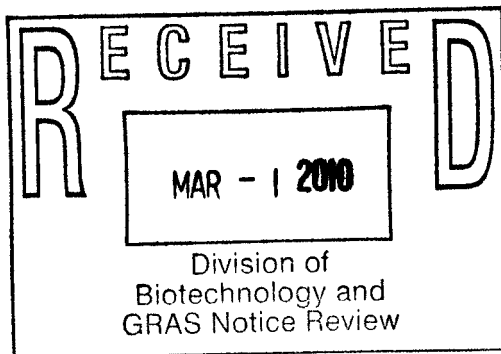


GR



Original Submission

000001.012



Archer Daniels Midland Company
Office of Compliance and Ethics
1001 North Brush College Road
Decatur, Illinois 62521

February 26, 2010

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

Re: GRAS Notification for the ADM/Burcon NutraScience Canola Protein Isolates Puratein® and Supertein™

Dear Dr. Martin:

Archer Daniels Midland (ADM)/Burcon NutraScience Corporation, by this letter and enclosed documents, is providing the Food and Drug Administration (FDA) notice in accordance with FDA proposed regulation at 62 Fed. Reg. 18938, 1980 (April 17, 1997), that it has determined, through scientific procedures, that the substances Cruciferin-rich canola protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) are Generally Recognized as Safe (GRAS) for food use, and in addition, exempt from the premarket approval requirements of the Food, Drug and Cosmetic Act.

The notification contains a listing of eight GRAS use categories for the incorporation as ingredients of these protein isolates into foods and their GRAS use is fully supported by published safety studies, generally available and accepted scientific data, and intake exposure calculations provided therein. This GRAS determination is also fully supported by a panel of experts, and their written concurrence is provided.

Respectfully,

(b) (6)

Luis A. Mejia, Ph.D.
Director, Regulatory and Scientific Affairs

LAM/jm

Enclosures: Four binders, each containing main body of dossier and Appendices 1 and 2

000001.013

**GRAS Notification for
Cruciferin-Rich
and Napin-Rich Protein Isolates
Derived from Canola/Rapeseed
(Puratein® and Supertein™)**

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

**Prepared by: Archer Daniels Midland Co. (ADM)
4666 Faries Parkway
Decatur, IL 62526**

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I - GRAS Exemption Claim

A) Claim of Exemption from the Requirements of Pre-Market Approval.

1) Background

Canola is a genetically improved variety of Rape (*Brassica napus*), an important oil seed crop member of the mustard family *Brassicaceae* (also known as *Cruciferae*). In the early 70s, conventional plant breeding of rape led to the development of rape varieties low in both glucosinolates and erucic acid, making them more suitable for food/feed consumption. This technological advancement allowed the introduction of canola (also known as oilseed rape or rapeseed) into the world market. Today, canola is the third largest oilseed crop in the world, only after soybean and palm, and represents a major cash crop in North America. Canada and the United States produce between 7 and 10 million metric tons of canola seed per year. Main uses of canola seed are extraction of canola oil for human consumption and production of the remaining canola meal for livestock feeding. Major consumers of canola oil and users of canola meal are the United States, Canada, Japan, Mexico, China, Pakistan, Taiwan and Europe. The use of canola meal in animal feeding is based on its nutritive value, conferred mainly by its protein content. However, canola/rapeseed proteins have not been widely used for human consumption.

The use of canola/rapeseed proteins as human foods has been suggested since the early 80's, not only for their nutritional value but also for their functional properties (Sosulski et al., 1983). However, inherent limitations in the seeds and technological constraints impaired its development. More recently, technological advances by Burcon NutraScience Corporation (Burcon NutraScience) and Archer Daniels Midland Company (ADM) have made possible the production of canola/rapeseed protein isolates suitable for human consumption as ingredients in a broad range of foods. One of these canola/rapeseed protein isolates consists mainly of the globulin storage protein called cruciferin (Puratein®) and the other of mainly the albumin storage protein called napin (Supertein™). Besides their nutritional value, these protein isolates possess unique functional properties similar to egg proteins, making them suitable for foods requiring egg protein as a functional ingredient, as well as for other food applications aimed to enhance their nutritional value.

From the regulatory point of view, the GRAS status of canola oil derived from *Brassica napus* or *Brassica campestris* was first acknowledged by FDA in 1985 (21 CFR-184.1555(c)). Later, a similar recognition for the canola oil derived from *Brassica juncea* was made in 2002 (GRAS Notice No. GRN 000033). More recently, in 2006, a qualified health claim was approved by the FDA for canola oil (Docket No. 2006Q-0091). However, the regulatory status and safety of canola/rapeseed proteins have not yet been established. Therefore, ADM and Burcon NutraScience

undertook a series of studies to evaluate the safety and affirm the GRAS status of the canola/rapeseed protein isolates indicated above to be marketed respectively under the trade names of Puratein® and Supertein™. This will be the subject of the present notification.

2) Scope of the Petition

Pursuant to proposed regulation 21 CFR 170.36 (62 FR 18938; April 17, 1997), and following established scientific procedures, ADM notifies the FDA of the GRAS self-affirmation of the crucifern-rich canola/rapeseed protein isolate ("Puratein®") and napin-rich canola/rapeseed protein isolate ("Supertein™") extracted from canola/rapeseed species, low in glucosinolates and erucic acid. The canola/rapeseed species include *Brassica napus*, *Brassica campestris* and *Brassica juncea*. These species are closely related and are similar in appearance and composition, including their low levels of erucic acid and glucosinolates. All of these species are used today for the production of canola oil for human consumption.

The proposed GRAS substances (Puratein® and Supertein™) will be used as food ingredients in powdered egg and egg substitutes, dairy products, processed meats, grain products, vegetable/fruit juices and beverages, salad dressings, protein supplement powders and meal replacement/nutritional bars.

3) GRAS Exemption Claim

Pursuant to the regulatory and scientific procedure established by proposed regulation 21 CFR 170.36 (62 FR 18938; april 17, 1997), ADM has determined, through scientific procedures, that its crucifern-rich canola/rapeseed protein isolates ("Puratein®") and napin-rich canola/rapeseed protein isolates ("Supertein™"), derived from canola/rapeseed species, are GRAS substances for the intended food applications indicated in Section IA-2 above and are, therefore, exempt from the requirement for pre-market approval established by the Federal Food, Drug and Cosmetic Act (21 CFR 170.36(C)(1). The basis for this GRAS affirmation is described in the following sections.

Signed:

(b) (6)

Luis A. Mejia, Ph.D.
Director, Regulatory and Scientific Affairs

February 26, 2010
Date

B) Name and Address of Notifier

Luis A. Mejia, Ph.D.
Director, Regulatory and Scientific Affairs
Archer Daniels Midland Company (ADM)
4666 East Faries Parkway
Decatur, IL 62526, U.S.A.
Tel.: 217-451-2201
E-mail: Luis.Mejia@adm.com

C) Name of GRAS Substance

The ingredients determined by ADM as GRAS are the cruciferin-rich canola/rapeseed protein isolate (minimum 80% cruciferin, as determined by HPLC-SEC) to be marketed under the trade name of Puratein® and the napin-rich canola/rapeseed protein isolate (minimum 80% napin, as determined by HPLC-SEC) to be marketed under the trade name of Supertein™.

For simplicity and convenience, throughout this document and in all supporting materials, cruciferin-rich canola/rapeseed protein isolate will also be referred to as cruciferin-rich protein isolate, or Puratein®. Napin-rich canola/rapeseed protein isolate will also be called napin-rich protein isolate, or Supertein™.

D) Intended Use

Each of the canola/rapeseed protein isolates have distinctive ingredient functionality properties. For example, while both can be added to transparent beverages, the cruciferin-rich protein isolate (Puratein®) has remarkable emulsifying/binding characteristics similar to egg proteins.

Depending on the suitability of each particular food application, either the cruciferin-rich protein isolate (Puratein®) or the napin-rich protein isolate (Supertein™), alone or in combination, will be used as food ingredients in the following food categories at the specified levels of addition indicated below. Foods containing the canola protein isolates (Puratein® and/or Cruciferin™) will be targeted to the general population, except children under 3 years of age. Therefore, infant foods or infant formulas are not included. Estimated exposure levels for all age groups, excluding children younger than 3 years, are presented in Section V-D.

	<u>Added up to*:</u>
Powdered egg/egg substitutes	60%
Dairy products	5%
Processed meats	2%
Grain products	2%
Fruit & vegetable juices/beverages	10%
Salad dressings	2%
Protein supplement powders	95%
Meal replacement/Nutritional bars	50%

* As is basis

E) Self-Limiting Levels of use

The use of Puratein® and Supertein™ as food ingredients is limited by the level that can technically be added to a given food without jeopardizing its quality and consumer acceptability.

F) Basis for GRAS Determination

Pursuant to 21 CFR-170.30, cruciferin-rich canola/rapeseed protein isolate (Puratein®) and napin-rich canola/rapeseed protein isolate (Supertein™) have been determined by ADM to be GRAS based on data generally available in the public domain pertaining to their safety, as discussed herein and in the accompanying documents, and on a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of the cruciferin-rich canola/rapeseed protein isolate (Puratein®) and the napin-rich canola/rapeseed protein isolate (Supertein™) as components of foods for human consumption. (Appendix 1. Expert Panel Report entitled: "Report of the Expert Panel on the Safety and Generally Recognized as Safe (GRAS) Status of the intended uses of Cruciferin-Rich and Napin-Rich Protein Isolates Derived from Canola/Rapeseed (Puratein® and Supertein™)"*.

*GRAS panel members were: G. Harvey Anderson, Ph.D., Professor of Nutritional Science and Physiology, University of Toronto, Canada; Joseph F. Borzelleca, Emeritus Professor of Pharmacology and Toxicology, Virginia Commonwealth University, U.S.A.; Paul Pencharz, M.B., Ch.B., Ph.D., FRCP, Professor of Pediatrics and Nutrition, University of Toronto, Canada; Steve Taylor, Ph.D., Professor of Food Science and Technology, University of Nebraska, U.S.A.

G) Availability of Information

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

Archer Daniels Midland Company
1001 Brush College Road
Decatur, IL 62521, U.S.A.

II - Product Identity and Specifications

A) Cruciferin-Rich Canola/Rapeseed Protein Isolate (Puratein®)

The cruciferin-rich canola/rapeseed protein isolate (Puratein®) is a protein fraction isolated from canola/rapeseed species containing a minimum of 80% globulin storage proteins called cruciferin, with the remainder being the albumin storage protein, napin (as determined by HPLC-SEC).

Specifications Cruciferin-Rich Protein Isolate (Puratein®)		Analytical Method
Appearance	Fine, brownish-yellow powder	
Protein (N x 6.25, d.b)	Minimum 90%	AOCS Ba 4e-93
Moisture	Maximum 9%	AOCS Bc 2-49
Ash	Maximum 6%	AOCS Ba 5a-49
Fat (ether method)	Maximum 2%	AOCS Bc 3-49
Glucosinolates	<30 µmol/g	ISO 9167-1
Erucic acid	<0.04%	AM-011 Ver. 5
Heavy metals (Cd, Pb, Hg, As)	<8 ppm	AM-011 Ver. 4
Lead	<0.5 ppm	AM-43 Ver. 1
<u>Microbiological Data</u>		
Standard plate count	Maximum 50,000 cfu/g	BAM Chap. 3
Salmonella	Negative (in 25 g)	BAM Chap. 5
<i>E. coli</i>	Negative (in 40 g)	BAM Chap. 4
<i>Staphylococcus aureus</i>	Negative (in 3 g)	BAM Chap. 12

B) Napin-Rich Canola/Rapeseed Protein Isolate (Supertein™)

The napin-rich canola/rapeseed protein isolate (Supertein™) is a protein fraction isolated from canola/rapeseed species containing a minimum of 80% albumin storage protein called napin, with the remainder being the globulin storage protein, cruciferin (as determined by HPLC-SEC).

Specifications Napin-Rich Protein Isolate (Supertein™)		Analytical Method
Appearance	Fine, light yellow powder	
Protein (N x 6.25, d.b)	Minimum 90%	AOCS Ba 4e-93
Moisture	Maximum 9%	AOCS Bc 2-49
Ash	Maximum 6%	AOCS Ba 5a-49
Fat (ether method)	Maximum 2%	AOCS Bc 3-49
Glucosinolates	<30 µmol/g	ISO 9167-1
Erucic acid	<0.04%	AM-011 Ver. 5
Heavy metals (Cd, Pb, Hg, As)	<8 ppm	AM-04 Ver. 4
Lead	<0.5 ppm	AM-43 Ver. 1
<u>Microbiological Data</u>		
Standard plate count	Maximum 50,000 cfu/g	BAM Chap. 3
Salmonella	Negative (in 25 g)	BAM Chap. 5
<i>E. coli</i>	Negative (in 40 g)	BAM Chap. 4
<i>Staphylococcus aureus</i>	Negative (in 3 g)	BAM Chap. 12

Typical Amino Acid Profiles (g/100g Protein*)

	Cruciferin-Rich Protein Isolate (Puratein®)	Napin-Rich Protein Isolate (Supertein™)
Aspartic acid + Asparagine	9.3	2.6
Threonine**	3.7	3.2
Serine	4.1	3.3
Glutamic acid + Glutamine	19.8	24.6
Proline	5.8	9.2
Glycine	5.4	4.3
Alanine	4.2	4.0
Cystine	1.6	4.5
Valine**	5.5	4.3
Methionine**	1.9	2.4
Isoleucine**	4.4	3.0
Leucine**	8.2	6.0
Tyrosine	4.1	1.4
Phenylalanine**	4.9	2.6
Histidine**	2.5	3.6
Lysine**	4.0	7.4
Arginine	7.2	5.8
Tryptophan**	2.0	1.4

* Determined by acid hydrolysis. Values are average of duplicate analyses.

**Essential Amino Acids

C) Data on Representative Lots

Five different production lots were analyzed with respect to the different parameters indicated in the specifications for both the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) derived from *Brassica napus*. The corresponding data are presented in the following Tables 1 and 2. Results show production consistency and compliance with specifications.

Table 1. Analytical Data on Representative Lots of the Cruciferin-Rich Protein Isolate (Puratein®)

	Lot 1 C300-C28-07	Lot 2 C300-E01-07	Lot 3 C300-E14-07	Lot 4 C300-E24-07	Lot 5 C300-F04-07	Specifications
Protein (N x 6.25, db), %	103	104	104	105	100	≥ 90%
Moisture, %	7.6	6.6	6.8	7.7	7.4	≤ 9%
Ash, %	3.0	3.0	2.8	3.3	2.9	≤ 6%
Fat, %	0.19	0.18	0.16	0.22	0.24	≤ 2%
Total Glucosinolates,						
μmol/g	1.4	1.3	1.8	1.1	2.5	< 30 μmol/g
Erucic acid, μg/g*	ND	ND	ND	ND	ND	< 0.04%
Heavy metals						
(Cd, Pb, Hg, As), ppm	0.04	0.07	0.04	0.10	0.02	< 8 ppm
Lead, ppm	ND	ND	ND	ND	ND	< 0.5 ppm
Std. plate count, cfu/g	1000	800	<100	300	700	≤ 50,000 cfu/g
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative	Negative	Negative

ND = Not Detected

* LD: 25 μg/g

Table 2. Analytical Data on Representative Lots of the Napin-Rich Protein Isolate (Supertein™)

	Lot 1 C200-C28-07	Lot 2 C200-D25-07	Lot 3 C200-E03-07	Lot 4 C200-E07-07	Lot 5 C200-F04-07	Specifications
Protein (N x 6.25, db), %	92	93	95	94	95	≥ 90%
Moisture, %	8.3	6.8	6.9	6.2	7.2	≤ 9%
Ash, %	6.0	3.7	3.1	3.8	3.8	≤ 6%
Fat, %	0.25	0.09	0.21	0.21	0.10	≤ 2%
Total Glucosinolates,						
μmol/g	0.9	0.8	0.4	0.4	1.0	< 30 μmo/gl
Erucic acid, , μg/g*	ND	ND	ND	ND	ND	< 0.04%
Heavy metals						
(Cd, Pb, Hg, As), ppm	0.06	0.07	0.06	0.02	0.03	< 8 ppm
Lead, ppm	ND	ND	ND	ND	ND	< 0.5 ppm
Std. plate count, cfu/g	<100	<100	<100	200	300	≤ 50,000 cfu/g
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative	Negative	Negative

ND = Not Detected

* LD: 25 μg/g

D) Detailed Typical Composition and Mass Balance

Table 3. Data from composite samples of the Cruciferin-Rich Protein Isolate (Puratein®) and the Napin-Rich Protein Isoalate (Supertein™) derived from <i>Brassica napus</i>				
	Puratein®		Supertein™	
	As Reported	%	As Reported	%
Protein (N x 6.25, as is)	94.9%	94.9	86.7%	86.7
Fat	0.22%	0.22	0.29%	0.29
Ash	3.36%	3.36	3.96%	3.96
Moisture	7.30%	7.30	7.1%	7.1
Carbohydrates				
Arabinose	0	0	52 mg/Kg	0.005
Xylitol	0	0	0	0
Xylose	0	0	559 mg/Kg	0.056
Fructose	164 mg/Kg	0.016	347 mg/Kg	0.034
Mannose	0	0	63 mg/Kg	0.006
Galactose	157 mg/Kg	0.016	267 mg/Kg	0.027
Glucose	319 mg/Kg	0.032	566 mg/Kg	0.057
Sucrose	1,369 mg/Kg	0.137	4,669 mg/Kg	0.467
Maltose	0	0	0	0
Isomaltose	0	0	0	0
Raffinose	85 mg/Kg	0.009	334 mg/Kg	0.033
Stachiose	58 mg/Kg	0.006	2,571 mg/Kg	0.257
Total Glucosinolates	1.22 µmol/g		0.80 µmol/g	
Erucic Acid	<25µg/g		<25µg/g	
Phytic Acid	3,221 mg/Kg	0.32	33,462 mg/Kg	3.34
Anions				
Chloride	19,212.9 mg/Kg	1.92	5,028.7 mg/Kg	0.50
Nitrites	NR	-	89.8 mg/Kg	0.01
Nitrates	59.3 mg/Kg	0.06	76.2 mg/Kg	0.08
Sulfates	388.1 mg/Kg	0.04	682.2 mg/Kg	0.07
Phosphates	124.9 mg/Kg	0.01	130.4 mg/Kg	0.01
Citrate	289.8 mg/Kg	0.03	622.3 mg/Kg	0.06
Purines (GMP Equivalence)	0.01 mg	0.01	0	0
Total Phenolic Acids*	4,025 µg/g	0.40	2,558 µg/g	0.26
TOTAL		108.8%		103.36%

*Sum of Sinapic (>90%), p-coumaric and ferulic acids. Others were not detected.

E) Stability of the Canola Protein Isolates Puratein® and Supertein™

The canola protein isolates Puratein® and Supertein™ have shown to be very stable. An on-going stability study revealed that the protein levels (N X 6.25) of retained samples of Puratein® and Supertein™ stored under ambient conditions (room temperature, 20-25°C) were maintained practically unchanged for a period of over two years. The average protein level of Puratein® blends analyzed during 2007 was 100.2% d.b. and 101.0% d.b. in February of 2010. For Supertein™, the average protein level of blends analyzed in 2007 was 91.9% d.b. and 91.8% d.b. in February 2010.

III - Manufacturing Process

The starting material for the isolation of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) is canola/rapeseed meal. Therefore, the overall production sequence, starting from the raw material (canola/rapeseed) includes two stages - the production of the defatted canola/rapeseed meal and the isolation of the proteins. The description of each of these stages and corresponding process flow diagram follows.

A) Canola/Rapeseed Meal Production Process

Canola/rapeseed is heat treated to inactivate enzymes and reduce the formation of undesirable breakdown products from the glucosinolates. The seed is partially dehulled then pressed to remove most of the oil resulting in the formation of an oil-laden press cake which is subsequently defatted by counter-current hexane extraction. The defatted meal is gently desolventized to become the starting material for the canola/rapeseed protein isolate production.

