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George Weston Foods Limited
ABN 45 008 429 632

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September 26, 2008

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BY:

Robert L. Martin, Ph.D.
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 Notice for Sweet Lupin Protein

Dear Dr. Martin:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in quadruplicate, as the notifier [George Weston Foods Limited, 1 Braidwood Street, Enfield, NSW, 2136, Australia], a Notice of the determination, on the basis of scientific procedures, that sweet lupin protein derived from sweet varieties of *Lupinus* spp. (lupin), produced by George Weston Foods Limited (GWF), as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and reviewed by an independent panel of experts in support of the safety of GWF's sweet lupin protein ingredient under the intended conditions of use, as well as *curricula vitae* evidencing the qualifications of the members of the panel of experts for evaluating the safety of food ingredients, also are enclosed.

I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Peter Schutz
Chief Executive

George Weston Technologies
A Division of George Weston Foods Limited
peter.schutz@gwf.com.au

Encl.

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SWEET LUPIN PROTEIN GRAS NOTICE

Prepared for:

Robert L. Martin, Ph.D.
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

Prepared by:

George Weston Foods Limited
1 Braidwood Street
Enfield, NSW, 2136
Australia

September 26, 2008

SWEET LUPIN PROTEIN GRAS NOTICE

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I GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

As defined herein, two protein ingredients derived from sweet lupin (referred to as lupin protein fraction 1 and lupin protein fraction 2 or sweet lupin protein fractions) have been determined by George Weston Foods Limited (GWF) to be Generally Recognized as Safe (GRAS) for use in a variety of traditional food products. This determination is based on scientific procedures, as described in the following sections, under the conditions of their intended use in food. Therefore, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act,* the use of sweet lupin-derived protein fractions in food as described below is exempt from the requirement of premarket approval.

Signed,

Peter Schutz
Chief

Date: 26 September 2008

George Weston
A Division of George Weston Foods Limited
peter schutz@gwf.com.au

B. Name and Address of Notifier

George Weston Foods Limited
1 Braidwood Street
Enfield, NSW, 2136
Australia

C. Common Name of the Notified Substance

Sweet lupin protein fractions, or sweet lupin protein fraction 1 and sweet lupin protein fraction 2

D. Conditions of Intended Use in Food

GWF intends to market 2 protein fractions derived from sweet varieties of *Lupinus* spp. (lupin) as food ingredients in various traditional food products intended for sale on the U.S. market. The intended food uses include baked goods and baking mixes, dairy product analogs, frozen

dairy desserts and mixes, gelatins, puddings, and fillings, jams and jellies, meat products, milk products, and soft candy. The sweet lupin protein fractions will be added to food products at use levels ranging from 1% to a maximum of 20%, depending on the proposed food use.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, protein fractions derived from sweet lupin have been determined by GWF to be GRAS on the basis of scientific procedures (U.S. FDA, 2008). This GRAS determination is based on data generally available in the public domain pertaining to the safety of lupin and sweet lupin-derived ingredients, including sweet lupin protein fractions, as discussed herein, and on a consensus among a panel of experts[1] [who are qualified by scientific training and experience to evaluate the safety of sweet lupin protein fractions as a component of food [see Appendix A, entitled, "**EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SWEET LUPIN-DERIVED INGREDIENTS FOR USE IN FOODS**"].

F. Availability of Information

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

George Weston Foods Limited
1 Braidwood Street
Enfield, NSW, 2136
Australia

Should the FDA have any questions or additional information requests regarding this notification, GWF will supply these data and information.

[1] The panel of experts consisted of Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Ashley S. Roberts, Ph.D. (Cantox Health Sciences International), and Prof. Stephen L. Taylor, Ph.D. (University of Nebraska).

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

A. Identity

Sweet lupin protein fractions are obtained by dehulling and milling/grinding the whole seed of sweet varieties of *Lupinus* spp. (lupin) followed by decanting and further processing. Four (4) species of lupin have been cultivated to include both a bitter variety and a 'sweet' variety, which is so-named due to its low alkaloid content (~0.001 to 0.002%), making the sweet varieties suitable for consumption by humans and livestock (Petterson, 1998)[2] (see Appendix B-1). The sweet varieties of lupin are of the following species: *L. angustifolia, L. albus*, *L. luteus*, and *L. mutabilis*.

B. Method of Manufacture

Sweet lupin protein fractions 1 and 2 are produced from the whole seed of sweet lupin. As mentioned, the species with sweet varieties of lupin used to manufacture the sweet lupin protein fractions include *L. angustifolia*, *L. albus*, *L. luteus*, and *L. mutabilis*. The seeds are received from growers and cleaned. The hull is removed from the seeds and the dehulled seeds (cotyledons or kernels) are dry-milled to produce sweet lupin flour. A portion of the sweet lupin flour is sold as the finished ingredient, while the remaining flour is utilized to produce the sweet lupin protein fractions and other ingredients manufactured by GWF.

A slurry is made with the wet-milled dehulled seeds or sweet lupin flour and potable water, and the pH of the slurry is adjusted and held for 45 minutes. The protein milk is separated from the fiber using a decanter. For production of the protein fractions, following separation of the protein from the kernel fiber fraction, the pH of the protein milk is adjusted, and then the protein milk is treated with enzymes and held for a period of 1 hour. The protein milk is then heated to greater than 70°C for a period of more than 5 minutes, and the pH is adjusted before separation of the protein mixture into 2 fractions through the use of a clarifier. The precipitate is washed and then spray-dried to give sweet lupin protein fraction 1, and the supernatant is concentrated, washed, and then spray-dried to give sweet lupin protein fraction 2. A schematic overview of the manufacturing process for sweet lupin protein fractions 1 and 2 is presented in Figure 1. All processing aids used in the manufacture of the sweet lupin protein fractions are used in compliance with appropriate federal regulations (see Table 1).

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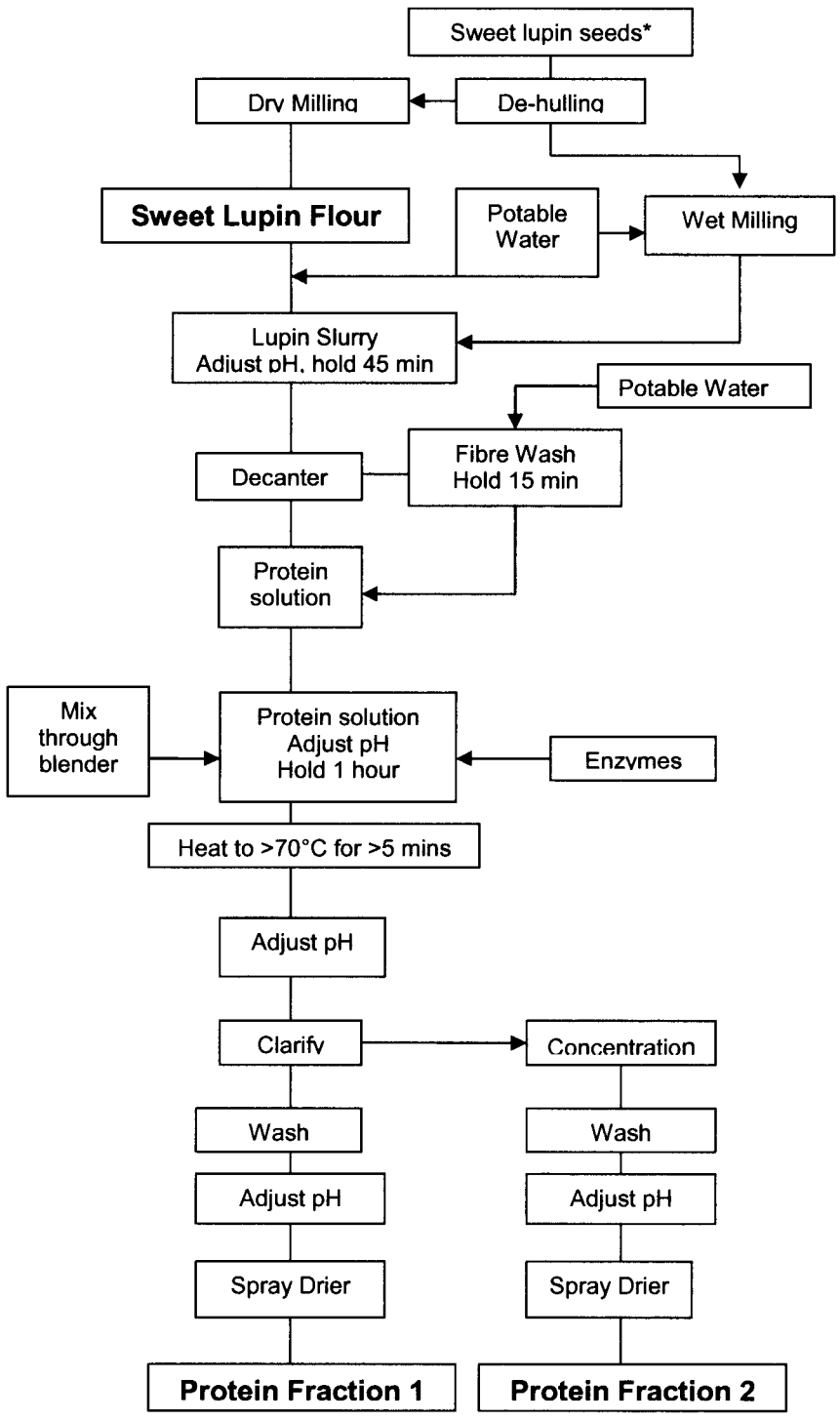
[2] Following a review of data on the safety of ingredients derived from sweet lupin, the Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom concluded that lupin seeds were safe for the production of human foods provided that the level of lupin alkaloids in the derived products did not exceed 200 mg/kg. A summary of the alkaloids identified in lupin is presented in the Appendix B-1.

Figure 1 Schematic Overview of the Manufacturing of Sweet Lupin Protein Fractions 1 and 2



* From sweet varieties of *L. angustifolia*, *L. albus*, *L. luteus*, and *L. mutabilis*

Table 1 List of Processing Aids Used In the Manufacture of the Sweet Lupin Protein Fractions

Processing Aids Used in the Manufacture of Sweet Lupin Protein Fractions	Reference to Appropriate Use in Food
Calcium hydroxide	21 CFR §184.1205 Calcium hydroxide
Phosphoric acid	21 CFR §182.1073 Phosphoric acid
Sodium Hydroxide	21 CFR §184.1763 Sodium hydroxide
Sulfuric acid	21 CFR §184.1095 Sulfuric acid
Pectinases, such as those prepared from *Aspergillus niger* var. [Rapidase® Press (DSM Food Specialties, Delft, The Netherlands) and PYR-FLO (Enzyme Solutions Pty. Ltd., Croydon, Australia)] or an alternative suitable enzyme with similar action permitted by FDA for use in the manufacture of foods.	GRN 000089 (U.S. FDA, 2002)
Carbohydrase preparation, such as Viscozyme® L (Novozymes A/S, Bagsvaerd, Denmark), a multi-enzyme complex prepared from *Aspergillus aculeatus* and comprising various carbohydrases, including arabinase, cellulose, *beta*-glucanase, hemicellulase, and xylanase, or an alternative suitable enzyme with similar action permitted by FDA for use in the manufacture of foods.. The enzyme meets appropriate food-grade specifications	Carbohydrases from *A. aculeatus* are listed in the Food Chemicals Codex (FCC) as appropriate enzyme preparations for use in foods (FCC, 2003)[a,b]
Protease, such as Corolase® LAP (AB Enzymes, Darmstadt, Germany), a protease obtained from *Aspergillus sojae* and with exclusively exopeptidase activities or an alternative suitable enzyme with similar action permitted by FDA for use in the manufacture of foods.	GRN 000010 (U.S. FDA, 1999a)[b]

CFR = Code of Federal Regulation; GRN = Generally Recognized as Safe (GRAS) Notice Number
[a] References to the permitted use by FDA of carbohydrases from *A. aculeatus* for use in foods were not identified.
[b] In the future manufacture of the sweet lupin protein ingredients, GWF may use an alternative suitable enzyme preparation permitted by FDA for use in foods.

C. Specifications for Food-Grade Material

Sweet lupin protein fractions 1 and 2 are produced in accordance with current Good Manufacturing Practices (cGMP), and in order to ensure consistent, safe products, GWF has established numerous food-grade specification parameters for the final preparations. These parameters comprise physical, chemical, and microbiological specifications, including a maximal alkaloid level of <200 ppm, as set forth by the Advisory Committee on Novel Foods and Processes (ACNFP, 1996) of the United Kingdom (UK), and a maximum phomopsins[3] level of 5 ppb, which is consistent with the maximum permitted value for human consumption of 5 µg phomopsins/kg seed established by Food Standards Australia New Zealand (FSANZ) and the Department of Health of the UK. The product specifications for sweet lupin protein fractions 1 and 2 are presented in Tables 2 and 3, respectively.

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[3] Phomopsins are toxins produced by fungi such as *Diaporthe toxica* or *Phomopsis leptostromiformis*, which grow on lupin plants, and phomopsin toxicity caused by phomopsin ingestion is called lupinosis (Allen, 1986; Morcombe *et al.*, 1992; ANZFA, 2001a).

