A	µg/kg		

		Results	Unit	LOQ	LOD
☆ SU05E	Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Arsenic (As)	0.011	mg/kg	0.005	
☆ SU05D	Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Lead (Pb)	<0.05	mg/kg	0.05	
☆ SU05G	Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod.				
	Cadmium (Cd)	0.046	mg/kg	0.005	
☆ SU007	Mercury (AAS) Method: BS EN 13806:2002				
	Mercury (Hg)	<0.005	mg/kg	0.005	

		Results	Unit	LOQ	LOD
☆ SU0SX	Heavy metals as Pb Method: GB 5009.74-2014				
	Heavy metals as Pb	<10	mg/kg	10	

SIGNATURE

Kevin Fu
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EXPLANATORY NOTE

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For and on behalf of Eurofins Technology Service (Qingdao) Co., Ltd.

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GRN 000948 Questions- Response

Enzyme-treated pea protein

Dear Ms. Hall,

RE: GRN 948- Enzyme Treated Pea Protein GRAS Notification

This responds to your email of February 1, 2021 regarding clarifications required for our GRAS Notification No. 000948 for Enzyme Treated Pea Protein submitted on behalf of Yantai Oriental Protein Tech Co., Ltd. We are providing a point-by-point response to your queries along with some relevant clarifications/discussion.

Chemistry

FDA Query 1: In Section 2.3, you indicated that "food grade enzymes are then added to break the protein aggregation." Please provide the conditions (time, temperature, and pH) for the enzymatic hydrolysis step in which the protein aggregation is broken into fragments of lower molecular weights.

Response: The protein aggregation is broken down by enzymes and can occur within a temperature range of 40 - 75^{o}C; the time ranges from 25 - 60 minutes depending on the temperature; and the pH range is approximately neutral.

FDA Query 2: In Section 2.3, you indicated that "The protein is isolated and subjected to flash evaporation..." Please provide detailed descriptions on how the enzyme-treated protein is isolated from the non-protein fraction (or aggregated protein) after treatment and provide information on the molecular weights of the enzyme-treated protein.

Response: The protein is separated from the non-protein fraction using methods and processes that are familiar with the art and protein chemistry. For example, depending on pH, etc., the starch is water-soluble which enables separation of the protein from the starch through filtration using different size screens or by centrifuging to separate the protein which may be further purified, if necessary. The molecular weight of the final protein product is on the range of 1,000 to 20,000 Daltons; the majority of the protein is over 5,000 Daltons.

FDA Query 3: In Sections 1.6 and 2.2, you indicated that "The protein content of the final product is ≥80%." You also presented two different values for protein (as is and dry matter basis) in Table 5 and the value for protein "as is" is less than 80%. Please clarify that the protein amount reported in other sections of the notice is on a dry matter basis.

Response: We confirm that protein amount reported in other sections of the notice is on a dry matter basis.

FDA Query 4: Please provide a statement indicating that all analytical methods are validated for their intended purpose.

Response: We confirm that all analytical methods employed are validated for their intended purposes.

Toxicology

FDA Query 5: It is generally known that pea protein is well tolerated by the majority of the population. However, people allergic to legumes may experience adverse reactions on consuming pea proteins. A recent paper (Lavine E, Ben-Shoshan M. J Allergy Clin Immunol Pract. 2019 Jul-Aug;7(6):2070-2071) discussed examples of the extremes of such adverse reaction, that is, anaphylaxis to pea protein.

a. Please discuss the information in the context of pea protein allergy, citing the frequency of such cases, as reported in the published literature.

b. Please discuss the safety of pea protein in the context of such information.

Response: Thank you for bringing this recent publication to our attention.

Lavine and Ben-Shorshan (2019) reported six children (age 1 to 14 years; 2 males, 4 females) with allergic reaction to foods that were confirmed to contain pea ingredient. Among the six children, four were also reported to be allergic to peanut, while two could eat peanuts or tree nuts freely. In most cases, the reaction was severe. Several (5 of 6) reacted to other leagues such as chickpeas, kidney beans, lentils and green peas. Majority had only mild or non-alarming signs of allergy to legumes before the sentinel event occurred to the pea protein ingredient. These investigators claimed that their findings support the observations that high concentrations of pea protein as compared with cooked peas themselves, may increase the chance of systemic reactions from small amounts of food ingested. Individuals with allergic reaction to peanuts and soy may also experience reactions to pea protein, though this is rare.