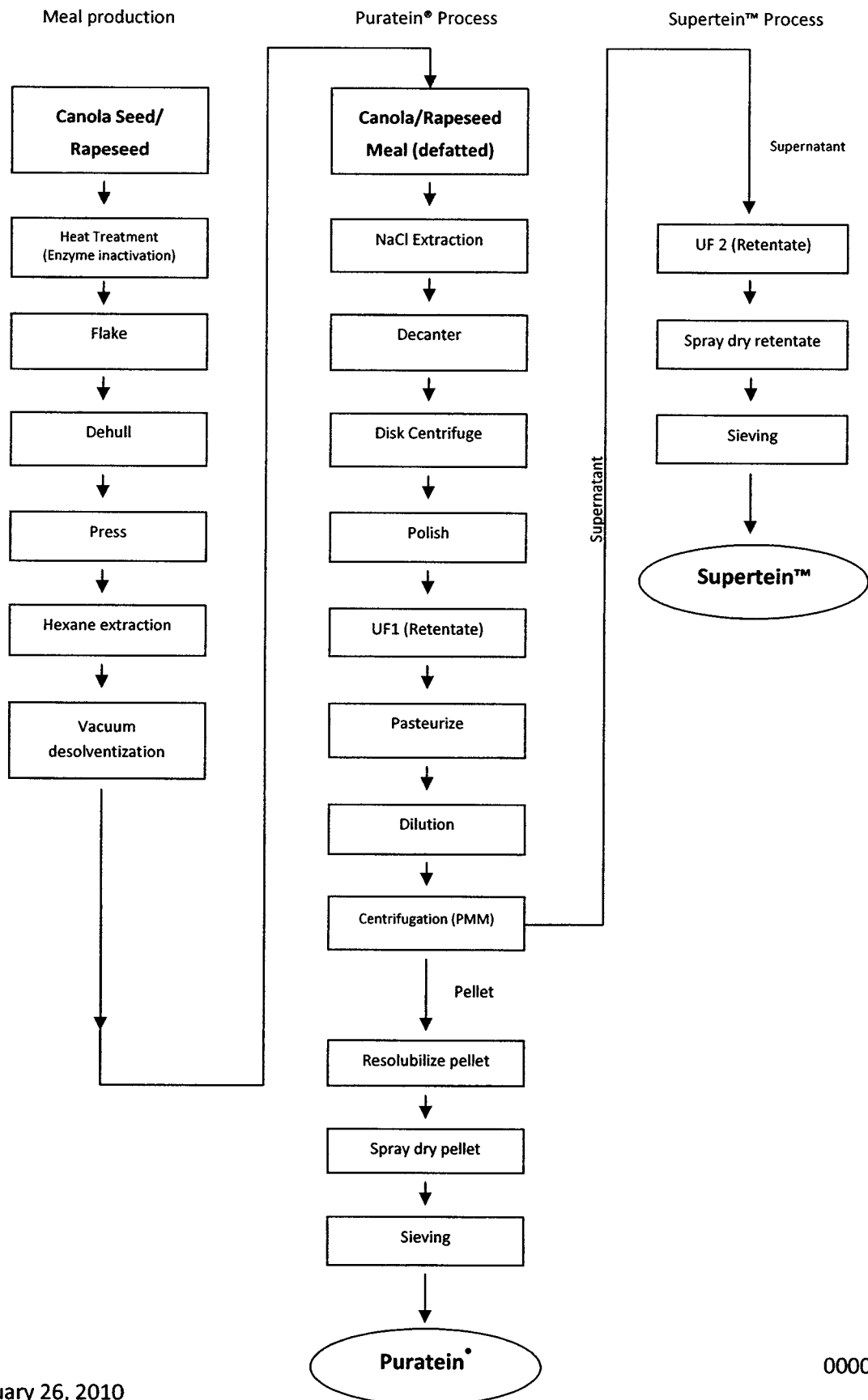
B) Canola/Rapeseed Protein Isolates Production Process

The canola/rapeseed meal contains two major protein fractions. The first is the globulin fraction, which is represented by cruciferin. The second is the albumin fraction, which is represented by napin. Cruciferin is the major protein in the seed (60% of the total protein) and it is conformed by six subunits with a total molecular weight of 300 kDa. Napin is a low molecular weight (MW: 14-14.9 kDa) protein composed of two disulfide-linked polypeptides (about 20% of the total protein).

Protein extraction involves solubilization in NaCl solution, several clarifications and filtrations then concentration using ultra-filtration with suitable membranes which allows the low molecular weight species to pass through (salt, carbohydrates, pigments and anti-nutritional factors). After the first ultra-filtration, the concentrated protein solution contains both cruciferin and napin protein fractions; the contaminants have passed through. The protein solution is pasteurized, cooled and diluted causing the globulin fraction (cruciferin) to precipitate as micelles (protein micellar mass, PMM) which are pelleted out by centrifugation; the pellets are then resolubilized, spray dried and sieved. The supernatant, which contains the albumin protein (napin), is further purified and subjected to a second ultra-filtration, retaining the protein while permitting the contaminants to pass through as permeate. The retentate is spray dried and sieved.

The canola/rapeseed protein isolates (Puratein® and Supertein™) used for analyses and toxicological evaluation reported in this document were manufactured from *Brassica napus*.

Canola/Rapeseed Protein Isolates Production Process Flow Chart



C) Potential Contaminants

1) Pesticide Residues

Both the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) were tested for the potential presence of 251 different pesticide residues from pesticides commonly used in a broad range of crops in the U.S.A. and Europe. None of these pesticides were detected in any of the samples (below limits of detection).

2) Solvent Residues

Hexane is the only organic solvent used in the manufacturing process of the protein isolates. Table 4 presents the levels of total hexane residues in different production lots of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™).

Table 4. Total Hexane Residues in the Protein Isolates

Cruciferin-Rich Protein Isolate (Puratein®)		Napin-Rich Protein Isolate (Supertein™)	
<u>Lot</u>	<u>Hexane (ppm)</u>	<u>Lot</u>	<u>Hexane (ppm)</u>
C300-C28-07	0.13	C200-C28-07	0.05
C300-E01-07	0.08	C200-D25-07	0.05
C300-E14-07	0.09	C200-E03-07	0.05
C300-E24-07	0.09	C200-E07-07	0.06
C300-F04-07	0.11	C200-F04-07	0.05
Composite	0.09	Composite	0.05

LOD: 0.01 ppm

Hexane is a permitted extraction solvent in the United States for the manufacture of foods for human consumption, up to levels of 60 ppm of n-hexane residues in finished products (21 CFR-172.894). On the other hand, ICH harmonized guidelines for pharmaceutical products classified hexane as class 2 solvent and accepts a maximum concentration of this substance in pharmaceutical products up to 290 ppm (ICH Harmonized Tripartite Guidelines, 1997). The levels of hexane residues found in both protein isolates are less than 1 ppm, far below the above indicated maximum limits. These hexane residue levels are also in compliance with maximum levels established by European Union regulations (Directives 92/115CEE, 97/60CE, 88/344/CEE).

3) Heavy Metals

Table 5 presents the analyses of heavy metals of most toxicological concern for different production lots of the cruciferin-rich and napin-rich protein isolates.

Table 5. Heavy Metals in the Cruciferin-Rich Protein Isolate (Puratein®) and Napin-Rich Protein Isolate (Supertein™)

	<u>Pb</u>	<u>Cd</u>	<u>As</u>	<u>Hg</u>
<u>Puratein® Lots</u>				
C300-C28-07	ND	0.04	ND	ND
C300-E01-07	ND	0.07	ND	ND
C300-E14-07	ND	0.04	ND	ND
C300-E24-07	ND	0.10	ND	ND
C300-F04-07	ND	0.02	ND	ND
Composite	ND	0.08	ND	ND
<u>Supertein™ Lots</u>				
C200-C28-07	ND	0.06	ND	ND
C200-D25-07	ND	0.07	ND	ND
C200-E03-07	ND	0.06	ND	ND
C200-E07-07	ND	0.02	ND	ND
C200-F04-07	ND	0.03	ND	ND
Composite	ND	0.04	ND	ND

ND = Not Detected. Pb LOD = 0.03 ppm. As and Hg LOD = 10 ppm.

These above results are in compliance with U.S. and European regulations.

4) Polycyclic Aromatic Hydrocarbons (PAHs)

The levels of main regulated markers of PAHs, in both cruciferin-rich and napin-rich protein isolate composite samples, are presented in Table 6.

Table 6. Benzo(a)pyrene and Benzo(a)anthracene in Cruciferin-Rich Protein Isolate (Puratein®) and Napin-Rich Protein Isolate (Supertein™)

	<u>Benzo (a) pyrene (ppb)</u>	<u>Benzo (a) anthracene (ppb)</u>
Puratein®	<0.1	<1
Supertein™	<0.1	<1

The limits of these carcinogenic compounds are not established in the U.S., but the European Union has established a maximum level of 2.0 ppb of Benzo (a) pyrene and Benzo (a) anthracene in oils and fats (EC No. 208/2005). The levels of these compounds in both protein isolates comply with this regulatory requirement.

5) Dioxins

Composite samples of cruciferin-rich protein isolate (Puratein®) and napin-rich protein isolate (Supertein™) were subjected to dioxin analysis. Total TEQ values, determined by using WHO toxic equivalent factors, were 0.134 pg/g for both protein isolates. This value is below the maximum level of <0.75 pg/g established by European Regulation (EC) No. 2375/2001.

6) Aflatoxins

Table 7 presents the levels of aflatoxins B₁, B₂, G₁ and G₂ for the cruciferin-rich protein isolate (Puratein®) and for the napin-rich protein isolate (Supertein™). No significant levels of these compounds were found. Other mycotoxins that can potentially be found in these type of products (Deoxinivalenol, fumosins and ochratoxin) were also undetected.

Table 7. Aflatoxin Levels in the Cruciferin-Rich (Puratein®) and Napin-Rich (Supertein™) Protein Isolates

<u>Aflatoxins (ppb)</u>				
	<u>B₁</u>	<u>B₂</u>	<u>G₁</u>	<u>G₂</u>
<u>Cruciferin-Rich Protein Isolate (Puratein®)</u>				
C300-C28-07	<1	<1	<1	<1
C300-E01-07	<1	<1	<1	<1
C300-E14-07	<1	<1	<1	<1
C300-E24-07	<1	<1	<1	<1
C300-F04-07	<1	<1	<1	<1
Composite	<1	<1	<1	<1
<u>Napin-Rich Protein Isolate (Supertein™)</u>				
C200-C28-07	<1	<1	<1	<1
C200-E01-07	<1	<1	<1	<1
C200-E14-07	<1	<1	<1	<1
C200-E24-07	<1	<1	<1	<1
C200-F04-07	<1	<1	<1	<1
Composite	<1	<1	<1	<1

7) Acrylamide

Composite samples of both cruciferin-rich and napin-rich protein isolates (Puratein® and Supertein™) were tested for their potential content of acrylamide, a toxicant of recent concern by the U.S. and European authorities. No significant levels

of this compound were found in any of these two canola protein fractions (<0.01 ppm).

D) Potentially Toxic Natural Components

1) Glucosinolates

Glucosinolates (GSL) are sulphur-containing molecules produced from amino acids by the secondary metabolism of plants (Nugon-Baudon & Rabot, 1994). Their occurrence is limited to some families of dicotyledonous angiosperms, such as the family cruciferae, which includes besides rapeseed, several commonly consumed edible plants like cabbage, brussel sprouts, cauliflower, broccoli, radish and mustard. Edible plants may contain up to 15 different GSLs and their total concentration ranges from 30-394 mg/100g fresh weight. As estimated by Sones et al. (1984), the amount of GSLs ingested by certain individuals could exceed 300 mg/day. GSLs themselves are not toxic. However, concern about the biological effect of GSL derivatives, produced from GSL by the action of the plant enzyme myrosinase, has led to the investigation of the profile and maximum safe limits in foods/feeds. The myrosinase enzyme hydrolyses the thioglucoside bond to release glucose and an unstable thiohydroximate-o-sulphonate, which is spontaneously transformed to yield sulphate and a wide range of potentially toxic GSL derivatives including isothiocyanates, nitriles, epithioalkanes, oxazolidinethions, 5-vinyloxazolidine-2-thione (VOT), thiocyanate ions and occasionally organic thiocyanates. Similarly, GSLs can also be metabolized by the intestinal microflora, producing the same toxic derivatives without the presence of myrosinase (Rabot et al., 1993). The gross toxic effects produced by GSL derivatives can be described as reduced feed intake, growth depression, impaired thyroid function, enlargement of target organs (liver, kidney, thyroid gland) and decreased egg production in birds (Rabot, 1993; Nugon-Baudon & Rabot, 1994). The intensity of these effects varies with animal species and the amount of GSL in their food. In humans, reduced iodine uptake by the thyroid gland has been reported and there is concern that high consumption levels of GSL could be goitrogenic (Langer et al., 1971). On the other hand, and contrary to this information, several authors have also suggested beneficial effects of glucosinolates and their breakdown products on cancer prevention (Lampe & Peterson, 2002).

The raw material used for the production of the canola protein isolates is not the traditional rapeseed containing high levels of GSLs (up to 200 µmol/g meal), but instead, canola seed or rapeseed which by design is low in GSL (<30 µmol/g meal). It is then expected that after processing, the GSL levels in the protein isolates would be insignificant. Table 8 presents the levels of most active GSL in the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™), including the total GSL content out of 15 different GSL identified.

Table 8. Glucosinolate Levels in the Cruciferin-Rich Protein Isolate (Puratein®) and Napin-Rich Protein Isolate (Supertein™)

Most Potentially Active Glucosinolates (GSL) in Different Lots of Puratein® and Supertein™ (µmol/g)					
	Progoitrin	Epiprogoitrin	Gluconapin	Gluco-brassicinapin	Total GSL *
Puratein®					
C300-C28-07	0.1991	0.0033	0.4891	0.3594	1.39
C300-E01-07	0.1752	0.0021	0.3643	0.3142	1.26
C300-E14-07	0.1406	0.0037	0.5019	0.6364	1.78
C300-E24-07	0.1613	0.0017	0.3905	0.2914	1.09
C300-F04-07	0.0889	0.0010	0.4392	1.2516	2.53
Composite	0.2129	0.0030	0.4115	0.3388	1.22
Supertein™					
C200-C28-07	0.2707	0.0050	0.2473	0.2318	0.95
C200-D25-07	0.2436	0.0051	0.1971	0.1870	0.80
C200-E03-07	0.1345	0.0014	0.1068	0.0651	0.39
C200-E07-07	0.1681	0.0030	0.0969	0.0537	0.41
C200-F04-07	0.2944	0.0064	0.2948	0.2168	1.02
Composite	0.1504	0.0052	0.2077	0.2320	0.80

*Total GSL includes the levels of 15 different identified GSLs

Based on toxicological data, the maximum allowed level of total GSL in canola meal is <30.0 µmol/g (USDA, GIPSA Standard 810.301). The levels of total GSL in both canola protein isolates are far below such cut-off point. Therefore, they are not of toxicological concern.

2) Thiocyanates and Nitriles

Thiocyanates, nitriles and 5-vinylloxazolidine-2-thione (VOT) (also known as goitrin) are end products of the action of the myrosinase enzyme on aliphatic GSLs. Isothiocyanates, thiocyanates and VOT are held responsible to interfere with the organic iodination of thyroxine precursors in the thyroid gland, thus leading in compensatory goiter (Nugon-Baudon & Rabot, 1994). Preferential targets of the nitrile derivatives seem to be the liver and kidneys. In experimental animals, the mechanism that underlies the toxicity of nitrile derivatives seems to be their ability to interact with reduced glutathione, thus leading to substantial alterations in tissue glutathione levels in the liver, kidney, adrenals and lungs. The toxic effect of nitriles manifest itself as hypertrophy of the target organs, disruption of the normal lobular structure of the liver and irregular proliferation of the bile duct. In regard to kidneys, nitriles produced rapid kidney lesions, along with elevated plasma levels of nitrogen, urea and creatinine, which would suggest functional alterations of the kidney. On the other hand, VOT (goitrin), which is derived from progoitrin, is considered the most potent goitrogen and its action is on the thyroid gland.

During the production process of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™), the myrosinase enzyme is inactivated by heating. This would prevent the formation of thiocyanates, nitriles or VOT from residual amounts that may be present in the protein isolates.

Total isothiocyanates and nitriles were analyzed in composite samples of Puratein® and Supertein™ as indicators of formation of GSL breakdown products. As expected, these compounds were not detected in either of the protein isolates. Table 9 shows the levels of isothiocyanates and Table 10 the nitrile levels.

Table 9. Total Isothiocyanates in the Cruciferin-Rich and Napin-rich Protein Isolates (Puratein® and Supertein™)

	<u>Composite Sample Run 1</u>	<u>Composite Sample Run 2</u>
<u>Puratein®</u>	ND	ND
<u>Supertein™</u>	ND	ND

LD: 0.1%

ND: None Detected

Table 10. Nitrile Levels in the Cruciferin-Rich and Napin-Rich Protein Isolates (Puratein® and Supertein™)

	<u>3-Butene Nitrile</u>	<u>4-Pentene Nitrile</u>	<u>5 Hexane Nitrile</u>	<u>Phenyl Acetonitrile</u>
<u>Puratein®</u>	ND	ND	ND	ND
<u>Supertein™</u>	ND	ND	ND	ND

ND = none detected. DL ≈ 20 mg/Kg

3) Erucic Acid

Erucic acid is a 22 carbon monounsaturated fatty acid present in small amounts in the lipid component of the canola seed. This compound has been associated with lipid and histological changes in the heart of experimental animals (Grice & Heggveit, 1983).

Traditional rapeseed varieties contain 22-60% erucic acid in their oils. Canola, on the other hand, is a rape variety that by design is low in erucic acid. Canola oil, for example, contains only 0.5-1% erucic acid, well below the 2% maximum limit set by the FDA (21 CFR 184.1555). The fact that the canola protein isolates Puratein® and Supertein™ contain only a trace amount of fat (<0.3%), means that any amount of erucic acid in these proteins would be negligible. Actual analyses of erucic acid in different production lots of the protein isolates (Puratein® and Supertein™) revealed undetectable levels of this fatty acid (below DL of 25 µg/g). In sum, the erucic acid content in both protein isolates (Puratein® and Supertein™) is insignificant and does not represent any toxicological concern.

4) Phytates

Phytic acid occurs as salts of calcium, magnesium and potassium (phytin) in the crystalline globoids inside protein bodies of rapeseed. Its content has been reported to be 2.0-4.0% in whole rapeseed, 2.0-5.0% in defatted meal, 5.0-7.5 in protein concentrates and <1 to 9.8% in protein isolates, depending on the method of protein isolation (Thompson, 1990). Regarding potential negative effects of phytates, there is concern that due to the strong cation binding capacity, high levels of phytic acid in the diet may decrease the bioavailability of essential minerals such as calcium, magnesium, zinc and iron, and thus impact mineral nutrition (Anderson et al. 1976; Jones, 1979; Larsen & Samdstrom, 1992; Weaver & Kannan, 2002). Phytates may also form complexes with proteins, interfering with their utilization (Hidvegi & Lasztity, 2002). Table 11 shows the levels of phytic acid in different production lots of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™.)