Table 2 Physical, Chemical, and Microbiological Specifications for Sweet Lupin Protein Fraction 1*

Specification Parameter	Specification	Method of Analysis[a]
Physical and Chemical		
Description	Light yellow-colored powder, free from foreign material and objectionable odors and flavors	Visual and olfactory inspection
Protein (TN x 6.25, % DSB m/m)	75 to 95	Australian Standard AS 2300.1.2.1-1991
Fat (% DSB m/m)	1 to 22[b]	Australian Standard AS 2300.1.3-1988
Total Carbohydrate (% DSB m/m)	0 to 5	By Difference[c]
Insoluble Dietary Fiber (% DSB m/m)	0 to 10	AOAC Official Method AOAC 985.29
Soluble Dietary Fiber (% DSB m/m)	0 to 3	AOAC Official Method AOAC 985.29
Moisture (% m/m)	1 to 6	Australian Standard AS 2300.1.1-1988
Ash (% DSB m/m)	1 to 3	Australian Standard AS 2300.1.5-1998
Alkaloids (ppm)	<200	GC-MS[d]
Phomopsins (ppb)	<5	Agrifood Technology Method TP/043
Cadmium (Cd) (ppm)	<0.1	ICP-MS Method IEL1STIM
Lead (Pb) (ppm)	<0.2	ICP-MS Method IEL1STIM
Microbiological		
Total plate count (CFU/g)	<40,000	Australian Standard AS 1766.2.1
Coliforms (CFU/g)	<100	Australian Standard AS 1766.2.3
Escherichia coli (CFU/g)	<10	Australian Standard AS 1766.2.3
Salmonella spp. (per 25 g)	Absent	AOAC Official Method 966.08, AOAC Official Method 2004.03
Yeasts and moulds (CFU/g)	<1,000	Australian Standard AS 1766.2.2
Staphylococcus spp. (CFU/g)	<10	Australian Standard AS 1766.2.4
Bacillus cereus (CFU/g)	<100	Australian Standard AS 1766.2.6
Listeria (per 25 g)	Absent	AOAC Official Method 999.06, AOAC Official Method 2004.06

* Derived from non-genetically modified clean de-hulled sweet lupin cotyledon.
AOAC = Association of Analytical Communities; CFU = Colony-forming units; DSB = dry solid basis; GC-MS = Gas chromatography/Mass spectrometry; ICP-MS = Inductively coupled plasma mass spectrometry; TN = total nitrogen
[a] For details of the methods of analyses please see http://www.aoac.org/ or http://www.standards.com.au/.
[b] The wide range in the specification for fat is due to the fact that defatted sweet lupin might at some point be used in the manufacture the lupin ingredients.
[c] By calculation: 100 – (Moisture + Fat + Protein + Ash + Dietary Fiber) = Total Carbohydrate
[d] Conducted at the Chemistry Centre of Western Australia

Table 3 Physical, Chemical, and Microbiological Specifications for Sweet Lupin Protein Fraction 2*

Specification Parameter	Specification	Method of Analysis[a]
Physical and Chemical		
Description	Fine cream- to white-colored powder, free from foreign material and objectionable odors and flavors	Visual and olfactory inspection
Protein (TN x 6.25, % DSB m/m)	70 to 95	Australian Standard AS 2300.1.2.1-1991
Fat (% DSB m/m)	0 to 5	Australian Standard AS 2300.1.3-1988
Total Carbohydrate (% DSB m/m)	0 to 6	By Difference[b]
Insoluble Dietary Fiber (% DSB m/m)	0 to 3	AOAC Official Method AOAC 985.29
Soluble Dietary Fiber (% DSB m/m)	4 to 15	AOAC Official Method AOAC 985.29
Moisture (% m/m)	2 to 8	Australian Standard AS 2300.1.1-1988
Ash (% DSB m/m)	1 to 4	Australian Standard AS 2300.1.5-1988
Alkaloids (ppm)	<200	GC-MS[c]
Phomopsins (ppb)	<5	Agrifood Technology Method TP/043
Cadmium (Cd) (ppm)	<0.1	ICP-MS Method IEL1STIM
Lead (Pb) (ppm)	<0.2	ICP-MS Method IEL1STIM
Microbiological		
Total plate count (CFU/g)	<40,000	Australian Standard AS 1766.2.1
Coliforms (CFU/g)	<100	Australian Standard AS 1766.2.3
Escherichia coli (CFU/g)	<10	Australian Standard AS 1766.2.3
Salmonella spp. (per 25 g)	Absent	AOAC Official Method 966.08, AOAC Official Method 2004.03
Yeasts and moulds (CFU/g)	<1,000	Australian Standard AS 1766.2.2
Staphylococcus spp. (CFU/g)	<10	Australian Standard AS 1766.2.4
Bacillus cereus (CFU/g)	<100	Australian Standard AS 1766.2.6
Listeria (per 25 g)	Absent	AOAC Official Method 999.06, AOAC Official Method 2004.06

* Derived from non-genetically modified clean de-hulled sweet lupin cotyledon.
AOAC = Association of Analytical Communities; CFU = Colony-forming units; DSB = dry solid basis; GC-MS = Gas chromatography/Mass spectrometry; ICP-MS = Inductively coupled plasma mass spectrometry; TN = total nitrogen
[a] For details of the methods of analyses please see http://www.aoac.org/ or http://www.standards.com.au/.
[b] By calculation: 100 – (Moisture + Fat + Protein + Ash + Dietary Fiber) = Total Carbohydrate
[c] Conducted at the Chemistry Centre of Western Australia

Product Analysis

Several lots of the manufactured products were analyzed to confirm that the manufacturing process produced consistent products within the physical and chemical parameters of each of the product specifications. The complete analyses for these batches are presented in Appendix B-2. The levels of alkaloids and phomopsins present in the sweet lupin protein fractions produced by GWF comply with the maximal established levels of <200 ppm and

5 ppb, respectively, and therefore, are expected not to produce any adverse effects on human health.

Pesticide Residues

The sweet lupin protein ingredients are derived from a raw agricultural product, and therefore, pesticide residue analysis was conducted on some of the sweet lupin-derived ingredients, including the sweet lupin flour, to determine potential pesticide residue levels in the final products. The sweet lupin protein fractions are manufactured from sweet lupin flour. Many of the potential pesticide residues present in sweet lupin flour are sparingly soluble in water, lending to inefficient concentrating of residues in the final material. Therefore, the results of pesticide residue analysis for sweet lupin flour are relevant to any potential pesticide residues in the sweet lupin protein fractions.

Maximum Residue Limits (MRL) for grain products in the U.S. were identified in Title 40 of the *Code of Federal Regulations* (CFR), and where data were available, the pesticide levels in the sweet lupin flour were determined to be below the levels established by the U.S. Environmental Protection Agency (U.S. EPA, 2007). Pesticide residues in sweet lupin flour also were compared with MRL established by the Australia New Zealand Food Standards Code (ANZ Food Standards Code) (FSANZ, 2005a) and/or by the Australian Pesticides and Veterinary Medicines Authority (APVMA, 2005) for grain and nut products. With the exception of some of the organochlorine compounds, the results of the analyses indicated that all tested residue components were below the MRL. With respect to the organochlorine compounds for which the sweet lupin flour did not meet the established MRL, the methods of analyses utilized for determining the levels of these compounds present in this ingredient involved limits of detection that were less sensitive than the established MRL, and hence the levels of these compounds may in fact comply with the regulatory standards. It is therefore expected that residues of pesticides that are present in the final products will not be of any concern. The results of the analysis of the pesticide residues in the sweet lupin flour in relation to identified MRL are presented in Appendix B-3.

Stability of Sweet Lupin Protein Fractions 1 and 2

The sweet lupin protein fractions 1 and 2 should be stored at room temperature (approximately 25°C) in a dry environment, and under proper storage conditions, the ingredients have a shelf life of 6 months. See Appendix B-4 for details on product stability.

III. SELF-LIMITING LEVELS OF USE

The use of sweet lupin protein fractions is self-limiting because the viscosity of the food matrix is increased significantly with concentrations above 20%. This increase in viscosity is more rapid than what occurs with other proteins, such as dairy proteins. Additionally, at high concentrations, the lupin protein has a distinctive flavor profile and a clinging dry mouthfeel. These characteristics limit the use of sweet lupin protein as an ingredient to levels below 30% of the food. Sweet lupin protein fractions 1 and 2 are intended to substitute for other sources of added protein.

IV. BASIS FOR GRAS DETERMINATION

A. Documentation to Support the Safety of Sweet Lupin Protein Fractions

The determination that sweet lupin protein ingredients (*i.e.*, sweet lupin protein fractions 1 and 2) are GRAS is on the basis of scientific procedures, and the information supporting the general recognition of the safe use of sweet lupin protein fractions includes:

- published scientific data on the background consumption of lupin and lupin-derived ingredients;
- the entirety of preclinical and human studies assessing the safety and nutritional value of lupin and lupin-derived ingredients; and
- data pertaining to the identity, intended use, and estimated intake of sweet lupin protein fractions 1 and 2.

Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the proposed uses of sweet lupin protein fractions 1 and 2 are safe and suitable and would be GRAS based on scientific procedures [see Appendix A, entitled, "**EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SWEET LUPIN-DERIVED INGREDIENTS FOR USE IN FOODS**]. A summary of these data is presented herein.

B. Estimated Intake of Sweet Lupin Protein Fractions 1 and 2

As mentioned, sweet lupin protein fractions 1 and 2 are intended for use in a variety of food products, including baked goods and baking mixes, dairy product analogs, frozen dairy desserts and mixes, gelatins, puddings, and fillings, jams and jellies, meat products, milk products, and soft candy. The individual proposed food uses and use levels are summarized in Table 4. As sweet lupin protein fraction 1 will be marketed for use in meat products, it is expected that this Notice also will be reviewed by the United States Department of Agriculture (USDA).

Table 4 Summary of the Individual Proposed Food Uses and Use Levels for Sweet Lupin Protein Fractions 1 and 2 in the United States

Food Category	Proposed Food Use	Serving Size (g or mL)	Maximum Use Level (%)
Sweet Lupin Protein Fraction 1			
Baked Goods and Baking Mixes	Bagels	55[a]	10.0
	Biscuits	55[a]	10.0
	Cakes	50	10.0
	Cookies	30 to 40[a]	10.0
	Cornbread, Corn Muffins, and Tortillas	55[a]	10.0
	Crackers	15 to 30[a]	10.0
	Croissants	55[a]	10.0
	English Muffins	50[a]	10.0
	French Toast, Pancakes, Waffles, and Crepes	85 to 110[a]	10.0
	Muffins and Popovers	55[a]	10.0
	Pastries	55 to 125[a]	10.0
	Pies	125[a]	10.0
	Quick Breads and Sweet Rolls	55[a]	10.0
	Soft Bread Sticks	55[a]	10.0
	Soft Pretzels	55[a]	10.0
	Yeast Breads and Rolls	60	10.0
Dairy Product Analogs	Condensed Milk Analogs[b]	20	15
	High Fat Powder	10	4
	Imitation Cheese	40	20.0
	Imitation Milk	250	4.0
	Milk Powder Analogs[b]	50	15
	Soy Milk Alternatives	250	4.0
Frozen Dairy Desserts and Mixes	Ice Cream	120[a]	20.0
Jams and Jellies	Spreadable Jelly	50[a]	3.0
Meat Products	Commercially Processed Meats and Sandwich Ingredients	55[a]	3.0
Milk Products	Fermented Milk Beverages	250	5.0
	Flavored Milk and Milk Drinks	250	5.0
	Milk-Based Meal Replacements	250	5.0
Soft Candy	Boiled Sweets	10	2.0
	Chocolate, Compound Chocolate	50	3.0
	Soft and Firm Jellies	30	3.0

Table 4 Summary of the Individual Proposed Food Uses and Use Levels for Sweet Lupin Protein Fractions 1 and 2 in the United States

Food Category	Proposed Food Use	Serving Size (g or mL)	Maximum Use Level (%)
Sweet Lupin Protein Fraction 2			
Baked Goods and Baking Mixes	Bagels	55[a]	10.0
	Biscuits	55[a]	10.0
	Cakes	50	10.0
	Cookies	30 to 40[a]	10.0
	Cornbread, Corn Muffins, and Tortillas	55[a]	10.0
	Crackers	15 to 30[a]	10.0
	Croissants	55[a]	10.0
	English Muffins	50[a]	10.0
	French Toast, Pancakes, Waffles, and Crepes	85 to 110[a]	10.0
	Muffins and Popovers	55[a]	10.0
	Pastries	55 to 125[a]	10.0
	Pies	125[a]	10.0
	Quick Breads and Sweet Rolls	55[a]	10.0
	Soft Bread Sticks	55[a]	10.0
	Soft Pretzels	55[a]	10.0
	Yeast Breads and Rolls	60	10.0
Dairy Product Analogs	Non-dairy Cream Substitutes and Coffee Whiteners	250	3.0
Gelatins, Puddings, and Fillings	Mousses and Meringues	125[a]	3.0
Jams and Jellies	Spreadable Jelly	50[a]	3.0
Soft Candies	Boiled Sweets	10	1.0
	Chocolate, Compound Chocolate	50	3.0
	Soft and Firm Jellies	30	3.0

[a] Serving size reported was based on Reference Amounts Customarily Consumed Per Eating Occasion (RACC) (21 CFR §101.12) (U.S. FDA, 2008). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACCs.

Humans and livestock have consumed lupin and lupin-derived ingredients for over 2,000 years, and various species and varieties of lupin have been historically cultivated in the Mediterranean region, northern Europe, South Africa, Australia, and New Zealand, and more recently in the southeastern United States. Sweet lupin has a crude protein level similar to that of soybeans, but contains much lower levels of potential anti-nutritional factors (ANFs), and hence has been recognized as a valuable protein source (Ballester *et al.*, 1980; Petterson and Crosbie, 1990; Petterson, 1995). Lupin-derived ingredients are permitted for use in food for human consumption in the European Union (EU) and Australia/New Zealand (Allen, 1992; Weston Technologies, personal communication, 2005). Lupins are enjoying application in Asia for modified traditional cultural dishes such as miso, tempeh, and tofu, as the yield of fermented products from lupin protein fractions has been reported to be greater than that for soybean (Petterson and Crosbie, 1990), and lupin flour is used in Europe in bread (up to 10%), pastas, cakes, and biscuits (up to 50%) (Belteky and Kovacs, 1984).

Despite the documented historical consumption of lupin, quantitative consumption data have not been identified.

The consumption of the sweet lupin protein fractions from all proposed food-uses was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2008). Depending on the particular food category, sweet lupin protein fractions are proposed for use as food ingredients at maximum levels of between 1 and 20%. The estimated daily consumption of the sweet lupin protein fraction ingredients from all proposed food uses at the proposed use-levels per serving was calculated on a g and g per kilogram body weight basis by population group.

The sweet lupin protein fractions are of similar composition, with both ingredients being composed of at least 70 to 95% protein, and thus their estimated intakes are discussed together. Consumption of foods in the proposed food-use categories was estimated to result in a maximum total population mean all-user intake of sweet lupin protein fraction 1 of 18.3 g/person/day (0.3 g/kg body weight/day), with a 90th percentile all-user intake of 35.3 g/person/day (0.7 g/kg body weight/day), and a maximum total population mean all-user intake of sweet lupin protein fraction 2 of 12.3 g/person/day (0.2 g/kg body weight/day), with a 90th percentile all-user intake of 23.4 g/person/day (0.4 g/kg body weight/day). The estimated intakes were highest for sweet lupin protein fraction 1 and, as the protein fractions have such similar composition, the intake estimates obtained for sweet lupin protein fraction 1 will be discussed herein.

Of the individual population groups, the greatest mean all-user intake of sweet lupin protein fraction 1 on an absolute basis was calculated to occur in male adults, at 20.9 g/person/day. Infants had the lowest estimated intake of sweet lupin protein fraction 1 on an absolute basis, with a mean all-users intake of 8.0 g/person/day. On a body weight basis, the mean all-user intake of sweet lupin protein fraction 1 was highest in infants and children (each at 0.7 g/kg body weight/day), and lowest in male and female adults (each at 0.2 g/kg body weight/day).

When heavy consumers (90th percentile) were assessed, all-user intakes of sweet lupin protein fraction 1 from all proposed food-uses was estimated also to be greatest in male adults (39.8 g/person/day), and lowest in infants (16.2 g/person/day) on an absolute basis. On a body weight basis, the highest all-user 90th percentile intakes of sweet lupin protein fraction 1 were estimated to occur in children and infants (1.3 g/kg body weight/day in each group), and the lowest were estimated to occur in male and female adults (0.5 g/kg body weight/day for each group). A summary of the estimated all-person and all-user mean and 90th percentile intakes of sweet lupin protein fraction 1 by individual population groups and for the total population is presented in Tables 5 and 6 on a g and g per kilogram body weight basis, respectively.

