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January 28, 2019

Via FedEx & CD-ROM

Dr. Susan Carlson
Director, Division of Biotechnology and GRAS
Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for Roquette Freres Pea Protein Isolate

Dear Dr. Carlson:

We respectfully submit the attached GRAS Notification on behalf of our client, Roquette Freres (Roquette) for pea protein isolate to be used as a concentrated, highly digestible protein source in various food categories (excluding infant formula) and as a binder and extender in meat and poultry products. The pea protein isolate will be used as a substitute for, and/or in conjunction with, other proteins in conventional food products, as well as in meal replacement and dry blend protein powder applications. Thus, the pea protein isolate will not contribute any additional exposure to protein and it is not intended to be used to replace the entire daily protein intake or as the sole source of protein in the diet for consumers. More detailed information regarding product identification, intended use levels, and the manufacturing and safety of the ingredient is set forth in the attached GRAS Notification.

Roquette has determined that their pea protein isolate is GRAS based on scientific procedures in accordance with 21 C.F.R. § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. § 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). Therefore, the use of the pea protein isolate as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notification are available for FDA review and copying at reasonable times at Keller and

Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

We look forward to the Agency's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Sincerely,

Evangelia C. Pelonis

GRAS Notification for Roquette's Pea Protein Isolate

Prepared for:

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

Submitted by:

Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001

Date:

January 28, 2019

GRAS Notice for Pea Protein Isolate

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LIST OF TABLES AND FIGURES

Part 1 – Signed statements and certification

(1) Applicability of 21 C.F.R. part 170, subpart E

We submit this generally recognized as safe (GRAS) notice in accordance with proposed 21 C.F.R. part 170, subpart E consisting of sections 170.203 through 170.285.

(2) Name and address of the notifier

Roquette Freres
Batiment Alpha 3
Lestrem 62080
FRANCE

All communications on this matter are to be sent to Counsel for the Notifier

Evangelia C. Pelonis
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1001 G Street, NW
Suite 500W
Washington DC 20005
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Fax: 202-434-4646
Email: pelonis@khlaw.com

(3) Name of the notified substance

Pea Protein Isolate is marketed under the trade name Nutralys®. It may also be described as "pea protein," "pea protein concentrate," or "concentrated pea protein."

(4) Applicable conditions of use of the notified substance

Pea Protein Isolate is intended for use as a concentrated, highly digestible protein source in foods, such as bakery products (e.g., bread, rolls, cakes, pasta), cereals, snack foods (e.g., chips, crackers, energy bars), ready-to-drink (RTD) beverages, soups, smoothies, fruit juices, protein beverages, dairy and dairy alternatives (e.g., yogurt, ice cream), meal replacements, nutritional bars, clinical nutrition, fruit and vegetable preparation, meat analog products, processed meat, dry blend protein products, extruded products, chocolate and confection compound coatings, non-chocolate confections, and as a binder and extender in meat and poultry applications. Infant formula is excluded from the intended uses. It is also intended for use in specialty foods intended to meet the protein requirements for sports activity or for

weight control. It is not intended to replace the entire daily protein intake or to be used as the sole source of protein in the diet.

Pea Protein Isolate is intended to be used as a binder and extender in the following meat and poultry applications: raw comminuted poultry, raw comminuted meat, sausage/hot dogs, and soups/stews/salad/similar products. Roquette has submitted an Acceptability Determination to the USDA Food Safety Inspection Service (FSIS) to show that the ingredient is suitable for use as a binder and extender in these applications.

A full list of the potential food applications and levels of intended use for Roquette's Pea Protein Isolate are discussed further in Part 3.

(5) Basis for the GRAS determination

Keller and Heckman LLP, on behalf of Roquette Freres, hereby notifies the Agency of its determination that Pea Protein Isolate is Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This GRAS conclusion is based on scientific procedures in accordance with 21 C.F.R. §170.30(a) and (b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. §170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016).

(6) Exclusion from premarket approval

Roquette has concluded that Pea Protein Isolate is GRAS and not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. It is respectfully submitted that this Notification establishes GRAS status for Pea Protein Isolate for use in food based on: (1) the long standing safe consumption of peas as food and the generally recognized safety of peas as traditionally used in food; (2) an analysis of contaminants; (3) the amino acid profile; (4) protein quality; (5) the animal studies that have been conducted on pea protein; and (6) the published safety data on Roquette's Pea Protein Isolate.

(7) Availability of data and information

The analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 C.F.R. § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

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(8) Applicability of FOIA exemptions

Roquette Freres is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential, or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

(9) FSIS/USDA – Use in Meat and/or Poultry

Roquette Freres intends to add Pea Protein Isolate to meat and/or poultry that come under U.S. Department of Agriculture (USDA) jurisdiction (21 C.F.R. § 170.270) and authorize FDA to send USDA any portion of this filing, which does not include any discussion of trade secrets.

(10) Certification

We certify on behalf of our client Roquette Freres that this GRAS conclusion is based on representative data from Roquette Freres required for the safety and GRAS status of the use of Pea Protein Isolate. To the best of our knowledge based on the information provided by Roquette Freres, this GRAS Notice (GRN) is a complete, representative, and balanced submission that includes all pertinent information known to the company concerning the evaluation of the safety and GRAS status of the use of the substance.

Signed:

1/28/2019

Evangelia C. Pelonis
Partner
Keller and Heckman LLP

Date:

Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

(1) Identity of the notified substance

Nutralys® is the trade name for Roquette's Pea Protein Isolate. The ingredient is purified from the dry common yellow pea *Pisum Sativum* which has been consumed as food for centuries. The Pea Protein Isolate is a pure, free-flowing, beige powder that functions as a protein source in foods, and as a binder and extender in meat and poultry products.

(a) Chemical and physical properties

Pea Protein Isolate is composed of a minimum of 80% protein; the remaining components are total extractible fat (9%), moisture (7%), fiber (1%), and other (salts, minerals) (3%). Pea proteins fall into two categories: globulins (55-65% of protein) and albumins (20-25% of protein). The major globulins consist of legumin, vicilin, and convicilin; the major albumins are pea albumin 1 and 2 (PA1 and PA2). There are also lesser amounts of lectins, protease inhibitors and lipoxygenases.[1]

(b) Amino acid analyses

The amino acid profile of Pea Protein Isolate is set forth in **Table 1** and is compared to the profile of unprocessed peas (source material). Roquette used the NF-EN-ISO 13903:2005 method to analyze the amino acid content. Pea Protein Isolate is a highly digestible protein with an 86% Protein Digestibility Corrected Amino Acid Score (PDCAAS) for children and 93% PDCAAS for adults.[2] Protein quality/digestibility is discussed in greater detail in Part 6.

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[1] Le Gall M., Quillien L., Seve B., Gueguen J. and J.P. Lalles (2007). *Weaned piglets display low gastrointestinal digestion of pea (Pisum sativum) lectin and pea albumen*. 2. J. Anim. Sci. 85:2972-2981.

[2] The PDCAAS is a method of evaluating protein quality based on both the amino acid requirements of humans and their ability to digest it; PDCAAS has been adopted as the preferred method to determine protein quality by the U.S. FDA and the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO); Boutrif E., Food Quality and Consumer Protection Group, Food Policy and Nutrition Division, FAO Rome, *Recent Developments in Protein Quality Evaluation*. Food, Nutrition and Agriculture, Issue 2/3, 1991, available at, http://www.fao.org/docrep/U5900t/u5900t07.htm.

Table 1: Comparison of Amino Acids of Pea and Roquette's Nutralys® Pea Protein Isolate

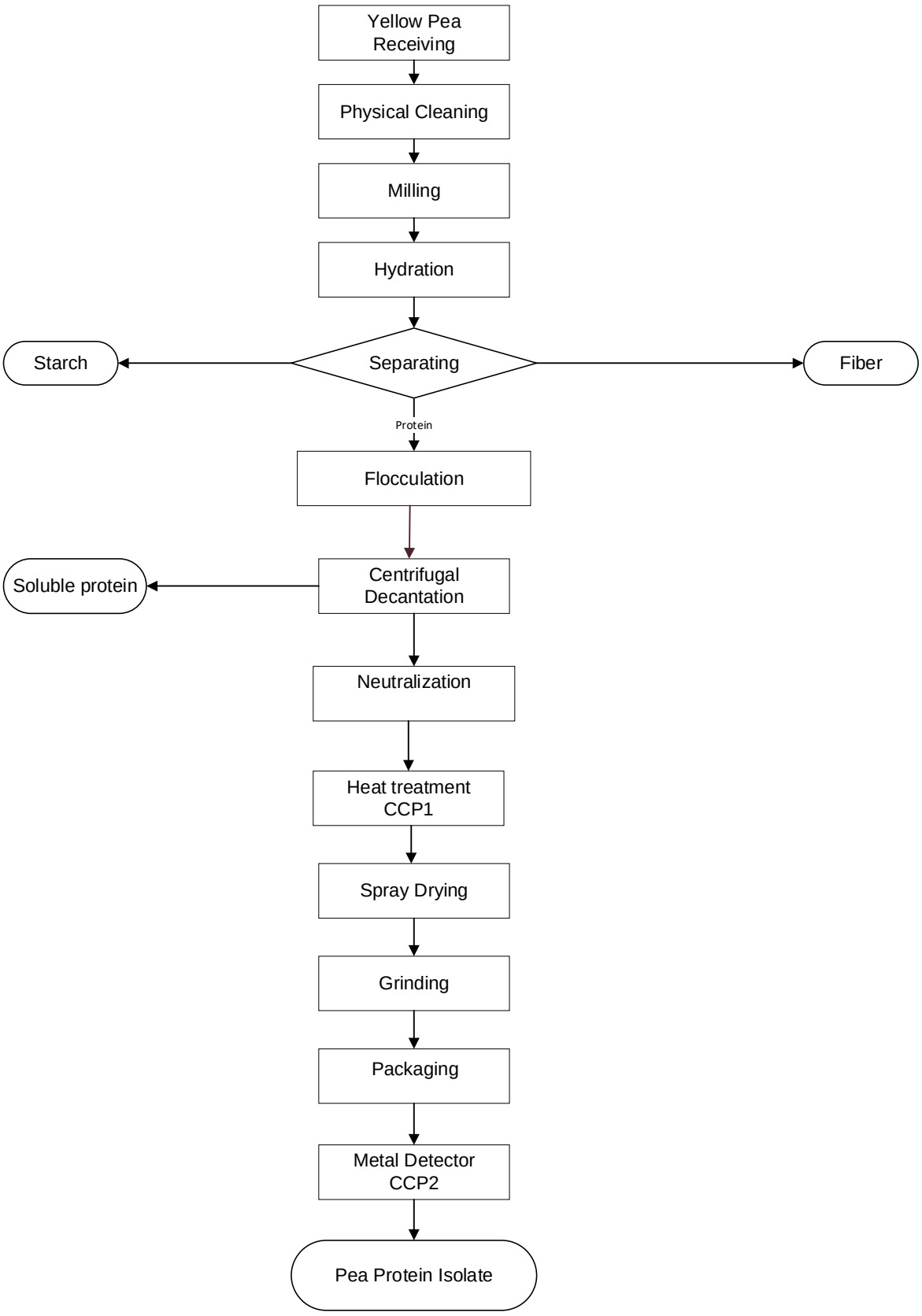
Nutrient	***Pisum sativum* (peas)***	**Nutralys® S85F**	**Nutralys® F85F**	**Nutralys® S85Plus**
	g/100g			
Aspartic acid	2.549	9.0	9.1	9.2
Glutamic acid	3.871	13.6	13.6	13.6
Alanine	1.049	3.3	3.4	3.4
Arginine	1.902	6.7	6.7	6.7
Cysteine	0.273	0.8	0.8	0.8
Glycine	1.012	3.2	3.2	3.2
Histidine	0.586	1.9	1.9	1.9
Isoleucine	0.983	3.6	3.6	3.6
Leucine	1.680	6.4	6.5	6.5
Lysine	1.771	5.8	5.8	5.9
Methionine	0.195	0.8	0.8	0.8
Phenylalanine	1.151	4.2	4.2	4.3
Proline	1.035	3.3	3.4	3.4
Serine	1.069	4.2	4.2	4.2
Threonine	0.813	3.0	3.1	3.1
Tyrosine	0.518	3.1	3.0	3.2
Valine	1.035	3.8	3.8	3.9
Tryptophan	0.159	0.7	0.7	0.8

* Source: United States Department of Agriculture. National Nutrient Database for Standard Reference, Nutrient data for 16085, Peas, split, mature seeds, raw. Release Apr. 1, 2018.