In general, there is a tendency for children to outgrow their food allergies, although this is not evident from the US survey of children in the case of peanuts, shellfish, and fin fish (Mark et al., 2020). The available information indicate that unlike peanuts, shellfish, and fin fish, the prevalence of soy allergy decreased by 60% when comparing the average prevalence for all ages with the prevalence for adolescents 14 to 17 years of age (Mark et al., 2020). Although there is no information about pea allergy in different age groups, it is likely that some children may outgrow pea allergy. Pea protein allergy has not been extensively studied.

Immunoglobulin E (IgE)-mediated food allergy is a significant public health issue that affects an estimated 3% to 10% of adults and 8% of children worldwide. The available information suggest that boiling or roasting decreases the IgE-binding capacity for legume allergens. Although the subject of this GRAS is subjected to flash evaporation and spray drying, it is not clear whether it will decrease the IgE binding capacity and similar to other products may contain higher levels of protein as compared to the cooked peas. Yantai, the manufacturer of pea protein, recognizes, the importance to clearly label products, as required by current food labelling laws, especially where

the product is one that the customer might not normally expect to contain peas or pea proteins. Yantai also recommend that the product indicate that peas are legumes and that people with peanut allergies should be cautious when introducing pea into their diet due to the possibility of a pea allergy. It is encouraged that patients and parents to read labels and educate themselves about the variety of foods that may contain pea protein ingredients.

A search of literature did not locate any additional information related to the prevalence of pea protein allergy in children. For those individuals who have a known allergy to widely used products that are safe for the general population, it has generally been FDA's recommendation that those sensitive individuals avoid those products that contain the ingredient.

References:

Lavine, E., Ben-Shoshan, M., 2019. Anaphylaxis to hidden pea protein: A Canadian pediatric case series. J Allergy Clin Immunol Pract. 7(6):2070-2071.

Mark, M., Carina, V., 2020. Recent Surveys on Food Allergy Prevalence. Nutrition Today 55(1) 22-29.

From: Madhu Soni
To: Hall, Karen
Subject: [EXTERNAL] RE: Regarding GRN 000948
Date: Thursday, March 4, 2021 9:33:38 AM

Good morning Ms. Hall,

I am responding to your below email query from your review team related to the intended use of enzyme-treated pea protein described in our GRAS notice GRN 948.

I have discussed this with our client (Yantai) and they have decided not to change the use levels of enzyme-treated pea protein. Given this, the use levels will remain same as described in our previous GRAS (GRN 608). If you have any questions, please let me know. We hope, this will help expedite the FDA review process. Given the marketing commitments, the client is looking forward to FDA completion of the review process. Thank you

Best regards

Madhu

Madhu Soni, *PhD, FACN, FATS*
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From: Hall, Karen [mailto:Karen.Hall@fda.hhs.gov]
Sent: Monday, March 1, 2021 3:43 PM
To: 'Madhu Soni' <sonim@bellsouth.net>
Subject: Regarding GRN 000948

Good Afternoon Dr. Soni,

After reviewing Yantai Oriental Protein Tech Co., Ltd.'s GRAS Notice 000948 for the intended use of enzyme-treated pea protein, we noted below that need to be addressed. Responses may be sent in an email or in a separate document. Please do not send a revised copy of the notice. We respectively request a response within 10 business days. If you are unable to complete the response within that time frame or have questions, please contact me to discuss further options at 240-402-9195 or via email.

Question

In Section 3.1.1. (p. 15), Yantai notes that they may change their use levels for enzyme treated pea protein similar to those described in GRN 000804 (except for use in meat and poultry). However, in GRN 000804, the proposed food uses and use levels for the pea protein are different from those stated on pp. 13-14 of GRN 000948, resulting in a

higher exposure estimate for pea protein. We note that each notice should stand alone and the exposure estimate should be reflective of all intended uses for the ingredient in that notice. If the intended use will be expanded/changed to include food categories in addition to those listed on pp. 13-14, then the intended use should be clearly stated and the dietary exposure estimate should be revised to reflect all of the intended uses of enzyme treated pea protein. Please clarify the intended use and dietary exposure for enzyme treated pea protein.

Kind Regards,

Karen

Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
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Karen.Hall@fda.hhs.gov