Table 5 Summary of the Estimated Daily Intake of Sweet Lupin Protein Fraction 1 from All Proposed Food Uses in the United States by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (g)		All-Users Consumption (g)	
				Mean	90th Percentile	Mean	90th Percentile
Infant	0 to 2	74.1	689	6.5	15.5	8.0	16.2
Children	3 to 11	99.7	1,283	18.9	34.7	19.0	35.0
Female Teenager	12 to 19	99.3	985	16.2	32.9	16.4	33.0
Male Teenager	12 to 19	99.1	990	20.3	37.6	20.5	37.6
Female Adult	20 and Up	99.4	2,116	16.4	32.2	16.5	32.4
Male Adult	20 and Up	99.2	1,914	20.6	39.8	20.9	39.8
Total Population	All Ages	96.5	7,977	17.9	35.1	18.3	35.3

Table 6 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Sweet Lupin Protein Fraction 1 from All Proposed Food Uses in the United States by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (g/kg body weight)		All-Users Consumption (g/kg body weight)	
				Mean	90th Percentile	Mean	90th Percentile
Infant	0 to 2	74.1	689	0.5	1.2	0.7	1.3
Children	3 to 11	99.7	1,283	0.7	1.3	0.7	1.3
Female Teenager	12 to 19	99.3	985	0.3	0.6	0.3	0.6
Male Teenager	12 to 19	99.1	990	0.3	0.6	0.3	0.6
Female Adult	20 and Up	99.4	2,116	0.2	0.5	0.2	0.5
Male Adult	20 and Up	99.2	1,914	0.2	0.5	0.2	0.5
Total Population	All Ages	96.5	7,977	0.3	0.7	0.3	0.7

The sweet lupin protein fractions are composed of at least 70 to 95% protein with lesser amounts of fiber (0 to 18% soluble and insoluble combined), other carbohydrates (0 to 6%), and fat (0 to 22%), the levels of which vary between the 2 protein products. These major components of the sweet lupin protein ingredients are macronutrients that are part of a normal human diet. As the sweet lupin protein fractions are composed mainly of protein, it is useful to compare the estimated intake of protein components from the sweet lupin protein ingredients to the reported background consumption of protein. The total U.S. population mean and 90th percentile daily intakes of protein were reported to be 75.2 and 114.0 g protein, respectively (USDA, 2000). The estimated total population all-user mean daily intakes of lupin protein fractions 1 and 2 (18.3 and 12.3 g, respectively) are well below the mean daily intakes of total protein from all dietary sources reported for the total population. Therefore, when considering the background intake of protein, the anticipated intake of

protein from the intended conditions of use of the sweet lupin protein fractions is not expected to pose any concern to human health.

C. Metabolic Fate of Sweet Lupin Protein Fractions

With respect to the metabolic fate of the sweet lupin protein fractions, the digestion and subsequent absorption, distribution, metabolism, and excretion of the ingredients is relevant to the metabolic fate of their macronutrient constituents. As mentioned, the sweet lupin protein fractions are composed of at least 70 to 95% protein with lesser amounts of fiber, other carbohydrates, and fat, the levels of which vary between the two protein products. The major macronutrients present in the sweet lupin protein fraction ingredients are expected to undergo normal metabolism. Following consumption, the protein components of the sweet lupin protein fraction ingredients are expected to be denatured in the stomach by acid and/or cleaved by enzymes, and the dietary fibers are expected to pass relatively intact into the large intestine following consumption, where they will be subjected to fermentation.

D. Preclinical Studies Pertaining to the Safe Consumption of Sweet Lupin Protein Fractions

Some traditional toxicological studies of oral exposure to lupin seed and/or ingredients derived thereof were identified in the published literature, including a subchronic oral toxicity study of lupin flour in rats, and these data support the safety of sweet lupin protein fractions 1 and 2 under the intended conditions of use. In addition, several studies designed to assess the nutritional equivalence of lupin seed and lupin-derived ingredients to other traditional seed crops and its acceptability for use as an alternate feed source for food-producing animals were identified, and these provide additional support for the safe consumption of GWF's sweet lupin protein fractions under the intended conditions of use. Summaries of these studies are presented below.

Acute Toxicity Studies

Sweet lupin and lupin fractions were reported to have low acute oral toxicity in rats, with reported oral LD_{50} values ranging from 750 to >4,000 mg/kg body weight for *L. angustifolia* and *L. albus* whole seed and seed fractions (Stobiecki *et al.*, 1993). No adverse effects were reported in rats following administration of a single gavage dose of conglutin γ, a lupin seed protein isolated from *L. albus*, at levels of up to 120 mg/kg body weight, the highest dose tested (Magni *et al.*, 2004). Moreover, lupin protein extracted from the seeds of *L. albus* and administered by gavage to male Sprague-Dawley rats for a period of 2 weeks at a dose of 250 mg lupin protein/kg body weight/day did not result in any adverse effects (Sirtori *et al.*, 2004).

Short-term Toxicity Studies

In a number of short-term toxicity studies conducted by one research group and using the same experimental design, male Hooded-Listar rats (4 to 20/group) were provided diets

containing *L. angustifolia* seed or protein fractions for 10 days (Rahman *et al.*, 1996a,b, 1997a). The diets included: one with whole lupin seeds (supplemented or unsupplemented with essential amino acids) providing 31.5 g lupin/kg body weight/day; 1 with a soluble (LPAD) and 1 with an insoluble (LPADI) aqueous dialyzed protein fraction, providing 13.0 and 10.9 g lupin/kg body weight/day, respectively; 1 with a non-dialyzed (LPAND) aqueous protein fraction providing 17.1 g lupin/kg body weight/day; 1 with a soluble (BUSOL) and 1 with an insoluble (BUDI) buffer-extracted fraction providing 11.9 and 10.4 g lupin/kg body weight/day; and 1 diet containing a dialyzed residue fraction (LMR) (*i.e.,* the fibrous material that is insoluble in both water and buffer) providing 14.9 g lupin/kg body weight/day. A diet containing lactalbumin was provided to a separate group of rats (control group) in each study. An additional 10-day study included rats supplemented or unsupplemented with whole lupin seed or LPADI in the diet at levels of 28 or 9.7 g whole seed and LPADI/kg body weight/day, respectively (Rahman, 2000). Observed effects from these studies, such as decreased body weight gain, increased urea, and decreased albumin, were suggested by the study authors to be due to a disturbance of normal protein utilization, which could have resulted from the amino acid deficiency of the lupin-containing diets, as lupin is known to contain low levels of essential amino acids, despite supplementation of the diets with amino acids. In a clinical study by Egaña *et al.* (1992), lupin protein digestibility was reported to be good, and therefore a disturbance of protein utilization is not likely to occur in humans consuming an average diet. Moreover, the reported increases in plasma urea remained within reported historical control values for rats (Sharp and LaRegina, 1998), the reported increases in serum alkaline phosphatase (AP) values in lupin-treated rats were not accompanied by significant differences in the alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT) levels compared to the control group, and liver lesions were not reported in any of the other reviewed dietary studies. Rahman (2000) evaluated spleen and thymus weights, and spleen weights were reported to be significantly reduced in the LPADI group compared to the all other groups. The authors suggested that uremia might have been the cause of the immunosuppression, characterized by a significant decrease in spleen weight. The results of the study by Rahman *et al.* (1996b) indicated that the stomachs of rats provided unsupplemented and supplemented whole lupin seed were distended due to undigested food material and their colons were reportedly enlarged compared to lactalbumin controls. Rats in the LMR group also were reported to have enlarged spleens and colons, although no changes in abdominal organs were reported in any of the other lupin groups. Sprague-Dawley rats that were provided approximately 11.1 g lupin (*L. albus*) protein/kg body weight/day for 10 days had significantly decreased relative rectum and total large intestine weights in comparison to rats that were provided a lactalbumin control diet (Caligari *et al.*, 2006). Similarly, Sprague-Dawley rats that were provided approximately 8.8 g lupin protein/kg body weight/day for 28 days had significantly decreased relative cecum and total large intestine weights in comparison to the control rats (Caligari *et al.*, 2006). Changes in kidney and intestinal weights were reported, as were gross and microscopic changes in the liver. Rahman *et al.* (1996a,b, 1997a) and Rahman (2000) did not discuss the significance of these effects, nor was the frequency of any of these effects reported. Furthermore, this study involved a number of different experimental parts, and it is unclear if organ weights

and gross and microscopic examinations were conducted in only 1 of the experimental study parts (resulting in examination of only 4 rats/group), or in 5 different experimental parts (resulting in examination of 4 to 20 rats/group). Nonetheless, as previously mentioned, the frequency of the observed effects was not reported, and effects in the lactalbumin control group were not always reported as a means for comparison. Gross and microscopic changes were reported in the livers of all lupin-treated groups; however, liver weights were not measured. Overall, the results of these studies are poorly reported and the significance of the observed effects is unclear.

Subchronic Toxicity Studies

A 90-day toxicity study in rats was identified in which rats were fed diets containing *L. angustifolia* lupin flour spiked with lupin alkaloids providing 0 (control), 250, 1,050, or 5,050 ppm of supplemental alkaloids (Butler *et al.*, 1996). The source of lupin used in this study was from the same agronomic area (*i.e.,* Australia) as GWF's lupin source. The control group of this study was provided a diet containing 13.2 g lupin flour (up to 33 g/kg body weight/day), which contained a background level of ~50 ppm alkaloids (6.6 mg alkaloids/kg body weight/day), a level similar to that present in the GWF sweet lupin-derived ingredients, and therefore the results of this group are considered relevant to the safety of sweet lupin flour, as well as the safety of the other sweet lupin-derived ingredients. The group of interest is the control group in this study; however, an 'untreated' group (*i.e.,* not provided lupin) was not available for which to compare results, and hence historical values in the rat were utilized to assess any potentially adverse effects resulting from 90-day dietary supplementation with lupin flour (Butler *et al.*, 1996). The historical values for control rats from the laboratory in which the study was conducted were sought without success, and hence the data of Sharp and LaRegina (1998) were used for the purpose of this assessment. No deaths or clinical signs of toxicity were reported, and there were no significant differences in biochemical or hematological parameters or organ weights in lupin-treated rats when compared to historical values in the rat. Furthermore, there were no histological findings in any of the organs evaluated. For the purpose of this assessment, a no-observed-effect level (NOEL) of 33 g/kg body weight/day was derived for lupin flour, which was the only dose of low-alkaloid lupin flour tested.

In an additional identified subchronic study, lupin protein isolated from *L. albus* and *L. luteus* administered in the diet to rats at levels of 6.3 and 6.88 g/kg body weight (20% of the diet), respectively, for a period of 112 days did not result in any changes in body weight gains, organ weights (including liver, kidney, heart, spleen, and adrenals), or gross pathology of the liver, kidney, or lungs (Ballester *et al.*, 1980).

Chronic Feeding Studies

Chronic studies in rats ranging from 700 to 800 days in duration and using *L. angustifolia* seed and seed fractions were identified in the available literature (Ballester *et al.*, 1980, 1984; Grant *et al.*, 1993, 1995). These studies were nutritional studies and not traditional toxicity studies; however, the results of these studies support the safety of dietary lupin and

lupin ingredients. Consumption of 5.6 g of whole *L. angustifolia* seed/kg body weight/day in the diet, increasing to a maximum intake of approximately 13.6 g/kg body weight/day after 15 weeks, was reported not to cause any adverse effects in rats when administered for up to 800 days (Grant *et al.*, 1993). Body weight gain was significantly reduced in lupin-treated rats compared to controls for the first 200 days, however, was not significantly different from controls for the remainder of the study. Furthermore, lupin seed was reported to have no significant effect on pancreatic weight or composition (Grant *et al.*, 1993). These same doses of whole lupin seed in the diet were reported to decrease body weight gain in rats dosed for up to 700 days (Grant *et al.*, 1995). Cecum and colon weights were significantly increased in lupin-fed rats compared to control rats after 700 days of feeding, which the authors stated was not mediated by either lectin or protease inhibitors, but rather may have been the result of volatile fatty acid production due to dietary fiber digestion in these organs.

Developmental and Reproductive Toxicity Studies

One study of the potential reproductive and developmental toxicity of lupin protein was identified in the literature. Dietary administration of 20% lupin protein isolated from *L. albus* administered to 3 generations of rats for 270 days each (providing 7 to 35.4 g lupin protein/kg body weight/day over the study duration) was reported to result in significantly decreased relative liver weights in both sexes in the second and third generation rats; however, these changes were not accompanied by any histological changes. No other effects on organ weights occurred and the lupin protein was reported to have no effect on either fertility or reproductive parameters in any of the generations (Ballester *et al.*, 1984).

Mutagenicity/Genotoxicity and Carcinogenicity Studies

Studies of the mutagenic/genotoxic potential of lupin or its fractions were not identified in the literature, nor were traditional carcinogenicity studies; however, as previously mentioned, chronic/life-time studies (*i.e.,* 700 and 800 days) in rats did not reveal any evidence of carcinogenicity in lupin-treated animals, and no signs of toxicity or decreases in body weight occurred (Grant *et al.*, 1993, 1995).

Nutritional Studies

Conflicting results were reported upon investigation of the potential effect of *L. angustifolia* seed and seed fractions on mineral absorption in rats, chickens, and pigs (Rubio *et al.*, 1994; Rahman *et al.*, 1997b; Olkowski *et al.*, 2005; Zraly *et al.*, 2006, 2007). Rubio *et al.* (1994) reported that dialyzed soluble and insoluble lupin protein fractions had no significant effect on absorption of calcium, phosphorus, or zinc in male Hooded-Listar rats, while in the same species, whole lupin seed and the LMR fraction significantly reduced phosphorus and zinc absorption due to the presence of phytate and insoluble non-starch polysaccharide fractions that are not present in protein fractions. Conversely, Rahman *et al.* (1997b) reported that equivalent doses of various lupin protein fractions, including dialyzed soluble and insoluble fractions, significantly reduced absorption of phosphorus, zinc, magnesium, and sodium (with no effects on calcium or potassium); however, these effects were not discussed by the

authors. Zraly *et al.* (2006) reported that plasma phosphorus concentrations in pigs were not significantly affected by the consumption of dehulled *L. angustifolia* seed meal for a period of 90 days, and the same authors also reported no significant changes in plasma calcium or phosphorus levels in pigs administered diets containing *L. albus* seed for 90 days (Zraly *et al.*, 2007). Additionally, no significant differences in plasma zinc concentrations were reported to occur in broiler chicks from the consumption of raw or dehulled *L. albus, L. luteus,* or *L. angustifolius* seed meal for a period of 21 days (Olkowski *et al.*, 2005). Plasma zinc concentrations in broiler chicks provided diets containing raw or dehulled lupin seed meal from *L. albus, L. luteus*, or *L. angustifolius* at doses of approximately 500 g/kg body weight/day (raw) or ranging from 252 to 650 g/kg body weight/day (dehulled) for 21 days were not significantly different from the values of control birds fed a soybean meal diet; however, plasma riboflavin concentrations were significantly increased in all lupin-fed chicks combined compared to the controls (Olkowski *et al.*, 2005).

Sweet lupin seeds are widely used in Australia as a source of protein and energy in livestock feeds, and hence, their nutritive value has been evaluated in various feeding studies in pigs and poultry. Overall, nutritional studies in pigs and chickens indicate that lupin feeds are generally well tolerated (Dunshea *et al.*, 2001; Rubio *et al.*, 2003; Steenfeldt *et al.*, 2003; Hammershoj and Steenfeldt, 2005; Martins *et al.*, 2005; Olkowski *et al.*, 2005; Zraly *et al.*, 2006, 2007); however, due to the generally low levels of both methionine and lysine in lupin (Petterson, 1998), feeds for pigs and poultry are more beneficial when they include multiple sources of protein, or supplemental amino acids (Edwards and van Barneveld, 1998). Transgenic lupin seeds have been reported to significantly improve the nutritive value of lupin as they have been modified to encode a protein which contains 16% methionine and 8% cysteine residues (Molvig *et al.*, 1997, 2003). Furthermore, digestible energy from lupin may be compromised by ANFs (*e.g.*, trypsin inhibitors)[4] by interfering with digestive enzymes in monogastrics (Edwards and van Barneveld, 1998). A summary of the results of the identified nutritional studies in pigs and poultry is provided below.