(2) Description of the method of manufacture

Pea Protein Isolate is extracted from the dry common yellow pea, *Pisum Sativum*, in a manufacturing process that involves a series of steps with mild conditions, without the use of organic solvents. The flow chart for the manufacturing of Pea Protein Isolate is shown below.

Figure 1: Pea Protein Isolate General Manufacturing Diagram



Upon receipt, the peas are physically cleaned and ground to remove hulls. These initial processes produce a pea flour, which is a mixture of protein, starch, fiber, sugar, and fat. Water is added to the pea flour and the pea starch and fiber are then removed. The protein goes through separation flocculation steps to adjust the pea protein at the isoelectric point (pI), which is where the proteins have the minimum solubility levels and are able to separate (isoelectric precipitation). The soluble pea protein (albumins) are then removed from the Pea Protein Isolate process. The pea protein is then coagulated, purified, and re-buffered to neutral pH. Following the extraction process, a heat treatment is used as a first critical control process (CCP1) and is conducted to effectuate microbial reduction and reduce moisture. Food grade enzymes from the exopeptidase and endopeptidase families are then used to enhance the pea protein isolate functionalities, such as a viscosity decrease. These added enzymes are destroyed with a thermal heat treatment (CCP1) before spray drying.

The function of the enzymes is to split pea proteins via hydrolysis. The hydrolysis of peptide bonds by proteases is called proteolysis. The above protease enzymes hydrolyze (breaks) the peptide bonds (linkages) in pea proteins; releasing lower molecular weight peptides of shorter chain length, and amino acids.

The final processing step includes drying the Pea Protein Isolate product in a spray dryer before it is packaged, tested through a metal detector as the second critical control process (CCP2), and stored.

The Pea Protein Isolate is produced in compliance with good manufacturing practices (GMPs) for the production of food pursuant to Subpart B in 21 C.F.R. Part 117, and adheres to all applicable requirements of the Foreign Supplier Verification Program (FSVP) in 21 C.F.R. Part 1, Subpart L.

(3) Specifications and identity

Rich in dietary protein and fiber, yellow peas offer many nutritional benefits. The nutritional composition of Pea Protein Isolate is provided in **Table 2**, where the nutritional composition of Pea Protein Isolate is also compared to the nutritional profile of raw or unprocessed peas.

Table 2: Nutritional Composition of Roquette's Pea Protein Isolate

Nutrient	Pea Protein Isolate (S85Plus)	Raw Pea – *Pisum Sativum**	Unit
Calories	400	364	Kcal/100g
Protein	80	23.12	g/100g
Total extractible fat	9	3.89	g/100g
Saturated fat	2	0.408	g/100g
Monounsaturated fat	2	0.615	g/100g
Polyunsaturated fat	5	1.022	g/100g
Cholesterol	0	0	mg/100g
Carbohydrate	0	61.63	g/100g
Sugars	0	3.14	g/100g
Dietary fiber	1	22.2	g/100g
Sodium	900	5	mg/100g
Potassium	300	852	mg/100g
Ash	3.9	-	g/100g
Moisture	7	8.69	g/100g

* Source: United States Department of Agriculture. National Nutrient Database for Standard Reference, Nutrient data for 16085, Peas, split, mature seeds, raw. Release 28.

(a) Nutralys® Pea Protein Isolate Variations

Nutralys® Pea Protein Isolate is manufactured in several particle sizes in order to suit a wide range of applications. The Pea Protein Isolate is produced within the range of specification with a protein target of 80% minimum for all the grades. These variations have no effect on the digestibility or availability of protein in the body.

The Nutralys® grades are summarized in **Table 3**, which details the specifications for three product lines (S85F, F85F, and S85Plus), including information about the levels of moisture, protein content, particle size, ash, pH, and microbiological values. Additionally, Roquette has provided analyses from three non-consecutive batches from three Pea Protein Isolates (S85F, F85F, and S85Plus) indicating a consistent manufacturing process as shown in **Tables 4 - 6**.

Table 3: Product Specifications for Roquette's Pea Protein Isolate

	Specification S85F	Specification F85F	Specification S85Plus	Internal Method	External Method
Physical - Chemical					
Appearance	Beige powder	Beige powder	Beige powder	MCL 086G	-
Loss on Drying	10% max.	10% max.	10% max.	MCL 002B	NF / ISO 1666 = Moisture determination
Protein Content (dry basis)	84% min.	83% min.	84% min.	MCL 030H	ISO 16634 = Total Nitrogen
Particle size on 200 µm	10% max.	10% max.	15% max.	MCL 110C	-
Ash	5%	5%	10%	MCL 010A	-
Poured Bulk Density	0.35-0.50 kg/L	0.45 kg/L no spec defined (indicative value)	0.35-0.60 Kg/L	MCL 095A	Direct Density measurement on powdery product
pH at 10% (w/w)	6.5-8.0	6.5-8.0	6.5-8.0	MCL 020P	-
Aqueous Solubility (pH 7)	55%	No spec defined	50%	FS-MCL 090K	-
Microbiological					
Total plate count	5000 cfu/g max.	5000 cfu/g max.	5000 cfu/g max.	MMC 2002A	NF EN ISO 4833 ISO 7218-A1
Yeasts	50 cfu/g max.	50 cfu/g max.	50 cfu/g max.	MMC 2003-A	ISO 7218/A1 NF V08-059
Moulds	50 cfu/g max.	50 cfu/g max.	50 cfu/g max.	MMC 2003-A	ISO 7218/A1 NF V08-059
Enterobacteriaceae	10 cfu/g max.	10 cfu/g max.	10 cfu/g max.	MMC 2005	ISO 7218/A1
Escherichia coli	Absent in 1g	Absent in 1g	Absent in 1g	MMC 2007-A	ISO 7218/A1
Salmonella	Absent in 25g	Absent in 25g	Absent in 25g	MMC 2010-H	AFNOR CERTIFICATION NF EN ISO 6579 / ISO 16140 AOAC = 050701
Staphylococcus aureus	Absent in 1g	Absent in 1g	Absent in 1g	MMC 2011-B	U.S.P, P.E , J.P
Bacillus Cereus	100 cfu/g max.	100 cfu/g max.	100 cfu/g max.	MMC 2028	NF EN ISO 7932

As evidenced by the above specifications, the variations among the product lines are minute. The differences are mostly attributed to the varying particle sizes and flocculation temperatures to suit a wide range of applications. For example, certain product lines are suited for sauces, caramel and chocolate compounds, or baking applications, whereas other product lines provide

better functionality for meat analog and dairy alternative products. However, the general formulation and safety analysis apply to all product lines.

Table 4: Analysis of Three Non-Consecutive Lots of Pea Protein Isolate, S85F

	Unit	**Method**	**Specification**	**S85F Lot#**	**S85F Lot#**	**S85F Lot#**
Appearance		MCL	Beige Powder	Conforms	Conforms	Conforms
Loss on Drying	%	MCL	10 max.	6.1	6.8	6.7
Protein Content (N x 6.25) (dry basis)	%	MCL	84 min.	81.0 commercial	79.5 commercial	80.4 commercial
Particle Size: Residue on 200 MIC	%	MCL	10 max.	3.3	2.7	7.0
Poured Bulk Density	kg/L	MCL	0.35-0.50 kg/L	0.46	0.43	0.47
pH at 10% (w/w)		MCL	6.5-8.0	7.4	7.4	7.5
Solubility (pH 7)	%	MCL	55	64.4	50.6	58.9
Heavy Metals						
Lead	mg/kg	Ext	< 0.2	< 0.01	0.02	< 0.01
Arsenic	mg/kg	Ext	< 0.2	< 0.10	< 0.10	< 0.10
Cadmium	mg/kg	Ext	< 0.2	0.04	0.06	0.04
Mercury	mg/kg	Ext	< 0.2	< 0.01	< 0.01	< 0.01
Mycotoxin						
Ochratoxin A	mg/kg	Ext	< 20	1.1	<1	6.3
Microbiological Analysis						
Total Count	cfu/g	MMC	5000 max.	1900	240	180
Yeasts	cfu/g	MMC	50 max.	< 10	< 10	< 10
Moulds	cfu/g	MMC	50 max.	< 10	< 10	< 10
Enterobacteriaceae	cfu/g	MMC	10 max.	< 10	< 10	< 10
E. coli	g	MMC	Absent in 1	Absent	Absent	Absent
Salmonella	g	MMC	Absent in 25	Conforms	Conforms	Conforms
Staphylococcus	g	MMC	Absent in 1	Absent	Absent	Absent

Aureus						
Bacillus Cereus	cfu/g	MMC	100 max.	< 10	< 10	< 10

Table 5: Analysis of Three Non-Consecutive Lots of Pea Protein Isolate, F85F

	Unit	**Method**	**Specification**	**F85F Lot#**	**F85F Lot#**	**F85F Lot#**
Appearance		MCL	Beige Powder	Conforms	Conforms	Conforms
Loss on Drying	%	MCL	10 max.	5.9	6.0	6.5
Protein Content (N x 6.25) (dry basis)	%	MCL	84 min.	81.3 commercial	82.0 commercial	80.3 commercial
Particle Size: Residue on 200 MIC	%	MCL	10 max.	4.2	2.6	3.1
Poured Bulk Density	kg/L	MCL	0.45 no specification defined (indicative value)	0.48	0.45	0.41
pH at 10% (w/w)		MCL	6.5-8.0	7.4	7.5	7.4
Solubility (pH 7)	%	MCL	No spec defined	32.7	38.0	43.9
Heavy Metals						
Lead	mg/kg	Ext	< 0.2	0.01	0.02	0.02
Arsenic	mg/kg	Ext	< 0.2	< 0.10	< 0.10	< 0.10
Cadmium	mg/kg	Ext	< 0.2	0.04	0.04	0.07
Mercury	mg/kg	Ext	< 0.2	< 0.01	< 0.01	< 0.01
Mycotoxin						
Ochratoxin A	mg/kg	Ext	< 20	0	0	<1
Microbiological Analysis						
Total Count	cfu/g	MMC	5000 max.	1500	400	20
Yeasts	cfu/g	MMC	50 max.	< 10	< 10	< 10
Moulds	cfu/g	MMC	50 max.	< 10	< 10	< 10
Enterobacteriaceae	cfu/g	MMC	10 max.	< 10	< 10	< 10
E. coli	g	MMC	Absent in 1	Absent	Absent	Absent