Table 11. Phytic Acid Levels of the Cruciferin-Rich and Napin-Rich Canola Protein Isolates (Puratein® and Supertein™)	
Puratein®	% Phytic Acid
C300-C28-07	0.13
C300-E01-07	0.44
C300-E14-07	0.51
C300-E24-07	0.23
C300-F04-07	0.15
Composite	0.32
Supertein™	
C200-C28-07	3.50
C200-D25-07	3.84
C200-E03-07	3.57
C200-E07-07	3.57
C200-F04-07	3.67
Composite	3.35

Although these phytic acid levels are contrastingly different between the cruciferin-rich protein isolate (Puratein®) (0.32%) and the napin-rich protein isolate (Supertein™) (3.35%), they are within the reported range of <1-9.8% phytates in rape protein isolates (Thompson, 1990) and below the 5.25% level found to cause adverse effects in rats (Anderson et al., 1976). In this latter study, several experiments were conducted to determine the affect of feeding various dietary levels of rapeseed flours containing either 4.97% or 5.25% phytates on growth, outcome of gestation, lactation and blood trace element concentrations in pregnant/lactating rats. In comparison to a casein containing diet, both diets containing rapeseed flour decreased food consumption, but only the diet

containing rapeseed flour with 4.97% phytate reduced weight gains of the mothers and corresponding pups. Furthermore, only the diet containing rapeseed flour with the highest (5.25%) phytate concentration reduced the plasma levels of zinc in the pregnant and non-pregnant rats. It was concluded that pregnant rats, and growing and adult rats fed 20% crude protein diets from rapeseed flour containing 5.25% phytate were deficient in zinc. Furthermore, that the zinc requirement at these phytate levels may be about 50% increased when feeding rapeseed diets. It needs to be noted in addition that the test material (rapeseed flour) used in this study had a higher phytate level than the napin-rich protein isolate (Supertein™) (5.25% Vs. 3.35%). It also contained about 9.8% crude fiber, which may have also contributed to the lower zinc bioavailability. Furthermore, this phytic acid level in Supertein™ is similar or lower to that reported for other commonly consumed foods (Reddy, 2002). For example, wheat bran that has entered the health consciousness of North America and Europe, contains 2-5.3% phytates. Rice bran contains 2.6-6.0% phytate. Nuts, such as Brazil nuts, contain more than 6% phytates. Inclusion of beans and lentils may lead to increased phytate ingestion, since many of these foods contain in excess of 2% phytate. Since phytate content in beans and bean products ranges from 0.17 to 9.15%, bean consuming countries like Mexico and Central America consume about 1666 to 2254 mg of phytate/day, while intake in the U.S. is only 631-1293 mg/day (excluding vegetarians) (Reddy, 2002). Another aspect that needs to be noted is that currently phytates are also considered desirable dietary substances. This is based on the observations that phytates can act as potent antioxidants (Burgess & Gao, 2002) and that they can also contribute to the prevention of some types of cancer, particularly of the colon (Jenab & Thompson, 2002).

We have also found, through our own toxicological studies, that the level of phytic acid in the napin-rich protein isolate (Supertein™) does not affect the plasma concentration of the trace mineral zinc in rats fed diets containing up to 20% napin-rich protein isolate (Supertein™), suggesting that the 3.35% phytate concentration in this protein isolate should not be of nutritional concern. The results of this investigation will be discussed in more detail in the toxicological section of this dossier (Section V-B1).

5) Phenolic Acids

Phenolic acids are ubiquitous in the plant kingdom, including all kinds of plant foods, and therefore are present in considerable amounts in the human diet. Main compounds are caffeic, chlorogenic, sinapic, ferulic and coumaric (King & Young, 1999). Rich sources of phenolic acids are blueberry (1,881-2,112 mg/Kg), cherry (290-1,280 mg/Kg), pear (44-1,270 mg/Kg), apple (2-258 mg/Kg), orange (21-182 mg/Kg), potato (100-190 mg/Kg) and coffee (56 g/Kg/Dry weight). Dietary exposure is then high and therefore, phenolic acids are considered in general safe. Thus, the

attention of these compounds has been focused primarily on their beneficial antioxidant effect.

Phenolic acids can also be found in rape and rapeseed products (Naczek et al., 1998). Main phenolic acid compound in rapeseed protein products is sinapic acid, which is found either in free or in esterified form (sinapine). Total content of phenolic acid (free and esterified) in various rapeseed products has been reported from 1.1 to 1.8% (Naczek et al., 1998). Main concerns about the presence of these substances in rapeseed products has been their negative impact on flavor, but not toxicity.

Phenolic acids (free and esterified) were determined in composite samples of Puratein® and Supertein™ using liquid chromatography. The analytes-of-interest were protocatechuic, p-hydroxybenzoic, vanillic, ferulic, gallic, syringic, caffeic, sinapic, p-coumaric and trans-2-hydroxycinnamic acids. Total level of phenolic acid in Puratein® was 0.40%, where sinapic acid (free and esterified) represented 93%. p-Coumaric and ferulic were present in significantly lesser amounts (2.9 and 4.1%, respectively). Other phenolic acids were not detected. Supertein™ contained 0.26% of total phenolic acids. Again, the greatest majority (96.3%) was sinapic acid. Levels of p-coumaric and ferulic acids were respectively only 2.1 and 1.6%. Other phenolic acids were not detected.

The concentration of sinapic acid alone (free and esterified) was 0.37% in Puratein® and 0.25% in Supertein™. Very recently, the safety of feeding sinapic acid to laying hens up to dietary levels of 0.5% has been demonstrated (Johnson et al., 2008). On the other hand, the beneficial anti-oxidant effects of sinapic acid from rapeseed/canola continue to appear in the literature (Thiyam, 2006).

IV - Nutrition and Metabolism

It is well understood that the nutritional value of dietary proteins can differ depending on their amino acid composition and digestibility. Protein digestibility of vegetable proteins (65-85%) is known to be less than protein from animal sources (>90%) (Delisle et al., 1983, Schaafsma, 2000). Additionally, many vegetable proteins are not complete sources of essential amino acids, making their overall quality, as assessed by protein efficiency ratio (PER) or protein digestibility corrected amino acid score (PDCAAS), less than animal proteins like milk and eggs (Jenkins and Mitchell, 1989).

Canola/rapeseed protein is mainly composed of a high molecular weight (12S) fraction, and a low molecular weight (1.7S or 2S) fraction (Schwenke, 1994). Both fractions are storage proteins. The 12S fraction, cruciferin, is a globular protein which has been reported to be rich in lysine and methionine (Bos et al., 2007), and the napin fraction (2S albumin) is a soluble protein containing high levels of glutamine, proline and cysteine (Bos et al., 2007). The relative

fractions of these storage proteins vary between rapeseed cultivars, with albumin (2S) levels ranging from 13 to 46% (Bos et al., 2007).

A) Protein Digestibility

Canola/rapeseed proteins, once digested into small peptides and amino acid components, are absorbed and metabolized as any other source of dietary protein. Dietary proteins are used in the daily maintenance of whole body protein content; as proteins are turned over, amino acids are oxidized, urea produced and nitrogen excreted. The rate of protein metabolism varies throughout the day and depends on diet composition and metabolic state of the individual. Amino acids required for the body to use as building blocks in endogenous protein production are normally achieved via the ingestion of food proteins. The process that all proteins undergo includes gastrointestinal digestion where dietary proteins are broken down into individual amino acids. Peptides and amino acids are then absorbed and transferred via the circulation to body organs for use in various metabolic pathways and production of structural proteins and enzymes. Approximately 30 to 40% of dietary nitrogen from dietary protein is used in the production of energy via anabolic pathways, with 17 to 25% of dietary nitrogen lost via oxidative metabolism (Young, 2001).

A recent study by Bos et al. (2007) examined in humans the digestibility and utilization of rapeseed protein using ¹⁵N-labeled rapeseed protein. In this study, five subjects were equipped with an intestinal tube at the jejuna level of the intestine and seven other at ileal level. All subjects ingested a mixed meal containing 27.3 g of ¹⁵N-labeled rapeseed protein which had a total energy content of 700 kcal. Dietary nitrogen kinetics was quantified in postprandial intestinal fluid, urine and blood samples. There was dietary nitrogen from undigested protein from both the 12S and 2S fractions at the ileal level of the intestine. Real ileal digestibility was $84 \pm 8.8\%$ on average, and the net postprandial protein utilization was $70.5 \pm 9.6\%$ (Bos et al., 2007). The real ileal digestibility was lower than other plant proteins in humans. Part of the reason for this is that rapeseed protein fractions are particularly resistant to hydrolysis (Bos et al., 2007). However, the overall net postprandial protein utilization of rapeseed protein of 70.5% was comparable to the lower range of other legume proteins and higher than wheat protein (Bos et al., 2007). Comparatively, soybean protein ingestion produces lower fecal nitrogen losses but higher urinary nitrogen losses than rapeseed protein. The findings of this study are in close agreement to studies of rapeseed meal done in pigs (Schone, 1992; Saur et al., 1986; Imbeah et al., 1988).

The specific protein digestibilities of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) were tested in a rat study sponsored by ADM/Burcon NutraScience conducted at Iowa State University (PI: R. MacDonald, 2008). Twenty-one Sprague Dawley rats were randomly assigned to each of three treatment groups (n=7/group) and fed either a diet containing zero protein, 10% protein from Supertein™ or 10% protein from Puratein® for 7 days. According to this

study, the napin-rich protein isolate (Supertein™) and cruciferin-rich protein isolate (Puratein®) had a true protein digestibility of 94% and 93%, respectively.

These values are indicative of good protein digestibilities of Supertein® and Puratein™ and are similar to those reported for wheat protein (91%), soy protein (95%) and cow's milk (95%) (Schaafsma, 2000). Since plant proteins are known to be less digestible than animal source proteins, it is then likely that processing improved their digestibility (Delisle, 1983).

B) Protein Biological Value

The amino acid profiles combined with the digestibility data allowed the calculation of the PDCAAS values of 0.61 (61%) and 0.64 (64%) for the napin-rich protein isolate (Supertein™) and the cruciferin-rich protein isolate (Puratein®), respectively (Table 12). As required for nutrition labeling purposes by the Code of Federal Regulations (21 CFR 101.9(7)ii), these values were calculated using the WHO/FAO standards from 1989 (WHO, 1989/FAO, 1990). The limiting amino acids in the napin-rich protein isolate (Supertein™) are leucine, phenylalanine, tyrosine and threonine; with phenylalanine-tyrosine being the most limiting. The cruciferin-rich protein isolate (Puratein®) is limited by the amount of lysine.

**Table 12. Calculated PDCAAS Values of the Napin-Rich
and Cruciferin-Rich Canola Protein Isolates
Supertein™ and Puratein®**

	WHO/FAO 1989 mg/g protein reference values (2-5 years old)	Supertein™		Puratein®	
		Amino acid (mg/g protein)	AAS (mg AA/ reference AA)	Amino acid (mg/g protein)	AAS (mg AA/ reference AA)
Histidine	19	35.4	1.865423	25.4	1.335221
Isoleucine	28	30.21	1.078826	44.4	1.586046
Leucine	66	60.1	0.910137	82.6	1.252238
Lysine	58	73.8	1.271775	39.7	0.684745
Methionine + Cysteine	25	68.4	2.734177	34.3	1.373418
Phenylalanine + Tyrosine	63	40.7	0.646611	89.2	1.416516
Threonine	34	31.8	0.935829	37.1	1.090531
Tryptophan	11	13.8	1.255361	20.1	1.822018
Valine	35	42.1	1.203354	54.9	1.568716
Limiting AAS			0.646611		0.684745
True Digestibility			0.94		0.93
PDCAAS (%)			60.7814		63.6813

$$PDCAAS \% = \frac{\text{mg of limiting amino acid in 1 g test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times \text{fecal true digestibility} \times 100$$

The PDCAAS scoring system has been updated in the FAO/WHO/UNU guidelines for 2002, altering the reference amount of specific amino acids and also dividing the requirement by age groups of children 1-2 years and 3-10 years (WHO/FAO, 2002). With the 2002 updated adjustments, the PDCAAS values for 1-2 year olds for Supertein™ and Puratein® will be 0.83 (83%) and 0.71 (71%), respectively; and for 3-10 year old the PDCAAS values will be 0.92 (92%) for Supertein™ and 0.77 (77%) for Puratein®. The newer FAO/WHO/UNU guidelines with updated reference values improves the PDCAAS values for these canola protein isolates. However, these improved values are not currently considered from the regulatory standpoint and are only reported here for academic purposes.

In relation to other indicators of protein quality, the protein efficiency ratio (PER) of rapeseed meal has been reported as 2.19 (Delisle, 1984). In a separate study, specific examination of rapeseed protein contained in flour and in the 12S and 2S fractions was examined and compared to that of casein. The authors found that the 12S fraction had the highest weight gain and food intake of the products, but a lower PER. Weight gain,

however, was significantly lower in animals consuming rapeseed protein in comparison to the casein group (see Table 13). These data show not only that the protein quality relative to rapeseed meal can be improved by processing, but also that in comparison to casein, food intake and weight gain are significantly reduced when feeding rapeseed protein. This subject will be further discussed in section V1b as part of the toxicological evaluation of Supertein™ canola protein isolate.

Table 13. Comparative PER Value for Rapeseed Protein Isolates

	Weight Gain (g)	Food Intake (g)	PER
Rapeseed protein flour	50.2 ^c	178.1 ^h	2.64 ^j
2S Fraction	54.5 ^{bc}	203.3 ^g	2.49 ^j
12S Fraction	65.5 ^c	254.0 ^f	2.21 ^k
Casein	102.7 ^a	278.3 ^e	3.23 ^l

Adapted from Delisle et al., 1983. Values with different superscripts are significantly different (p<0.05).

V - Basis for GRAS Affirmation

A) Safety Overview

1) History of Consumption

Ancient civilization in Asia and along the Mediterranean recorded hundreds of years ago the use of rapeseed oil in oil lamps for illumination and later as cooking oil (Shahidi, 1990). Early records indicate that rapeseed was cultivated in India over 3,000 years ago. It was then introduced to China around the time of Christ. Although its cultivation was started in the 13th century in Europe, its industrial use was not widespread until the superior quality of the oil as a lubricant was realized. The consumption of rapeseed products in the Western world is more recent and has been limited chiefly to the use of the oil for cooking and salads and as canola meal in the livestock industry. Edible rapeseed oil extracts were first put in the market in 1956-57, but these suffered from several unacceptable characteristics. Rapeseed oil had a distinctive taste and a disagreeable greenish color due to the presence of chlorophyll. It also contained a high concentration of erucic acid, a compound known to cause heart lesions in experimental animals. The rapeseed meal was not particularly appealing either to livestock, due to high levels of sharp-tasting toxic compounds called glucosinolates.

All of these limitations for the consumption of rape products have been practically eliminated by significant genetic improvements of rape, particularly in regard to the high content of erucic acid and glucosinolates. Plant breeders in Canada, where rapeseed had been grown since the 1940's, worked extensively to improve the quality characteristics of the plant. In the 1960's, Canadian scientists

used selective breeding to develop rapeseed varieties low in erucic acid. During 1974-1978, a rapeseed variety, low in both erucic acid and glucosinolates, was developed and named canola. Today, the most important and common canola varieties come from either *Brassica napus* or *Brassica campestris*. Since then, rapeseed oil, low in erucic acid, was recognized as GRAS in 1985 by the U.S. FDA (21 CFR 184.1555(c)) and became a commonly used oil for cooking and salads. This GRAS status has been later extended to canola oil from *Brassica juncea*. Today it is also known that technological processes to manufacture rapeseed products eliminate further significant amounts of anti-nutritional factors. For example, isolation of canola proteins has been found to eliminate up to 95% of glucosinolates, 92% of phytic acid and 100% of tannic acid (Mansour, 1993).

The use of canola meal, containing about 35% canola protein, has been a common practice around the world in livestock production for many years in a broad range of animal species. Particularly after the development of low erucic acid/low glucosinolate rapeseed (canola) in the mid-1970s, canola meal has been used extensively in poultry, pig, cattle and fish diets. The inclusion levels depend on the animal specie and physiological state. In poultry diets, inclusion levels of canola meal range from 5% in chick starters to 20% in turkey growers. In pigs, canola meal in the diet is used at levels from 5% in pig starters to no-limit in hog grower/finisher and during gestation. Recommended maximum inclusion levels of canola meal in cattle diets is "no limit", including calf, dairy and beef feedlot. In aquaculture, canola meal is used in salmon/trout diets (inclusion level 20%), catfish (inclusion level 30%), tilapia (inclusion level 25%) and prawns (inclusion level 15%). In compliance with the corresponding AAFCO definition of this feed ingredient (Product 71.77, canola meal), this canola meal contains less than 30 µmol/g of GSLs, a maximum of 12% crude fiber and the oil component less than 2% erucic acid (Official Publication, Association of American Feed Control Officials Incorporated, 2001). Typical composition of the canola meal (not specified by AAFCO) consists of 35% crude protein (N X 6.25%), 3.5% fat, 6.1% ash, 12% crude fiber, 4% phytic acid and 16 µmol/g of GSLs (Hickling, 2001).

The use of canola/rapeseed protein as human food has been suggested since the early 1980s (Sosulski, 1982). However, its early use has been constrained by technological limitations for the production of good quality protein isolates, particularly in relation to acceptable organoleptic properties and to possession of low levels of anti-nutritional factors such as glucosinolates and erucic acid. Thus, within the last 10 years the consumption of canola protein as a food ingredient has been limited to only a few food products manufactured and marketed on a very low scale in Canada and to some degree in Japan and the U.S.A.. The majority of available products in the market contain canola protein hydrolysates added as an ingredient to products such as processed meats (Olymel Co., Lafleur Co., Canada), cheeses (Olymel Co., Canada), partially prepared turkey main dish dinners (Maple Leaf, Canada), pizza (Casa di Mama, Dr. Oetker Co., Canada), stuffing cooking ingredients (Grissol Co., Canada), beef base cooking ingredient (Oxo, Unilever, Canada) and bagels (Crunch

Frenzi, Culinor Inc., Canada). Other products contain canola seeds as a component of seasoning blends (Lemon Pepper Seasoning, Hy's of Canada) or as a component of spices (Shichimi, Japan). Food product companies in Canada also offer canola seeds for food use (Italco Food Products, Inc.). In the U.S.A., hydrolyzed canola protein has been added to crackers (Beta Brands, U.S.A.) (MINTEL gn timer, database).

In the context of the history of consumption of canola products in the human diet (except canola oil), it also needs to be considered that many other edible plant products of the Brassicaceae family containing the same anti-nutritional factors as rapeseed, such as broccoli (47-121 mg GSL/100g), cauliflower (14-208 mg GSL/100g), cabbage (39-70 mg GSL/100g), turnip (99-230 mg GSL/100g), radish (44-252 mg GSL/100g), etc. have been safely consumed for centuries, and some of them are now recognized as having desirable health benefits.

2) Literature Review - Overview

Most safety studies have been focused on determining the feasibility of use and maximum tolerable limits of either rapeseed meal or rapeseed protein concentrates in different animal species such as poultry, swine, ruminants and fish (Burel et al., 2000). Based on this, recommended dietary inclusion levels are those described in the previous Section V-A, 1. Main observed deleterious effects at high inclusion levels have included impaired growth, reduced appetite, alterations in thyroid metabolism and reduced mineral bioavailability. These negative effects have been attributed to the presence of chiefly high levels of glucosinolates, erucic acid and/or phytates (Anderson, 1976; Rabot, 1993). Adverse effects in humans has only been observed circumstantially as an allergic reaction by dust inhalation of one worker at a feed processing plant in Europe (Monsalve, 1997).

It is also clear in all these studies that negative effects are related to the levels of anti-nutritional factors in the diets. Studies using rapeseed products have shown more deleterious effects, while studies using genetically improved canola products containing low levels of anti-nutritional factors have shown practically no adverse impact (Loew et al., 1976). In addition, modern technologies used in processing have practically eliminated the majority of the remaining anti-nutritional factors in canola such as glucosinolates, phytates and tannates (Mansour et al., 1993). For example, the levels of glucosinolates, erucic acid and phytates in the ADM/Burcon canola protein isolates (Puratein® and Supertein™) are much lower than the levels of these compounds in rapeseed products tested in the published literature in which negative effects have been reported. For instance, if traditional rapeseed products were used in the studies, traditional rapeseed meal contains 100-250 µmol/g of GSL, while canola contains only 18-30 µmol/g. Similarly, the erucic acid content in the oil of traditional rapeseed is 22-60%, in canola it is now less than 2%.