Administration of whole *L. angustifolia* seed or kernel to pigs *via* the diet during a 14-day feeding study, which provided a dose of 16 g lupin seed or kernel/kg body weight/day, was reported not to produce any adverse effects (Dunshea *et al.*, 2001). Similarly, doses of 8.8 g of whole *L. angustifolia* or *L. albus* seed or kernel/kg body weight/day provided for 14 days did not result in any adverse effects (Dunshea *et al.*, 2001). Feed intake was increased in both intact and ileorectal anastomosed pigs provided doses of 10.64 and 10.30 g *L. angustifolia* seed/kg body weight/day, respectively, in the diet for a period of 3 weeks compared to pigs provided nutritionally-equivalent cholesterol-enriched casein control diets; however, there was no significant difference in body weight gains between groups (Martins *et al.*, 2005). The difference in feed intake was likely due to unpalatability of the cholesterol-enriched control diet. Significantly decreased relative liver weights were reported in pigs

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[4] See Section F for further discussion of the phytonutrient components identified in lupin and their relevance to the safety of sweet lupin protein fractions.

provided lupin protein compared to control pigs; however, there were no significant differences in gallbladder weight between groups (Martins *et al.*, 2005). Body weight gain was not affected following administration of 13.2 and 14.3 g of *L. angustifolia* seed and kernel/kg body weight/day, respectively, in the diet to pigs for 28 days, but was significantly decreased with doses of 10.2 and 10.9 g/day of *L. albus* seed and kernel, respectively, in the diet for the same period of time (Dunshea *et al.*, 2001). Neither lupin diet had any significant effect on liver weight. In another feeding study, histological examination of the livers and kidneys of pigs revealed no gross lesions following administration of 1 of 3 different diets containing a combination of 2 different varieties of *L. angustifolia* seed levels providing doses of up to 26.9 g lupin seed/kg body weight/day and up to 23.7 mg alkaloids/kg body weight/day for a period of 7 weeks (Godfrey *et al.*, 1985). Body weight gain and feed conversion in Large White x Landrace pigs were not affected by the administration of diets containing approximately 2.3 g dehulled lupin (*L. angustifolius*) seed meal/kg body weight/day for a period of 90 days (Zraly *et al.*, 2006), although significant decreases in plasma glucose and calcium, and a significant increase in total protein were reported. Body weight gain, feed intake, and plasma and calcium levels were not affected in hybrid P x (Du x LW x L) pigs administered a diet containing approximately 4.1 g lupin (*L. albus*) seed/kg body weight/day for 90 days compared to animal or soy protein-fed controls (Zraly *et al.*, 2007) or in (LW x L) x D pigs fed 5.4 and 5.1 g/kg body weight/day raw and extruded lupin (*L. albus*), respectively, for 42 days (Prandini *et al.*, 2005).

Performance and some biochemical measures of toxicity were evaluated in (LW x L) x D piglets (16 males and 12 females/group, average initial body weight of 10.4 kg) weaned at 28 days of age and administered basal diets supplemented with 170 g/kg raw or extruded lupin (*L. albus*) seeds (providing approximately 5.4 and 5.1 g/kg body weight/day raw and extruded lupin, respectively) *ad libitum* for a period of 42 days (Prandini *et al.*, 2005). No significant differences in total bilirubin or ALAT, ASAT, or AP activity were reported to occur in blood samples taken from the lupin-fed pigs at Day 42 compared to the controls; however, significant decreases in total protein and urea were reported to occur in the lupin-fed pigs compared to the controls. In 2 studies of longer duration (90 days), total protein, albumin, AP, ASAT, and ALAT levels were not significantly different between control pigs and pigs administered a diet containing approximately 2.3 g dehulled lupin (*L. angustifolius*)/kg body weight/day or a diet containing 4.1 g lupin (*L.* albus) seed/kg body weight/day (Zraly *et al.*, 2006, 2007).

L. albus seed (unsupplemented) provided to pigs at levels of 20.7 or 31% in the diet significantly reduced growth rates, although this effect was not observed in animals provided a lower level (10.3% lupin in the diet) or in pigs provided 31% lupin in the diet supplemented with 0.2% lysine (duration not specified) (King, 1981). The authors reported that 20.7 and 31% lupin diets were deficient in lysine and therefore growth rates were significantly reduced compared to control pigs (King, 1981). *L. albus* is therefore not recommended for use in pig feeds due to recognized reductions in feed intake and depressed growth rates (Edwards and van Barneveld, 1998).

Feed intake was reduced in hens provided up to 25% lupin seed in the diet for 11 weeks (providing 15 g lupin seed/kg body weight/day), although there were no significant effects on body weight (Hammershoj and Steenfeldt, 2005). Broiler chickens dosed with 1,006 g lupin seed/kg body weight/day in the diet for 14 days were reported to have decreased body weight gain, although there was no effect on feed consumption. The authors attributed the effects on body weight to a lack of appropriate digestive enzymes in chickens (Steenfeldt *et al.*, 2003). Rubio *et al.* (2003) reported that whole lupin seed provided in chicken feed at levels of up to 1,540 g/kg body weight/day for 21 days decreased both feed intake and body weight gain; however, these effects were not observed with dehulled lupin seeds consumed at a level of 1,347 g/kg body weight/day for 21 days. Olkowski *et al.* (2001) examined the effects of raw, autoclaved, and dehulled lupin (*L. angustifolius*) seed meal (approximately 546, 445, and 584 g/kg body weight/day, respectively) in the diet of broiler chicks for a period of 21 days and reported significantly decreased feed intake and body weight gain in all lupin-fed chicks. Raw lupin seed meal from *L. albus, L. luteus*, or *L. angustifolius* at doses of approximately 500 g/kg body weight/day (raw) or ranging from 252 to 650 g/kg body weight/day (dehulled) for 21 days also resulted in significantly decreased feed intakes and growth rates compared to the controls. Dehulling was reported to significantly increase body weight gain, but the level remained significantly lower than the control group (Olkowski *et al.*, 2005). Conversely, Ross 308 broiler chicks provided dehulled lupin seeds of the variety JUNO (*L. luteus*) at an average level of 16.4 g/kg body weight/day for 40 days reached body weights similar to those of the control chicks fed a diet containing soy extract; chicks provided dehulled lupin seeds of the variety SONET (*L. angustifolius*), however, had significantly decreased final body weights (Suchy *et al.*, 2006).

Significant dose-dependent increases in relative gizzard weights were reported in Leghorn chicks receiving diets containing whole lupin (*L. albus*) seeds at levels of up to 70% for a period of 14 days, and Leghorn chicks that received diets containing dehulled lupin seeds supplemented with lupin hulls had significant increases in relative intestinal organ weight and length compared to chicks that received the lupin diet without the addition of hulls (Brenes *et al.*, 2002). Similarly, it was reported by the same authors that broiler chicks fed diets containing 35 and 45% whole lupin (*L. albus*) (approximately 599 and 762 g lupin/kg body weight/day) for 6 weeks had significantly increased relative weights of the crop, proventriculus, gizzard, and duodenum compared to the control group receiving a wheat-soy diet (Brenes *et al.*, 2002), and significant increases in the size of the duodenum, jejunum, and ileum were reported in broiler chicks fed diets containing either 40% raw (approximately 500 g/kg body weight/day) or 35% dehulled (approximately 425, 252, and 650 g/kg body weight/day for *L. albus, L. luteus*, and *L. angustifolius*, respectively) lupin seed meal for 21 days when compared to chicks fed a soybean meal control diet (Olkowski *et al.*, 2005); however, the relative weights of the liver, pancreas, gizzard, and heart were not significantly different from control values in Ross 308 broiler chicks fed wheat- and barley-based diets containing up to 20% lupin (*L. luteus*) for 6 weeks (Orda *et al.*, 2006). Enlargement of some gastrointestinal organs may be interpreted as a physiological adaptation to overcome ANFs present in the lupin-based diets (Olkowski *et al.*, 2005).

E. Studies in Humans

Data relating specifically to the safety of the sweet lupin protein ingredients consumed by human volunteers were not identified; however, safety data were reported by Gattás Zaror *et al.* (1990) and Egaña *et al.* (1992) following consumption of *L. albus* flour-enriched products by healthy volunteers. As the sweet lupin protein fractions are derived from lupin flour, these data are presented to support the safety of the sweet lupin protein ingredients.

In a crossover study, Gattás Zaror *et al.* (1990) provided one 150 g cookie/day, with or without lupin flour (providing 35 and 0 g lupin flour/day and containing 13.3 and 0 g lupin protein, respectively) for a treatment period of 60 days. No compound-related changes were reported in any of the biochemical or hematological parameters tested [*i.e.*, hematocrit, hemoglobin, prothrombin, uric acid, urea nitrogen, bilirubin, glutamic-pyruvic transaminase (GPT), ASAT, blood lipids, and creatinine], although body weight was significantly increased in both groups. The authors reported that lupin flour was well tolerated by the subjects. Egaña *et al.* (1992) supplemented the diet of young men (n=9) with lupin flour derived from *L. albus*, providing a dose of 0.4, 0.6, or 0.8 g lupin protein/kg body weight/day for a period of 10 days, which would correspond to 28, 42, and 56 g/day for the average 70 kg person. Nitrogen digestibility, complete blood count, serum total protein, albumin, urea nitrogen, globulin, ASAT, ALAT, cholesterol, and triglycerides were evaluated at the end of the study period, although hematological parameters were only measured in the low- and high-dose groups. Nitrogen digestibility was reported to range between 78.8 and 70.2%. The only significant hematological change reported was a significant increase in urea nitrogen in the high-dose group (0.8 g lupin protein/kg body weight/day) compared to the low-dose group (0.4 g lupin protein/kg body weight/day). The authors reported that lupin-containing diets were well tolerated by the subjects and were without adverse effects.

In an open-label trial designed to assess the effects of lupin on coronary artery disease, 16.75 g lupin protein (derived from *L. albus*)/day provided to otherwise healthy, chronically-smoking, volunteers with moderate hypercholesterolemia for a period of 90 days was well tolerated and resulted in significant reductions in total cholesterol, low-density lipoprotein (LDL) cholesterol, blood glucose, homocysteine, high sensitivity C-reactive protein (hsCRP), urinary F_2-isoprostane/creatinine excretion, systolic blood pressure (SBP), and diastolic blood pressure (DBP) (Naruszewicz *et al.*, 2006).

F. Other Data Pertaining to the Safety of Sweet Lupin Protein Fractions

Various phytonutrients (*i.e.*, oligosaccharides, phenolics and condensed tannins, trypsin inhibitors, phytic acid, saponins, and lectins) occur naturally in *L. angustifolia*, *L. albus,* and *L. luteus* at very low levels and are comparable to levels present in other grain legume species (Petterson, 1998). The possible effects of exposure to these compounds under the intended conditions of use of the sweet lupin protein ingredients of GWF are discussed below. Moreover, lupin has recently been recognized as a potential food allergen, and

therefore, the possible allergenicity of sweet lupin protein fractions 1 and 2 also has been considered and is discussed below.

Other Phytonutrient Components

As a result of their natural presence in lupin, possible additional components occurring in the final sweet lupin protein fraction ingredients are oligosaccharides, phenolics and condensed tannins, trypsin inhibitors, phytic acid, saponins, and lectins. (See Appendix B-5 for results of analysis). These compounds are reported to occur naturally in sweet lupin varieties at very low levels and are comparable to levels found in other grain legume species (Petterson, 1998).

The oligosaccharides present in lupin belong to the raffinose family and are considered to be ANFs because they cannot be metabolized by monogastrics (Petterson, 1998). Oligosaccharides occur naturally in sweet lupin at levels of 5.2 to 11.87% (Petterson, 1998). Following batch analysis, the level of oligosaccharides in the sweet lupin protein ingredients was determined to be 0.4% (dry solid basis) in each protein ingredient. Based on the estimated total population all-user 90th percentile intake of sweet lupin protein fraction 1 (35.3 g/person/day), a maximum intake of 142 mg oligosaccharide/person/day was calculated. Considering that the method of calculating the estimated intakes of the ingredients of the lupin ingredients under the recommended conditions of use is 'worst-case', the actual intake of oligosaccharides will likely be much lower, and hence is not expected to produce adverse effects on human health.

Phenolic compounds are reported to have the potential to bind iron and decrease iron absorption (Disler *et al.*, 1975; Brune *et al.*, 1989; Hurrell *et al.*, 1999), and condensed tannins have an affinity for binding proteins (Ricardo da Silva *et al.*, 1991; Vallet *et al.*, 1994; Santos-Buelga and Scalbert, 2000). The background dietary intake of phenolics (as flavonoids) from various sources, such as coffee, cocoa, red wine, and many fruits, was reported to be 1,000 mg/day, with condensed tannin intakes of 250 to 460 mg/day (Kühnau, 1976; Santos-Buelga and Scalbert, 2000). Following batch analysis, the sweet lupin protein fractions were determined to contain phenolics at levels up to 0.307%, which occurred in sweet lupin protein fraction 2, corresponding to a maximum exposure of ~72 mg/person/day, which is a small increase over the background daily intake, and is expected to have no effect upon the iron absorption. The level of condensed tannins as a component of the overall total phenolic(s) levels identified in the sweet lupin protein fraction ingredients (<0.05%) is negligible, and hence is expected not to produce any adverse effects on human health.

Trypsin inhibitors are present in whole lupin seed (<0.01 to 0.29 mg/g protein) (Petterson, 1998) at levels which are several-fold lower than the amount occurring naturally in soybeans (34.30 to 56.14 mg/g protein), soy protein isolates (1.11 to 4.49 mg/g protein), and commercial infant soy formulas (2.2 to 15.5 mg/g protein) (Peace *et al.*, 1992). The authorized health claim on the association between soy protein and reduced risk of coronary heart disease (CHD) includes a qualifying level of a total daily intake of 25 g soy protein for CHD risk reduction claim (U.S. FDA, 1999b). Using a reported level of up to 4.49 mg trypsin

inhibitors/g soy protein isolate, individuals consuming 25 g soy protein/day could be exposed to levels of trypsin inhibitors of up to 1,400 mg/day. Analysis of sample batches of sweet lupin protein fractions 1 and 2 indicated the highest levels of trypsin inhibitors to occur in sweet lupin protein fraction 2 with levels of up to 0.94 mg/g, which would provide an exposure of ~22 mg trypsin inhibitors/person/day. This level is approximately 63 times less than the estimated exposure to trypsin inhibitors from consumption of 25 g soy protein/day. Therefore, the levels of trypsin inhibitors present in the sweet lupin protein fraction ingredients are anticipated not to produce any adverse effects on human health.

Phytate is an ANF that can form insoluble complexes with cations, such as calcium and zinc, making them less available for absorption and utilization (Petterson, 1998). Phytate occurs naturally in sweet lupin at levels of 0.58 to 0.96% (Petterson, 1998), and is present in soybeans in the range of 1 to 2% (Wang and Wixon, 1999). In a review of studies investigating phytate isolated from soybeans and other studies of the effects of soy protein on iron and zinc status, the FDA concluded that evidence of potential adverse effects of phytic acid is equivocal, and noted that many other factors affect the absorption of these minerals (U.S. FDA, 1999b). Analysis of sample batches of sweet lupin protein fractions 1 and 2 indicated levels of phytate of up to 0.31%, which occurred in sweet lupin protein fraction 2, and which would provide an exposure of ~73 mg phytate/person/day. This level is approximately 3-fold less than the estimated exposure to phytate from soybean, and therefore the levels of phytate present in the lupin ingredients are anticipated not to produce any adverse effects on human health.