Salmonella	g	MMC	Absent in 25	Conforms	Conforms	Conforms
Staphylococcus Aureus	g	MMC	Absent in 1	Absent	Absent	Absent
Bacillus Cereus	cfu/g	MMC	100 max.	< 10	< 10	< 10

Table 6: Analysis of Three Non-Consecutive Lots of Pea Protein Isolate, S85Plus

	Unit	**Method**	**Specification**	**S85Plus Lot#**	**S85Plus Lot#**	**S85Plus Lot#**
Appearance		MCL	Beige Powder	Conforms	Conforms	Conforms
Loss on Drying	%	MCL	10 max.	6.2	6.3	6.1
Protein Content (N x 6.25) (dry basis)	%	MCL	84 min.	79.4 commercial	80.2 commercial	81.2 commercial
Particle Size: Residue on 200 MIC	%	MCL	10 max.	3.5	7.5	7.3
Poured Bulk Density	kg/L	MCL	0.35-0.60	0.45	0.50	0.53
pH at 10% (w/w)		MCL	6.5-8.0	7.0	6.9	7.0
Solubility (pH 7)	%	MCL	50	50.0	50.0	50.0
Heavy Metals						
Lead	mg/kg	Ext	< 0.2	0.02	0.02	0.03
Arsenic	mg/kg	Ext	< 0.2	< 0.10	< 0.10	< 0.10
Cadmium	mg/kg	Ext	< 0.2	0.08	0.06	0.06
Mercury	mg/kg	Ext	< 0.2	< 0.01	< 0.01	< 0.01
Mycotoxin						
Ochratoxin A	mg/kg	Ext	< 20	<1	2.6	0
Microbiological Analysis						
Total Count	cfu/g	MMC	5000 max.	500	300	50
Yeasts	cfu/g	MMC	50 max.	< 10	< 10	< 10
Moulds	cfu/g	MMC	50 max.	< 10	< 10	< 10
Enterobacteriaceae	cfu/g	MMC	10 max.	< 10	< 10	< 10
E. coli	g	MMC	Absent in 1	Absent	Absent	Absent

Salmonella	g	MMC	Absent in 25	Conforms	Conforms	Conforms
Staphylococcus Aureus	g	MMC	Absent in 1	Absent	Absent	Absent
Bacillus Cereus	cfu/g	MMC	100 max.	< 10	< 10	< 10

(4) Stability Data

The general stability of Nutralys® Pea Protein Isolate was assessed by studying three lots of S85Plus (Lots) for a total of 24 months at a temperature of < 30°C. The storage conditions varied depending on lot type. For example, B1-type lots were stored in bag type 106, whereas B2-type lots were stored in bag type 249.

Based on the results of the study, no significant degradation was observed over the 24-month period. Thus, the results of the stability assessment demonstrate that Nutralys® Pea Protein Isolate, stored in all tested storage conditions, is stable for at least 24 months. **Table 7** summarizes the results from the stability tests for Nutralys® S85Plus (Lots).

Table 7: Stability Data for Nutralys® S85Plus

Criteria	**Specification**					
Appearance	Conforms (C)					
Dry Substance	90% min					
Protein Content (%/DS)	83% / DS min.					
Ash	10% max.					
pH in solution	6.5 – 7.5					
Arsenic	0.20 ppm max.					
Lead	0.20 ppm max.					
Cadmium	0.10 ppm max.					
Mercury	0.02 ppm max.					
Total aerobic plate count CFU/g	10,000 max.					
Yeasts CFU/g	100 max.					
Molds CFU/g	100 max.					
Escherichia coli absent/g	Conforms (C)					
Salmonellae absent/25g	Conforms (C)					
Lot						
Date	T0	T0+3 months	T0+6 months	T0+12 months	T0+18 months	T0+24 months
Appearance (C)	C	C	C	C	C	C

Dry Substance (%)	95.1	95.4	94.8	94.4	93.5	93.3
Protein Content (%/DS)	84.3	-	83.8	83.6	83.5	83.2
Ash	5.2	-	-	5.1	5.1	5.0
pH in solution	7.0	6.9	6.9	6.9	6.9	6.9
Total Aerobic Plate Count (CFU/g)	450	-	-	<100	<100	<100
Yeasts (CFU/g)	<10	-	-	<10	<10	<10
Molds (CFU/g)	<10	-	-	<10	<10	<10
Lot						
Date	T0	T0+3 months	T0+6 months	T0+12 months	T0+18 months	T0+24 months
Appearance (C)	C	C	C	C	C	C
Dry Substance (%)	95.1	95.0	93.9	93.1	92.2	92.2
Protein Content (%/DS)	84.3	-	83.7	84.6	83.6	83.1
Ash	5.2	-	-	5.1	5.0	5.0
pH in solution	7.0	6.9	6.9	6.9	6.9	6.8
Total Aerobic Plate Count (CFU/g)	450	-	-	<100	<100	<100
Yeasts (CFU/g)	<10	-	-	<10	<10	<10
Molds (CFU/g)	<10	-	-	<10	<10	<10
Lot						
Date	T0	T0+3 months	T0+6 months	T0+12 months	T0+18 months	T0+24 months
Appearance (C)	C	C	C	C	C	C
Dry Substance (%)	95.5	94.9	93.9	93.0	92.3	92.2
Protein Content (%/DS)	83.8	-	83.5	84.7	82.7	83.3
Ash	5.4	-	-	5.4	5.4	5.2
pH in solution	6.9	7.0	7.1	7.0	7.0	7.0
Total Aerobic Plate Count (CFU/g)	1800	-	-	250	1000	950
Yeasts (CFU/g)	<10	-	-	<10	<10	<10
Molds (CFU/g)	<10	-	-	<10	<10	<10

(5) Contaminants

Roquette monitors the raw peas and the pea protein ingredient for pesticide residues – the pea protein ingredient is monitored at a minimum of three times per year. Roquette also monitors for the presence of mycotoxins, such as Ochratoxin A. Monitoring for Ochratoxin A is conducted on all batches of the pea protein, and monitoring for other mycotoxins, including aflatoxins, zearalenone, and fumonisins, are conducted at least three times per year.

The results of each product variation's three batch analyses for the contaminants that are monitored in the pea protein ingredient are provided below in **Tables 8 - 10** and indicate that the pea protein does not contain any of these contaminants at levels of concern.

Table 8: Contaminant Analysis for S85F Lot# [redacted]

Property	**Unit**	**Method**	**Specification**	**S85F Lot#** [redacted]	**S85F Lot#** [redacted]	**S85F Lot#** [redacted]
Pesticide Residue Analysis						
Chlorpyrifos-methyl	mg/kg	§64 LFBG L00.00-34, mod.	-	ND	ND	ND
Deltamethrine	mg/kg	MOC3/05	-	ND	ND	ND
Pirimiphos-methyl	mg/kg	MOC3/25	-	ND	ND	ND
Pyrimethanil	mg/kg	MOC3/25	-	ND	ND	ND
Tebuconazole	mg/kg	MOC3/25	-	ND	ND	ND
Mycotoxins						
Ochratoxin A	µg/kg	MOC3111	-	1.1 ± 0.3	Below LOQ <1	6.3 ± 1.3

*ND = Not detected

Table 9: Contaminant Analysis for F85F Lot#

Property	Unit	Method	Specification	F85F Lot#	F85Fs Lot#	F85F Lot#
Pesticide Residue Analysis						
Chlorpyrifos-methyl	mg/kg	§64 LFBG L00.00-34, mod.	-	ND	ND	ND
Deltamethrine	mg/kg	MOC3/05	-	ND	ND	ND
Pirimiphos-methyl	mg/kg	MOC3/25	-	ND	ND	ND
Pyrimethanil	mg/kg	MOC3/25	-	ND	ND	ND
Tebuconazole	mg/kg	MOC3/25	-	ND	ND	ND
Mycotoxins						
Ochratoxin A	µg/kg	MOC3111	-	ND	ND	Below LOQ <1

*ND = Not detected

Table 10: Contaminant Analysis for S85Plus Lot#

Property	Unit	Method	Specification	S85Plus Lot#	S85Plus Lot#	S85Plus Lot#
Pesticide Residue Analysis						
Chlorpyrifos-methyl	mg/kg	§64 LFBG L00.00-34, mod.	-	ND	ND	ND
Deltamethrine	mg/kg	MOC3/05	-	ND	ND	ND
Pirimiphos-methyl	mg/kg	MOC3/25	-	ND	ND	ND
Pyrimethanil	mg/kg	MOC3/25	-	ND	ND	ND
Tebuconazole	mg/kg	MOC3/25	-	Below LOQ <0.01	Below LOQ <0.01	ND
Mycotoxins						
Ochratoxin A	µg/kg	MOC3111	-	Below LOQ <1	2.6 ± 0.6	ND

*ND = Not detected

Part 3 – Dietary exposure

(1) Estimate of dietary exposure

Pea Protein Isolate is intended for use as an ingredient in various food categories at levels ranging from 1 to 90 percent. The Pea Protein Isolate will be used as a substitute for, and/or in conjunction with, other proteins (such as soy protein, whey protein, and animal derived protein) in bakery products, cereals, snack foods, ready-to-drink (RTD) beverages, soups, smoothies, fruit juices, protein beverages, dairy and dairy alternatives, meal replacements, nutritional bars, clinical nutrition, fruit and vegetable preparation, meat analog products, processed meat, dry blend protein powders, extruded products, chocolate and confection compound coatings, non-chocolate confections, and as a binder and extender in meat and poultry applications at levels ranging from 1 to 90%. A list of the proposed food uses and use-levels for the Pea Protein Isolate is provided below in **Table 11**.

Table 11: List of Proposed Food Uses and Use-Levels for Roquette's Pea Protein Isolate

Product category	**Use Level**
Bakery products: breads, rolls, bars, cakes, pasta, cookies	5-10 %
Cereals: cold cereals, oatmeal, cereal bars	1-30 %
Snack Foods: chips, crackers, energy bars	2-30%
Ready-to-drink (RTD) beverages, soups, smoothies, fruit juices, high protein beverages	3-50%
Dairy and Dairy Alternatives: cheeses, spreads, creamers, yogurt, drinkable yogurts, ice cream, refrigerated desserts, frozen desserts, milks, dips, whipped toppings	2-20%
Meal Replacement/Nutritional bars	10-30%
Meat Analogs	10-30%
Processed Meat	2-7%
Dry Blend Protein powders	20-90%
Extruded Products: pea crisps	30-90%
Chocolate and Confection Compound Coatings	10-25%
Non-Chocolate Confection (chewy candies, gummies)	2-30%

The Pea Protein Isolate will be added to food products as a protein substitute and therefore will not contribute any additional exposure to protein for consumers. We do not realistically expect that the actual consumption of foods containing the Pea Protein Isolate would result in a daily consumption greater than the Daily Reference Value (DRV) of 50 g/day of protein for adults and

children 4 or more years of age. Additionally, the Institute of Medicine (IOM) used the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 to estimate the background dietary intakes of protein for the US population.[3] The mean adult protein intake ranged from 56-104 g/day, depending on the age group. At the 90th percentile, adult protein intakes ranged from 76 g/day to 142 g/day. Thus, the IOM has established a Dietary Reference Intakes (DRIs) for protein of 56 g/day for adult males and 46 g/day for adult females.

We do not realistically expect that the actual consumption of foods containing Roquette's Pea Protein Isolate would result in a daily consumption of greater than the DRV or DRI for protein. Most of the population's intake of protein is, and will remain, in the form of unprocessed foods, including meat, poultry, fish and legumes. Moreover, as noted above, for the processed foods to which the proteins will be added, there are competitive products in the market.