In the published literature previous to 2009, there were only two identified toxicological feeding studies in rats and dogs which addressed the safety of protein containing rapeseed products; specifically, rapeseed protein concentrate flours (Loew et al., 1976) and a rape protein isolate and corresponding extraction residue (Plass, 1992). In Loew's study (Loew et al., 1976), high protein concentrate flours made from rapeseed and containing low levels of glucosinolates were fed to growing dogs and rats for 90 days in semisynthetic diets at levels of 20 and 40% (to provide about 10 and 20% protein, respectively). Control groups received only casein. Only a low order of antithyroid activity was detected in the flours used. Measure of thyroid ¹³¹I uptake, serum thyroxine concentration, thyroid weights and histology remained essentially normal. In the rats, serum glutamic-pyruvic transaminase activity was decreased and packed cell volume and erythrocyte counts increased slightly. No treatment-associated abnormalities were noted in the dogs. It was concluded that the antithyroid effects of the protein containing flours were substantially reduced compared to previous studies using other rapeseed products which probably contained high levels of glucosinolates.

In Plass' study (Plass, 1992), the protein isolate was fed to rats at levels of 2.5, 5 and 10% for four weeks. Body weight gains and food intake were recorded weekly. Clinical chemistry analyses, hematology, urinalysis, organ weights and histology were performed terminally. All animals appeared healthy during the feeding period. No treatment-related differences in appearance or behavior were observed. Furthermore, there was no effect on growth or goitrogenic activity. Abnormalities in the liver and kidney were noted at the 10% protein isolate level, but similar observations were also made in the 10% casein control group. The levels of alanine aminotransferase (ALAT) decreased in animals fed the test material but a similar change was also experienced by the casein fed group. The GSL levels of the test materials, rape protein isolate and rapeseed extraction residue, were low (less than 10 µg/g) and phytate levels were not reported.

Since there were no studies available on the safety of specifically the canola/rapeseed protein isolates Puratein® and Supertein™, ADM/Burcon NutraScience conducted a series of toxicological, allergenicity and genotoxicity studies to assess the safety of these products. The results and interpretation of these investigations recently published by Mejia et al. (2009a &b) will be the subject of the following section.

B) ADM/Burcon NutraScience Conducted Toxicological Studies

During 2007-2008, ADM/Burcon NutraScience sponsored several toxicological studies aimed to assess the safety of the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) as food ingredients for human consumption. They included:

- i) A rat 13-week sub-chronic oral toxicity study for the cruciferin-rich protein isolate (Puratein®) conducted by WIL Research Laboratories, Ashland, Ohio.
- ii) A rat 13-week sub-chronic oral toxicity study for the napin-rich protein isolate (Supertein™) conducted by WIL Research Laboratories, Ashland, Ohio.
- iii) Reverse mutation studies for the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) conducted by Notox Laboratories, The Netherlands (subcontracted by WIL Research Laboratories).
- iv) Micronucleus test in bone marrow cell studies conducted by Notox Laboratories, The Netherlands, for the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) (subcontracted by WIL Research Laboratories).
- v) Gene mutation test studies for the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) conducted by Notox Laboratories, The Netherlands (subcontracted by WIL Research Laboratories).
- vi) Potential allergenicity study conducted by the University of Nebraska, Lincoln, Nebraska.

1) 13-Week Subchronic Studies in Rats

1A) 13-Week Oral (Dietary) Toxicity Study of the Cruciferin-Rich Protein Isolate (Puratein®) in Rats (Mejia et al., 2009a).

This study (WIL Research Labs, Study-642002) was conducted in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice Regulations (21 CFR, Part 58), the standard operating procedures of WIL Research Laboratories and in accordance with FDA Redbook 2000 guidelines. A full report, including raw data (6 volumes, 1914 pages) is available electronically on a computer disk (CD) and can be provided upon request. The objective of the study was to evaluate the possible toxic effects of the cruciferin-rich canola protein isolate (Puratein®) when fed as a protein source at various dietary levels to rats for a period of 13 weeks.

The study included four groups of young (\approx 7 weeks old) Sprague Dawley (CrI:CD(SD) rats. Each group consisted of 20 animals/sex/group. The rats in each group were fed ad libitum for 91 days with an AIN-93G-based protein-free diet (Purina Test Diet® 5T6X, PMI Nutrition International) (Reeves et al., 1993; Reeves, 1997) added respectively with, 5%, 10% and 20% Puratein® (test article) or 20% Vitamin-free Casein (VF-Casein) (control article). Diets in all groups were formulated

by means of a computer program to contain similar levels of protein and of all other nutrients. Protein levels were adjusted to 18% in all groups using VF-casein. The same program also assured that all diets met NRC micronutrient requirements. (National Research Council, 1995).

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for 10 animals/sex/group prior to the initiation of dose administration, and for the same animals during study week 12. Ophthalmic examinations were performed during study weeks 2 and 11. Clinical pathology evaluations (hematology and serum chemistry) were performed on the same 10 animals/sex/group on study days 14, 45 and 91-93. Coagulation and urinalysis evaluations were conducted on these same animals prior to the scheduled necropsy. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals in the control and high-dose groups. All gross lesions were examined.

One male from the 5% Puratein® group and one control group female were found dead on study days 23 and 86, respectively. The control group female died of urinary tract obstruction, most likely secondary to calculi. The cause of death of the male could not be determined and was not considered to be test article-related. There were no test article-related effects on body weight gains, food consumption, ophthalmology examinations or functional observational battery parameters which included home cage, handling, open field, sensory and neuromuscular parameters. There were no test article-related effects on coagulation, serum chemistry or urinalysis parameters. There were no test article-related effects on macroscopic findings.

As illustrated in Table 14, slightly higher thyroid/parathyroid weight ratios were noted in the 20% Puratein® treated group males and females.

Table 14. Thyroid/Parathyroid Ratios at Different Levels of the Cruciferin-Rich Protein Isolate (Puratein®) in the Diet (From Table 32 in Original Report)					
<u>MALES:</u>	<u>0% (Control)</u>	<u>5%</u>	<u>10%</u>	<u>20%</u>	<u>% Δ Vs Control</u>
Thyroid/Parathyroid (g)	0.0213 ± 0.00449	0.0202 ±0.00272	0.0228 ±0.00416	0.0252* ±0.00559	+18.3
Thyroid/Parathyroid (g/100g BW)	0.004 ±0.0008	0.004 ±0.0005	0.004 ±0.0008	0.004 ±0.0008	0.0
<u>FEMALES:</u>					
Thyroid/Parathyroid (g)	0.0144 ±0.00349	0.0155 ±0.00398	0.0155 ±0.00301	0.0173 ±0.00410	+20.1
Thyroid/Parathyroid (g/100g BW)	0.004 ±0.0011	0.005 ±0.0014	0.005 ±0.0010	0.006** ±0.0010	+50.0

* p <0.05

** p <0.01

However, there were no correlating histopathologic changes and the values of the ratios in all groups were within the WIL Research Laboratories historical normal control range. In addition, as it can be seen on the table, the statistical significance is not consistent between absolute and relative values in males vs. females, suggesting a random outcome. Therefore, this observation does not have toxicological relevance and was not considered an adverse effect.

It was concluded in this study that daily administrations of the cruciferin-rich protein isolate (Puratein®) as part of dietary admixes at dose levels of 5%, 10% and 20% w/w to Sprague Dawley rats for 13 weeks was very well tolerated. There were no Puratein® related effects on body weights, food consumption, clinical observations, functional observational battery parameters, motor activity assessment, clinical pathology parameters or ophthalmic examinations. There were no test article-related findings noted during the histopathological examination. The only observation suggestive of being test article related was a slightly higher thyroid/parathyroid ratio noted in the 20% cruciferin-rich protein isolate (Puratein®) treated group males and females. Based on the lack of correlating histopathological changes and the fact that absolute and relative thyroid/parathyroid weights were within the WIL historical control range, the effect on thyroid weights was not considered adverse. Therefore, no-observed-effect level (NOEL) for dietary administration of Puratein® to Crl:CD(SD) rats for 13 weeks was 10% w/w whereas the no-observed-adverse-effect level (NOAEL) was 20% w/w, which was equivalent to 11.24 g/kg BW/day for males and 14.11 g/kg BW/day for females.

1B) A 13-Week Oral (Dietary) Toxicity Study of the Napin-Rich Canola Protein Isolate (Supertein™) in Rats (Mejia et al. 2009b).

This study (WIL Research Labs, Study-642001) was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice Regulations (21 CFR, Part 58), the WIL Laboratories Standard Operating Procedures and in accordance with FDA Redbook 2000 guidelines. A full report, including raw data (6 volumes, 1846 pages) is available electronically on a computer disk (CD) and can be provided upon request. The objective of this study was to evaluate the possible toxic effects of the napin-rich canola protein isolate (Supertein™) when fed as a protein source at various dietary levels to rats for a period of 13 weeks.

The study included four groups of young (\approx 7 week old) Sprague Dawley Crl:CD(SD) rats. Each group consisted of 20 animals/sex/group. The rats in each group were fed ad libitum for 91 days with an AIN-93G based protein free diet (Purina Test Diet® 5T6X, PMI Nutrition International) (Reeves et al., 1993; Reeves, 1997) added respectively with 5%, 10% and 20% Supertein™ canola protein isolate (test article) or 20% Vitamin Free Casein (VF-Casein) (Control Article). Diets in all groups were formulated by means of a computer program to contain the same levels of protein and of all other nutrients. Protein levels were adjusted to 18% using VF-casein. The same program assured that all diets met NRC micronutrient requirements (National Research Council, 1995).

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for 10 animal/sex/group prior to the initiation of dose administration, and for the same animals during study week 12. Ophthalmic examinations were performed during study weeks 2 and 11. Clinical pathology evaluations (hematology and serum chemistry) were performed on the same 10 animals/sex/group on study days 14, 45 and 91. Coagulation and urinalysis evaluations were conducted on these animals prior to the scheduled necropsy. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals in the control and high-dose groups. All gross lesions were examined.

One male rat from the 10% napin-rich protein isolate (Supertein™) group was found dead on day 23 of the study. The cause could not be determined from gross and microscopic examination and was considered incidental and unrelated to the exposure to the test article. All remaining animals survived to the scheduled necropsies.

There were no test article-related effects on ophthalmology examination and functional observational battery parameters which included home cage, handling, open field, sensory and neuromuscular parameters. There were no test article-related effects on hematology, serum chemistry and urinalysis parameters. There were no test article-related macroscopic or microscopic findings or changes in organ weights.

Lower body weights and body weight gains, when compared to control values, were observed in the 10% Supertein™ treated males and the 20% Supertein™ treated males and females. Lower food consumption, most notable during the first week of the study, were noted in all groups of Supertein™ treated males, as well as in the 10% and 20% Supertein™ treated females. It was then hypothesized that the lower food intake was probably due to a poor acceptability of the diets.

The potential reasons for the observed lower body weight gains and reduced food intake, particularly at the beginning of the study, in animals consuming diets containing the test article was first investigated by conducting a separate preference or acceptability study of the napin-rich protein isolate (Supertein™) vs. the cruciferin-rich (Puratein®) and casein in rats. This investigation, conducted at Iowa State University (R. McDonald, P.I., 2008; ADM/Burcon internal report), consisted of offering ad libitum choices of diets containing either 20% casein vs. similar levels of each of the protein isolates. Food intake and body weight were monitored. It was found in this study that rats preferred the diet containing casein vs. the protein isolates (Supertein™ or Puratein®). More importantly, that the napin-rich protein isolate (Supertein™) was significantly less preferred than either the cruciferin-rich protein isolate (Puratein®) or casein. This indicated that at least in part, the observed reduced body weight gain in the rats consuming the napin-rich protein isolate (Supertein™) was related to a lower food intake caused by its poor palatability, which led to a lower acceptability. High phytates have been linked to poor palatability of rape seed products. The napin-rich protein isolate, Supertein™, has higher levels of phytates ($\approx 3.5\%$) than the cruciferin-rich protein isolate, Puratein® ($<0.5\%$). It can then be hypothesized that the poor acceptability of the diets for the rats in the 13-week toxicological study of Supertein™ was caused by the presence of phytates. Since phytates in rape products have also been reported to impair the bioavailability of minerals, particularly zinc (Anderson et al., 1976) and that zinc deficiency inhibits food intake and growth (Hambidge et al., 1986), additional blood samples were taken at the end of the 13-week toxicological study to determine plasma zinc concentration in subgroups of animals from each of the four groups of rats. The data is presented in the Table 15.

Table 15. Plasma Zinc Levels in Rats Fed Diets Containing Supertein™		
Group	N	Zinc (µg/L)
1 (0% Supertein™)	6	1,119.5 ± 74.1
2 (5% Supertein™)	6	1,095.5 ± 112.1
3 (10% Supertein™)	6	1,127.5 ± 139.5
4 (20% Supertein™)	6	1,136.2 ± 121.4

Not statistically different (F = 0.87)

As it can be observed on the table, the plasma concentration of zinc was not significantly affected by consuming the napin-rich protein isolate (Supertein™), even at the 20% dietary level. This suggests that zinc bioavailability was not impaired by Supertein™. These results are in agreement with those found by Liu et al. in experimental animals in which phytic acid did not alter mineral bioavailability from a rapeseed concentrate containing 1.26% phytic acid (Liu et al., 1982).

It was concluded in this 13-week toxicological study that daily administrations of Supertein™ as dietary admixes at dose levels of 5%, 10% and 20% w/w to Sprague Dawley rats was very well-tolerated. The only effect of Supertein™ noted at the dietary levels of 10% (males only) and 20% was evidenced by statistically significant lower body weight gains for the entire duration of the study which was probably due to a low palatability of the test material. In spite of lower body weight gains, the mean body weights of all Supertein™ treated groups were within WIL historical control range. The reduction in body weight gains coincided with reduced food consumption (g/kg BW/day) mainly during the first week of the study for the 10% test article treated males and 20% test article treated males and females. Therefore, the lower weight gain in the 10 and 20% test article treated groups was not considered an adverse event. Based on this information, it was estimated that the no-observed-effect level (NOEL) for dietary administration of the napin-rich protein isolate (Supertein™) to Sprague Dawley Crl:CD(SD) rats for 13 weeks was 5% w/w, and the no-observed-adverse-effect level (NOAEL) 20% w/w, equivalent to 12.46 g/kg BW/day for males and 14.95 g/kg BW/day for females.

2) Genotoxicity Studies

Genotoxicity studies for the cruciferin-rich protein isolate (Puratein®) and the napin-rich protein isolate (Supertein™) were conducted by Notox Laboratories, in the Netherlands, in compliance with the Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997), which conforms to the United States Food and Drug Administration Good Laboratory Practice Regulations and the Environmental Protection Agency Good Laboratory Practice Regulations.

i) Reverse Mutation Assays

Cruciferin-Rich Protein Isolate (Puratein®)

The objective of this study was to evaluate the mutagenic activity of the cruciferin-rich Protein Isolate (Puratein®) in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat). A complete internal report of this study is available.

Puratein® was tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and in the *Escherichia coli* reverse mutation assay with a tryptophan-requiring strain of *Escherichia coli* (WP₂uvrA). The test was performed in two independent experiments in the presence and absence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and β -naphthoflavone). The test substance was suspended in ethanol.

In the dose range finding test, Puratein® was tested to concentrations of 5000 μ g/plate in the absence and presence of S9-mix in the strains TA100 and WP₂uvrA. Puratein® precipitated on the plates at the dose level of 5000 μ g/plate. The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decrease in the number of revertants was observed.

Based on the results of the dose range finding test, Puratein® was tested in the first mutation assay at a concentration range of 100 to 5000 μ g/plate in the absence and presence of 5% (v/v) S9-mix in tester strains TA1535, TA1537 and TA98. In an independent repeat of the assay with additional parameters, Puratein® was tested at the same concentration range as the first assay in the absence and presence of 10% (v/v) S9-mix in tester strains TA1535, TA1537, TA98, TA100 and WP₂uvrA. Puratein® precipitated on the plates at the top dose of 5000 μ g/plate. The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decrease in the number of revertants was observed.

Puratein® did not induce a significant dose-related increase in the number of revertant (His⁺) colonies in each of the four tester strains (TA1535, TA1537, TA98 and TA100) and in the number of revertant (Trp⁺) colonies in tester strain WP₂uvrA both in the absence and presence of S9-metabolic activation. These results were confirmed in an independently repeated experiment.

In this study, the negative and strain-specific positive control values were within Notox Laboratory historical control data ranges, indicating that the test

conditions were adequate and that the metabolic activation system functioned properly.

The concentrations analyzed in the formulations were in agreement with target concentrations (i.e. between 99% and 114% of target).

Based on the results of this study, it was concluded that the cruciferin-rich protein isolate (Puratein®) was not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

Napin-Rich Protein Isolate (Supertein™)

The objective of this study was to assess the mutagenic activity of the napin-rich protein isolate (Supertein™) in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat). A complete internal report of this investigation is available.

Supertein™ was tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and in the *Escherichia coli* reverse mutation assay with a tryptophan-requiring strain of *Escherichia coli* (WP₂uvrA). The test was performed in two independent experiments in the presence and absence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and β -naphthoflavone). The test substance was suspended in ethanol.

In the dose range finding test, Supertein™ Canola Protein Isolate was tested up to concentrations of 5000 μ g/plate in the absence and presence of S9-mix in the strains TA100 and WP₂uvrA. Supertein™ precipitated on the plates at the dose level of 5000 μ g/plate. The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decreases in the number of revertants was observed.

Based on the results of the dose range finding test, Supertein™ was tested in the first mutation assay at a concentration range of 100 to 5000 μ g/plate in the absence and presence of 5% (v/v) S9-mix in tester strains TA1535, TA1537 and TA98. In an independent repeat of the assay with additional parameters, Supertein™ was tested at the same concentration range as the first assay in the absence and presence of 10% (v/v) S9-mix in tester strains TA1535, TA1537, TA98, TA100 and WP₂uvrA. Supertein™ precipitated on the plates at the top dose of 5000 μ g/plate. The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decrease in the number of revertants was observed.

Supertein™ did not induce a significant dose-related increase in the number of revertant (His⁺) colonies in each of the four tester strains (TA1535, TA1537, TA98 and TA100) and in the number of revertant (Trp⁺) colonies in tester strain WP₂uvrA both in the absence and presence of S9-metabolic activation. These results were confirmed in an independently repeated experiment.

In this study, the negative and strain-specific positive control values were within Notox Laboratory historical control data ranges, indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

The concentrations analyzed in the formulations were in agreement with target concentrations (i.e. between 88% and 115% of target).

Based on the results of this study, it was concluded that the napin-rich protein isolate (Supertein™) was not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

ii) Micronucleus Test in Bone Marrow Cells

Cruciferin-Rich Protein Isolate (Puratein®)

Puratein® was tested in the Micronucleus Test in mice to evaluate its genotoxic effect on erythrocytes in bone marrow. A full internal report of this study is available.

The test substance (Puratein®) was suspended in Milli-Q water. In this study, male animals were dosed once by oral intubation with a vehicle control (Milli-Q water) or with 2000 mg Puratein® (two dose groups at 24 and 48 hours sampling time) per kg body weight. A positive control group was dosed with 50 mg/kg body weight of cyclophosphamide (CP). In total 4 dose groups were used, each consisting of 5 animals. No treatment related mortality and clinical signs were noted in all animals treated with Puratein® or in the control animals.

Bone marrow of the groups treated with Puratein® was sampled 24 or 48 hours after dosing. Bone marrow of the negative and positive control groups was harvested 24 and 48 hours after dosing, respectively.

The concentrations analyzed in the formulations of Puratein® were in agreement with target concentrations (i.e. between 100% and 114% of target).

No increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals treated with Puratein®.

The incidence of micronucleated polychromatic erythrocytes in the bone marrow of all negative control animals was within the historical solvent control data range. Cyclophosphamide, the positive control substance, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes. Hence, both criteria for an acceptable assay were met.

The groups that were treated with Puratein® showed no decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, which reflects a lack of toxic effects of this compound on erythropoiesis. The groups that were treated with cyclophosphamide showed an expected decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, demonstrating toxic effects on erythropoiesis.

It was concluded in this study that the cruciferin-rich protein isolate (Puratein®) was not clastogenic in the micronucleus test under the experimental conditions used in the investigation.