Saponins are considered to be ANFs because they can lyse red blood cells (RBCs). Saponins occur naturally in *L. albus* at negligible levels and in *L. angustifolia* at levels of 480 to 730 ppm (Petterson, 1998). These compounds also are present in soybeans at levels in the range of 1 to 5 mg/g dry weight (Anderson and Wolf, 1995; Wang and Wixon, 1999). Saponins have been consumed for many years as part of the human diet without reports of ill effects. Additionally, saponins are poorly absorbed and are considered to be of low oral toxicity (Price *et al.*, 1987; Wang and Wixon, 1999). Moreover, no adverse effects were reported in chicks, rats, or mice fed concentrations of saponins from soy that were 3- to 5-fold greater than a typical soybean meal diet (Ishaaya *et al.*, 1969). Following batch analysis, the levels of saponins in the sweet lupin protein fraction ingredients were determined to range up to 0.065% (dry solid basis), the highest level of which was identified in sweet lupin protein fraction 2, and which would provide an exposure of ~15 mg saponins/person/day. This level is at least 7-fold less than the background levels of saponin reported in *L. angustifolia*.

Although lectin has been reported to be present naturally in lupin, lectin activity was not detected in either *L. angustifolia* or *L. albus* following conventional agglutination assay procedures using a wide variety of red blood cell types (Petterson, 1998). Similarly, batch analysis by GWF indicated that there was no lectin activity following agglutination assays with both sheep and horse red blood cells, and therefore, the potential presence of lectin in

the sweet lupin protein fraction ingredients is anticipated not to present any concerns on human health.

Potential Allergenicity

Lupin allergy has recently been recognized with documented cases of anaphylaxis and other allergic reactions following lupin consumption (Hefle *et al.*, 1994; Matheu *et al.*, 1999; Novembre *et al.*, 1999; Moneret-Vautrin *et al.*, 2004; Smith *et al.*, 2004; Radcliffe *et al.*, 2005; Rotiroti *et al.*, 2007; Wassenberg and Hofer, 2007). Lupin has recently been added to the list of commonly allergenic foods in the European Union but does not have such status in other parts of the world. Additionally, cross-reactivity between lupin protein allergens and allergens in peanuts has been reported (Hefle *et al.*, 1994; Moneret-Vautrin *et al.*, 1999; Kanny *et al.*, 2000; Fæste *et al.*, 2004; Wüthrich *et al.*, 2004; Costa *et al.*, 2005; Magni *et al.*, 2005a,b; Peeters *et al.,* 2007), although such cross-reactions only occur in a fraction (perhaps as high as 20%) of peanut-allergic individuals. Furthermore, lupin allergy can occur independent of peanut allergy (Peeters *et al.,* 2007). As expected, the results of *in vitro* studies using GWF's ingredients indicate that not all peanut-allergic individuals would be sensitive to these lupin-based ingredients (Nordlee, unpublished, 2004). The sweet lupin protein fractions contain between 70 and 95% protein and are potentially allergenic, although thermal processing of lupin has been reported to decrease the allergenic potential of lupin-derived ingredients (Álvarez-Álvarez *et al.,* 2005).

Under the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA), if a packaged food product contains, or contains any ingredients derived from, one of the eight major allergenic foods, namely milk, eggs, fish, crustacean shellfish, tree nuts, wheat, peanuts, and soybeans, the presence of the allergenic ingredient must be identified in plain English in the list of ingredients or it should be stated adjacent to the list of ingredients that the product contains the allergenic ingredient. Although lupin is not one of the eight major allergenic foods, there have been documented cases of allergic responses to the consumption of lupin. Therefore, GWF will take steps to ensure that the presence of lupin is identified on all products that contain the sweet lupin protein ingredients in order to notify consumers and to attempt to prevent exposure in sensitive populations. In order to ensure that lupin is identified on all end product labels for products containing sweet lupin protein fractions 1 or 2 either as direct ingredients or as incidental additives, GWF will indicate on the specification sheets for the sweet lupin protein fractions that the presence of lupin should be disclosed either to the food additive manufacturers' customers and/or to the end product manufacturer so that lupin will be included on the end product label. Under FALCPA there are no labeling requirements for food ingredients that may elicit responses in individuals who are allergic to one of the eight major food allergens (*i.e.*, there is no requirement for the labeling of the cross-reactive ingredients). As there are currently no requirements for the labeling of ingredients that may be cross-reactive, GWF intends to ensure that lupin is included on the end product label for all products containing the sweet lupin protein ingredients either as direct ingredients or as incidental additives rather than include a statement regarding cross-reactivity. It is therefore expected that the labeling of foods to

which the sweet lupin protein ingredients are added should alert the lupin-allergic consumer to the presence of lupin.

G. Summary and Basis for GRAS Conclusion

The results of the identified animal and human studies of whole sweet lupin seed and lupin fractions have been determined by GWF not to indicate any potential for adverse effects in humans following consumption of the sweet lupin protein ingredients under the intended conditions of use. Sweet lupin protein fractions 1 and 2 are composed mainly of protein, with lower levels of fiber, fat, and other carbohydrates, and all macronutrient components of the sweet lupin protein ingredients have a history of consumption as part of a normal diet, with estimated intakes of protein from the intended conditions of use that are within range of the background consumption of protein from various dietary sources. Therefore, following a critical evaluation of scientific data generally available in the public domain that pertain to the safety of lupin and sweet lupin-derived ingredients, including sweet lupin protein fractions 1 and 2, under the intended conditions of use, and derivation of a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of ingredients as components of food that sweet lupin protein fractions 1 and 2 would be safe and suitable for use under the proposed conditions and also would be generally recognized as such by other experts, GWF has concluded that the sweet lupin protein fractions are GRAS under the intended conditions of use on the basis of scientific procedures.

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APPENDIX A

APPENDIX A

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SWEET LUPIN-DERIVED INGREDIENTS FOR USE IN FOODS

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF SWEET LUPIN-DERIVED INGREDIENTS FOR USE IN FOODS

September 16, 2008

INTRODUCTION

At the request of George Weston Foods Limited (GWF), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information relevant to the safety of lupin and sweet lupin-derived ingredients , and determine whether the intended use as food ingredients of 6 ingredients derived from sweet varieties of *Lupinus* spp. (lupin), including lupin flour, 2 lupin protein fractions, and 3 lupin fiber products, are safe and suitable and would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Ashley S. Roberts, Ph.D. (Cantox Health Sciences International), and Prof. Stephen L. Taylor, Ph.D. (University of Nebraska). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data pertaining to the safety of lupin and sweet lupin-derived ingredients compiled from the literature and other published sources through July 2007 by Cantox Health Sciences International. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Weston Technologies, a division of GWF. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of sweet lupin and sweet lupin fractions.

Following independent, critical evaluation of such data and information, the Panel convened on 15 September 2005 and unanimously concluded that the intended uses in traditional foods described herein of ingredients derived from sweet lupin, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. In August of 2007, the Panel evaluated additional data made publicly available since their initial meeting, and in March of 2008 they reviewed an amendment to the initially proposed food uses and use-levels. Subsequently, the Panel reaffirmed their consensus of the safety and suitability and the GRAS status of the intended uses of GWF's sweet lupin-derived ingredients. A

summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

SUMMARY AND BASIS FOR GRAS

GWF intends to market ingredients derived from sweet lupin as food ingredients in various traditional food products such as bakery products, breakfast cereals, and beverages, in the United States. There are 4 species with sweet lupin varieties, namely *L. angustifolia*, *L. albus*, *L. luteus* and *L. mutabilis*, which are 'sweet' due to their low alkaloid content (Petterson, 1998). Lupin and ingredients derived thereof have been consumed by humans and livestock for over 2,000 years, and various species and varieties of lupin have been historically cultivated in the Mediterranean region, northern Europe, South Africa, Australia, and New Zealand, and more recently in the south-eastern United States (Gladstones, 1970; IPK Gatersleben, 2002). Moreover, lupin-derived ingredients are permitted for use in food for human consumption in the European Union and Australia/New Zealand (Allen, 1992; Weston Technologies, personal communication, 2005). Despite the documented historical consumption of lupin, quantitative consumption data has not been identified.

The sweet lupin ingredients are manufactured in accordance with cGMP, and include Sweet Lupin Flour, which is produced by Weston Milling, and Sweet Lupin Protein Fractions 1 and 2, Sweet Lupin Kernel Fibers 1 and 2, and Sweet Lupin Hull Fiber, which are produced by GWF. Essentially, the 6 lupin ingredients are derived from the whole seed of sweet lupin. Sweet Lupin Flour and Sweet Lupin Hull Fiber are obtained by dehulling and milling/grinding the whole lupin seeds. The protein and kernel fiber fractions require further processing to yield the final ingredients. In order to ensure consistent products, GWF has established numerous chemical and microbiological specification parameters for the final preparations, and batch samples are routinely assayed to verify that the specifications are met, ensuring a safe and consistent product. The sweet lupin-derived flour, protein, and fiber ingredients produced by GWF are intended to replace a portion of other sources of flour, protein, and fiber, and due to the self-limiting properties of the ingredients, such as viscosity and baking properties and/or sensory characteristics, the levels of substitution of the flour will be in the range of 10 to 25%, and the levels of use of the protein and fiber ingredients will be up to 20% (see Attachment 2). The ingredients are stable when stored at room temperature (approximately 25°C) in a dry environment, with a shelf life of 6 months.

The consumption of each sweet lupin-derived ingredient from all proposed food uses was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2008), which provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States. Under the conditions of intended use, the total population all-user mean and 90th percentile intake of Sweet Lupin Flour was estimated to be 39.8 g/person/day (0.7 g/kg body weight/day) and 75.3 g/person/day (1.5 g/kg body weight/day), respectively. The protein fractions contain the same amount of protein (70 to 95%), and of the 2 lupin protein ingredients, Sweet Lupin Protein Fraction 1 has the highest estimated intake, with mean and 90th percentile total population all-user intakes of 18.3 g/person/day (0.3 g/kg body

weight/day) and 35.3 g/ person/day (0.7 g/kg body weight/day), respectively. Sweet Lupin Kernel Fibers 1 and 2 are intended to be used in the same food categories and at the same levels and were estimated to have a total population all-user mean intake of 35.3 g/person/ day (0.6 g/kg body weight/day), and an estimated 90th percentile all-user intake of 64.9 g/ person/day (1.2 g/kg body weight/day), from all proposed food-uses. The total population all-user mean and 90th percentile intakes of Sweet Lupin Hull Fiber were estimated to be 14.9 g/person/day (0.3 g/kg body weight/day) and 28.1 g/person/day (0.5 g/kg body weight/day), respectively, under the intended conditions of use.

The sweet lupin-derived ingredients are composed mainly of varying levels of protein and fiber, with lower levels of fat and carbohydrate, all of which have a long history of consumption as part of a normal diet (Harwood, 1991; IOM, 2002a,b; USDA, 2005a,b,c), and hence, are expected to undergo normal metabolism. Consumption of wheat flour in the United States was reported to be 165 g/person/day (Wheat Foods Council, 2005), and the total population U.S. mean and 90th percentile intakes of protein and fiber were reported to be 75.2 and 114.0 g protein, respectively, and 15.1 and 24.7 g fiber, respectively (IOM, 2002a,b). Background consumption of the major macronutrients of the sweet lupin ingredients from various dietary sources are within range of those estimated from the intended conditions of use of each of Sweet Lupin Flour, Sweet Lupin Protein Fractions 1 and 2, Sweet Lupin Kernel Fibers 1 and 2, and Sweet Lupin Hull Fiber.

Some of the ingredients may be utilized in the same food categories; therefore, for completeness of the data, an all-user intake of sweet lupin based on all intended food-uses of all of GWF's sweet lupin-derived ingredients also was estimated, providing total population all-user intake mean and 90th percentile level estimates of 92.6 and 158.5 g/day, respectively. The method used to calculate the daily dietary intakes under the intended conditions of use is considered to be 'worst case', as it incorporates several conservative assumptions, such as the assumption that all of the ingredients will be used in all of the food use categories at the highest level of use at the same time, which is highly unlikely, and therefore, the total population all-user estimated daily intakes are considered to be gross-overestimates and it is expected that the actual exposure to lupin from all of the sweet lupin-derived ingredients will be much less.

The safety assessment of lupin and sweet lupin-derived ingredients is based on the known metabolism of the macro-components of lupin, several short- and long-term preclinical toxicity studies and nutritional studies supporting the tolerability of these ingredients, as well as several human studies investigating the effect of sweet lupin-derived ingredients on parameters such as safety, glycemic and insulinemic response, bowel function, and palatability, which demonstrated that lupin was well tolerated.

Subchronic toxicity studies in rats using lupin seed and seed fractions ranged from 10 days to 13 weeks in duration and dietary administration of lupin-derived ingredients at doses of 9.7 to 57 g/kg body weight/day resulted in few significant effects on physical, biochemical, and hematological parameters (Fudiyansyah *et al.*, 1995; Butler *et al.*, 1996; Rahman *et al.*, 1996a,b, 1997a,b; Rahman, 2000; Caligari *et al.*, 2006; Pilvi *et al.*, 2006). Observed effects,

such as decreased body weight gains, increased urea, and decreased albumin, were suggested by study authors to be due to a disturbance of normal protein utilization, which could have resulted from the amino acid deficiency of diets containing lupin seed or lupin fractions, as lupin is known to contain low levels of the essential amino acids, lysine and methionine. The reported increases in plasma urea remained within reported historical control values for rats (Sharp and LaRegina, 1998) and similar effects on body weight gains were not observed in studies in which rats were provided adequate amino acid-supplemented diets. No significant differences in biochemical or hematological parameters, organ weights, or histopathology were reported in rats provided up to 33 g lupin flour/kg body weight/day for a period of 90 days (Butler *et al.*, 1996). Reported increases in serum alkaline phosphatase values in lupin-treated rats were suggested by the study authors to be a result of liver necrosis; however, there were no significant differences in the alanine aminotransferase or aspartate aminotransferase levels in the lupin-treated animals compared to the control group (Rahman *et al.*, 1996a), and liver lesions were not reported in any of the other reviewed dietary studies. A toxicity study in which lupin protein extracted from the seeds of *L. albus* was administered by gavage to male Sprague-Dawley rats for a period of 2 weeks at a dose of 250 mg/kg body weight/day did not result in any adverse effects (Sirtori *et al.*, 2004).

Chronic studies in rats using *L. angustifolia* seed and seed fractions ranging from 700 to 800 days in duration were identified in the available literature. Consumption of up to 13.6 g whole *L. angustifolia* seed/kg body weight/day was reported not to cause any adverse effects in rats when administered in the diet for up to 800 days (Grant *et al.*, 1993), although levels of whole lupin seed in the diet were reported to decrease body weight in rats dosed for up to 700 days (Grant *et al.*, 1995). Additionally, cecum and colon weights were significantly increased in rats after 700 days of feeding; however, the authors attributed this effect to volatile fatty acid production due to dietary fiber digestion in these organs (Grant *et al.*, 1995). Dietary administration of lupin protein from *L. albus* and *L. luteus* to rats at levels of 6.3 and 6.88 g/kg body weight (20% of the diet), respectively, for a period of 112 days did not result in any changes in body weights, organ weights, or gross pathology (Ballester *et al.*, 1980), and 20% lupin protein (isolated from *L. albus*) administered in the diet to 3 generations of rats for 270 days each did not result in adverse effects on either fertility or reproductive parameters in any of the three generations (Ballester *et al.*, 1982, 1984).