[3] *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, INSTITUTE OF MEDICINE (2005), http://www.nationalacademies.org/hmd/~/media/Files/Activity%20Files/Nutrition/DRI-Tables/3_RDA%20AI%20AMDR%20Values_Total%20Water%20and%20Macronutr.pdf?la=en.

Part 4 – Self-limiting levels of use

The use of Pea Protein Isolate as a food ingredient is limited by the level that can technically be added to a given food without jeopardizing its quality and consumer acceptability. In addition, use is limited by cost of the ingredient.

Part 5 – Experience based on common use in food before 1958

The statutory basis for the conclusion of GRAS status of the Pea Protein Isolate in this document is not based on common use in foods before 1958. The GRAS determination is based on scientific procedures. However, as described below, the pea protein source material, peas, has been commonly used in foods prior to 1958.

Part 6 – Narrative

(1) Introduction

The conclusion that Roquette's Pea Protein Isolate is GRAS under the conditions of its intended use in specific conventional food and beverage products is based on (1) the composition and manufacturing process of the pea protein, (2) the intended uses that result in safe dietary exposure, and (3) the safety information on pea and pea protein.

(2) Existing Clearances for Pea Protein

Pea protein has been recognized as GRAS by FDA for use as an ingredient, formulation aid, and texturizer in baked goods, 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%.

Further, unhydrolyzed and hydrolyzed pea protein has been recognized as GRAS by FDA for use as an ingredient in bakery products, snack foods, beverages (including nutritional beverages), soups, dairy products, dry instant milk shake mixes and protein drinks, instant powdered nutritional beverages, processed meat products, vegetarian food products/meat analogues, and meal replacement/nutritional bars at levels ranging from 2-90% of the finished food. Effective GRAS notices pertaining to pea and pea protein are described in **Table 12** below.

Table 12: GRAS Notices for Pea Ingredients

Year	Clearance
2018	GRN 788, Pea protein; For use as an ingredient in conventional foods; FDA has no questions.
2016	GRN 608, Pea protein concentrate; For use as an ingredient in conventional foods; FDA has no questions
2016	GRN 581, Unhydrolyzed and hydrolyzed pea protein; FDA has no questions
2014	GRN 525, Pea fiber; For use as an ingredient in conventional foods; FDA has no questions
2006	GRN 182, Pea protein isolate; For use as a filling agent in wine making; FDA has no questions

(3) Safety of Pea

Peas, *Pisum Sativum*, both yellow and green varieties, have a safe and extraordinary long use as food in virtually all countries of the world. The earliest archaeological finds of peas come from neolithic Syria, Turkey and Jordan, and peas were cultivated in the Egyptian delta area by 4800-4400 BC.[4] Peas also have played a prominent role in the science of genetics. Gregor Mendel determined, inter alia, that that the yellow color of *Pisum Sativum* was a recessive trait which only appeared when the dominant green color trait was not present. The yellow pea cultivar of *Pisum Sativum* is popular in Europe and traditionally sold after dehulling as dried "split peas," and made into soups or purées. The green variety, or garden pea, was common in the American colonies by 1600 and Thomas Jefferson grew more than 30 cultivars of peas in his gardens at Monticello.[5] Different cultivars have approximately the same overall composition, subject to the varietal influences of climate, season and soil quality.

(a) Anti-Nutrient Factors (ANFs) in Peas

Peas, in addition to starch and protein, contain significant levels of a variety of anti-nutrient factors (ANFs) including, protease inhibitors, lectins, tannins, saponins, phytic acid (phytates) and α-galactosides. These ANFs are not generally potent toxicants *per se* and are not hazardous at the normal levels in peas, but they can reduce the nutritive value of peas. ANFs primarily reduce the digestibility of the protein and other essential nutrients by binding strongly with them and making them less bioavailable. This reduces the caloric benefit of the peas as well as the amount of utilized protein and is particularly important when peas are used as the protein supplements for animal feed. Protease inhibitors, for example, of which trypsin inhibitors are the best known, reduce the effectiveness of enzymes responsible for breakdown of vicilin and convicilin, two of the major proteins in peas.[6] Phytates are strong chelators of important

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[4] Zohary D. and Hopf M. (2000). *Domestication of Plants in the Old World*, third edition. Oxford: University Press. ISBN 978-0-19-850356-9 p. 105–107.

[5] Kafka, B. (2005) "Vegetable Love" New York, Artisan.

[6] Perrot, C., Quillien, L., Gueguen, J. and Legoux, A. (1999). *Identification by immunoblotting of pea (Pisum sativum) proteins resistant to in vitro enzymatic hydrolysis.* Sciences des Alimants. 19: 377-390.

minerals such as calcium, iron and zinc, and therefore can contribute to mineral deficiencies in people by the sequestration of these minerals thus reducing their bioavailability.

These ANFs as well as the relatively lower levels of sulfur containing amino acids in legume protein tend to reduce its nutritional utilization compared to proteins of animal origin. Monogastric animals in particular do not have the enzymes capable of dealing with these ANFs and while peas and other legumes are used as feed supplements, several approaches have been used to remove or inactivate ANFs, thus, increasing the bioavailability of the supplements. Some of these processes include: cultivar selection, chemical or physical treatment, soaking, thermal treatments, irradiation, and protein fractionation.[7] Of these, the combination of cultivar selection and hydrothermal processing of pea protein isolates has proved effective at lowering the level of the more important ANFs in peas very significantly. **Table 13** shows the improvement obtained both by the selection of cultivars lowest in anti-nutrients and by the processes used to make Roquette's Pea Protein Isolate.

[7] Fernandez-Quintela, A., Macarulla, M.T., Del Barrio, A.S. and Martinez, J.A. (1997). *Composition and properties of protein isolates obtained from commercial legumes grown in northern Spain*. Plant Foods for Human Nutrition. 51: 331-342, 1997.

Table 13: Reduction in Anti-Nutrient Factors (ANF) of Pea Protein Isolate

Anti-Nutrients	Normal ANF Levels in Unselected peas	Reduction via Cultivar Selection (Yellow peas)	Final Reduction by Extraction Process
Tannins	0.016 – 0.033%	Low tannin varieties. Present in hull	Reduced by dehulling (< 10 ppm)
Phytates	0.72-1.23% (>50% of phosphorous)	-	1.2 % of phosphorous
Trypsin inhibitors	6-15 TIU/mg sample	2.5 TIU/mg sample	<2.5 TIU/mg protein
Lectins	0.1- 0.3%	0.1-0.3% Non-toxic *	0.1-0.3%
Saponins	-	10 ppm in yellow peas	Reduced by heat < 10 ppm
α-galactosides	2.3 -9.6% [8, 9,10]	-	0.5 -1.0%[+]

[#] References supporting the listed data are found in the section on anti-nutrients
* Lectins in yellow peas are recognized as non-toxic (3rd European conference on Grain Legumes 1998)
+ α-galactosides consisting only of stachyose and verbascose. Raffinose is not given.

In extracting the pea protein from peas, a potential nutritional hazard might arise if these ANFs were somehow concentrated into the final protein isolate. Because this does not occur, and since the Pea Protein Isolate is lower in these ANFs than the peas themselves, it is significantly more digestible and more complete as a protein source than the whole pea. Another source of potential toxicity might occur if the processing temperatures were high enough to produce conformational changes in the protein or alterations in the amino acids themselves. But, as the processing conditions are mild, this cannot occur. For both these reasons Pea Protein Isolate is at least as safe as peas themselves and a significantly more digestible protein source. Below we provide a short discussion of the issues with various ANFs and why the ANFs are not an issue in the Pea Protein Isolate.

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[8] Frias, J., Concepcion, V.V., Kozlowska, H., Gorecki, R., Honke, J., and C.L., Hedley (1996). *Evolution of soluble carbohydrates during the development of pea, fava bean and lupin seeds.* Zeitschrift fur Lebensmitteluntersuchung und-Forschung A. 203: (1) 27-32.

[9] Urbano, G., Lopez- Jurado, M. *et al* (2005). *Nutritional assessment of raw and germinated pea (Pisum Sativum L.) protein and carbohydrate by in vitro and in vivo techniques.* Nutrition 21: 230-239.

[10] Martinez-Villaluenga, C., Frias, J., Vidal-Valverde, C. (2008). *Alpha-galactocides: Antinutritional factors or functional ingredients.* Critical Reviews in Food Science and Nutrition, 48: 301-316.

i. Tannins

Tannins are astringent and bitter plant polyphenols that are capable of interacting with proteins, by binding with them and reducing their digestibility.[11] Examples of tannins are the gallotannins which produce gallic acid and sugars upon hydrolysis, and the proanthocyanidins which are resistant to hydrolysis. The levels of tannins in peas typically range from 162– 325 ppm dry matter.[12] Several adverse nutritional effects may be associated with tannins, including depression of food intake, complexation with digestive enzymes, thus, interfering with normal digestion, and local and systemic toxicity.[13] Tannins may also complicate metallic cofactors or enzymes. In general, a principle effect of the ingestion of tannins is a reduction in the digestibility of the protein component of the diet.

Several studies have shown that such effects require significant amounts of dietary tannin, far more than the small amounts found in dietary levels of pea protein.[14] Most of the tannins are in external fibers and are eliminated in the bran and not carried over into the protein fraction

ii. Phytates

Phytic acid (known as inositol hexakisphosphate (IP6) or phytate when in salt form) is the principal storage form of phosphorous in many plant tissues especially bran and seeds.[15] It accounts for 50–80% of the total phosphorus in different cereals. Phosphorous in phytate form is, in general, not bioavailable to non-ruminant animals because they lack the digestive enzyme, phytase which is required to separate phosphorus from the phytate molecule. Phytate is also a strong chelator of important minerals such as calcium, iron and zinc, and therefore can contribute to mineral deficiencies in people by the sequestration of these minerals thus reducing their

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[11] Glick, Z. and Josyln, M.S. (1970). *Effect of tannic acid and related compounds on the absorption and utilization of protein in the rat.* J. Nutr. 100: 156.

[12] Wang, X., Warkentin, T.D., Briggs, C.J., Oomah, B.D., Campbell, C.G. and S. Woods. (1998). *Tot-5.2al phenolics and condensed tannins in field pea (Pisum Sativum L.) and grass pea (Lethrus sativus).* Euphytica 101: (1) 97-102.

[13] Fahey Jr., G.C. and H.G., Jung 1989. *Phenolic compounds in forages and fibrous feedstuffs.* P. R. Cheeke (ed). Toxicants of plant origin. Vol. IV Phenolics. pp. 123-190. CRC Press, Inc. Florida.

[14] See id.