Napin-Rich Protein Isolate (Supertein™)

Supertein™ was tested in the Micronucleus Test in mice, to evaluate its genotoxic effect on erythrocytes in bone marrow. A full internal report of this investigation is available.

The test substance (Supertein™) was suspended in Milli-Q water. In this study, male animals were dosed once by oral intubation with a vehicle control (Milli-Q water) or with 2000 mg Supertein™ (two dose groups at 24 and 48 hours sampling time) per kg body weight. A positive control group was dosed with 50 mg/kg body weight of cyclophosphamide (CP). In total 4 dose groups were used, each consisting of 5 animals. No treatment related mortality and clinical signs were noted in all animals treated with Supertein™ or in the control animals.

Bone marrow of the groups treated with Supertein™ was sampled 24 or 48 hours after dosing. Bone marrow of the negative and positive control groups was harvested 24 and 48 hours after dosing, respectively.

The concentrations analyzed in the formulations of Supertein™ were in agreement with target concentrations (i.e. between 89% and 106% of target).

No increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals treated with Supertein™.

The incidence of micronucleated polychromatic erythrocytes in the bone marrow of all negative control animals was within the historical solvent control

data range. Cyclophosphamide, the positive control substance, induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes. Hence, both criteria for an acceptable assay were met.

The Supertein™ treated group showed no decrease in the ratio of polychromatic to normochromatic erythrocytes compared to vehicle controls at the 24 hours sampling time, indicating lack of adverse effect of this substance on erythropoiesis. At 48 hours sampling time, only two out of five animals (numbers 11 and 15) in the Supertein™ group treated with 2000 mg/kg body weight showed a clear decrease in the ratio of polychromatic to normochromatic erythrocytes, compared to the vehicle controls. This observation is not related to chromosomal aberration and only suggested a potential adverse effect on erythropoiesis; a possibility not supported by our 13-week rat feeding study of Supertein™ that showed no alteration on hematological parameters (Mejia et al., 2009b). In addition, the available literature on the toxicological effects of rape protein products does not indicate any decrease in erythropoiesis in rats and dogs (Loew et al., 1976) and in rats (Plass et al., 1992).

The group treated with cyclophosphamide showed the expected decrease in the ratio of polychromatic to normochromatic erythrocytes in all animals compared to vehicle control animals, indicating a clear adverse effect on erythropoiesis.

It was concluded in this genotoxic evaluation that the napin-rich protein isolate (Supertein™) was not clastogenic in the micronucleus test under the experimental conditions used in the study.

iii) Gene Mutation Test with Mouse Lymphoma Cells

Cruciferin-Rich Protein Isolate (Puratein®)

The purpose of this study was to evaluate the mutagenic activity of Puratein® in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat) based on the induction of forward mutations at the thymidine-kinase locus (TK-locus). A full internal report of this investigation is available. The test was performed in two independent experiments in the absence and presence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and β -naphthoflavone). In the first experiment, Puratein® was tested up to concentrations of 1000 μ g/ml in the absence and presence of 8% (v/v) S9-mix. The incubation time was 3 hours. In the second experiment, Puratein® was again tested up to concentrations of 1000 μ g/ml, but in the absence and presence of 12% (v/v) S9-mix. The incubation times were 24 hours and 3 hours for incubations in the absence and presence of S9-mix, respectively.

In the mutation assays, no toxicity was observed even when Puratein® was tested up to the precipitating dose level of 1000 µg/ml.

Mutation frequencies in cultures treated with positive control chemicals were increased by 14- and 9.5 fold by Methyl Methanesulfonate (MMS) in the absence of S9-mix, and by 16- and 13-fold for CP in the presence of S9-mix. It was therefore concluded that the test conditions, both in the absence and presence of S9-mix, were appropriate and that the metabolic activation system (S9-mix) functioned properly.

In the absence of S9-mix, Puratein® did not induce a significant increase in the mutation frequency in the first experiment. This result was confirmed in an independent repeat experiment with modifications in the duration of treatment time.

In the presence of S9-mix, Puratein® did not induce a significant increase in the mutation frequency in the first experiment. This result was confirmed in an independent repeat experiment with modifications in the concentration of S9 for metabolic activation.

The concentrations analyzed in the formulations were in agreement with target concentrations (i.e. between 90% and 102% of target).

It was concluded in this investigation that the cruciferin-rich protein isolate (Puratein®) was not mutagenic in the mouse lymphoma L5178Y test system under the experimental conditions used in the study.

Napin-Rich Protein Isolate (Supertein™)

The objective of this experiment was to evaluate the mutagenic activity of Supertein™ in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat) based on the induction of forward mutations at the thymidine-kinase locus (TK-locus). The corresponding internal report is available. The test was performed in two independent experiments in the absence and presence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and β-naphthoflavone). The test substance was suspended in ethanol.

In the first experiment, Supertein™ was tested up to concentrations of 2000 µg/ml in the absence and presence of 8% (v/v) S9-mix. The incubation time was 3 hours. In the second experiment, Supertein™ was again tested up to concentrations of 2000 µg/ml, but in the absence and presence of 12% (v/v) S9-mix. The incubation times were 24 hours and 3 hours for incubations in the absence and presence of S9-mix, respectively.

In the mutation assays, no toxicity was observed, even when Supertein™ was tested up to the precipitating dose level of 2000 µg/ml.

Mutation frequencies in cultures treated with positive control chemicals were increased by 24- and 9.0-fold by Methyl Methanesulfonate (MMS) in the absence of S9-mix, and by 18- and 13-fold for CP in the presence of S9-mix. It was therefore concluded that the test conditions, both in the absence and presence of S9-mix, were appropriate and that the metabolic activation system (S9-mix) functioned properly.

In the absence of S9-mix, Supertein™ did not induce a significant increase in the mutation frequency in the first experiment. This result was confirmed in an independent repeat experiment with modifications in the duration of treatment time.

In the presence of S9-mix, Supertein™ did not induce a significant increase in the mutation frequency in the first experiment. This result was confirmed in an independent repeat experiment with modifications in the concentration of the S9 for metabolic activation.

The concentrations analyzed in the formulations were in agreement with target concentrations (i.e. between 88% and 115% of target).

It was concluded in the investigation that the napin-rich protein isolate (Supertein™) was not mutagenic in the mouse lymphoma L5178Y test system under the experimental conditions used in the study.

3) Potential Allergenicity Study of the Cruciferin-Rich Protein Isolate (Puratein®) and the Napin-rich Protein Isolate (Supertein™)

Allergic reactions from the ingestion of canola/rapeseed seed based ingredients have not been described in the scientific literature. However, allergies have been reported to related *Brassicaceae* species, particularly mustard and other proteins containing 2S albumin fractions (napins) in its composition. Mustard allergy is practically non-existent in the United States (Robotham et al. 2005). Therefore, in this country mustard labeling is not even required (FALCPA, Food and Drug Administration, 2004). However, because of possible cross reactivity of the canola proteins with other known allergenic proteins, the purpose of this study, conducted by the University of Nebraska (R. Goodman, P.I., 2008), was to investigate the potential allergenicity of Supertein™ and Puratein®. Such potential was evaluated by:

a) Conducting a literature search to review the historical food uses of plant materials

from members of the mustard family and identifying studies indicating allergic risk associated with canola or rape seeds (*Brassica sp.*), foods derived from canola seed or from food sources that are highly similar to canola.

- b) Determining the amino acid sequences of the major proteins in Puratein® and Supertein™ using liquid chromatography/mass spectrometry (LC/MSMS).
- c) Evaluating the potential risks of allergic cross-reactivity of using Puratein® and Supertein™ in foods by comparing their amino acid sequences with known allergenic proteins found in bio-informatic databases.
- d) Testing the stability of proteins in Puratein® and Supertein™ upon the action of pepsin using a standardized assay as an indication of potential risk of food allergy.

A complete internal report of this investigation is available and can be provided upon request.

Our literature review revealed that although the mustard seed is frequently consumed as a spice or condiment, only a small number of scientific articles have been published on mustard allergy. In the United States, there was only one scientific publication that mentioned allergy to mustard (Robotham, 2005). The aim of this study was to characterize the allergens in cashew nut and their potential cross-reactivity with other nuts based on homology. In this investigation, only two of the 40 cashew allergic subjects studied claimed to have food allergy to mustard. However, there was no confirmation of allergy to mustard or canola in any of the patients. Only in Europe, mustard allergy seems to be more common and there are several reports of allergy to mustard or related *Brassica sp.* from studies in France, Spain and Finland.

For the biochemical studies, samples from five production lots and one composite (pooled) lot each for Supertein™ and Puratein® were first analyzed for similarity. The protein content of the dry powdered samples was first determined by nitrogen analysis using LECO instrumentation. Supertein™ contained 85.7% and Puratein® 93.0% protein (N X 6.25, As Is). Expected protein molecular weights for the main proteins were confirmed under both reducing and non-reducing SDS-PAGE. Protein identities of the composite samples (amino acid sequences) were confirmed by LC/MSMS of isolated bands of protein from one-dimensional SDS-PAGE gels. The amino acid sequence results of isolated Supertein™ matched multiple isomers or variants of albumin storage proteins called napins. Isolated bands of Puratein® included multiple isomeric forms of the globulin storage proteins called cruciferins. Based on gel staining patterns and image analysis, napins represented approximately 84% of Supertein™. The cruciferins represented approximately 74% of Puratein®. The Puratein® samples were also found to contain a small amount of detectable bands corresponding to the MW of napins and in fact some peptide fragments of

napins based on highly sensitive LC-MS/MS. Similarly, the napin bands contained some fragments of cruciferins.

The LC-MS/MS sequence data did not provide full-length protein sequences for any of the proteins. However, based on analysis of peptides from each band, using the Scaffold program and data supplied by Colorado State University, who participated in this study, specific NCBI protein sequences entries of full-length or nearly full-length napins and cruciferins were matched with 95% probability of identification. Seven napins (2S albumins) and eight cruciferins (cupins) were found by the program based on peptide analysis. Each of these (15 sequences) were compared to sequences of known allergens using AllergenOnline.com, version 8.0 (with full-length and sliding 80 amino acid segment FASTA comparisons) to find the most similar allergenic proteins. As expected, the highest identity matches were to mustard allergens from *Brassica juncea* and *Sinapis alba*. The seven proteins identified as napins in Supertein™ were between 75 and 94% identical to each other and between 50% and 95% identical to allergens listed as *Sinapis alba* or *Brassica sp.* in AllergensOnline version 8.0 based on overall FASTA alignments. The Puratein® major proteins were cruciferins 11S (12S) globulin seed storage proteins. Seven peptides matched mustard 11S (12S) globulins from *Brassicus napus* while one matched an 11S (12S) albumin from *Raphanus sativum* (another species of mustard) and based on Clustal W align with between 74% and 94% identities. All eight of these proteins matched two cruciferins (cupins) from *Sinapis alba* at from 50% to > 90% identity by full-length FASTA. The multiple cruciferin isomers were greater than 70% identical and they closely matched the sequences of allergenic 11S (12S) storage proteins from *Sinapis alba*, or yellow mustard (sometimes referred to as white mustard).

These protein fractions were then subjected to digestion in pepsin to evaluate their stability based on the protocol in Thomas et al. (2004). A number of potent food allergens have been demonstrated to be stable in this assay and it is thought to be at least partly predictive for potential sensitization. The pepsin was diluted to 10 units of activity per microgram of test protein. The mass ratio of pepsin to protein was approximately 3:1 under these conditions, with the specific lot of pepsin. Separate digestion assays were performed at both pH 1.2 and 2.0 to consider potential differences in stability. The specific pepsin solutions were assayed for activity in an independent test digesting a standard amount of hemoglobin. The end-point of the assay was taken as the time required to achieve approximately 90% reduction in the stained protein band in SDS-PAGE, compared to the time zero, undigested sample. The results of this study demonstrate that the Supertein™ was stable for pepsin digestion at both pH 1.2 and 2.0 up to the time interval of 60 minutes. This stability indicates a heightened risk of this protein sensitizing some individuals through dietary exposure. Whereas Puratein® protein fraction was rapidly digested in pepsin at both pH 1.2 and 2.0. No stable degradation bands were found to result from digestion, however, the minor bands representing napin within

the Puratein® sample, were still visible even after 60 minutes on pepsin degradation, mimicking the stability of the napin bands in the Supertein™ digests.

In summary, the proteins in Supertein™ and Puratein® shared high sequence identities with allergenic mustard seed proteins from yellow and Indian mustard seeds. The identities were sufficiently high to suspect allergic cross-reactivity for those individuals with allergies to yellow or Indian mustards. While both Puratein® and Supertein™ also shared moderately high identities (30%-40%) with seed storage allergens of some tree nuts and legumes, these would not be of additional concern as the identity matches were similar to those found between the allergens of yellow and Indian mustard and the tree nuts and there are no clear reported cases of clinical cross-reactivity between the mustards and nuts. The rapid digestion of cruciferin proteins in Puratein® indicated that it is less likely to cause potential new sensitizations compared to the napins in Supertein™.

C) Potential Adverse Effects of Consuming Cruciferin-Rich Canola Protein Isolate (Puratein®) and Napin-Rich Canola Protein Isolate (Supertein™)

Due to immuno cross-reactivity between canola/rapeseed proteins and mustard proteins, the possibility exists of allergic reactions on mustard sensitive individuals when consuming canola/rapeseed proteins. However, in the United States, this possibility is very low because mustard allergy is not a common event. In this country, only one study in the published literature merely indicates that two of 40 subjects studied claimed food allergy to mustard, but there was no evidence of IgE or demonstrated allergy to canola or mustard for any subject (Robotham et al., 2005). As a reflection of this epidemiological situation, mustard allergy is currently not included in the U.S. allergen labeling regulations (FALCPA, Food and Drug Administration, 2004). However, any concern of allergic reaction to Puratein® or Supertein™ could be minimized, for example, by indicating in the ingredient list of products containing these proteins that they are part of the "mustard family". Other adverse effects are unlikely to occur. The canola protein isolates, Puratein® and Supertein™, have been shown to contain glucosinolates at levels far below those at which toxicity in animals has been reported. Based on toxicity data, the maximum allowed limit of glucosinolate in canola/rapeseed products is <30 µmol/g; the levels of glucosinolates in composite samples of Puratein® is only 1.2 µmol/g, and for Supertein™ 0.8 µmol/g. Undesirable breakdown products of glucosinolates are not present. Erucic acid is not detected in any of the protein isolates, either. Puratein® and Supertein™ canola protein isolates are also devoid of toxic contaminants. In addition, genotoxicity studies using multiple standardized methods have shown that Puratein® and Supertein™ canola protein isolates are in addition not mutagenic and not clastogenic. The literature also indicates that canola proteins are not teratogenic (Sharpe et al., 1975). Sub-chronic 13-week rat studies of Puratein® and Supertein™ also revealed no adverse effects at the highest level tested (Mejia et al., 2009a & b). The slightly higher thyroid/parathyroid ratios observed in animals on the

20% Puratein® diet were not correlated with histopathological findings, their statistical significance was inconsistent between absolute and relative weights, and in-between male and female animals. In addition, these higher values were within the range of WIL Laboratories' historical control values. All these factors indicate that the apparent higher levels of thyroid/parathyroid ratios in the 20% Puratein® group lack biological meaning. The lower weight gain observed in the animals fed the Supertein™ diet is clearly related to a poor acceptability of the diet due to taste. It also needs to be considered that the protein quality of Supertein™ (PDCAAS: 0.61) is lower than that of casein (PDCAAS: 1.0). Therefore, a lower food efficiency, manifested as inferior weight gain, would be expected. This, in fact, occurred in the study reported by Delisle et al., in which rats consuming rapeseed proteins gained less weight than the casein control (Delisle et al., 1983). In sum, the observed lower weight gain in the Supertein™ was due to palatability of the test article and also probably to a lower nutritional quality of the canola protein, but not to safety. Therefore, this cannot be considered as an adverse effect.

D) Estimated Consumption of the Cruciferin-Rich and Napin-Rich Protein Isolates from Proposed Food Categories

The proposed food categories and corresponding levels of addition of the cruciferin-rich protein isolate (Puratein®) and/or the napin-rich protein isolate (Supertein™) are as follows:

<u>Food Category</u>	<u>Up to*:</u>
Powdered egg/egg substitute	60%
Dairy products	5%
Processed meats	2%
Grain products	2%
Fruit & vegetable juices/beverages	10%
Salad dressings	2%
Protein supplement powders	95%
Meal replacement/nutrition bars	50%

* As is basis

Based on these inclusion levels, the potential consumption of Puratein® and Supertein™ was estimated in the U.S. population using the 1999-2004 NHANES databases. Mean consumption values and corresponding 90th percentiles for age groups from 3-6 years, 7-12 years, 12-19 years and > 20 years of age are presented in Table 16. Consumption figures are the sum of protein inclusions from the consumption of foods in the 8 categories. Children below 3 years of age were not considered because the intended use of the canola protein isolates is not as an ingredient in infant foods or infant formulas. A full report, including exposure levels, food categories and specific food items within each category which will be added with the canola protein isolates Puratein® and/or Supertein™, is presented as Appendix 2.

Table 16. Consumption Estimates of Puratein®/Supertein™ in the U.S. Population			
Age Group	n	Mean	90th Pctl.
3-6 years	1908	25.7*	60.9
	1857	(1.3)**	(3.1)
7-12 years	3207	25.8	68.7
	3175	(0.7)	(1.9)
12-19 years	5215	32.2	89.7
	5161	(0.5)	(1.4)
≥ 20 years	12469	20.9	57.9
	12230	(0.3)	(0.8)

Based on 1999-2004 NHANES database

* g/person/day

** g/Kg BW/day

According to the Institute of Medicine (IOM), the RDA for protein for men and women is 0.80 g/Kg BW/day (IOM, 2005). This is equivalent to 56 g/day for a 70 Kg reference person.

Due to insufficient data, a tolerable upper intake level (UL) was not established by the IOM. However, the IOM report indicated that based on the 1994-96, 1998 Continuing Survey of Food Intake by Individuals (CSFII), the highest mean intake of protein from diet for any gender and life stage was estimated as 104 g/d for men aged 19-30 y. For a 70 Kg reference person, this would equate to 1.5 g/Kg BW/day. This group had the highest reported protein intake at the 99th percentile of 190 g/d, equivalent to 2.7 g/Kg BW/day (IOM, 2005).

The estimated daily mean consumption values of the protein isolates presented here are within the highest reported protein intakes reported by IOM in the U.S. population. Furthermore, both the mean consumption values and 90th percentiles for all age groups are far below the NOAEL value of 11.24 g/kg BW/day for the cruciferin-rich protein isolate (Puratein®) and of 12.46 g/kg BW/day for the napin-rich protein isolate (Supertein™).

It also needs to be noted that the estimated mean consumption of the canola/rapeseed protein isolates for children (3-12 years) is about 25 g/person/day. From the nutritional point of view, this represents half of the 50 g of protein Daily Reference Value for children older than 4 years (21 CFR-109.9 (iii)). It could then be assumed that the other 25 g of protein provided by the rest of the diet will complement the protein quality of the canola/rapeseed protein isolates. Therefore, the canola/rapeseed protein isolates, like any other plant derived proteins, will not be detrimental to the overall quality of the children's diet.