Sweet lupin seeds (*L. angustifolia* and *L. albus*) are widely used in Australia as a source of protein and energy in livestock feeds, as they are cost-competitive with a number of other protein sources (Edwards and van Barneveld, 1998). These 2 species may be used with equal success in all livestock with the exception of pigs, where *L. albus* is not recommended for use in feed due to reduced feed intake and depressed growth rates (Edwards and van Barneveld, 1998). Pigs and poultry (monogastrics) require specific levels of individual amino acids in their diets (Edwards and van Barneveld, 1998), and due to low levels of both methionine and lysine in sweet lupins, feeds for pigs and poultry are more beneficial when they include multiple sources of protein or supplemental amino acids (Edwards and van Barneveld, 1998). Additionally, non-starch polysaccharides (NSP) have been suggested to

interfere with digestive enzymes in these monogastric species, causing a large difference between net energy content and digestible energy content (Edwards and van Barneveld, 1998). Equivocal results on feed consumption and body weights have been reported in nutritional studies ranging from 5 to 90 days in pigs and chickens consuming lupin seed or kernel, or lupin protein fractions at dietary levels providing doses of 2.3 to 1,540 g/kg body weight/day (King, 1981; Godfrey *et al.*, 1985; Dunshea *et al.*, 2001; Olkowski *et al.*, 2001, 2005; Brenes *et al.*, 2002; Rubio *et al.*, 2003; Steenfeldt *et al.*, 2003; Hammershoj and Steenfeldt, 2005; Martins *et al.*, 2005; Mieczkowska *et al.*, 2005; Prandini *et al.*, 2005; Bielecka *et al.,* 2006; Orda *et al.*, 2006; Suchy *et al.*, 2006; Zraly *et al.*, 2006, 2007), and up to 210 days in cows (dose not provided) (Krapivina and Vashchekin, 2006); however, overall, lupin-containing feeds were generally well-tolerated and without adverse effects. Dehulled lupin seeds are now used in pig feeds because dehulling is reported to greatly improve gross energy digestibility (Wigan *et al.*, 1994), and dehulling also was reported to be beneficial for poultry (Brenes *et al.*, 1993). Studies evaluating the potential effect of 9.4 to 10.25 g lupin/kg body weight/day on the reproductive performance of cows (Axelsen, 1980) indicated no adverse effects, and a lack of effect on ovulation in ewes was reported by Pearse *et al.* (1991).

Conflicting results were reported upon investigation of the potential effect of lupin seed and seed fractions on mineral absorption (Rubio *et al.*, 1994; Rahman *et al.*, 1997b; Olkowski *et al.*, 2005; Zraly *et al.*, 2006, 2007). Rubio *et al.* (1994) reported that dialyzed soluble and insoluble aqueous lupin protein fractions had no significant effect on absorption of calcium, phosphorus, or zinc in rats, while whole lupin seed and the dialyzed residue fraction (*i.e.,* the fibrous material that is insoluble in both water and buffer) significantly reduced phosphorus and zinc absorption due to the presence of phytate and insoluble NSP fractions which typically are not present in protein fractions. Conversely, Rahman *et al.* (1997b) reported that slightly higher doses of various aqueous lupin protein fractions, including dialyzed soluble and insoluble fractions, significantly reduced absorption of phosphorus, zinc, magnesium, and sodium in rats (with no effects on calcium or potassium); however, these effects were not discussed by the authors. Olkowski *et al.* (2005) reported that diets containing raw or dehulled lupin (*L. albus, L. luteus,* or *L. angustifolius*) seed meal did not affect plasma zinc levels in broiler chicks but significantly reduced plasma calcium levels, and Zraly *et al.* (2006) reported that dehulled lupin (*L. angustifolius*) seed meal in the diets of pigs resulted in significant decreases in plasma calcium but no changes in plasma phosphorus, whereas *L. albus* seed in the diet of pigs did not have any significant effect on plasma calcium or phosphorus levels (Zraly *et al.*, 2007).

The results of nutritional studies in humans using ingredients derived from *L. angustifolia* indicated that the lupin ingredients were well tolerated in healthy volunteers (Petterson *et al.*, 1994; Johnson *et al.*, 2003; Archer *et al.*, 2004; Hall and Johnson, 2004; Hall *et al.*, 2005a,b; Johnson *et al.*, 2006; Lee *et al.*, 2006; Naruszewicz *et al.*, 2006; Smith *et al.*, 2006; Joray *et al.*, 2007). The identified studies ranged in length from a single dose to 28 days and included daily doses of 9 to 37.4 g kernel fiber and 7.7 to 264 g lupin flour derived from *L. angustifolia*. A dose of 35 g lupin flour from *L. albus* provided to young adults for a period

of 60 days was well tolerated and was without compound-related changes in biochemical or hematological parameters (Gattás Zaror *et al.*, 1990). A daily intake of 28 to 56 g of lupin protein from lupin flour also was reported to be well tolerated in 9 healthy men for a period of 10 days, with the only significant change being an increase in urea nitrogen in the high-dose group compared to the low-dose group (Egaña *et al.*, 1992). Consumption of 16.75 g of *L. albus*-derived lupin protein/day by otherwise healthy, chronically-smoking, volunteers with moderate hypercholesterolemia for a period of 90 days was well tolerated and resulted in significant reductions in total cholesterol, low-density lipoprotein cholesterol, blood glucose, homocysteine, high sensitivity C-reactive protein, urinary F_2-isoprostane/creatinine excretion, systolic blood pressure, and diastolic blood pressure (Naruszewicz *et al.*, 2006). Some of the doses used in these studies exceed the estimated intakes for Lupin Flour, Lupin Protein Fractions 1 and 2, Lupin Kernel Fibers 1 and 2, and Lupin Hull Fiber. There are no indications from the published literature that the intended uses of sweet lupin-derived ingredients would result in any adverse health effects in humans.

High levels of dietary alkaloids, natural toxicants in plants, may be toxic (Bradbury *et al.*, 2004). Although some species of lupin can contain 'high' levels (2 to 3%) of alkaloids (bitter lupins) (Reinhard *et al.*, 2006), the sweet varieties have low alkaloid levels (typically <0.02%), making them suitable for consumption by humans and livestock (Petterson, 1998). The Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom (ACNFP, 1996) established a limit for alkaloid levels in lupin seed fit for human consumption of 200 mg alkaloids/kg seed. Additionally, a tolerable level of exposure to lupin alkaloids for humans of 35 µg/kg body weight/day was tentatively established by Australia New Zealand Food Authority (ANZFA) (2001a). The lupin ingredients produced by GWF comply with the maximal alkaloid level set forth by ACNFP, as evidenced by the product specifications and batch data analyses, and hence the levels of alkaloids present in the sweet lupin-derived ingredients are expected not to produce any adverse effects on human health. The presence of other phyto-components in the ingredients of GWF, specifically oligosaccharides, phenols and condensed tannins, trypsin inhibitors, saponins, phytic acid, and lectins, are negligible and are not anticipated to impact the safety of the sweet lupin-derived ingredients. Lupinosis, which can occur as a result of the ingestion of lupin plants contaminated with phomopsins, which are toxins produced by fungi, has been reported in livestock that grazed on infected plants (Allen, 1986; Morcombe *et al.*, 1992; ANZFA, 2001b), although cases of lupinosis in humans have not been identified (Lowen *et al.*, 1995). Analysis of GWF's sweet lupin-derived ingredients indicated compliance with maximum tolerable levels of 5 µg/kg final product, as set forth by Food Standards Australia New Zealand (FSANZ) (ANZFA, 2001b), and therefore, there is no concern for the potential occurrence of lupinosis in consumers of products containing these ingredients.

Lupin allergy has recently been recognized with documented cases of anaphylaxis and other allergic reactions following lupin consumption (Hefle *et al.*, 1994; Matheu *et al.*, 1999; Novembre *et al.*, 1999; Moneret-Vautrin *et al.*, 2004; Smith *et al.*, 2004; Radcliffe *et al.*, 2005; Rotiroti *et al.*, 2007; Wassenberg and Hofer, 2007). Lupin has recently been added to the list of commonly allergenic foods in the European Union but does not have such status in

other parts of the world. Additionally, cross-reactivity between lupin protein allergens and allergens in peanuts has been reported (Hefle *et al.*, 1994; Moneret-Vautrin *et al.*, 1999; Kanny *et al.*, 2000; Fæste *et al.*, 2004; Wüthrich *et al.*, 2004; Costa *et al.*, 2005; Peeters *et al.*, 2007), although such cross-reactions only occur in a fraction (perhaps as high as 20%) of peanut-allergic individuals. Furthermore, lupin allergy can occur independent of peanut allergy (Peeters *et al.,* 2007). As expected, the results of *in vitro* studies using GWF's ingredients indicate that not all peanut-allergic individuals would be sensitive to these lupin-based ingredients (Nordlee, unpublished, 2004). While the levels of protein in these different lupin-based ingredients is variable, each of the ingredients is potentially allergenic, and therefore, GWF will take steps to ensure that the presence of lupin is identified on all products that contain their sweet lupin-derived ingredients in order to notify consumers and to attempt to prevent exposure in sensitive populations. As there are currently no requirements for the labeling of ingredients that may be cross-reactive, GWF intends to ensure that lupin is included on the end product label for all products containing the sweet lupin-derived ingredients either as direct ingredients or as incidental additives rather than include a statement regarding cross-reactivity. It should therefore be expected that the labeling of foods to which the sweet lupin-derived ingredients are added should alert the lupin-allergic consumer to the presence of lupin.

The results of pre-clinical and human studies of whole sweet lupin seed and lupin fractions do not indicate any potential for adverse effects in humans consuming these ingredients under the intended conditions of use. A critical evaluation of the data and information summarized in this report supports the safety and suitability and the GRAS status based on scientific procedures of the intended uses of Sweet Lupin Flour, Sweet Lupin Protein Fractions 1 and 2, Sweet Lupin Kernel Fibers 1 and 2, and Sweet Lupin Hull Fiber meeting appropriate food-grade specifications and manufactured consistent with cGMP.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses in traditional foods of ingredients derived from sweet lupin, meeting appropriate food-grade specifications presented herein and produced consistent with current Good Manufacturing Practices (GMP), are safe and suitable.

We further conclude that the intended uses in traditional foods of ingredients derived from sweet lupin, meeting appropriate food-grade specifications presented herein and produced consistent with current GMP, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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APPENDIX A
ATTACHMENT 1

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APPENDIX A
ATTACHMENT 2

ATTACHMENT 2

INTENDED FOOD USES AND USE LEVELS OF SWEET LUPIN-DERIVED INGREDIENTS

Table A-2 Intended Food Uses and Use Levels of Sweet Lupin-Derived Ingredients

Food Category	Proposed Food-Use	Sweet Lupin Ingredient	Maximum Use Level
Baked Goods and Baking Mixes	Bagels; Biscuits; Cakes; Cookies; Cornbread, Corn Muffins, and Tortillas; Crackers; Croissants; English Muffins; French Toast, Pancakes, Waffles, and Crepes; Muffins and Popovers; Pastries; Pies; Quick Breads and Sweet Rolls; Soft Bread Sticks; Soft Pretzels; and Yeast Breads and Rolls	Flour	25%
		Protein Fraction 1 & 2, Kernel Fibers 1 & 2, Hull Fiber	10%
Grains Products and Pastas	Macaroni and Noodle Products	Flour	25%
Dairy Product Analogs	Condensed Milk Analogs[a]; Milk Powder Analogs[a]	Protein Fraction 1	15%
	High Fat Powder; Imitation Milk; Soy Milk Alternatives	Protein Fraction 1	4%
	Imitation Cheese	Protein Fraction 1	20%
	Non-dairy Cream Substitutes and Coffee Whiteners	Protein Fraction 2	3%
Frozen Dairy Desserts and Mixes	Ice Cream	Protein Fraction 1	20%
Gelatins, Puddings, and Fillings	Mousses and Meringues	Protein Fraction 2	3%
Jams and Jellies	Spreadable Jelly	Protein Fraction 1 & 2	3%
		Kernel Fibers 1 & 2	2%
Meat Products	Commercially Processed Meats and Sandwich Ingredients	Protein Fraction 1	3%
Milk Products	Fermented Milk Beverages; Flavored Milk and Milk Drinks; Milk-Based Meal Replacements	Protein Fraction 1	5%
Soft Candy	Boiled Sweets	Protein Fraction 1	2%
		Protein Fraction 2, Kernel Fibers 1 & 2	1%
	Chocolate, Compound Chocolate	Protein Fraction 1 & 2, Kernel Fibers 1 & 2	3%
	Soft and Firm Jellies	Protein Fraction 1 & 2	3%
		Kernel Fibers 1 & 2	2%
Beverages and Beverage Bases	Carbonated Beverages	Kernel Fibers 1 & 2	3%
	Energy, Sports and Isotonic Drinks	Kernel Fibers 1 & 2	10%
Breakfast Cereals	Instant and Regular Hot Cereals; Ready-to-Eat Breakfast Cereals	Kernel Fibers 1 & 2	20%
		Hull Fiber	10%
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks	Kernel Fibers 1 & 2	3%

[a] No food codes were found for these categories, thus surrogate codes were chosen to represent the category. Analogues of these types were not found thus milk codes were used.

APPENDIX B

APPENDIX B

SUMMARY OF ANALYTICAL DATA, CERTIFICATES OF ANALYSIS, AND OTHER COMPOSITIONAL INFORMATION

APPENDIX B-1

APPENDIX B-1

SUMMARY OF THE ALKALOIDS IDENTIFIED IN *LUPINUS* SPP.

SUMMARY OF THE ALKALOIDS IDENTIFIED IN *LUPINUS* SPP.

High levels of dietary alkaloids, natural constituents in plants, may be toxic. Although some species of lupin can contain 'high' levels (2 to 3%) of alkaloids (bitter lupins), the sweet varieties have low alkaloid levels (~0.001 to 0.002%), making them suitable for consumption by humans and livestock. Alkaloids present in *L. angustifolia* comprise mainly lupanine (42 to 59%), 13-hydroxylupanine (24 to 45%), and angustifoline (7 to 15%) (Wink *et al.*, 1995), which are derivatives of quinolizidine (Petterson, 1998). *L. albus* contains <100 mg alkaloids/kg whole seed, the majority of which is lupanine (~70%), followed by albine (~15%) and lesser amounts of 13-hydroxylupanine, sparteine, and multiflorine (Petterson and Mackintosh, 1994; Zdunczyk *et al.*, 1994; Wink *et al.*, 1995). The alkaloid profile of *L. luteus* is composed almost entirely of lupanine (60%) and sparteine (~30%) (Wink *et al.*, 1995), while *L. mutabilis* contains several different alkaloids, the main ones being lupanine, sparteine, and 13-hydroxylupanine (Petterson, 1998).

Following a review of the literature, a limit for alkaloid levels in lupin seed fit for human consumption of 200 ppm was set by the Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom (ACNFP, 1996). Additionally, a tolerable level of exposure to lupin alkaloids for humans of 35 μg/kg body weight/day has tentatively been established by the Australia/New Zealand Food Authority (ANZFA) (ANZFA, 2001). The sweet lupin protein fractions produced by GWF comply with the maximal alkaloid level of <200 ppm set forth by the ACNFP, as evidenced by the product specifications and batch data analyses, and hence the levels of alkaloids present in the sweet lupin protein fractions are expected not to produce any adverse effects on human health.