[15] Committee on Food Protection, Food and Nutrition Board, National Research Council (1973). *"Phytates" Toxicants Occurring Naturally in Foods*. National Academy of Sciences. pp. 363–371.

bioavailability. Phytate also acts as an acid, chelating the vitamin niacin (B_3) which is basic, and may contribute to vitamin B_3 deficiency (pellagra).[16] The level of phytate in peas was found to range from 0.72-1.23 %.[17]

iii. α-galactosides

α-galactosides are a family of polysaccharides (produced in plant seeds) composed of one sucrose unit linked by α−1,6 molecular bonds to several galactose units. Next to sucrose itself they are the most abundant soluble sugars in the plant kingdom. The α-galactosides in peas include raffinose, stachyose, and verbascose. The human intestinal tract does not have the enzyme α-galatosidase (α-GAL), capable of splitting these oligosaccarides; instead they are acted on by anaerobic bacteria in the colon which produce gases and cause flatulence. Frias et al (1995) found that the α-galactoside levels of peas increased as they matured reaching 3.8% in mature pea seeds.[18] After processing to make the protein isolate, the total carbohydrate is reduced from 45% to 0% and the protein increased from 25% to 80%. Before processing, the carbohydrate consists mainly of polysaccharides; including the α-galactosidides, stachyose, verbascose and raffinose. After processing the combined level of stachyose and verbascose in Nutralys® is reduced to <0.5%. The small percentage of indigestible polysaccharides remaining after processing would not be expected to cause significant flatulence.[19]

iv. Protease Inhibitors

Protease inhibitors, of which trypsin inhibitors are the most well-known, are chemicals that reduce the availability of proteases, the enzymes essential to the breakdown of protein in the stomach and intestines.[20] Field peas (*Pisum Sativum*) contain trypsin and chromotrypsin

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[16] Reddy, N.R. and Sathe, S.K. (2002). *Food Phytates*. Boca Raton, CRC Press.

[17] Hidvegi, M. and Lasztity, R. (2002) *Phytic acid content of cereals and legumes and interaction with proteins*. Periodica Polytechnica Ser Chem Eng. 46: (1-2) 59-64.

[18] Frias, J., Vidal-Valverde, C., Kozlowska, H., *et al* (1995). *Evolution of soluble carbohydrates during the development of the pea, fava bean and lupin seeds.* Zeitschrift fur Lebensmittleluntersuchungund forschung A, 203:(1) 27-32.

[19] Seve P., Kerros C., *et al* (1989) Effect of the extraction of α-galatacides from toasted or raw soybean on dietary nitrogen and fat utilization in the young pig. In: "Recent Advances in Research in Antinutritional Factors in Legume Seeds" , pp 276-280 Huisman J, van der Poel, TFB, Liner IE., Editors, Purdic, Wageningen.

[20] Grosjean F., Et al (2000). *Ileal digestibility of protein an amino acids of feed peas with different trypsin inhibitor activity in pigs.* Canadian J. of Animal Science.

inhibitors. These form complexes with pancreatic proteases and inactivate them.[21] Trypsin inhibitor activity (TIA) of 17 spring-sown field pea cultivars grown in New Zealand ranged from 0.33 to 0.75 TIA/mg dry matter (DM).[22] These values were much lower than those reported for most European pea cultivars, which are typically in the 5-10 trypsin inhibitor units (TIU)/mg DM range.[23]

v. Saponins

Saponins are glycosides of triterpenes, steroids or steroidal alkaloids. They can be found in plants and marine organisms.[24] After hydrolysis with acids, strong bases or enzymes, saponins yield an aglycone, which is either a triterpenoid or steroid, and glycosides or uronic acids.[25] The toxicity of saponins is related to their surfactant properties, resulting in a soap like or foam-forming activity. When given intravenously saponins are hemolytic and toxic to warm blooded animals. Studies in rats and mice have shown that saponins are not significantly absorbed when ingested and acute oral doses produce only local effects rather than systemic toxicity.[26] Saponins have been detected in peas at levels from 0.15-0.18%. Processing peas by heat or water extraction reduces the saponin levels. Doses as small as 100 ppm have no detectable toxic effects.[27] The level of saponins in Nutralys® is less than 10 ppm and, thus, of no concern.

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[21] Le Gall, M., Quillien, L., Seve, B., Gueguen, J. and J.P., Lalles (2007). *Weaned piglets display low gastrointestinal digestion of pea (Pisum sativum) lectin and pea albumen.* 2. J. Anim. Sci. 85:2972-2981.

[22] One trypsin inhibitor unit (TIU) will decrease the activity of 2 trypsin units by 50% where one trypsin unit will hydrolyze 1.0 μmole of N-α-benzoyl-DL-arginine p-nitroanilide (BAPNA) per min at pH 7.8 at 25 °C.

[23] Guillamón, E., Pedrosa, M.M. Burbano, C., Cuadrado, C., et al. (2008). *The trypsin inhibitors present in seed of different grain legume species and cultivar.* Food Chemistry 107:(1) 2008, pp. 68-74.

[24] Rao, A.V., and Gurfinkle, D.M. (2000) *Saponins in human health*, Chp. 26 in "Saponins in Food, Feedstuffs and Medicinal Plants", Proceedings of the Phytochemical Society of Europe, Edited by W. Oleszek and A. Marston.

[25] Birk,Y. (1969) *Saponins*, In "Toxic Constituents of Plant Poisons", Chp. 7, Food Science and Technology, A Series of Monographs, Academic Press, New York.

[26] George, A.J. (1965). *Legal status and toxicity of saponins.* Food Cosmetic. Toxicol. 3. 85-91.

[27] Birk,Y. (1969) *Saponins*, In "Toxic Constituents of Plant Poisons", Chp. 7, Food Science and Technology, A Series of Monographs, Academic Press, New York.

vi. Lectins

Lectins (haemagglutinins) are proteins with the capability of binding sugars present as glycoproteins and the agglutination of red blood cells. They are present in virtually all plant derived protein, but typically in an inactive form. Some active lectins can also bind to the mucosa of the intestinal wall damaging the epithelial cells, depressing nutrient absorption, reducing activity of brush border enzymes, and causing hyper secretion of endogenous protein with the shedding of damaged cells. This does not occur with pea lectins. Pea protein consists of approximately 55-65% globulins and 20-25% albumins. Lectin is one of the albumins. Studies on pigs show that pea lectin is not digested in the GI tract and has no antibody reactivity.[28] Pea lectin was recognized as nontoxic by the 3rd European conference on Grain Legumes (1998).

(b) Protein Quality and Digestibility

i. Sulfur-Containing Amino Acids

Sulfur-containing amino acids are the limiting amino acids in peas, resulting in peas having a protein digestibility score below that typical of a complete protein with an adequate percentage of amino acids.[29] When whole peas are mixed with other proteins that contain sulfur containing amino acids, the digestibility is enhanced. For these reasons, when peas are used as feed supplements it is usually recommended that they are complemented with other protein sources or supplemented with methionine or cystine. This concern does not exist for Roquette's Pea Protein Isolate, which is produced by methods that significantly enhances the sulfur-containing amino acids.

During the manufacture of Roquette's Pea Protein Isolate, the sulfur-containing amino acids are enhanced to a combined level of 2.1%.[30] This compares favorably with that in the FAO adult reference protein of 2.2%. Pea Protein Isolate would therefore be useful as protein extenders in

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[28] Le Gall M., Quillien L., Seve B., Gueguen J. and J.P. Lalles (2007). *Weaned piglets display low gastrointestinal digestion of pea (Pisum sativum) lectin and pea albumen*. 2. J. Anim. Sci. 85:2972-2981.

[28] Corbett, R.R. (1997). *Peas as a protein and energy source for ruminants.* Alberta Agriculture Food and Rural Development, https://wcds.ualberta.ca/wcds/wp-content/uploads/sites/57/wcds_archive/Archive/1997/ch18-97.htm.

[29] The content of methionine and cystine is approximately 2.4g/16g N. Bramsnaes F. and Olsen S. (1979) *Development of Field Pea and Fava Bean Proteins*. J. Am. Oil Chemists Soc. 56: 450 - 454. Using the FAO reference protein, the digestibility for the field pea is from 50 -60%.

[30] Roquette Nutralys® Pea Protein Technical Bulletin, page 13.

meat products and protein enrichment in baked goods and could also be used as the major source of protein in the diet. The essential amino acid content of Roquette's Pea Protein Isolate is provided in **Table 1**.

ii. Protein Quality

The nutritional value of proteins can differ depending on their essential amino acid composition and digestibility. In 1989, a joint FAOWHO Expert Consultation on Protein Quality Evaluation (FAO/WHO, 1990)[31] concluded that protein quality could be assessed by a comparison of its amino acid composition, corrected for digestibility, with a reference protein meeting the essential amino acid requirements of the pre-school aged child. In 1993, FDA decided to use the protein digestibility corrected amino acid score (PDCAAS) over the older Protein Efficiency Ratio (PER) to measure protein quality.[32] The FDA gave three reasons for rejecting the PER method and adopting the PDCAAS in 1993: (1) PDCAAS is based on human amino acid requirements, which makes it more appropriate for humans than a method based on the amino acid needs of animals; (2) the PDCAAS is recommended by the FAO/WHO; and (3) values obtained by the two methods differ so that their simultaneous use on different foods would not allow for comparison.[33]

The protein quality assay yields the PDCAAS and it requires knowing the amino acid composition and the true digestibility of the protein.

True digestibility (TD) is defined as the difference between intake of nitrogen and output of fecal nitrogen. This is expressed as a percentage of nitrogen intake where fecal nitrogen is corrected for metabolic fecal nitrogen loss as measured using a protein-free diet; or:
(Eq. 1)

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31 FAO of the United Nations, "Protein Quality Evaluation, Report of a Joint FAO/WHO Expert Consultation," FAO of the United Nations, Rome, and WHO, Geneva, 1990.

32 See 21 C.F.R. § 101.9(c)(7).

33 58 Fed. Reg. 2079 at 2103 (Jan. 6, 1993).

$$\text{True Digestibility} = \frac{\text{Nitrogen Intake} - (\text{Fecal Nitrogen} - \text{Fecal Metabolic Nitrogen})}{\text{Nitrogen Intake}}$$

In order to calculate Nitrogen Intake, the dried weight of the uneaten food is subtracted from the initial amount of food given and multiplied by the percent amino acid of the feed in question. To calculate Fecal Nitrogen, the weight and amino acid content of the feces is determined using the Kjeldahl method. The Fecal Metabolic Nitrogen is equal to the amount of amino acid in the feces of rats fed a protein-free diet.

Amino acid composition of protein is determined by hydrolyzing the protein into its component amino acids and then separating, identifying and quantitating the amino acids using HPLC.[34] The number of milligrams of a given amino acid was determined from one gram of Nutralys® S85M and one gram of the reference food.

(Eq. 2)

$$\text{Amino Acid Score} = \frac{\text{mg of amino acid in 1 g of NUTRALYS S85M protein}}{\text{mg of amino acid in 1 g of reference protein}}$$

FAO/WHO reference values for pre-school children, 2-5 years of age (1991) were used in the denominator of Equation 2. In 2008, WHO/FAO released a new version of the reference values for 3-10 year-old children and the adult, which are also reported in the analysis.[35]

iii. Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

Of the amino acid scores derived above for Nutralys®, the amino acid with the lowest amino acid score is the limiting amino acid. This limiting amino acid score is then multiplied by the True Digestibility of Nutralys® S85M, resulting in the PDCAAS score.

(Eq. 3)

$$\text{PDCAAS} = \frac{\text{mg of first limiting amino acid in 1 g study product protein}}{\text{mg of the same amino acid in 1 g reference protein}} \times \text{TD}$$

The amino acid scores for Nutralys® S85M are given in the Table below. The amino acid scores for Methionine+Cystine, the limiting amino acid, is 0.84 with the 1991 standard for 2-5 year-old

[34] See SPRIM Analysis Report (V3), Assessing Protein Quality of Nutralys® S85M Using Protein Digestibility Corrected Amino Acid Score (PDCAAS), December 30, 2009.

[35] See Table 2.3 in SPRIM Analysis Report.

children, 0.88 with the 2008 standard for 3-10 year-old children, and 0.95 with the 2008 standard for adults.