VI) Summary of Basis for GRAS Determination

ADM/Burcon NutraScience has determined the Generally Recognized As Safe (GRAS) status of the cruciferin-rich canola protein isolate (Puratein®) and the napin-rich canola protein isolate (Supertein™) based on the following:

- The published toxicological studies by Loew et al. (1976) and Plass et al. (1992) in rats and dogs using testing materials with similar composition to the canola protein isolates Puratein® and Supertein™. In these studies, no toxicologically relevant treatment effects were observed at the highest doses tested.
- The history of safe use of rapeseed/canola meal, containing about 35% canola protein, in animals raised for human consumption, including cattle, swine, poultry and fish.
- The canola protein isolates (Puratein® and Supertein™) are manufactured under Good Manufacturing Practices and meet appropriate food grade specifications. Potential natural toxicants such as glucosinolates and erucic acid, as well as potential contaminants such as pesticide residues, solvent residues, heavy metals, dioxins, aflatoxins, polycyclic aromatic hydrocarbons and acrylamides are either absent (non-detected) or below toxicological and regulatory allowed limits.
- The genotoxicity studies conducted by ADM/Burcon of the canola protein isolates (Puratein® and Supertein™), using standardized methods, show no mutagenicity or clastogenicity.
- The two pivotal sub-chronic toxicological studies of Puratein® and Supertein™ canola protein isolates conducted by Mejia et al. (2009a & b) showing no adverse effect when feeding these substances to rats for 13 weeks up to a level of 20% in the diet. The NOAEL's were, respectively, 20% for both proteins, the highest inclusion level tested.
- The estimated protein consumption data for Puratein® and Supertein™ in the U.S. population is within the mean higher limits of protein intakes reported by the Institute of Medicine (IOM), and both, mean consumption values and 90th percent values are far below (approximately <10X) the NOAEL's of the protein isolates.
- The fact that mustard allergy is not common in North America and, therefore, the risk of allergy produced by cross-reactivity with canola proteins is low.
- The unanimous conclusions reached through scientific procedures, by a panel of experts, qualified by scientific training and experience, that the canola protein isolates Puratein® and Supertein™ are Generally Recognized As Safe (GRAS) for the intended uses when manufactured and used in accordance with current good manufacturing practices (cGMP) and, meeting appropriate food grade specifications (Appendix 1).

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Appendix 1

Expert GRAS Panel Report



**REPORT OF THE EXPERT PANEL ON THE SAFETY AND THE GENERALLY
RECOGNIZED AS SAFE (GRAS) STATUS OF THE INTENDED USES OF
CRUCIFERIN-RICH AND NAPIN-RICH PROTEIN ISOLATES DERIVED FROM
CANOLA/RAPESEED (PURATEIN® AND SUPERTEIN™)**

09 February 2010

INTRODUCTION

ADM convened an Expert Panel (the "Panel") of independent biomedical scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients and food, to conduct an independent, critical and comprehensive evaluation of the available data and information on Cruciferin-rich and Napin-rich protein isolates (Puratein® and Supertein™) produced by ADM/Burcon as part of their partnership. The specific objective was to determine whether the intended uses of ADM/Burcon's Cruciferin-rich and Napin-rich protein isolates derived from canola/rapeseed (Puratein® and Supertein™) are safe and suitable and are Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the following qualified experts: Prof. G. Harvey Anderson, Ph.D. (University of Toronto), Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Prof. Paul Pencharz, M.B., Ch.B., Ph.D., FRCP (University of Toronto), and Prof. Steve Taylor, Ph.D. (University of Nebraska).

ADM conducted a comprehensive search of the scientific literature on canola/rapeseed protein isolates through July, 2008 and made this available to the Panel. ADM also provided information on the identity, manufacturing, specifications, batch analyses, potentially toxic natural contaminants, nutrition and metabolism, intended uses and levels, estimated daily intakes, safety/toxicity data and potential adverse effects, including potential allergenicity of Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates derived from canola/rapeseed. This information was submitted to the Panel as a dossier ("GRAS Affirmation of Cruciferin-Rich and Napin-Rich Protein Isolates Derived from Canola/Rapeseed (Puratein® and Supertein™)"). The Panel, independently and collectively, critically evaluated the dossier and other information deemed appropriate.

Members of the Panel conferred by telephone and then convened in Chicago on 11/12 September, 2008, with technical experts from ADM. Following a critical review and discussion of the materials evaluated and information provided by ADM at the meeting, the Panel went into executive session and unanimously concluded that the intended uses of ADM/Burcon's Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates derived from canola/rapeseed, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications presented in the dossier, are safe and

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suitable and are GRAS based on scientific procedures. Subsequently the Panel was asked by ADM/Burcon to update their evaluation of the published literature up to January 2010 as it relates to the safety of the canola proteins.

A summary of the information evaluated and the basis for the determination that the intended uses of Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates derived from canola/rapeseed, are safe and suitable and GRAS based on scientific procedures are presented below.

BASIS FOR GRAS DETERMINATION

Background

Cruciferin-rich (Puratein®, minimum 80% cruciferin) and Napin-rich (Supertein™, minimum 80% napin) protein isolates derived from canola/rapeseed were developed by ADM/Burcon for use as ingredients in various food categories at levels from 2% in processed meats, grain products and salad dressings to 95% in protein supplement powders. Each protein isolate has unique functional properties and each protein isolate may be used singly or in combination. The Cruciferin-rich isolate contains a minimum of 80% globulin storage proteins called cruciferins with the remainder being the albumin storage protein, napin. The Napin-rich isolate contains a minimum of 80% albumin storage proteins called napins with the remainder being the globulin storage protein, cruciferin.

Manufacturing Process

Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates are derived from canola/rapeseed species low in glucosinolates and erucic acid. The canola/rapeseed species include *Brassica napus*, *Brassica campestris* and *Brassica juncea*. These species are closely related and are similar in appearance and composition, including their low levels of glucosinolates and erucic acid.

The manufacturing process for the protein isolates involves production of the defatted canola/rapeseed meal and isolation of the proteins. The corresponding process flow diagram is presented as Figure 1. Canola/rapeseed is first treated to reduce the formation of undesirable breakdown products from the glucosinolates. The seed is dehulled and then pressed to remove most of the oil resulting in the formation of an oil-reduced press cake which is subsequently defatted by counter-current hexane extraction. The defatted meal is gently desolventized to become the starting material for the canola/rapeseed protein isolate production.

The canola/rapeseed meal contains two major protein fractions, a globulin fraction, represented by cruciferin, and an albumin fraction, represented by napin. Cruciferin is the major protein in the seed (60% of the total protein) and is conformed by six subunits with a total molecular

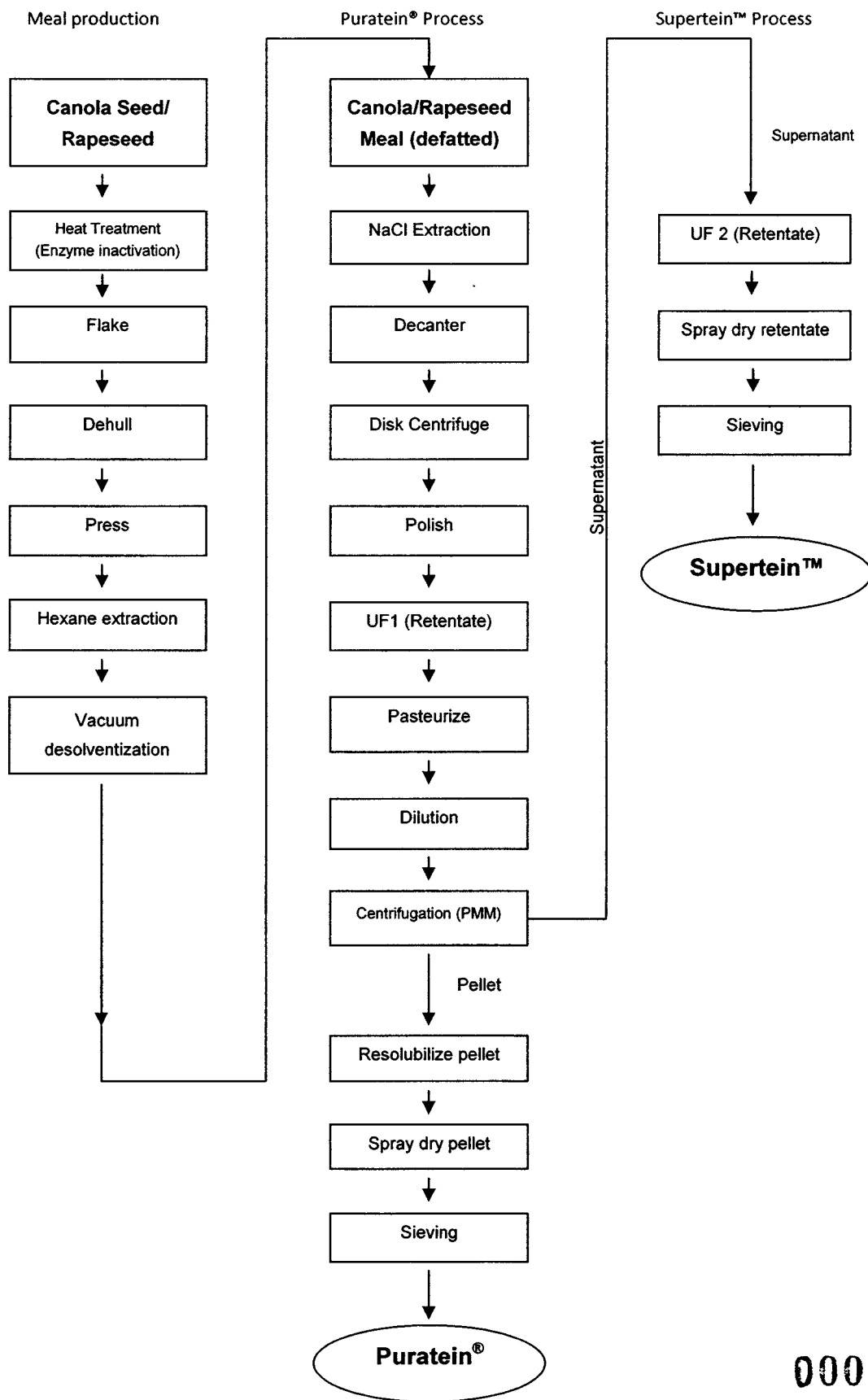


weight of 300 kDa. Napin is a minor protein in the seed (~20% of the total protein) and is composed of two disulfide-linked polypeptides with a molecular weight of 14-14.9 kDa. Protein extraction involves solubilization in NaCl solution, several clarifications and filtrations then concentration using ultra-filtration with suitable membranes which allows the low molecular weight species to pass through (salt, carbohydrates, pigments and anti-nutritional factors). After the first ultra-filtration, the concentrated protein solution contains both cruciferin and napin protein fractions; the contaminants have passed through. The protein solution is pasteurized, cooled and diluted causing the globulin fraction (cruciferin) to precipitate as micelles (protein micellar mass, PMM) which are pelleted out by centrifugation; the pellets are then resolubilized, spray dried and sieved (Puratein®). The supernatant, which contains the albumin protein (napin), is further purified and subjected to a second ultra-filtration, retaining the protein while permitting the contaminants to pass through as permeate. The retentate is spray dried and sieved (Supertein™).

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Fig. 1. Canola/Rapeseed Protein Isolates Production Process Flow Chart



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Product Specifications

ADM/Burcon has established specifications for both products (Tables 1 and 2). Batch analyses confirm that consistent products can be manufactured (Tables 3 and 4). Analyses are also conducted for potential contaminants including pesticides, solvent residues, heavy metals, polycyclic hydrocarbons, dioxins, aflatoxins, acrylamide, glucosinolates, thiocyanates and nitriles, erucic acid, phytates, and phenolic acids. Levels detected are below levels of toxicological concern.

Table 1. Specifications Cruciferin-Rich Protein Isolate (Puratein®)		Analytical Method
Appearance	Fine, brownish- Yellow powder	
Protein (N x 6.25, d.b)	Minimum 90%	AOCS Ba 4e-93
Moisture	Maximum 9%	AOCS Bc 2-49
Ash	Maximum 6%	AOCS Ba 5a-49
Fat (ether method)	Maximum 2%	AOCS Bc 3-49
Glucosinolates	<30 µmol/g	ISO 9167-1
Erucic acid	<0.04%	AM-011 Ver. 5
Heavy metals (Cd, Pb, Hg, As)	<8 ppm	AM-011 Ver. 4
Lead	<0.5 ppm	AM-43 Ver. 1
Microbiological Data		
Standard plate count	Maximum 50,000 cfu/g	BAM Chap. 3
Salmonella	Negative (in 25 g)	BAM Chap. 5
<i>E. coli</i>	Negative (in 40 g)	BAM Chap. 4
<i>Staphylococcus aureus</i>	Negative (in 3 g)	BAM Chap. 12

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Table 2. Specifications Napin-Rich Protein Isolate (Supertein™)		Analytical Method
Appearance	Fine, light yellow powder	
Protein (N x 6.25, d.b)	Minimum 90%	AOCS Ba 4e-93
Moisture	Maximum 9%	AOCS Bc 2-49
Ash	Maximum 6%	AOCS Ba 5a-49
Fat (ether method)	Maximum 2%	AOCS Bc 3-49
Glucosinolates	<30 µmol/g	ISO 9167-1
Erucic acid	<0.04%	AM-011 Ver. 5
Heavy metals (Cd, Pb, Hg, As)	<8 ppm	AM-04 Ver. 4
Lead	<0.5 ppm	AM-43 Ver. 1
Microbiological Data		
Standard plate count	Maximum 50,000 cfu/g	BAM Chap. 3
Salmonella	Negative (in 25 g)	BAM Chap. 5
<i>E. coli</i>	Negative (in 40 g)	BAM Chap. 4
<i>Staphylococcus aureus</i>	Negative (in 3 g)	BAM Chap. 12

Table 3. Analytical Data on Representative Lots of the Cruciferin-Rich Protein Isolate (Puratein®)						
	Lot 1 C300-C28-07	Lot 2 C300-E01-07	Lot 3 C300-E14-07	Lot 4 C300-E24-07	Lot 5 C300-F04-07	Composite JRRRCB- 071107
Protein (N x 6.25, db), %	103	104	104	105	100	102
Moisture, %	7.6	6.6	6.8	7.7	7.4	7.4
Ash, %	3.0	3.0	2.8	3.3	2.9	3.4
Fat, %	0.19	0.18	0.16	0.22	0.24	0.2
Total Glucosinolates, µmol/g	1.4	1.3	1.8	1.1	2.5	1.2
Erucic acid, µg/g*	ND	ND	ND	ND	ND	ND
Heavy metals (Cd, Pb, Hg, As), ppm	0.04	0.07	0.04	0.10	0.02	0.08
Lead, ppm	ND	ND	ND	ND	ND	ND
Std. plate count, cfu/g	1000	800	<100	300	700	2000
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative	Negative	Negative

ND = Not Detected

* LD: 25 µg/g

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**Table 4. Analytical Data on Representative Lots of the Napin-Rich Protein Isolate (Supertein™)**

	Lot 1 C200-C28-07	Lot 2 C200-D25-07	Lot 3 C200-E03-07	Lot 4 C200-E07-07	Lot 5 C200-F04-07	Composite JRRRCA- 071107
Protein (N x 6.25, db), %	92	93	95	94	95	93
Moisture, %	8.3	6.8	6.9	6.2	7.2	7.1
Ash, %	6.01	3.7	3.1	3.8	3.8	4.0
Fat, %	0.25	0.09	0.21	0.21	0.10	0.31
Total Glucosinolates,						
μmol/g	0.9	0.8	0.4	0.4	1.0	0.8
Erucic acid, , μg/g*	ND	ND	ND	ND	ND	ND
Heavy metals						
(Cd, Pb, Hg, As), ppm	0.06	0.07	0.06	0.02	0.03	0.04
Lead, ppm	ND	ND	ND	ND	ND	ND
Std. plate count, cfu/g	<100	<100	<100	200	300	600
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative	Negative	Negative

ND = Not Detected

* LD: 25 μg/g

Functionality, Intended Uses and Use-Levels and Exposure

Each of the canola/rapeseed protein isolates has distinctive ingredient functionality properties. For example, while both can be added to transparent beverages, the Cruciferin-rich protein isolate (Puratein®) has remarkable emulsifying/binding characteristics similar to egg proteins.

Depending on the suitability of each particular food application, either the Cruciferin-rich protein isolate (Puratein®) or the Napin-rich protein isolate (Supertein™), alone or in combination, will be used as food ingredients in the following food categories at the specified levels of addition indicated below. The intended levels of use of the cruciferin-rich (Puratein®) and/or the Napin-rich (Supertein™) protein isolates derived from canola/rapeseed range from 2% in processed meats, grain products, and salad dressings; to 5% in dairy products; 10% in fruit & vegetable juices/beverages; 50% in meal replacement/nutrition bars; 60% in powdered egg/egg substitute products and 95% in protein supplement powders. For nutritional considerations, the nutritional quality of Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates should be reviewed in conjunction with other proteins that may be contained in the formulated product.

Based on the above inclusion levels and using the 1999-2004 NHANES databases, mean estimated daily intakes of Cruciferin-rich (Puratein®) and Napin-rich (Supertein™) protein isolates derived from canola/rapeseed (in grams/person/day and g/kg bw/day) for the U.S. population are: 25.7 (1.3) in 3-6 years olds, 25.8 (0.7) in 7-12 year olds, 32.2 (0.5) in 12-19 year olds and 20.9 (0.3) in >20 year olds. The 90th percentile values are: 60.9 (3.1), 68.7 (1.9),

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89.7 (1.4), and 57.9 (0.8), respectively. According to the Institute of Medicine (IOM), the RDA for protein for men and women is 0.80 g/kg bw/day or 56 g/day for a 70 kg person (Institute of Medicine, 2005). A tolerable upper limit (TUL) was not established by IOM but indicated in the same publication that the highest reported mean intake of protein from the diet has been estimated at 104 grams/day for males aged 19-30 years (1.5 g/kg bw/day for a 70 kg person). This group also had the highest reported protein intake at the 99th. percentile of 190 g/day, equivalent to 2.7 g/Kg BW/day. The estimated mean consumption values for the canola proteins presented here are within the range of protein intake values reported by the IOM for the U.S. population (Institute of Medicine, 2005).

Safety Assessment

History of Consumption

Rapeseed was cultivated in India at least 3000 years ago. It was introduced to China around the time of Christ. It was cultivated in Europe in the 13th century. Rapeseed has been grown in Canada since 1936 and in the United States since the 1940s. The consumption of rapeseed/canola products in the Western world is more recent and has been limited chiefly to the use of oil for cooking and salads and as canola meal in the livestock industry. Edible rapeseed oil extracts were first introduced on a limited scale into the marketplace in 1956-57 but these suffered from several unacceptable characteristics. Rapeseed oil had a distinctive taste and a greenish color due to the presence of chlorophyll. It also contained a high concentration of erucic acid which had been reported to cause heart lesions in experimental animals. Livestock did not find rapeseed meal appealing apparently due to the presence of sharp-tasting potentially toxic glucosinolates. Selective breeding has resulted in the development of rapeseed low in erucic acid and glucosinolates, now known as canola. Advances in technology have resulted in the virtual elimination of anti-nutritional factors. The most important and common canola varieties come from *Brassica napus*, *Brassica campestris*, and *Brassica juncea*. Low erucic acid canola oil was recognized as GRAS in 1985. Canola/rapeseed proteins have not been widely used in human foods, although Sosulski (1983) suggested their use since 1983.

Animal Studies

Loew, et al. (1976) reported on the safety of dietary rapeseed protein concentrate flours (RSF) in male and female Wistar rats and male and female beagle dogs fed the flours for 92 consecutive days. "In each experiment [rat, dog], 20 and 40 % of the total dietary protein was supplied by the rapeseed flour (RSF); the balance was supplied as casein. Control groups received only casein." "Only a low order of anti-thyroid activity was detected in the flours used." "No treatment-associated abnormalities were noted in the dogs." "The flours used in the present study in growing dogs and rats elicited only slight histological changes and no



significant alterations in ^{131}I uptake of thyroxine concentrations in serum." (Loew, et al. 1976). There were no consistent, treatment-related, dose-dependent, statistically significant adverse effects on any of the parameters reported. The No-Observed-Adverse-Level (NOAEL) in both rats and dogs was 40%, the highest concentration tested.