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000115

APPENDIX B-2

BATCH ANALYSES OF SWEET LUPIN PROTEIN FRACTIONS

BATCH ANALYSES OF SWEET LUPIN PROTEIN FRACTIONS 1 AND 2

In order to develop the commercial manufacturing process for the protein ingredients, sample batches of sweet lupin protein fractions were manufactured on pilot-scale equipment. Several lots of the products were analysed to verify that the pilot process produced consistent products within the physical and chemical parameters of each of the product specifications. Summaries of the chemical analyses of batches of sweet lupin protein fractions 1 and 2 are presented in Tables B-2-1 and B-2-2.

Table B-2-1 Summary of the Chemical Analyses of Batches of Sweet Lupin Protein Fraction 1

Specification Parameter	Specification	Manufacturing Sample and/or Date					
		1087	1233	1621	1816	1835	1924
Protein (% DSB)	75 to 95	81.4	78.9	85.0	82.7	82.2	83.4
Fat (% DSB)	1 to 22	18.3	22.0	19.6	21.9	21.0	21.7
Total Carbohydrate (%)	0 to 5	<0.1	N/A	N/A	N/A	N/A	<0.1
Insoluble Dietary Fibre (% DSB)	0 to 10	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Soluble Dietary Fibre (% DSB)	0 to 3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Moisture (%)	1 to 6	4.3	1.3	3.6	1.7	1.9	4.2
Ash (% DSB)	1 to 3	1.7	1.6	1.3	1.2	1.2	1.5
		No. 1037 (10/03/05)	1037 (03/03/08)		1736 (03/03/08)	2340 (03/03/08)	
Alkaloids (ppm)[a]*	<200	20	1		1	4	
Cadmium (ppm)[b]	<0.1	0.06*	N/A		N/A	N/A	
Lead (ppm)[b]	<0.2	0.14*	N/A		N/A	N/A	

* = Measured as received
DSB = Dry solid basis; N/A = Not available
[a] Total alkaloids were measured by the Department of Industry and Resources Chemistry Centre [Western Australia (WA)] using Gas Chromatography/Mass Spectrometry
[b] Measured by the Department of Industry and Resources Chemistry Centre (WA) using Inductively coupled plasma mass spectrometry (ICP-MS) Method IEL1STIM
See Attachment B-2-1 for certificates of analyses.

Table B.2-2 Summary of the Chemical Analyses of Batches of Sweet Lupin Protein Fraction 2

Specification Parameter	Specification	Manufacturing Sample (Date)		
		1034 (09/03/05)	1086 (17/03/05)	1728 (21/06/06)
Protein (% DSB)	70 to 95	77.7	90.0	92.2
Fat (% DSB)	0 to 5	4.4	0.4	1.1
Total Carbohydrate (%)	0 to 6	N/A	<0.1	N/A
Insoluble Dietary Fibre (% DSB)	0 to 3	1.8	<0.1	<0.1
Soluble Dietary Fibre (% DSB)	4 to 15	14.1	7.6	4.8
Moisture (%)	2 to 8	6.0	7.8	7.5
Ash (% DSB)	1 to 4	1.7	3.4	1.8
		No. 1046 (11/03/05)	1813 (19/07/06)	2331 (27/06/07)
Alkaloids (ppm)[a]	<200	30* 30**	5*	2*
Cadmium (ppm)[b]	<0.1	0.06*	N/A	N/A
Lead (ppm)[b]	<0.2	0.14*	N/A	N/A

• = Measured as received; ** = Measured on a dry solid basis
DSB = Dry solid basis; N/A = Not available
[a] Total alkaloids were measured by the Department of Industry and Resources Chemistry Centre [Western Australia (WA)] using Gas Chromatography/Mass Spectrometry
[b] Measured by the Department of Industry and Resources Chemistry Centre (WA) using Inductively coupled plasma mass spectrometry (ICP-MS) Method IEL1STIM
See Attachment B-2-1 for certificates of analyses.

Microbiological parameters were not assessed for any of the pilot batches, as GWF currently are not able to simulate the conditions that will be used in the commercial production with respect to microbiological contamination. This is due to the fact that the trials are conducted with each unit operation separated from the next unit of operation of the process and, instead of piping the material to the next unit of operation, it is manually transferred in buckets. The limited scale of the facility means that the material is held overnight or in buckets prior to the next operation rather than undergoing a continuous process. The time constraints using an off-site pilot plant have meant that, although the plant is cleaned, the required CIP (Clean-In-Place) procedure is not performed during the pilot process. In commercial-scale production, a Hazard Analysis Critical Control Point (HACCP) plan will be followed to ensure that the products comply with the product specifications. The HACCP plan will include the points in the process that are Quality Control Points and Critical Control Points to ensure conformity to physical, chemical, and microbiological parameters of each ingredient. The Critical Control Points ensure that when the process is maintained within specified manufacturing conditions at each point, the end products will be within the established product specifications. Material that is outside of the product specifications will be downgraded for use in livestock feed or discarded.

ATTACHMENT B-2-1

000119

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Kensington Vic 3031
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PO Box 81
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Phone (03) 8371 7600 Fax (03) 9372 2013

LABORATORY REPORT
on
MISCELLANEOUS POWDER

Date: 03/06/05

Our ref: 24MAR05/50176/MISCP

FOR: GEORGE WESTON FOODS LTD.
(WESTON TECHNOLOGIES)
PO BOX 1
ENFIELD NSW 2136

SHERRY DUCKWORTH

Account: W20

COPY:

SAMPLE DETAILS:
Date Received - 24/03/05
Origin - -
Code/Ref. - 1087 DISCHARGE 6.5(H)
Order number -
No.of samples - 1
Package Type -

REPORTS:
STD

TEST	RESULTS		DTS METHOD No.
MOISTURE %m/m	4·3		MOIS 01 12.99
FAT %m/m	17·5		FATS 07 12.99
PROTEIN (TN x 6.25) %m/m	77·9		PROT 01 02.01
ASH %m/m @ 550°C	1·6		ASHS 04 04.93
TOTAL CARBOHYDRATE % m/m	<0·1	BY DIFFERENCE	TCAR 01 07.93
DIETARY FIBRE % (INSOLUBLE)	<0·1		DIET 02 03.93
DIETARY FIBRE % (SOLUBLE)	<0·1		DIET 02 03.93

S. Nolan
Technical Manager

000120

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Flemington Vic 3031
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LABORATORY REPORT
on
MISCELLANEOUS POWDER

Date: 18/07/05

Our ref: 24JUN05/79771/MISCP

FOR: GEORGE WESTON FOODS LTD.
(WESTON TECHNOLOGIES)
PO BOX 1
ENFIELD NSW 2136

SHERRY DUCKWORTH

Account: W20

COPY:

SAMPLE DETAILS:
Date Received - 24/06/05
Origin - -
Code/Ref. - 1233 DISCHARGE + HOM
Order number -
No.of samples - 1
Package Type -

REPORTS:
STD

TEST	RESULTS	DTS METHOD No.
MOISTURE %m/m	1·3	MOIS 01 12.99
FAT %m/m	21·7	FATS 07 12.99
PROTEIN (TN x 6.25) %m/m	77·9	PROT 01 02.01
ASH %m/m @ 550°C	1·6	ASHS 04 04.93
DIETARY FIBRE % (INSOLUBLE)	<0·1	DIET 02 03.93
DIETARY FIBRE % (SOLUBLE)	<0·1	DIET 02 03.93

S. Nolan
Technical Manager

000121

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Postal Address:
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Fax: (03) 9372 2012

LABORATORY REPORT on MISCELLANEOUS POWDER

Date: 21/03/2006
Our Ref: DTS0604983
Report No: 22909 Final

FOR: GEORGE WESTON FOODS LTD

Sherry Duckworth

P O Box 1
ENFIELD NSW 2136

Date received: 6/03/2006
Origin:
Code/Ref: 1621 DISCHARGE 6.5

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD
06MAR06/133814		
Moisture	3.6 % m/m	MOIS 01 12.99
Fat	18.9 % m/m	FATS 07 12.99
Protein (TN x 6.25)	81.9 % m/m	PROT 01 02.01
Non Protein Nitrogen	0.061 % m/m	NITF 01 03.03
Dietary Fibre - Soluble	<0.1 % m/m	DIET 02 03.93
Dietary Fibre - Insoluble	<0.1 % m/m	DIET 02 03.93

Shane Nolan
Technical Manager

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LABORATORY REPORT on SOY PRODUCTS

Date: 27/03/2006
Our Ref: DTS0606212
Report No: 23294 Final

FOR: GEORGE WESTON FOODS LTD

Sherry Duckworth

P O Box 1
ENFIELD NSW 2136

Date received: 23/03/2006
Origin:
Code/Ref: 1621 DISCHARGE 6.5

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD
23MAR06/144929		
Ash @ 550°C	1.3 % m/m	ASHS 04 04.93

ADDITIONAL TESTING ON DTS0604983

Shane Nolan
Technical Manager

DTS FOOD LABORATORIES

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LABORATORY REPORT on MISCELLANEOUS POWDER

Date: 10/08/2006
Our Ref: DTS0613736
Report No: 33741 Final

FOR: GEORGE WESTON FOODS LTD

P O Box 1
ENFIELD NSW 2136

Sherry Duckworth

Date received: 21/07/2006
Origin:
Code/Ref: 1816 DISCHARGE

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD
21JUL06/213284		
Moisture	1.7 % m/m	MOIS 01 12.99
Fat	21.5 % m/m	FATS 07 12.99
Protein (TN x 6.25)	81.3 % m/m	PROT 01 02.01
Non Protein Nitrogen	0.043 % m/m	NITF 01 03.03
Ash (@ 550°C)	1.2 % m/m	ASHS 01 04.93
Dietary Fibre - Soluble	<0.1 % m/m	DIET 02 03.93
Dietary Fibre - Insoluble	<0.1 % m/m	DIET 02 03.93

Shane Nolan
Technical Manager

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LABORATORY REPORT on MISCELLANEOUS POWDER

Date: 10/08/2006
Our Ref: DTS0613738
Report No: 33742 Final

FOR: GEORGE WESTON FOODS LTD

Sherry Duckworth

P O Box 1
ENFIELD NSW 2136

Date received: 21/07/2006
Origin:
Code/Ref: 1835 DISCHARGE

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD
21JUL06/213286		
Moisture	1.9 % m/m	MOIS 01 12.99
Fat	20.6 % m/m	FATS 07 12.99
Protein (TN x 6.25)	80.6 % m/m	PROT 01 02.01
Non Protein Nitrogen	0.042 % m/m	NITF 01 03.03
Ash (@ 550°C)	1.2 % m/m	ASHS 01 04.93
Dietary Fibre - Soluble	<0.1 % m/m	DIET 02 03.93
Dietary Fibre - Insoluble	<0.1 % m/m	DIET 02 03.93

Shane Nolan
Technical Manager

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LABORATORY REPORT on MISCELLANEOUS POWDER

Date: 30/01/2007
Our Ref: DTS0700283
Report No: 46663
Final

FOR: GEORGE WESTON FOODS LTD **Sherry Duckworth**

P O Box 1
ENFIELD NSW 2136

Date received: 8/01/2007
Origin:
Code/Ref: 1924 DISCHARGE

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD I
08JAN07/312870		
Moisture	4.2 % m/m	MOIS 01 12.99
Fat	20.8 % m/m	FATS 07 12.99
Protein (TN x 6.25)	79.9 % m/m	PROT 01 02.01
Non Protein Nitrogen	0.301 % m/m	NITF 01 03.03
Ash (@ 550°C)	1.4 % m/m	ASHS 01 04.93
Free Fat (fat basis)	31.2 % m/m	FRFA 01 04.93
Total Carbohydrate	<0.1 % m/m	TCAR 01 07.93
Dietary Fibre - Soluble	<0.1 % m/m	DIET 02 03.93
Dietary Fibre - Insoluble	<0.1 % m/m	DIET 02 03.93

Fred Irvine
Systems Manager

07D304
S. F. Wang
9222 3040

Sherry Duckworth
Weston technologies
1 Braidwood Street
Enfield NSW 2136

3rd March 2008

Attention : Sherry Duckworth

REPORT ON THE ANALYSIS OF ELEVEN SAMPLES RECEIVED ON 19 FEBRUARY 2008

Sampling procedure and sample history

Eleven samples of lupin products were received for alkaloid analysis.

Test Method

Total alkaloids determined by GC method SP1.

Results

Lab No 07D304	Client No.	Total Alkaloid %ar
001	1037	0.001
002	1048	0.006
003	1017	0.013
004	2102	0.028
005	2331	0.002
006	2340	0.004
007	090907	0.050
008	1813	0.005
009	2505	0.004
010	1736	0.001
011	1737	<0.001

%ar = Percent as received.

This report relates specifically to the sample(s) as received.

N E ROTHNIE
CHIEF
FOOD AND BIOLOGICAL CHEMISTRY

000127

CHEMISTRY
CENTRE

Your Ref: 3409 & 3414
Our Ref: Lab. No 04D505/1-6
Enquiries: SF Wang
9222 3040

Ms Sherry Duckworth
Project Manager
Weston Technologies
Braidwood Street
Enfield NSW 2136, Australia
PO Box 1, Enfield 2136

19 December 2005

REPORT ON THE ANALYSIS OF SIX LUPIN PRODUCT SAMPLES
Sample History

Six lupin product samples were received for analysis of total alkaloids, total phenolic, oligosaccharides, trypsin inhibitor, lead, cadmium, phytic acid, lectin and total saponins.

Lab Code	Sample No
04D505_001	103
04D505_002	1017
04D505_003	1037
04D505_004	1046
04D505_005	1071
04D505_006	1090

Test Methods

Total alkaloids by in-house GC-MS method.
Oligosaccharides by method SP7.
Total phenolics by folin-ciocalten.
Trypsin inhibitor by method SP 9.
Cd (ICP-MS) = Cadmium, Cd by ICP-MS method IELE1STIM.
Pb (ICP-MS) = Lead, Pb by ICP-MS method iELE1STIM.
Phytic acid by in house GC-MS method.
Lectin: Haemagglutinin activity by in house method
Saponins by in house GC-MS method
mg/kgar = milligrams per kilogram as received.
%ar = per cent as received.

GOVERNMENT OF WESTERN AUSTRALIA
04D505 3-Jun-08

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Western Australia 6004
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abn: 40 991 805 705

CHEMISTRY
CENTRE

Results

Lab No 04D	Total alkaloids %ar	Oligosac %ar	Total Phenolics %ar	Trypsin inhibitor mg/g ar	Cd (ICP-MS) mg/kgar
505-001	0.012	5.6	0.324	1.45	0.04
505-002	0.008	1.5	0.168	2.48	0.03
505-003	0.002	0.4	0.125	0.099	0.06
505-004	0.003	0.4	0.307	0.94	0.06
505-005	0.0005	0.2	0.066	0.17	0.03
505-006	0.001	0.6	0.126	0.15	0.02

Lab No 04D	Pb (ICP-MS) mg/kgar	Phytic Acid %ar	Lectin (sheep)	Lectin (Horse)	Saponin (Total) %ar
505-001	0.13	0.50	0	0	<0.003
505-002	0.20	0.19	0	0	0.026
505-003	0.14	0.31	0	0	0.065
505-004	0.14	0.69	0	0	0.295
505-005	0.14	0.17	0	0	0.226
505-006	0.12	0.16	0	0	0.056

Lectin figures presented indicate the highest dilution that agglutination is still observed, a 0 result indicates no haemagglutinin activity.