Table 14: Amino Acid Content in Roquette's Pea Protein Isolate (Nutralys® S85M)

Amino Acid	Amino Acid (mg/g crude protein)				Amino Acid Score		
	Nutralys® S85M	2-5 year (1991)	3-10 year (2008)	Adult (2008)	2-5 year (1991)	3-10 year (2008)	Adult (2008)
Histidine	25	19	16	15	1.32	1.56	1.67
Isoleucine	45	28	31	30	1.61	1.45	1.50
Leucine	84	66	61	59	1.27	1.38	1.42
Lysine	72	58	48	45	1.24	1.50	1.60
Methionine+Cystine	21	25	24	22	**0.84**	**0.88**	**0.95**
Phenylalanine+Tyrosine	93	63	41	38	1.48	2.27	2.45
Threonine	39	34	25	23	1.15	1.56	1.70
Tryptophan	10	11	7	6	0.91	1.43	1.67
Valine	50	35	40	39	1.43	1.25	1.28

The PDCAAS of the test protein for each rat is the product of the amino acid score for the limiting amino acid and the TD values obtained from each rat in the last 5 days (Day 7-11) of the study. All 150 individual animal values of PDCAAS for Nutralys® S85M protein are calculated and listed in the SPRIM Report.

The individual parameters required for the Total Digestibility (TD) calculation are listed in the SPRIM Report. Three different diets were fed to three groups of rats. The TD values for the Nutralys® diet (T) and two reference diets, methionine-supplemented (R1) and un-supplemented casein (R2) were calculated according to equation 1. The mean Total Digestibility values and the standard deviations for T, R1 and R2, are given below:

Table 15: Mean and Standard Deviation for Total Digestibility (TD) Values

Feed Group		Mean Value of TD (%)	Standard Deviation of TD (%)	95% Confidence Limits LL	UL
T	Nutralys® S85M	97.268	1.5816	0.969457	0.977570
R1	Methionine-suppl. Nutralys® S85M feed	97.337	1.5283	0.976127	0.984239
R2	Unsupplemented casein reference feed	98.018	1.2175	0.968620	0.976733

Combining the 3 limiting amino acid scores with the average TD values gives the PDCAAS score for Nutralys® S85M: **81.70%** (1991 standard, 2-5 year old), **85.11%** (2008 standard, 3-10 year old) and **92.85%** (2008 standard, adult). The digestibility of the two reference diets are shown for comparison. The digestibility of the two Nutralys® diets are essentially identical and only marginally less digestible than the casein reference feed.

(4) Safety of Pea Protein Isolate

(a) Animal Nutritional Studies on Pea Protein

Many studies have been conducted in animals to investigate the digestive process of pea proteins, to determine the utility of the protein as an animal feed, to investigate the impact of various treatments to lessen the concentration of ANFs, and to compare the nutritional quality of pea protein with soybean, fava bean, and other protein sources. Taken together, these studies include thousands of animals on high doses of both whole peas and pea protein for sustained periods without observed toxic effects. The only adverse effects have been related to the limited digestibility of some field pea cultivars when used to provide the major fraction of protein for developing monogastric animals.

Pea protein has been successfully substituted for soybean protein in diets fed to late lactation cows in a study conducted at the University of Alberta. Cows fed for six months on a diet containing up to 100% of their protein from field peas produced milk in equivalent quantity and quality as those on soy protein diets.[36] Field peas can also be an alternative to soybean meal in diets for non-ruminant animals, but diets that incorporate field peas usually depress growth,

[36] Corbett R.R. (1997). *Peas as a protein and energy source for ruminants.* Alberta Agriculture Food and Rural Development, available at, https://wcds.ualberta.ca/wcds/wp-content/uploads/sites/57/wcds_archive/Archive/1997/ch18-97.htm.

especially in young animals. This has generally been attributed to the presence of anti-nutrients, especially protease inhibitors and tannins, which are not digested in monogastric animals. The results of studies in weanling piglets showed that while pea globulins are well digested, PA2 and PA (albumins) are only partially digested, and lectin is totally resistant to gastrointestinal tract digestion.[37] The study indicates that when whole field peas are used in pig diets, cultivars with a higher globulin to albumin ratio should be used. In comparison, the digestibility of a pea protein isolate, from which trypsin inhibitors were removed, was found to be equivalent to a soy protein diet. The minor differences among the digestive parameters of three rat diets containing protein from either meat, soy isolate or pea isolate sources were more attributable to the quality of the overall diet then to the particular protein source.[38] A study by Urbano et al. shows that even mild treatment of raw pea flour to produce pea protein isolate can radically reduce the level of ANFs.[39] Mild hydrothermal treatment (pH = 5, at 37^{O}C for 60 min) resulted in the reduction of trypsin inhibitor activity from 8.59 TIU/mg of DM to 2.45 TIU/mg of DM; the reduction of phytate from 339mg/100 g DM to 75 mg/100g DM; and the reduction of α-galactosides from 5.15% of DM to 0.86 % of DM. The dehulling process to produce the pea flour from the pea seeds already removed the bulk of the tannins.

(b) Animal Safety Studies on Nutralys® Pea Protein Isolate

Since no experimental animal studies had been published on the safety of pea protein isolate an acute oral and a 90-day oral toxicity study in Wistar rats was conducted by Roquette Frères. In addition, a skin sensitization study and a genotoxicity battery were conducted. The results of the studies fully confirm the expectation from the traditional food use of peas that pea protein isolate is safe when used in food at the proposed levels. Detailed discussions of the experimental

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[37] Le Gall M., Quillien L., Seve B., Gueguen, J. and J.P., Lalles (2007). *Weaned piglets display low gastrointestinal digestion of pea (Pisum sativum) lectin and pea albumen*. 2. J. Anim. Sci. 85:2972-2981.

[38] Lhoste E.F., Mouzon B., Andrieux C. et al. (1998). *Physiologic effects of a pea protein isolate in Gnotobiotic rats: Comparison with a soybean isolate and meat.* Ann Nutr. Metab. 42: 44-54.

[39] Urbano G., Aranda P., Gómez-Villalva F., et al. (2003). *Nutritional evaluation of pea (Pisum sativum) protein diets after mild hydrothermal treatment and with and without added phytase*. J Agric and Food Chem. 51: 2415-2420.

procedures and the results may be found in the cited published scientific papers and one unpublished Study Report (lymph node assay).

i. Acute oral study

Three female Wistar rats and three CD1 female mice were administered Nutralys® Pea Protein Isolate by oral intubation at 2000 mg/kg.bw.[40] The studies were conducted according to the Organization for Economic Cooperation and Development (OECD) Guideline 423.[41] None of the animals exhibited any signs of dullness, abnormal body posture, tremors, seizures; restlessness, weight gain decrement or any other signs of toxicity. The results showed that an oral dose of 2000 mg/kg.bw of pea protein isolate did not produce toxicity in any of the treated animals, and that the LD_{50} of pea protein isolate taken orally was higher than 2000 mg/kg.bw. According to OECD Guideline 423, substances that have a LD_{50} higher than 2000 mg/kg.bw orally can be considered nontoxic.[42]

ii. 90-day oral study

Wistar rats of both sexes were fed Nutralys® Pea Protein Isolate in their diets at doses of 25,000 ppm, 50,000 ppm and 100,000 ppm, according to OECD Guideline 408.[43] After acclimation, animals were randomly distributed, into six groups (consisting of 10 males and 10 females per group) namely: control (0.0 ppm), low-dose (25,000 ppm), intermediate-dose (50,000 ppm), high-dose (100,000 ppm), satellite control (0.0 ppm) and satellite high-dose (100,000 ppm). The test substance was administered daily by a mixture with the diet for a period of 90 days. Food intake and water of the animals were measured once daily and reported weekly**.** Body weight of the

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[40] Aouatif C., Looten P., Srinivasaan M. and Srinivas A. (2013) Acute oral toxicity of pea protein isolate (Nutralys) in Wistar rats and Cd1 mouse. *Journal of Toxicology and Health, Photon*:103, 180-184. ISJN: 22947439.

[41] OECD Guideline For The Testing Of Chemicals, Acute Oral Toxicity – Acute Toxic Class Method, 423, December 2001, available at, http://www.oecd-ilibrary.org/docserver/download/9742301e.pdf?expires=1381948814&id=id&accname=guest&checksum=77566C918CE529FD0E00E8FF10AB2D28.

[42] See id.

[43] Aouatif C., Looten P., Srinivasan M., Srinivas A., Murkunde Y.V. (2013) Subchronic toxicological effects of pea protein isolate (Nutralys) on wistar rats: A ninety-day dietary. *Journal of Toxicology and Health Photon* 103:225-233; OECD Guideline For The Testing Of Chemicals, Repeated Dose 90-day Oral Toxicity Study in Rodents, 408, September 1998, available at, http://www.oecd-ilibrary.org/docserver/download/9740801e.pdf?expires=1381948914&id=id&accname=guest&checksum=95462ABA3510B3B9495C90EA05006176.

animals was measured once weekly. Rats in the satellite groups were given the control diet without the test item for an additional 28 days to evaluate any possible withdrawal effects. All animals were individually observed once daily for clinical signs. All animals were observed for functional observational parameters (FOB) prior to the administration of the test substance, during the 13th week for the main groups and, during the 17th week for the satellite groups.

During the feeding period, there were no deaths or signs of toxicity on gross observation that were attributable to the ingestion of Nutralys® Pea Protein Isolate. Feed consumption and weight gain during the study was found to be normal. The absolute and relative organ weights in the rats were normal, except an increase in the absolute weight of the spleen in females and decrease of the testes organ in male rats of the high and low dose respectively. These minimal alterations were attributed to intra -animal variation as these changes were not dose dependent.

Gross pathological examination did not reveal any changes due to pea protein isolate in the groups tested of either sex. Further, no adverse histopathological findings were observed in any of the treated animals of either sex. Minor, but statistically significant changes occurred in a few biochemical assays, e.g., AST, BUN, glucose and triglycerides. These changes were not test compound dose dependent and appeared to be spurious. Pea protein isolate administration in rats did not alter normal liver or kidney function or produce any hematological alterations.

Ophthalmoscopic examination was normal, and data from the functional observational battery tests did not reveal any neurological toxicity induced by pea protein isolate dietary administration. The NOAEL of Nutralys® Pea Protein Isolate in Wistar rats can be defined as 100,000 ppm of diet (equivalent to 8,726 mg/kg.bw/day for males and 9,965 mg/kg.bw/day for females. Based on these findings the authors concluded that pea protein can be considered as non-toxic when administered as a food ingredient.

iii. Local Lymph Node Assay (LLNA)

The LLNA is designed to measure the proliferation of lymphocytes that are induced by a skin sensitizing agent.[44] The agent is applied topically to the skin on the ear lobes of mice at three different concentrations for 5 consecutive days. On day 6, tritiated methyl thymidine was given intravenously to the mice, the auricular lymph nodes were excised and the degree of lymphocyte proliferation measured. The incorporation of radioactive tritiated methyl thymidine into the DNA of the cells is used as an index of proliferation. Dose levels of 25%, 10% and 5% of pea protein isolate failed to produce the minimal x3 stimulation index. The EC_{50} value was not calculable and hence the pea protein isolate was considered as non-skin sensitizer at 25% under the conditions of the test.[45]

iv. Mutagenicity assays

Ames Test - Pea protein isolate was assessed for its mutagenic potential with five tester strains of Salmonella typhimurium (TA100, TA102, TA1535, TA98, and TA1537, in presence and in absence of metabolic activation.[46] The assay was performed according to OECD Guideline 471.[47] Five test concentrations of 312.5, 625, 1250, 2500, and 5000 g/plate with 10% S9 and without S9 along with solvent and positive controls were chosen for mutagenicity evaluation in the five tester strains.