Plass, et al. (1992) fed male, out bred Shoen: WIST/II rats 0, 2.5, 5 or 10% rapeseed protein isolate (RPI), 2.5, 5 or 10% rapeseed extraction residue (RER) or 10% casein. "Microscopic examinations revealed abnormalities in liver and kidneys of animals at the 10% RPI, RER and casein levels. The absolute liver weights were reduced in the 5 and 10% RER groups and in the 10% RPI group. The relative kidney weights were reduced at all RER levels and in the 2.5% RPI group. Antithyroid activity of the rapeseed products was not noted." "As a preliminary evaluation it is concluded from these results that feeding RPI up to a dietary level of 5% and RER at a level of 2.5% for 4 weeks did not produce any changes of toxicological significance in male rats." (Plass, et al, 1992). There were no consistent, treatment-related, dose-dependent, statistically significant adverse effects on any of the parameters reported. Although the authors report a NOAEL for RPI (without using this acronym) of 5%, this level is more appropriately a No Observed Effect Level (NOEL) since liver effects were reported at 10% but these were not adverse and not related to treatment since they were seen in control rats. Therefore, a NOAEL of 10% is more appropriate.

The subchronic oral toxicity of ADM/Burcon's Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) was respectively evaluated in male and female Sprague-Dawley Crl: CD (SD) rats (Mejia et al, 2009a; Mejia et al, 2009b). The animals received the protein isolates as dietary admixtures for 90 consecutive days at levels of 0, 5, 10, or 20%; controls received 20% vitamin-free casein diet. All diets were balanced, that is, they were adjusted to contain similar levels of protein and all other nutrients. Protein levels were adjusted to 18% in all groups using vitamin free-casein. These studies were conducted consistent with GLP Guidelines and FDA Redbook 2000 guidelines.

In the Cruciferin-rich protein isolate (Puratein®) study, the consumption of the test article (Puratein®) for 13 weeks was well tolerated (Mejia et al, 2009a). There were no Puratein® related adverse effects on body weights, food consumption, clinical observations, functional observational parameters, motor activity, clinical pathology or ophthalmic examinations. There were no test article-related findings noted during the histopathology examination. The only observation suggestive of being test article-related was a slightly higher thyroid/parathyroid weight ratio noted in the male and female rats consuming 20% Cruciferin-rich protein isolate (Puratein®). Based on the lack of correlating histopathological changes and the fact that absolute and relative thyroid/parathyroid weights were within the testing laboratory historical control range, this observation was not considered adverse. Therefore, the NOAEL for dietary administration of Puratein® for 13 weeks to rats was 20% w/w, the highest level tested; equivalent to 11.24 g/Kg BW/day for males and 14.11 g/Kg BW/day for females.



In the toxicological study of the Napin-rich protein isolate (Supertein™), there were no test article-related effects on ophthalmology examinations and functional observational parameters which included home cage, handling, open field, sensory and neuromuscular parameters (Mejia et al.2009b). In addition, there were no test article-related effects on haematology, serum chemistry and urinalysis parameters. There were no test article-related macroscopic or microscopic findings . However, lower body weights and body weight gains, when compared to control values, were observed in the 10% Supertein™ treated males and 20% Supertein™ treated females. Lower food consumption, most notable during the first week of the study, were noted in all groups of Supertein™ treated males , as well as in the 10% and 20% Supertein™ treated females. The potential reasons for the observed lower body weights and reduced food intake in animals consuming Supertein™ was first investigated by conducting a separate palatability study of the Napin-rich protein isolate (Supertein™) vs. the Cruciferin-rich protein isolate (Puratein®) and casein in rats. This investigation conducted at Iowa State University (R. McDonald, P.I. 2008, internal ADM/Burcon Report), consisted of offering *ad libitum* choices of diets containing either 20% casein vs. similar levels of each of the protein isolates. Food intake and body weights were monitored. It was found in this study that the animals preferred the diet containing casein vs. the protein isolates (Supertein™ or Puratein®). More importantly , that the Napin-rich protein isolate (Cruciferin) was significantly less preferred than either the cruciferin-rich protein isolate (Puratein®) or casein. This indicated that at least in part, the observed reduced body weight gain in the rats consuming the Napin-rich protein isolate (Supertein™) was related to a lower food intake caused by its poor palatability which led to a lower acceptability. Another considered factor was the relative higher level of phytates in Supertein™ (about 3.5%) vs. Puratein® (< 0.5%). High phytates have been linked to poor palatability of rapeseed products and impairment of mineral bioavailability, particularly zinc (Anderson, et al, 1976). Zinc deficiency in experimental animals also may inhibit food intake and growth (Hambidge et al, 1986). In order to evaluate the effect of consuming the test article on zinc nutrition of the animals, blood samples were taken at the end of the 13 weeks of study in a subgroup of rats to determine plasma zinc concentrations. The plasma concentration of this parameter was not altered by consuming Napin-rich-protein isolate (Supertein™) indicating that zinc was not a limiting factor in the growth performance of the rats. In conclusion, since the lower body weight was associated to a lower food intake (g/Kg/BW/day), particularly at the beginning of the study, and the mean weights of the test article-treated-animals were within the historical control range of the testing laboratory, this observation was not considered an adverse effect of Supertein™. The lower weight gain was most probably due to a lower acceptability of the test material. Therefore, the NOAEL for the dietary administration of Napin-rich protein isolate (Supertein™) to rats for 13 weeks was 20%, the highest level tested. This corresponds to 12.46 g/Kg BW/day for males and 14.95 g/Kg BW for females.



Genotoxicity Studies.

The following genotoxicity tests were conducted with both Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™): reverse mutation assays (*E. coli* and *Salmonella typhimurium*), mouse bone marrow micronucleus test, and gene mutation test in mouse lymphoma cells. All studies were conducted consistent with GLP Guidelines and appropriate OECD Guidelines. It was concluded that ADM/Burcon's Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) were not mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays; not clastogenic in the mouse micronucleus test; and not mutagenic in the mouse lymphoma L5178Y test system.

Allergenicity

Since allergic reactions have been reported following the ingestion of canola/rapeseed and allergies have been reported to related Brassicae species, especially mustard and other proteins containing 2S albumin fractions (napins), the potential allergenicity of Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) was evaluated by Goodman, et al. (2008) at the University of Nebraska (2008, ADM/Burcon Internal Report). Amino acid sequences of protein fractions in Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) were compared to the amino acid sequences of known allergens. Resistance of Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) to pepsin digestion was also evaluated. The proteins in Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™) shared high sequence identities with allergenic mustard seed proteins from yellow and Indian mustard seeds. The identities were sufficiently high to suspect allergic cross-reactivity for those individuals with allergies to yellow or Indian mustard. The rapid digestion of cruciferin proteins in Cruciferin-rich protein isolate (Puratein®) indicated that it is less likely to cause potential new sensitizations compared to the napins in Napin-rich canola protein isolate (Supertein™). Mustard allergy in the US is not common. A literature search revealed that in North America there was only one paper that merely indicated that two of 40 subjects allergic to cashew claimed food allergy to mustard but there was no immunological confirmation to allergy of either mustard or canola (Robotham et al, 2005). As a reflection of this epidemiological situation, mustard allergy is currently not included in the U.S. allergen labelling regulations (FALCPA, Food and Drug Administration, 2004). However, proper labelling could address this issue.

Human Studies

There are no reported human studies on ADM/Burcon's Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™).



Summary

The safety and suitability and the GRAS status of the intended uses of ADM/Burcon Cruciferin-Rich and Napin-Rich Protein Isolates (Puratein® and Supertein™) are based on:

- The published studies of Loew et al. (1976) and Plass et al. (1992) on materials substantially equivalent to Cruciferin-Rich and Napin-Rich Protein Isolates (Puratein® and Supertein™) (Table 5).
- The history of safe use of rapeseed meal in animals raised for human consumption including cattle, pigs, chickens, turkeys and fish;
- The genotoxicity studies conducted by ADM/Burcon of the Cruciferin-rich (Puratein®) and the Napin-rich (Supertein™) canola protein isolates showing no mutagenesis, teratogenicity or clastogenicity.
- The more recently published pivotal sub-chronic (90 day) toxicological studies by Mejia et al (2009a and b) of ADM/Burcon's Cruciferin-rich and Napin-rich Protein Isolates (Puratein® and Supertein™) conducted in male and female Sprague-Dawley rats with NOAELs of 20 % Cruciferin-rich and Napin-rich protein Isolates (Puratein® and Supertein™).



Table 5. Compositional comparison of the rapeseed products used in Plass et al. and Loew et al. studies and in ADM/Burcon studies on Cruciferin-rich protein isolate (Puratein®) and Napin-rich canola protein isolate (Supertein™).

	Plass et al. 1992			Loew et al. 1976		ADM/Burcon	
	REM	RPI	RER	Flour of Experiment 1	Flour of Experiment 2	Supertein	Puratein
Protein (%), N*6.25	40.2	94.2-96.7	28.8	63.4	48.5	86.7	94.9
Fat (%)	not reported	not reported	not reported	0.9	20.68	0.29	0.22
Ash (%)	not reported	not reported	not reported	8.21	7.36	3.96	3.36
Progoitrin (umole/g)	64.92	<0.026 - <0.051	<0.0257	not reported	not reported	0.1504	0.2129
Total glucosinolates *	not reported	not reported	not reported	0.0903	0.003	0.00235	0.00466
VOT, free (ug/g)	88	<5-<10	<5	287	20	n/a	n/a
Butenyl ITC (ug/g)	2400	n.d.	n.d.	242	6	n.d.	n.d.
Pentenyl ITC (ug/g)	346	n.d.	n.d.	287	n.d.	n.d.	n.d.
Phenylethyl ITC (ug/g)	140	n.d.	n.d.	not reported	not reported	n.d.	n.d.
Butenyl nitrile (mg/g)	not reported	not reported	not reported	n.d.	n.d.	n.d.	n.d.
Pentenyl nitrile (mg/g)	not reported	not reported	not reported	0.006	0.005	n.d.	n.d.

* as % butyl ITC by wt. (calculated from gluconapin for Supertein and Puratein)

n.d. = not detected

n/a = not analysed

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CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information currently available and summarized in the GRAS dossier prepared by ADM/Burcon, and other information deemed appropriate, and conclude that the intended uses of ADM/Burcon Cruciferin-rich and Napin-rich protein isolates derived from canola/rapeseed (Puratein® and Supertein™), manufactured and used in accordance with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications, are safe and suitable.

We, the Expert Panel, further conclude that the intended uses of ADM/Burcon Cruciferin-rich and Napin-rich protein isolates derived from canola/rapeseed (Puratein® and Supertein™), manufactured and used in accordance with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications, are Generally Recognized as Safe (GRAS) based on scientific procedures supported by the subchronic toxicity studies of ADM/Burcon Cruciferin-rich and Napin-rich protein isolates derived from canola/rapeseed (Puratein® and Supertein™), the history of safe use of canola meal in animal feeds and the safe history of the use of canola oil in preparing foods for human consumption.

It is the opinion of the Expert Panel that other experts, qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

The Panel's determination that the intended uses of ADM/Burcon Cruciferin-rich and Napin-rich protein isolates derived from canola/rapeseed (Puratein® and Supertein™) are GRAS based on scientific procedures was re-enforced following its subsequent evaluation of the recent literature review by ADM/Burcon.

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Appendix 2

**Estimated Consumption and Food
Categories to be added with
Cruciferin-Rich and Napin-Rich
Canola Protein Isolates,
Puratein® and Supertein™**

Estimation of Dietary Intake of Cruciferin-Rich and Napin-Rich Canola/Rapeseed Protein Isolates from Proposed Food Categories in U.S. Population

By Sam Z. Sun, Ph.D.

Office of Compliance and Ethics, Archer Daniels Midland Company

July 31, 2008

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Estimation of Dietary Intake of Canola/Rapeseed Protein Isolates (Puratein &Supertein) from Proposed Food Categories in U.S. Population

By Sam Sun, Ph.D.

Office of Compliance and Ethics, Archer Daniels Midland Company

The purpose of this intake estimation of canola/rapeseed protein was to provide potentially dietary intake levels (means and 90 percentiles) of added canola/rapeseed protein isolates, commercially named 'Supertein or Puratein', from the consumption of the proposed food items in variety of food categories; and further, evaluate its safety-GRAS status. The National Health and Nutrition Examination Survey (NHANES) 1999-2004 Data (1) and the SAS software (SAS System, V9.1, SAS Institute, Cary, NC) were used in this intake estimation..

The major working steps in this estimation include: to recognize food categories and items in which the canola/rapeseed protein isolates are proposed to be added in (totally 8 categories or groups of foods, see below); to calculate intake amounts from each selected food items and individual intake daily by grams and by grams per kilogram of body weight from all recognized food items consumed; and to determine population intake means, standard deviations, and ninety percentile by age groups with weighted sample sizes. The population was grouped by age ranges of 3-6, 7-12, 13-19, and 20 and over years of age. The population of children aged under 3 was not included because the canola protein will not be added into infant foods. The suggested levels of addition of the canola/rapeseed protein isolates in each food category are: 2% for selected processed meat products, baking food and salad dressings; 5% for selected dairy products; 10% for selected beverages and fruit/vegetable juices; 50% for meal replacements and high protein bars; 60% for egg substitute or egg powdered mixture; and 95% for protein powders.

The daily intake means and 90 percentiles of the added canola/rapeseed protein isolates (Puratein and Supertein) per grams and grams/kg body weight (grams/kg-bw) are shown in the table below. The 'N' represents the number of subject. The '90pctl' in the table is the 90th percentile of intake amounts in grams as is commonly used in food consumption evaluations. Each selected food item and its corresponding code in the databases are listed as part of this report.

**Canola/Rapeseed protein isolates intake estimation, NHANES 1999-2004
databases, weighted data**

Age groups	N	Label	Mean	Std Dev	90th Pctl
preschoolers 3-6	1908	grams/day/person	25.709	33.366	60.931
		grams/day/kg-bw	1.3228	1.758	3.136
kids 7-12	3207	grams/day/person	25.817	32.861	68.669
		grams/day/kg-bw	0.718	0.960	1.886
teenagers 13-19	5215	grams/day/person	32.177	49.680	89.754
		grams/day/kg-bw	0.502	0.783	1.368
adults 20 & over	12469	grams/day/person	20.903	37.489	57.851
		grams/day/kg-bw	0.271	0.490	0.752

Reference

- Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey (NHANES) Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 1999-2004, <http://www.cdc.gov/nchs/nhanes.htm>.

Food Items Proposed to be added with Canola/Rapeseed Protein Isolates (Puratein and Supertein)

* Added percentage of protein isolate.

List	Food code	Percent*	category	Food name
Dairy Products				
1	11612000	5	Dairy	Instant breakfast, powder, milk added
2	11613000	5	Dairy	Instant breakfast, powder, sweetened with low calorie sweetener
3	11621000	5	Dairy	Diet beverage, liquid, canned
4	11622000	5	Dairy	Diet beverage, powder, milk added
5	11622000	5	Dairy	Diet beverage, powder, milk added
6	11622010	5	Dairy	Diet beverage, powder, reconstituted with skim milk
7	11622010	5	Dairy	Diet beverage, powder, reconstituted with skim milk
8	11631000	5	Dairy	High calorie beverage, canned or powdered, reconstituted
9	11631000	5	Dairy	High calorie beverage, canned or powdered, reconstituted
10	11832000	5	Dairy	Meal replacement, protein type, milk- and soy-based, powdered, not r
11	11835000	5	Dairy	Meal replacement or nutritional supplement, Cambridge diet formula,
12	11835100	5	Dairy	Meal replacement, Amway's Nutrilite brand Positrim Drink Mix, powder
13	11835150	5	Dairy	Dynatrim, meal replacement, powder
14	11835200	5	Dairy	Lose-it (Nanci), meal replacement, powder
15	12200100	5	Dairy	Cream substitute, NS as to frozen, liquid, or powdered
16	12210400	5	Dairy	Cream substitute, powdered
17	12210410	5	Dairy	Cream substitute, light, powdered
18	12220000	5	Dairy	Whipped topping, nondairy, NS as to canned, frozen, or made from
19	12220300	5	Dairy	Whipped cream substitute, nondairy, made from powdered mix
20	12220400	5	Dairy	Whipped cream substitute, nondairy, lowfat, low sugar, made from
21	14420000	5	Dairy	Cheese spread, NFS
22	14420100	5	Dairy	Cheese spread, American or Cheddar cheese base
23	14420140	5	Dairy	Cheese spread, American or Cheddar cheese base, lowfat, low sodium
24	14420160	5	Dairy	Cheese spread, Swiss cheese base
25	14420200	5	Dairy	Cheese spread, cream cheese or Neufchatel base
26	14420300	5	Dairy	Cheese spread, pressurized can
27	14501010	5	Dairy	Imitation cream cheese
28	14502010	5	Dairy	Imitation cheese, American or cheddar type
29	14502040	5	Dairy	Imitation cheese, American or cheddar type, low cholesterol
30	14503010	5	Dairy	Imitation cheese spread
32	14504010	5	Dairy	Imitation mozzarella cheese

33	14640300	5	Dairy	Cheese spread sandwich
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Processed meat products

34	25210110	2	Meat	Frankfurter, wiener, or hot dog, NFS
35	25210120	2	Meat	Frankfurter or hot dog, breaded, baked
36	25210150	2	Meat	Frankfurter or hot dog, cheese-filled
37	25210160	2	Meat	Frankfurter or hot dog, bacon and cheese-filled
38	25210170	2	Meat	Frankfurter or hot dog, chili-filled
39	25210210	2	Meat	Frankfurter or hot dog, beef
40	25210220	2	Meat	Frankfurter or hot dog, beef and pork
41	25210230	2	Meat	Frankfurter or hot dog, beef and pork, lowfat
42	25210250	2	Meat	Frankfurter or hot dog, meat and poultry, fat free
43	25210280	2	Meat	Frankfurter or hot dog, meat and poultry
44	25210310	2	Meat	Frankfurter or hot dog, chicken
45	25210410	2	Meat	Frankfurter or hot dog, turkey
46	25210510	2	Meat	Frankfurter or hot dog, low salt
47	25210610	2	Meat	Frankfurter or hot dog, beef, lowfat
48	25210700	2	Meat	Frankfurter or hot dog, meat & poultry, lowfat
49	25220110	2	Meat	Beef sausage, brown and serve, links, cooked
50	25220120	2	Meat	Beef sausage, smoked, stick
51	25220130	2	Meat	Beef sausage, smoked
52	25220310	2	Meat	Bockwurst
53	25220350	2	Meat	Bratwurst, cooked
54	25220360	2	Meat	Bratwurst, with cheese
55	25220390	2	Meat	Bologna, beef, lowfat
56	25220400	2	Meat	Bologna, pork and beef
57	25220410	2	Meat	Bologna, NFS
58	25220420	2	Meat	Bologna, Lebanon
59	25220430	2	Meat	Bologna, beef
60	25220440	2	Meat	Bologna, turkey
61	25220450	2	Meat	Bologna ring, smoked
62	25220460	2	Meat	Bologna, pork
63	25220470	2	Meat	Bologna, beef, lower sodium
64	25220480	2	Meat	Bologna, chicken, beef, and pork
65	25220490	2	Meat	Bologna, with cheese
66	25220500	2	Meat	Bologna, beef and pork, lowfat
67	25220650	2	Meat	Chicken and beef sausage, smoked
68	25221420	2	Meat	Pork sausage, brown and serve, cooked
69	25221450	2	Meat	Pork sausage rice links, brown and serve, cooked
70	25221470	2	Meat	Pork and beef sausage, brown and serve, cooked
71	25221650	2	Meat	Smoked link sausage, pork
72	25221660	2	Meat	Smoked link sausage, pork and beef
73	25221680	2	Meat	Smoked sausage, pork