Commercial standards of lupin saponins are not commercially available. The alternative standard purchased from a private company is not sufficiently pure for definitive analysis, affecting total saponin accuracy. Definitity analysis may be achieved with the provision of additional time and the isolation expense that is reauired to prepare specific and pure standards.

If you have any inquires regarding these results, please contact Shao Fang Wang.
These results apply specifically to the sample as received.

NEIL ROTHNIE
CHIEF
FOOD & BIOLOGICAL CHEMISTRY
LABORATORY

S F WANG
Chemist and Research Officer
FOOD & BIOLOGICAL CHEMISTRY
LABORATORY

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000129

GOVERNMENT OF WESTERN AUSTRALIA

04D505 3-Jun-08

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Phone: (03) 8371 7600 Fax: (03) 9372 2013

LABORATORY REPORT
on
MISCELLANEOUS PRODUCT

Date: 03/05/05

Our ref: 15MAR05/47080/MISS

FOR: GEORGE WESTON FOODS LTD.
(WESTON TECHNOLOGIES)
PO BOX 1
ENFIELD NSW 2136

SHERRY DUCKWORTH

Account: W20

COPY:

SAMPLE DETAILS:
Date Received - 15/03/05
Origin - -
Code/Ref. - 1034 CENTRATE UF pH 4.5
Order number -
No.of samples - 1
Package Type -

REPORTS:
STD

TEST	RESULTS	DTS METHOD No.
MOISTURE %m/m	6·0	MOIS 01 12.99
FAT %m/m	4·1	FATS 07 12.99
PROTEIN (TN x 6.25) %m/m	73·0	PROT 01 02.01
ASH %m/m @ 550°C	1·6	ASHS 04 04.93
DIETARY FIBRE % (INSOLUBLE)	1·7	DIET 02 03.93
DIETARY FIBRE % (SOLUBLE)	13·3	DIET 02 03.93

S. Nolan
Technical Manager

000130

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LABORATORY REPORT
on
MISCELLANEOUS POWDER

Date: 03/06/05

Our ref: 24MAR05/50175/MISCP

FOR: GEORGE WESTON FOODS LTD.
(WESTON TECHNOLOGIES)
PO BOX 1
ENFIELD NSW 2136

SHERRY DUCKWORTH

Account: W20

COPY:

SAMPLE DETAILS:
Date Received - 24/03/05
Origin - -
Code/Ref. - 1086 CENTRATE 6.5
Order number -
No.of samples - 1
Package Type -

REPORTS:
STD

TEST	RESULTS		DTS METHOD No.
MOISTURE %m/m	7·8		MOIS 01 12.99
FAT %m/m	0·4		FATS 07 12.99
PROTEIN (TN x 6.25) %m/m	83·0		PROT 01 02.01
ASH %m/m @ 550°C	3·1		ASHS 04 04.93
TOTAL CARBOHYDRATE % m/m	<0·1	BY DIFFERENCE	TCAR 01 07.93
DIETARY FIBRE % (INSOLUBLE)	<0·1		DIET 02 03.93
DIETARY FIBRE % (SOLUBLE)	7·0		DIET 02 03.93

S. Nolan
Technical Manager

000131

DTS FOOD LABORATORIES

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LABORATORY REPORT on MISCELLANEOUS POWDER

Date: 05/07/2006
Our Ref: DTS0612173
Report No: 31076 Final

FOR: GEORGE WESTON FOODS LTD

Sherry Duckworth

P O Box 1
ENFIELD NSW 2136

Date received: 23/06/2006
Origin:
Code/Ref: 1728 CENTRATE (1)

Order Number:
No of samples: 1
Package Type:

TEST	RESULTS	METHOD
23JUN06/198669		
Moisture	7.5 % m/m	MOIS 01 12.99
Fat	1.0 % m/m	FATS 07 12.99
Protein (TN x 6.25)	85.3 % m/m	PROT 01 02.01
Non Protein Nitrogen	1.232 % m/m	NITF 01 03.03
Ash (@ 550°C)	1.7 % m/m	ASHS 01 04.93
Dietary Fibre - Soluble	4.4 % m/m	DIET 02 03.93
Dietary Fibre - Insoluble	<0.1 % m/m	DIET 02 03.93

Shane Nolan
Technical Manager

APPENDIX B-3

SUMMARY OF THE RESULTS OF ANALYSIS OF PESTICIDE RESIDUES IN SWEET LUPIN FLOUR AND CORRESPONDING IDENTIFIED MAXIMUM RESIDUE LIMITS (MRL) FOR VARIOUS FOOD PRODUCTS

Table B-3-1 Pesticide Residues Identified In Sweet Lupin Flour and Corresponding Identified Maximum Residue Limits (MRL) For Various Food Products

Food Product	U.S. FDA MRL (ppm)[a]	FSANZ MRL (ppm)[b,c]	Sweet Lupin Flour (ppm)
Organochlorine Profile (Analyzed by MS)			**< 0.05**
Cereal grain	Not available		
Dichlorodiphenyltrichloroethane (DDT)	Not available	0.10	Complies
Chlordane (cis and trans)	Not available	0.020	MRL below LOD
Oxychlordane	Not available	0.020	MRL below LOD
Aldrin	Not available	0.020	MRL below LOD
Dieldrin	Not available	0.020	MRL below LOD
Endrin	Not available	No limit set	NA
Endosulfan	0.1	0.20	Complies
Lindane	Not available	0.50	Complies
Hexachlorocyclohexanes (HCH)	Not available	0.10	Complies
Heptachlor	Not available	0.020	MRL below LOD
Heptachlor epoxide	Not available	0.020	MRL below LOD
Hexachlorobenzene (HCB)	Not available	0.050	Complies
Dichlorvos			**< 0.02**
Cereal grain	0.5	5	Complies
Peanut	Not available	2	Complies
Wheat bran, unprocessed	Not available	10	Complies
Wheat germ	Not available	10	Complies
Chlorpyrifos Methyl			**< 0.02**
Cereal grains (barley, oat, wheat, not rice)	6	10	Complies
Lupin (dry)	NA	10	Complies
Wheat bran, unprocessed	Not available	20	Complies
Wheat germ	Not available	30	Complies
Fenitrothion			**< 0.02**
Cereal grains	30	10	Complies
Wheat bran, unprocessed	Not available	20	Complies
Wheat germ	Not available	20	Complies
Pirimiphos Methyl			**< 0.02**
Barley	Not available	7	Complies
Bran of cereal grain, unprocessed	Not available	20	Complies
Maize	8	7	Complies
Oats	Not available	7	Complies
Peanut	Not available	5	Complies
Rye	Not available	10	Complies
Sorghum	8	10	Complies
Triticale	Not available	10	Complies
Wheat	Not available	10	Complies
Wheat germ	Not available	30	Complies

Table B-3-1 Pesticide Residues Identified In Sweet Lupin Flour and Corresponding Identified Maximum Residue Limits (MRL) For Various Food Products

Food Product	U.S. FDA MRL (ppm)[a]	FSANZ MRL (ppm)[b,c]	Sweet Lupin Flour (ppm)
Malathion			**< 0.02**
Cereal grains	8	8	Complies
Peanut	8	8	Complies
Wheat bran unprocessed	Not available	20	Complies
Piperonyl Butoxide			**< 0.02**
Cereal Bran unprocessed	Not available	40	Complies
Wheat germ	Not available	50	Complies
Cereal grains	20[d] 8[e]	20	Complies
Oilseed	Not available	8	Complies

FSANZ = Food Standards Australia New Zealand; LOD = Limit of detection; MS = Mass spectrometry; NA = not applicable; U.S. FDA = Food and Drug Administration;
[a] U.S. EPA, 2007
[b] *Australia New Zealand Food Standards Code, Standard 1.4.2 (FSANZ, 2005)*
[c] APVMA, 2005
[d] MRL established for post-harvest barley, buckwheat, rice, rye and wheat
[e] MRL established for post-harvest oat and sorghum

APPENDIX B-4

APPENDIX B-4

STABILITY OF SWEET LUPIN PROTEIN FRACTIONS 1 AND 2

STABILITY OF SWEET LUPIN PROTEIN FRACTIONS 1 AND 2

In order to evaluate the stability of the sweet lupin protein fraction ingredients under the intended storage conditions, GWF assessed various compositional parameters (*i.e.*, solid, fat, protein, ash, and fibre contents) of batches of sweet lupin protein fractions 1 and 2 at a temperature of 25°C over periods of 16 to 24 weeks. The analyses indicated that the composition of each of the protein fractions did not vary significantly over the duration of the study and were within range of the established limits of each of the product specifications at the end of the study period. The results of this study are presented in the table below.

Table B-3-1 Results of the Storage Stability Study of Sweet Lupin Protein Fractions 1 and 2

Date of Sample	Code Number	Storage Temperature	Storage Duration (weeks)	Parameter (%, Dry solid basis)					
				Solids	Fat	Protein	Ash	Dietary Fibre	
								Insoluble	Soluble
Sweet Lupin Protein Fraction 1									
Feb-05	924	25°C	0	96	21	79	3	14	<0.1
			24	94	20	79	3	3	<0.1
Feb-05	957	25°C	0	95	20	78	2	10	<0.1
			24	95	22	78	2	2	<0.1
Mar-05	1037	25°C	0	96	20	77	2	14	<0.1
			16	95	22	79	2	2	<0.1
Sweet Lupin Protein Fraction 2									
Feb-05	983	25°C	0	93	1	67	4	<0.1	13
			24	92	1	68	4	0.5	10
Mar-05	1045	25°C	0	94	9	72	2	8	5
			16	94	9	72	2	0.9	10

APPENDIX B-5

SUMMARY OF THE ANALYSES OF OLIGOSACCHARIDES, PHENOLICS AND CONDENSED TANNINS, TRYPSIN INHIBITORS, PHYTIC ACID, SAPONINS, AND LECTINS PRESENT IN ONE BATCH OF SWEET LUPIN PROTEIN FRACTIONS 1 AND 2

Table B-5-1 Summary of the Analyses of Phytonutrients Present in One Batch of Sweet Lupin Protein Fractions 1 and 2

Parameter	Sweet Lupin Protein Fraction 1		Sweet Lupin Protein Fraction 2	
	%, AR	%, DSB	%, AR	%, DSB
Oligosaccharides	0.4	0.4	0.4	0.4
Phenolics[a]	0.125	0.126	0.307	0.308
Trypsin Inhibitors[b]	0.10	NR	0.94	NR
Tannins	<0.05	NR	<0.05	NR
Phytic Acid	0.19	NR	0.31	NR
Lectin Activity (Sheep)[c]	0	NA	0	NA
Lectin Activity (Horse)[c]	0	NA	0	NA
Saponin	0.026	NR	0.065	NR

AR = as received; DSB = dry solids basis; NA, not applicable; NR, not reported
[a] Expressed as Mark Tannic acid equivalent
[b] Trypsin inhibitor reported in mg/g, as received.
[c] Lectin measured by hemagglutinin activity. Figures indicate the highest dilution where agglutination is still observed. A zero (0) result indicates no activity. Cells from both sheep and horse were tested with all results equal to zero.

SUBMISSION END

AM

Carlson, Susan

From: Sherry.Duckworth@gwf.com.au
Sent: Friday, October 31, 2008 7:29 AM
To: Carlson, Susan
Subject: Suggested names for lupin protein fractions - GRAS Notices

Dear Susan

After some discussion we would like to propose the following suggestions:

Lupin Protein 1: pH dependent Soluble Protein

Lupin Protein 2: Soluble Protein

Thanks
Sherry

"Carlson, Susan" <Susan.Carlson@fda.hhs.gov>
29/10/2008 03:21 AM

To <Sherry.Duckworth@gwf.com.au>
cc
Subject RE: Names for lupin protein fractions - GRAS Notices

Dear Sherry,

Thank you for your prompt response. My chemistry colleagues have advised me that chemically descriptive names would be more useful for our purposes. They have proposed that what you have designated as Lupin Protein 1 Fraction be referred to as Acid Insoluble Lupin Protein. For what you refer to as Lupin Protein 2 Fraction they suggest Acid Soluble Lupin Protein.

Please let me know if these proposed names are accurate.

As for your acknowledgement letters, yes, you will receive them soon. They have not been sent out yet. I can send you an e-mail when they do.

Thank you,
Susan

Susan Carlson, Ph.D.
General Health Scientist
.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review

000144

-----Original Message-----
From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Monday, October 27, 2008 10:51 PM
To: Carlson, Susan
Subject: Names for lupin protein fractions - GRAS Notices

Dear Susan

Peter forwarded your email to me. In future he has asked that I be the point of contact at George Weston Foods for correspondence regarding the progress of our submission notices.

In regard to the naming of the proteins we understand that your request is
to be able to differentiate the 2 proteins with names other than 1 and 2.

Our suggestion would be to call the proteins:

Lupin Protein 1: Emulsifying Lupin Protein

Lupin Protein 2: Foaming and Emulsifying Lupin Protein

We were expecting to receive a letter to confirm the arrival of the GRAS notices at the FDA office. Has this been sent as yet?

Thanks
Sherry

Sherry Duckworth
Project Manager
Research and Technology
George Weston Technologies
(A Division of George Weston Foods)
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Enfield NSW 2136
Australia
Tel: + 61 2 9764 8160
Fax: + 61 2 9742 5959
MOB: +61 0419 412 398
Email: sherry_duckworth@gwf.com.au

----- Forwarded by Peter Schutz/WT/NSW/GWF on 23/10/2008 05:16 PM -----

	"Carlson, Susan" <Susan.Carlson@fda.hhs.gov>
To	<peter.schutz@gwf.com.au>
	22/10/2008 04:51 AM
cc	
Subject	Names for lupin protein fractions

000145

Dear Mr. Schutz,

My name is Susan Carlson and I am with the U.S. Food and Drug Administration (FDA). We recently received three GRAS Notices from you for various lupin products. We have a minor question regarding the lupin protein product.

For administrative purposes, we would like to use more descriptive names for what you have designated as lupin protein fractions 1 and 2. We would like to invite you to make some suggestions for brief, technically descriptive names that would distinguish between lupin protein fractions 1 and 2. For example, could solubility characteristics be used as part of the name? Any suggestions or comments would be appreciated.

Sincerely,
Susan Carlson

Susan Carlson, Ph.D.
General Health Scientist
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review

Telephone: 301-436-1253
E-mail: Susan.Carlson@fda.hhs.gov

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000146

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000147

AM

George Weston Foods Limited

ABN 45 008 429 632

SENT *VIA* INTERNATIONAL EXPRESS POST

December 10, 2008

Robert L. Martin, Ph.D.
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: Withdrawal of Generally Recognized As Safe (GRAS) Exemption Notices for Sweet Lupin Fiber, Sweet Lupin Flour, and Sweet Lupin Protein

Dear Dr. Martin:

This letter is to inform you that we would like to withdraw our GRAS Exemption Notices for Sweet Lupin Fiber, Sweet Lupin Flour, and Sweet Lupin Protein, which were forwarded to your office on September 26, 2008.

Thank you for your kind attention to this matter. Please contact me should you have any questions regarding the withdrawal of these Notices.

Sincerely,

(b)(6)

Peter Schutz
Chief Executive

George Weston Technologies
A Division of George Weston Foods Limited
peter.schutz@gwf.com.au

RECEIVED
DEC 16 2008
(b)(6)

000151

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