There was no concentration related or reproducible increase in the number of revertant colonies in the employed test concentrations in any of the tester strains. No two- or threefold increase in the

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[44] Srinvas A. (2012) Unpublished Study Report International Institute of Biotechnology and Toxicology (IIBAT), Tamil Nadu, India.

[45] The EC_{50} value is the estimated concentration required to induce a threshold positive response.

[46] Aouatif C., Looten P., Parvathi M.V.S., Raja Ganesh S., and Paranthaman V. (2013) Genotoxicological Evaluation of NUTRALYS Pea Protein Isolate, ISRN Toxicology, Volume 2013, Article ID 817353, http://dx.doi.org/10.1155/2013/817353.

[47] OECD Guideline For The Testing Of Chemicals, Bacterial Reverse Mutation Test, 471, July 1997, available at, http://www.oecd-ilibrary.org/docserver/download/9747101e.pdf?expires=1381948987&id=id&accname=guest&checksum=21F57B1D56A0AACE5CBBF182F0B36130.

means of the revertant counts was observed in the test concentrations in all tester strains with and without S9. Positive controls exhibited a significant multifold increase in revertant counts ($P < 0.05$, Dunnett's test). The negative result indicated that under these experimental conditions pea protein was nonmutagenic in the Ames Salmonella typhimurium reverse mutation assay

In- Vitro Chromosomal Aberration Test - Pea protein isolate was evaluated for its capacity to induce structural and numerical aberrations in cultured human peripheral blood lymphocytes. The peripheral blood was obtained from three healthy adult (>30 years age) nonsmoking male volunteers, without any recent history of illness, as is the guideline requirement. Informed consent was obtained from each donor. The assay was performed according to OECD Guideline 473.[48]

The percentage aberrations of all Pea Protein Isolate treated cultures were not significantly different from the concurrent solvent control cultures. Positive controls exhibited a significant increase in the percentage aberrations ($P < 0.05$, Dunnett's test). The authors concluded that, under the conditions of the test, pea protein isolate did not induce genotoxic response in human lymphocytes when tested up to concentrations inducing acceptable levels of cytotoxicity.

In Vivo Micronucleus Assay - To detect genotoxic potential of pea protein isolate in vivo, mouse micronucleus assay was performed by assessing the induction of micronuclei in polychromatic erythrocytes (PCEs) and determining the ratio of immature and mature erythrocytes in bone marrow cells, in compliance with the OECD Guideline 474.[49] Healthy male and female CD1 mice of 6–8 weeks of age were used for the study as per the guideline's specification. A range finding study was performed with doses of 320, 800, and 2000 mg/kg.bw employing 2 mice/sex/dose with concurrent vehicle control. The mice were treated orally and administered a single treatment with a 24-hour

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[48] OECD Guideline For The Testing Of Chemicals, In Vitro Mammalian Chromosome Aberration Test, 473, July 1997, available at, http://www.oecd-ilibrary.org/docserver/download/9747301e.pdf?expires=1381949079&id=id&accname=guest&checksum=2E508090FBCD170CEE03FB0DEA9952FB.

[49] OECD Guideline For The Testing Of Chemicals, Mammalian Erythrocyte Micronucleus Test, 474, July 1997, available at, http://www.oecd-ilibrary.org/docserver/download/9747401e.pdf?expires=1381949181&id=id&accname=guest&checksum=2B38AA57AC7C98CE73CE5CFF50041800.

sacrifice time point. A limit test was performed administering single- and two-day treatments (24 hours apart) with the highest dose 2000 mg/kg.bw.

No mortality was observed in any of the groups. In the preliminary test there was a mild dose-dependent increase in the PCE : NCE ratio observed in females (PCE : NCE ratio of >1) without disturbance in cellularity, and a ratio of >1 was observed in males at 800 mg/kg.bw., exhibiting a similar trend to that of the concurrent vehicle control.[50] In the limit test no evident increase in the frequencies of MN-PCE (micronucleated polychromatic erythrocytes) were observed in the dose group compared to that of the concurrent vehicle control groups at all time points of sacrifice. The authors concluded that pea protein isolate was nongenotoxic in single- and two-day treatments under the test conditions employed.

(c) Summary of Safety Studies

Feeding Nutralys® Pea Protein Isolate to rats, daily, for 90 days did not induce any toxicological changes. Clinical signs, body weights, food and water consumption, and hematological, blood biochemical and urinalysis were comparable with concurrent control animals. Organ weights, gross and histological examinations did not indicate any systemic toxicity induced by pea protein isolate consumption. The NOAEL was greater than 10% in the diet equivalent to 8,726 mg/kg.bw/day for male rats and 9,965 mg/kg.bw/day for female rats respectively. Pea protein isolate was found to be non-mutagenic and non-genotoxic at the conditions employed in Ames test, *in vitro* chromosomal aberration test, and *in vivo* micronucleus test.

(d) Pea Allergenicity

According to the Food Allergen Labeling and Consumer Protection Act of 2004, peas are not a major allergen.[51] Nor are they considered one of the "big eight" allergens, as determined by the

[50] PCE- polychromatic erythrocyte; NCE Normochromatic erythrocyte.

[51] The Food Allergen Labeling and Consumer Protection Act of 2004 (Title II of Pub. Law 108-282) (FALCPA) amended the Food, Drug, and Cosmetic Act (FD&C Act) to require more complete labeling of foods that contain the eight most common food allergens or ingredients derived from them. The eight most common allergens defined in Section 201(qq)(1) of the FD&C Act are: (1) milk; (2) eggs; (3) fish; (4) Crustacean shellfish; (5) tree nuts; (6) wheat; (7) peanuts; and (8) soybeans.

Food and Agriculture Organization (FAO) of the United Nations.[52] Indeed, neither the United States nor the European Union require peas to be labeled as a potential food allergen.

Epidemiological data on the prevalence of pea allergy in the general population is not readily available, however, among food allergic patients, it is estimated there is a pea allergy prevalence of 1%.[53] Thus, it is reasonable to assume that pea allergies are rare in the general population.

Vicilin (Pis S1) and convicilin (Pis s2) are the major allergens in pea protein and simultaneous food allergies to peas and other legumes may reflect cross-reactivity between the allergenic proteins of these legumes. [54] But this does not necessarily correlate with clinical hypersensitivity. The rates reported in the studies identified from the literature should be considered with caution given the small number of subjects involved in most studies.

No differences in the protein content, protein types, or allergenicity can be found between yellow and green varieties. The low prevalence of pea allergy does not affect our GRAS conclusion. Further, the ingredient will be identified on food product labels so that any pea allergic consumer will be aware of its presence in a food.

(e) Conclusions

For reasons ranging from health and environmental, proteins of plant origin have been gaining interest as an alternative to animal-derived proteins. Pea Protein Isolate can be used as a substitute for, and/or in conjunction with, other proteins (such as soy protein, whey protein, and animal derived protein) in conventional food products, as well as in sports nutrition and meal

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[52] Taylor S.L. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, *Emerging problems with food allergens*, available at, http://www.fao.org/docrep/003/x7133m/x7133m03.htm.

[53] Final Report on Allergenicity of Yellow Pea Protein, DHI Denmark, Final Report January 2008. Received from Roquette Freres. The report concludes that since the prevalence of adult food allergy in the U.S. is approximately 4%, one can estimate the prevalence of pea allergy in adults as 0.04 x 0.01 = 0.0004 or about 4 in 10,000. It would probably be less than 1/1000 in children.

[54] Cross reactions to pea proteins in people with allergies to soy and peanuts are not common, despite the fact that there is considerable cross-reactivity with specific IgE tests. Ibanez, et al (2003) Legume cross reactivity, Allergol Immunopath (Madrid) 31(3) 151-161.

replacement applications. Pea Protein Isolate will be used as a food ingredient, formulation aid, nutrient supplement, stabilizer/thickener, and texturizer in food products.

The Pea Protein Isolate will be added to food products as a protein substitute and therefore will not contribute any additional exposure to protein for consumers. We do not realistically expect that the actual consumption of foods containing the Pea Protein Isolate would result in a daily consumption greater than the Daily Reference Value (DRV) of 50 g/day of protein for adults and children 4 or more years of age. Additionally, the Institute of Medicine (IOM) used the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 to estimate the background dietary intakes of protein for the US population.[55] The mean adult protein intake ranged from 56-104 g/day, depending on the age group. At the 90th percentile, adult protein intakes ranged from 76 g/day to 142 g/day, and the IOM established a Dietary Reference Intakes (DRIs) for protein of 56 g/day for adult males and 46 g/day for adult females. Based on our conservative estimate, it is unlikely the consumption of foods with Pea Protein Isolate will result in a consumption greater than the DRI.

Substituting with Pea Protein Isolate in conventional foods will not result in a significant increase of protein intake, and therefore, it is deemed safe. In addition, Pea Protein Isolate as a directly consumed protein in sports nutrition or meal replacement applications will not have an impact on the overall protein intake since it is used to substitute the protein from other sources, i.e., animals or whey. Most of the population's protein intake will remain in the form of unprocessed foods, including meat, poultry, fish, and legumes.

Based on a critical evaluation and analysis of the information and literature available on Pea Protein Isolate discussed above, it is concluded that there is reasonable certainty that Pea Protein Isolate is safe under the intended conditions of use and is also Generally Recognized as Safe (GRAS), by scientific procedures for use as a concentrated, highly digestible protein source in food.

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[55] *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, INSTITUTE OF MEDICINE (2005).

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Bonnette, Richard

From: Pelonis, Evangelia C. <pelonis@khlaw.com>
Sent: Friday, March 15, 2019 11:14 AM
To: Bonnette, Richard
Subject: Pea protein isolate submission dated January 28, 2019 - FSIS information
Attachments: Suitability Data for Pea Protein.pdf

Dear Richard,

As discussed, we are providing the suitability data for Roquette's Pea Protein Isolate as an Appendix to the GRAS Notice that was submitted to FDA on January 28, 2019. Please find attached the suitability data for meat and poultry applications. This Appendix contains confidential business information that, in accordance with the Freedom of Information Act (5 U.S.C. § 552), should not be disclosed to the public. Under Part 1 we are authorizing FDA to share this information with the U.S. Department of Agriculture (USDA) Food Safety Inspection Service (FSIS).

Regards,

Eve Pelonis

Evangelia C. Pelonis
Partner
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1001 G Street NW, Suite 500 West | Washington, DC 20001

KELLER AND HECKMAN LLP
SERVING BUSINESS THROUGH LAW AND SCIENCE

From: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Sent: Tuesday, February 26, 2019 3:46 PM
To: Pelonis, Evangelia C. <pelonis@khlaw.com>
Subject: Pea protein isolate submission dated January 28, 2019 - FSIS information

Hello Eve,
Your submission on for pea protein isolate submitted on behalf of Roquette Freres is making its way through our prefiling evaluation. I believe you've already corresponded a bit with some of my colleagues about this submission. I have what I think is a minor procedural issue to mention. We noticed that you've separately provided information to FSIS regarding safety and suitability for the use of this substance in USDA regulated products for their evaluation. Before we can file the submission as a GRAS notice, we will need to add the FSIS-relevant material to the GRAS submission here. You can email the information to my attention and I will append it to the submission and we can move forward with filing. Let me know if you have any questions.
Regards,
Richard

Response to FDA Questions regarding GRN 851 (pea protein isolate)
Submitted to FDA on December 11, 2019

1. On p. 8, you state that food grade enzymes from the exopeptidase and endopeptidase families are used to enhance the pea protein isolate functionalities. The protein content of the hydrolysates and peptide fraction can vary considerably in the final products, which could be attributed to differences in the peptide cleavage specificity of the enzymes used in the process. Therefore, the current description is too broad. Please identify the enzymes used and clarify the use of the food grade enzymes from the exopeptidase and endopeptidase families.

The enzymes are used to enhance the pea protein isolate functionalities. The first enzyme is a concentrated food grade enzyme preparation from the exopeptidase family with a lower activity endopeptidase. The second enzyme is a powdered food grade enzyme from the exopeptidase family (aminopeptidase) and is derived from a highly concentrated fungal proteolytic food grade enzyme, with low alpha amylase activity and significant amino peptidase activity for debittering. The highly concentrated fungal proteolytic food grade enzyme is generally recognized as safe (GRAS) as per GRN 90. Both enzymes are prepared from enzymes that have GRAS status and both enzymes are manufactured consistent with the FCC/JECFA/WHO/FAO recommendations for enzymes used in food processing.

2. On p. 8, you state that the protease enzymes hydrolyze the peptide bonds in pea proteins, releasing lower molecular weight peptides of shorter chain length. The enzymatic hydrolysis of pea protein can release peptides exhibiting various bioactivities, and the molecular weight range of peptides present in the hydrolysates differs according to the protease used for hydrolysis. Please describe the peptide size distribution of the pea protein hydrolysates in order to characterize/support your pea protein hydrolysates and peptide fractions.

The enzymatic hydrolysis of pea protein releases peptides with different functions and bioactivity, depending on whether the enzymes used for hydrolysis are from exopeptidase or endopeptidase families. Protein hydrolysis refers to the breakdown of proteins into amino acids and smaller weight peptides with shorter chain length (as described in Figure 1). The pea protein has been partially hydrolyzed and the peptides have not been filtered, in order to obtain a mixture with smaller peptides and larger protein.

The molecular weight distribution (MWD) profile reflects the functional and nutritional properties of the protein hydrolysates. This MWD profile is commonly measured using Gel Permeation Chromatography (GPC). Using this method, peptides are separated according to their size and shape, as related to their molecular weight. The results of the pea protein hydrolysate, Nutralys S85Plus, are generally represented as a percent (%) distribution of the molecular weight classes: MW <1000; 1,000-3,000; 3,000-6,000; 6,000-18,000 and >18,000 Dalton (see Figure 2).

Figure 1: Protein hydrolysis with endoprotease and exopeptidase

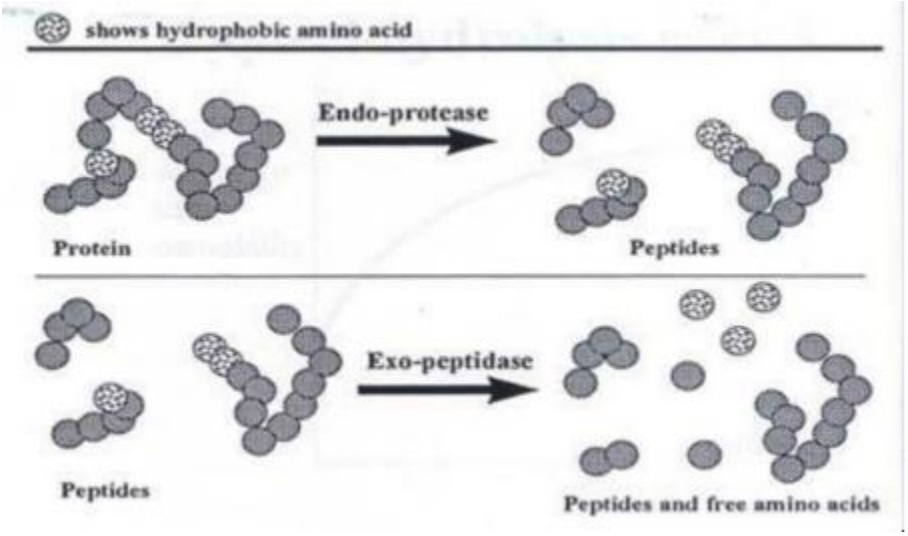

Figure 2: Molecular Weight Distribution profile (MWD) of the protein

	W279N	W272N
MW Dalton	%	%
PM > 18000	24,0	24,0
18000 > PM > 6000	11,0	13,0
6000 > PM > 3000	9,1	10,2
3000 > PM > 1000	23,6	26,1
PM <1000	32,3	26,8

3. On pp. 9-10, you indicate there are 3 different final products (Nutralys®S85F, Nutralys®F85F, Nutralys®S85Plus). Please describe the differences in the functional properties (i.e., solubility, viscosity, emulsifying, and gelling properties) for these three products. If you modified the manufacturing conditions, such as the enzyme-induced hydrolysis step, please clarify how you modified the proteolysis conditions (e.g., enzymes/substrate, pH, and temperature) to achieve the specific differences in the functionalities and particle sizes of the 3 different final products.

Figure 3 highlights some of the functional differences between Nutralys S85F, Nutralys F85F, and Nutralys S85Plus. The differences are mainly due to the flocculation temperature (used to change the level of the protein denaturation, which is associated with the protein solubility properties.

Industrial hydrolysates are classified into three groups: (1) low (<10%) degree of hydrolysis (DH) for improved functional properties; (2) variable (usually high) DH for taste enhancement; and (3) high (>10%) DH for use in

nutritional supplements and medical diets. High DH is also known as extensive DH. The Nutralys S85Plus is hydrolyzed at a low DH in order to change specific properties that are linked to solubility and/or viscosity. The main difference between Nutralys S85Plus and the other two Nutralys products (S85F and F85F) is the viscosity, which is lower in comparison as a result of the Molecular Weight Distribution evolution from higher molecular weight to lower molecular weight.

The particle sizes for Nutralys S85F, F85F and S85Plus are provided in Tables 4, 5, and 6 of GRAS Notice 851. The particle sizes are within the same range and do not impact the functionalities of the pea protein.

Figure 3: Nutralys functional properties

Mean Reference Value	Solubility (pH7) (%)	Emulsion Capacity (ml oil/g)	Whippability (%)	Viscosity (40s-1) (Pa.s)	Gel (Pa)
NUTRALYS S85F	55	570	190	0,54	218
NUTRALYS F85F	40	550	180	0,47	152
NUTRALYS S85Plus	50	150	340	0,01	155

4. On p. 18, you indicate that your pea protein is intended to be used in "clinical nutrition" and "fruit and vegetable preparations." However, we note that these food categories are not included in the list of food categories in Table 1 (p. 18). For completion of the submission, please revise Table 1 to include these food categories with the proposed use levels and examples of the types of products in these food categories.

We have revised Table 11 to add "clinical nutrition" and "fruit and vegetable preparations" as product categories. Both food categories have a use level of 3-50%.

Revised Table 11: List of Proposed Food Uses and Use-Levels for Roquette's Pea Protein Isolate

Product category	Use Level
Bakery products: breads, rolls, bars, cakes, pasta, cookies	5-10 %
Cereals: cold cereals, oatmeal, cereal bars	1-30 %
Snack Foods: chips, crackers, energy bars	2-30%
Ready-to-drink (RTD) beverages, *fruit and vegetable preparations* (soups, smoothies, fruit juices, fruit puree and fillings), high protein beverages, *clinical nutrition foods*	3-50%
Dairy and Dairy Alternatives: cheeses, spreads, creamers, yogurt, drinkable yogurts, ice cream, refrigerated desserts, frozen desserts, milks, dips, whipped toppings	2-20%
Meal Replacement/Nutritional bars	10-30%

Meat Analogs	10-30%
Processed Meat	2-7%
Dry Blend Protein powders	20-90%
Extruded Products: pea crisps	30-90%
Chocolate and Confection Compound Coatings	10-25%
Non-Chocolate Confection (chewy candies, gummies)	2-30%

5. In the subchronic (90-day) study (Aouatif et al., 2013), several significant toxicological findings were noted in the recovery high dose (HD) groups on day 119. In the satellite HD male rats, there was a significant increase in AST and prothrombin time; but not in female rats. Triglyceride, urea, blood urea nitrogen (BUN) and potassium levels were significantly higher in the satellite HD female rats compared to satellite control females; whereas glucose levels were significantly lower in the satellite HD female rats compared to controls.

It is apparent that the absolute magnitudes of these effects were minimal, no corresponding histopathological changes were reported, and the effects did not occur in both sexes. Therefore, these effects could be regarded as random and without toxicological relevance. However, neither the study authors nor the notifier have made such statements. Please provide a statement to this effect to close the loop as this is important in evaluating the safety of your pea protein isolate.

We agree that it is apparent that the absolute magnitudes of these effects were minimal, no corresponding histopathological changes were reported, and the effects did not occur in both sexes. Therefore, these effects could be regarded as random and without toxicological relevance.

6. The Notifier cited previous GRAS notices on various forms of pea protein (GRNs 000182, 000525, 000581, 000608, and 000788) that FDA had no questions about.
Please provide an updated literature search results for pea protein isolate since the last GRAS notice FDA received for this ingredient.

Prior to submission of the GRAS Notice, we conducted a literature search through January 2019. After receipt of FDA's questions, we conducted an additional literature search through November 2019 and did not find any published studies that raise safety concerns regarding pea protein isolate.

7. Under pea allergenicity, please discuss the findings from Richard et al. (2015) publication which addresses cross-reactivity of a new food ingredient, dun pea (Pisum sativum var. arvense), with legumes, and risk of anaphylaxis in legume allergic children.

Richard et al. (2015) evaluated the cross-reactivity between dun pea and other legumes. The study goal of the study was to determine whether new food technologies impact allergenicity. The results showed that patients 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. In addition, analysis of dun pea allergens suggested that protein epitopes were presented differently in dun pea seeds, isolate, and flour. Immunoblots of serum from a patient who had allergy to all

legumes since infancy (HE) with the same amount of protein amount for each extract illustrate the difference of IgE reactivity with seed, flour and isolate extracts. For example, (1) allergenic profiles were different between flour and isolate, (2) IgE recognized the 9 kDa proteins in dun pea seed but not in flour or isolate, (3) 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 (4) seed and flour differentially inhibited IgE binding to 28 kDa and 50 kDa proteins in seed. Based on these observations, it was hypothesized that manufacturing processes may be different for the two types of ingredients, thus modifying the allergenicity of native proteins.

In the case of GRN 851, the pea protein isolate is derived from the dry common yellow pea Pisum Sativum. The common or usual name of the pea protein isolate will always include reference to pea, therefore consumers will always be aware of the presence of pea in foods.

8. Please clarify if the Local Lymph Node Assay (LLNA) is reported in the Srinvas A. (2012) unpublished study report, because it is unclear from the GRAS notice where this comes from.

The Local Lymph Node Assay (LLNA) is reported in "Pea Protein Isolate: Local Lymph Node Assay in CBA/CaOlaHSD mice," an unpublished study report on pea protein isolate conducted by the International Institute of Biotechnology and Toxicology (IIBAT), a test facility located in Tamil Nadu, India in 2012 (Study No. 11741). The study was sponsored by Roquette Freres, conducted according to OECD guideline 429 and GLP, and A. Srinvas served as the study director.