74	25221850	2	Meat	Turkey sausage, smoked
75	25221860	2	Meat	Turkey sausage, reduced fat, brown and serve, cooked
76	25221880	2	Meat	Turkey, pork, and beef sausage, reduced fat, smoked
77	25221890	2	Meat	Turkey, pork, and beef sausage, lowfat, smoked
78	25230210	2	Meat	Ham, sliced, prepackaged or deli, luncheon meat
79	25230220	2	Meat	Ham, sliced, low salt, prepackaged or deli, luncheon meat
80	25230230	2	Meat	Ham, sliced, extra lean, prepackaged or deli, luncheon meat
81	25230310	2	Meat	Chicken or turkey loaf, prepackaged or deli, luncheon meat
82	25230790	2	Meat	Turkey ham, sliced, extra lean, prepackaged or deli, luncheon
83	25230800	2	Meat	Turkey ham
84	25240000	2	Meat	Meat spread or potted meat, NFS
85	25240000	1	Meat	Meat spread or potted meat, NFS
86	25240110	1	Meat	Chicken salad spread
87	25240220	1	Meat	Ham salad spread
88	25240310	1	Meat	Roast beef spread
89	25240320	1	Meat	Corned beef spread
113	28345010	2	Meat	Chicken or turkey soup, cream of, canned, NS as to made with milk or
114	28345020	2	Meat	Chicken or turkey soup, cream of, canned, made with milk, reduced so
115	28345030	2	Meat	Chicken or turkey soup, cream of, canned, made with water, reduced s
116	28345040	2	Meat	Chicken or turkey soup, cream of, canned, undiluted, reduced sodium
117	28345110	2	Meat	Chicken or turkey soup, cream of, NS as to prepared with milk or wat
118	28345120	2	Meat	Chicken or turkey soup, cream of, prepared with milk
119	28345130	2	Meat	Chicken or turkey soup, cream of, prepared with water
120	28345140	2	Meat	Chicken or turkey soup, cream of, canned, undiluted
121	28345160	2	Meat	Chicken and mushroom soup, cream of, prepared with milk
122	28350110	2	Meat	Crab soup, NS as to tomato-base or cream style
123	28350210	2	Meat	Clam chowder, NS as to Manhattan or New England style
124	28355110	2	Meat	Clam chowder, New England, NS as to prepared with water or milk
125	28355120	2	Meat	Clam chowder, New England, prepared with milk
126	28355130	2	Meat	Clam chowder, New England, prepared with water
127	28355140	2	Meat	Clam chowder, New England, canned, reduced sodium, ready-to-serve
128	28355210	2	Meat	Crab soup, cream of, prepared with milk
129	28355410	2	Meat	Shrimp soup, cream of, NS as to prepared with milk or water
130	28355420	2	Meat	Shrimp soup, cream of, prepared with milk
131	28355430	2	Meat	Shrimp soup, cream of, prepared with water

Powdered egg substitute

132	33000100	60	Egg	Egg substitute, NS as to powdered, frozen, or liquid
133	33102010	60	Egg	Scrambled egg, made from powdered mixture

Protein supplement powders

134	41430000	95	Legume/nuts	Protein powder, NFS
135	41430010	95	Legume/nuts	Protein supplement, powdered

Meal replacement/nutrition bars

136	41430100	50	Legume/nuts	Formulated diet meal, powdered, soy protein isolate, with herbs
137	41430200	50	Legume/nuts	Meal replacement or supplement, soy- and milk-base, powder, reconsti
138	41430310	50	Legume/nuts	Protein diet powder with soy and casein
139	41435010	50	Legume/nuts	High protein bar, soy base
140	41435110	50	Legume/nuts	High protein bar, candy-like, soy and milk base
141	41435200	50	Legume/nuts	High protein bar, cookie type, soy and milk base

Grain products

142	50030000	2	Grain	Biscuit mix, dry
143	51000100	2	Grain	Bread, NS as to major flour
144	51000110	2	Grain	Toast, NS as to major flour
145	51000180	2	Grain	Bread, made from home recipe or purchased at a bakery, NS as to
146	51000190	2	Grain	Bread, made from home recipe or purchased at a bakery, toasted, NS
147	51000200	2	Grain	Roll, NS as to major flour
148	51000230	2	Grain	Roll, NS as to major flour, toasted
149	51000250	2	Grain	Roll, made from home recipe or purchased at a bakery, NS as to major
150	51000260	2	Grain	Roll, made from home recipe or purchased at a bakery, toasted, NS as
151	51000300	2	Grain	Roll, hard, NS as to major flour
152	51000400	2	Grain	Roll, bran, NS as to type of bran
153	51000410	2	Grain	Roll, bran, NS as to type of bran, toasted
154	51101000	2	Grain	Bread, white
155	51101010	2	Grain	Bread, white, toasted
156	51101050	2	Grain	Bread, white, made from home recipe or purchased at a bakery
157	51101060	2	Grain	Bread, white, made from home recipe or purchased at a bakery, toasted
158	51102010	2	Grain	Bread, white with whole wheat swirl
159	51102020	2	Grain	Bread, white with whole wheat swirl, toasted
160	51105010	2	Grain	Bread, Cuban
161	51105040	2	Grain	Bread, Cuban, toasted
162	51106010	2	Grain	Bread, Native, Puerto Rican style (Pan Criollo)
163	51106020	2	Grain	Bread, Native, Puerto Rican style, toasted (Pan Criollo)
164	51106100	2	Grain	Bread, Native water, Puerto Rican style (Pan de agua)
165	51106200	2	Grain	Bread, lard, Puerto Rican style (Pan de manteca)
166	51106210	2	Grain	Bread, lard, Puerto Rican style, toasted (Pan de manteca)
167	51106300	2	Grain	Bread, caressed, Puerto Rican style (Pan sobao)
168	51106310	2	Grain	Bread, caressed, Puerto Rican style, toasted (Pan sobao)
169	51107010	2	Grain	Bread, French or Vienna
170	51107040	2	Grain	Bread, French or Vienna, toasted
171	51109010	2	Grain	Bread, Italian, Grecian, Armenian
172	51109040	2	Grain	Bread, Italian, Grecian, Armenian, toasted
173	51109100	2	Grain	Bread, pita
174	51109110	2	Grain	Bread, pita, toasted

175	51109150	2	Grain	Bread, pita with fruit
176	51109200	2	Grain	Bread, pita with fruit, toasted
177	51110010	2	Grain	Bread, batter
178	51111010	2	Grain	Bread, cheese
179	51111040	2	Grain	Bread, cheese, toasted
180	51113010	2	Grain	Bread, cinnamon
181	51113100	2	Grain	Bread, cinnamon, toasted
182	51115010	2	Grain	Bread, cornmeal and molasses
183	51115020	2	Grain	Bread, cornmeal and molasses, toasted
184	51119010	2	Grain	Bread, egg, Challah
185	51119040	2	Grain	Bread, egg, Challah, toasted
186	51119100	2	Grain	Bread, lowfat, 98% fat free
187	51119110	2	Grain	Bread, lowfat, 98% fat free, toasted
188	51121010	2	Grain	Bread, garlic
189	51121040	2	Grain	Bread, garlic, toasted
190	51121110	2	Grain	Bread, onion
191	51121500	2	Grain	Bread, gluten
192	51121510	2	Grain	Bread, gluten, toasted
193	51122000	2	Grain	Bread, reduced calorie and/or high fiber, white or NFS
194	51122010	2	Grain	Bread, reduced calorie and/or high fiber, white or NFS, toasted
195	51122050	2	Grain	Bread, reduced calorie and/or high fiber, Italian
196	51122060	2	Grain	Bread, reduced calorie and/or high fiber, Italian, toasted
197	51122100	2	Grain	Bread, reduced calorie and/or high fiber, white or NFS, with fruit
198	51122110	2	Grain	Bread, reduced calorie and/or high fiber, white or NFS, with fruit
199	51122300	2	Grain	Bread, white, special formula, added fiber
200	51122310	2	Grain	Bread, white, special formula, added fiber, toasted
201	51122400	2	Grain	Bread, white, special formula, high calcium
202	51123010	2	Grain	Bread, high protein
203	51123020	2	Grain	Bread, high protein, toasted
204	51126010	2	Grain	Bread, milk and honey
205	51126020	2	Grain	Bread, milk and honey, toasted
206	51127010	2	Grain	Bread, potato
207	51127020	2	Grain	Bread, potato, toasted
208	51129010	2	Grain	Bread, raisin
209	51129020	2	Grain	Bread, raisin, toasted
210	51130510	2	Grain	Bread, white, low sodium or no salt
211	51130520	2	Grain	Bread, white, low sodium or no salt, toasted
212	51131010	2	Grain	Bread, salt-rising
213	51131040	2	Grain	Bread, salt-rising, toasted
214	51133010	2	Grain	Bread, sour dough
215	51133020	2	Grain	Bread, sour dough, toasted
216	51134000	2	Grain	Bread, sweetpotato
217	51135000	2	Grain	Bread, vegetable

218	51135010	2	Grain	Bread, vegetable, toasted
219	51140100	2	Grain	Bread, dough, fried
220	51150000	2	Grain	Roll, white, soft
221	51150100	2	Grain	Roll, white, soft, toasted
222	51151060	2	Grain	Roll, white, soft, made from home recipe or purchased at a bakery
223	51152000	2	Grain	Roll, white, soft, reduced calorie and/or high fiber
224	51152100	2	Grain	Roll, white, soft, reduced calorie and/or high fiber, toasted
225	51153000	2	Grain	Roll, white, hard
226	51153010	2	Grain	Roll, white, hard, toasted
227	51154510	2	Grain	Roll, diet
228	51154550	2	Grain	Roll, egg bread
229	51154560	2	Grain	Roll, egg bread, toasted
230	51154600	2	Grain	Roll, cheese
231	51155000	2	Grain	Roll, French or Vienna
232	51155010	2	Grain	Roll, French or Vienna, toasted
233	51156500	2	Grain	Roll, garlic
234	51157000	2	Grain	Roll, hoagie, submarine
235	51157010	2	Grain	Roll, hoagie, submarine, toasted
236	51158100	2	Grain	Roll, Mexican, bolillo
237	51159000	2	Grain	Roll, sour dough
238	51160000	2	Grain	Roll, sweet
239	51160010	2	Grain	Roll, sweet, toasted
240	51160100	2	Grain	Roll, sweet, cinnamon bun, no frosting
241	51160110	2	Grain	Roll, sweet, cinnamon bun, frosted
242	51161000	2	Grain	Roll, sweet, with fruit, no frosting
243	51161020	2	Grain	Roll, sweet, with fruit, frosted
244	51161030	2	Grain	Roll, sweet, with fruit, frosted, diet
245	51161050	2	Grain	Roll, sweet, with nuts, frosted
246	51161070	2	Grain	Roll, sweet, with fruit, frosted, fat free
247	51161100	2	Grain	Roll, sweet, with fruit and nuts, no frosting
248	51161150	2	Grain	Roll, sweet, with fruit and nuts, frosted
249	51161200	2	Grain	Roll, sweet, with nuts, no frosting
250	51161250	2	Grain	Roll, sweet, no topping, Mexican (Pan Dulce)
251	51161260	2	Grain	Roll, sweet, crumb topping, Mexican (Pan Dulce)
252	51161270	2	Grain	Roll, sweet, sugar topping, Mexican (Pan Dulce)
253	51161280	2	Grain	Roll, sweet, with raisins and icing, Mexican (Pan Dulce)
254	51165000	2	Grain	Coffee cake, yeast type
255	51165060	2	Grain	Coffee cake, yeast type, made from home recipe or purchased at a bak
256	51165100	2	Grain	Coffee cake, yeast type, fat free, cholesterol free, with fruit
257	51166000	2	Grain	Croissant
258	51166100	2	Grain	Croissant, cheese
259	51166200	2	Grain	Croissant, chocolate
260	51166500	2	Grain	Croissant, fruit

261	51166700	2	Grain	Croissant, nut
262	51167000	2	Grain	Brioche
263	51168000	2	Grain	Bread, Spanish coffee
264	51180010	2	Grain	Bagel
265	51180020	2	Grain	Bagel, toasted
266	51180030	2	Grain	Bagel, with raisins
267	51180040	2	Grain	Bagel, with raisins, toasted
268	51180080	2	Grain	Bagel, with fruit other than raisins
269	51180090	2	Grain	Bagel, with fruit other than raisins, toasted
270	51182010	2	Grain	Bread stuffing
271	51182020	2	Grain	Bread stuffing made with egg
272	51184000	2	Grain	Bread sticks, hard
273	51184010	2	Grain	Bread stick, soft
274	51184020	2	Grain	Bread stick, NS as to hard or soft
275	51184030	2	Grain	Bread stick, soft, prepared with garlic and parmesan che
276	51184100	2	Grain	Bread stick, hard, low sodium
277	51185000	2	Grain	Croutons
278	51186010	2	Grain	Muffin, English
279	51186020	2	Grain	Muffin, English, toasted
280	51186100	2	Grain	Muffin, English, with raisins
281	51186120	2	Grain	Muffin, English, with raisins, toasted
282	51186130	2	Grain	Muffin, English, cheese
283	51186140	2	Grain	Muffin, English, cheese, toasted
284	51186160	2	Grain	Muffin, English, with fruit other than raisins
285	51186180	2	Grain	Muffin, English, with fruit other than raisins, toasted
286	51201010	2	Grain	Bread, whole wheat, 100%
287	51201020	2	Grain	Bread, whole wheat, 100%, toasted
288	51201060	2	Grain	Bread, whole wheat, 100%, made from home recipe or purchased at bake
289	51201070	2	Grain	Bread, whole wheat, 100%, made from home recipe or purchased at bake
290	51201110	2	Grain	Bread, whole wheat, 100%, with raisins
291	51201120	2	Grain	Bread, whole wheat, 100%, with raisins, toasted
292	51201150	2	Grain	Bread, pita, whole wheat, 100%
293	51201160	2	Grain	Bread, pita, whole wheat, 100%, toasted
294	51202000	2	Grain	Muffin, English, whole wheat, 100%
295	51202020	2	Grain	Muffin, English, whole wheat, 100%, toasted
296	51202050	2	Grain	Muffin, English, whole wheat, 100%, with raisins
297	51202060	2	Grain	Muffin, English, whole wheat, 100%, with raisins, toasted
298	51204010	2	Grain	Bread, wheat germ
299	51204020	2	Grain	Bread, wheat germ, toasted
300	51207010	2	Grain	Bread, sprouted wheat
301	51207020	2	Grain	Bread, sprouted wheat, toasted
302	51208000	2	Grain	Bagel, whole wheat, 100%
303	51208010	2	Grain	Bagel, whole wheat, 100%, toasted

304	51208100	2	Grain	Bagel, whole wheat, 100%, with raisins
305	51208110	2	Grain	Bagel, whole wheat, 100%, with raisins, toasted
306	51220000	2	Grain	Roll, whole wheat, 100%
307	51220010	2	Grain	Roll, whole wheat, 100%, toasted
308	51220030	2	Grain	Roll, whole wheat, 100%, made from home recipe or purchased at baker
309	51220040	2	Grain	Roll, whole wheat, 100%, made from home recipe or purchased at baker
310	51300110	2	Grain	Bread, whole wheat, other than 100% or NS as to 100%
311	51300120	2	Grain	Bread, whole wheat, other than 100% or NS as to 100%, toasted
312	51300140	2	Grain	Bread, whole wheat, other than 100% or NS as to 100%, made from home
313	51300150	2	Grain	Bread, whole wheat, other than 100% or NS as to 100%, made from home
314	51300180	2	Grain	Bread, puri or poori (Indian puffed bread), whole wheat, other than
315	51300210	2	Grain	Bread, whole wheat, NS as to 100%, with raisins
316	51300220	2	Grain	Bread, whole wheat, NS as to 100%, with raisins, toasted
317	51300610	2	Grain	Bread, whole wheat, NS as to 100%, low sodium or no salt
318	51300620	2	Grain	Bread, whole wheat, NS as to 100%, low sodium or no salt, toasted
319	51301010	2	Grain	Bread, wheat or cracked wheat
320	51301020	2	Grain	Bread, wheat or cracked wheat, toasted
321	51301040	2	Grain	Bread, wheat or cracked wheat, made from home recipe or purchased at
322	51301050	2	Grain	Bread, wheat or cracked wheat, made from home recipe or purchased at
323	51301120	2	Grain	Bread, wheat or cracked wheat, with raisins
324	51301130	2	Grain	Bread, wheat or cracked wheat, with raisins, toasted
325	51301510	2	Grain	Bread, wheat or cracked wheat, reduced calorie and/or high fiber
326	51301520	2	Grain	Bread, wheat or cracked wheat, reduced calorie and/or high fiber, to
327	51301540	2	Grain	Bread, French or Vienna, whole wheat, other than 100% or NS as to 10
328	51301550	2	Grain	Bread, French or Vienna, whole wheat, other than 100% or NS as to 10
329	51301600	2	Grain	Bread, pita, whole wheat, other than 100% or NS as to
330	51301610	2	Grain	Bread, pita, whole wheat, other than 100% or NS as to 100%, toasted
331	51301620	2	Grain	Bread, pita, wheat or cracked wheat
332	51301630	2	Grain	Bread, pita, wheat or cracked wheat, toasted
333	51301700	2	Grain	Bagel, wheat
334	51301710	2	Grain	Bagel, wheat, toasted
335	51301750	2	Grain	Bagel, whole wheat, other than 100% or NS as to 100%
336	51301760	2	Grain	Bagel, whole wheat, other than 100% or NS as to 100%, toasted
337	51301800	2	Grain	Bagel, wheat, with raisins
338	51301810	2	Grain	Bagel, wheat, with raisins, toasted
339	51301820	2	Grain	Bagel, wheat, with fruit and nuts
340	51301830	2	Grain	Bagel, wheat, with fruit and nuts, toasted
341	51301900	2	Grain	Bagel, wheat bran
342	51301910	2	Grain	Bagel, wheat bran, toasted
343	51302010	2	Grain	Bread, wheat bran
344	51302020	2	Grain	Bread, wheat bran, toasted
345	51302050	2	Grain	Bread, wheat bran, with raisins
346	51302060	2	Grain	Bread, wheat bran, with raisins, toasted

347	51302500	2	Grain	Muffin, English, wheat bran
348	51302510	2	Grain	Muffin, English, wheat bran, toasted
349	51302520	2	Grain	Muffin, English, wheat bran, with raisins
350	51302530	2	Grain	Muffin, English, wheat bran, with raisins, toasted
351	51303010	2	Grain	Muffin, English, wheat or cracked wheat
352	51303020	2	Grain	Muffin, English, wheat or cracked wheat, toasted
353	51303030	2	Grain	Muffin, English, whole wheat, other than 100% or NS as to 100%
354	51303040	2	Grain	Muffin, English, whole wheat, other than 100% or NS as to 100%, toast
355	51303050	2	Grain	Muffin, English, wheat or cracked wheat, with raisins
356	51303060	2	Grain	Muffin, English, wheat or cracked wheat, with raisins, toasted
357	51303070	2	Grain	Muffin, English, whole wheat, other than 100% or NS as to 100%, with
358	51303080	2	Grain	Muffin, English, whole wheat, other than 100% or NS as to 100%, with
359	51306000	2	Grain	Bread stick, hard, whole wheat, NS as to 100%
360	51320010	2	Grain	Roll, wheat or cracked wheat
361	51320020	2	Grain	Rolls, wheat or cracked wheat, toasted
362	51320040	2	Grain	Roll, wheat or cracked wheat, made from home recipe or purchased
363	51320050	2	Grain	Roll, wheat or cracked wheat, made from home recipe or purchased
364	51320500	2	Grain	Roll, whole wheat, NS as to 100%
365	51320510	2	Grain	Roll, whole wheat, NS as to 100%, toasted
366	51320530	2	Grain	Roll, whole wheat, other than 100% or NS as to 100%, made from home
367	51320540	2	Grain	Roll, whole wheat, other than 100% or NS as to 100%, made from home
368	51401010	2	Grain	Bread, rye
369	51401020	2	Grain	Bread, rye, toasted
370	51401030	2	Grain	Bread, marble rye and pumpernickel
371	51401040	2	Grain	Bread, marble rye and pumpernickel, toasted
372	51401060	2	Grain	Bread, rye, reduced calorie and/or high fiber
373	51401070	2	Grain	Bread, rye, reduced calorie and/or high fiber, toasted
374	51401200	2	Grain	Muffin, English, rye
375	51401210	2	Grain	Muffin, English, rye, toasted
376	51404010	2	Grain	Bread, pumpernickel
377	51404020	2	Grain	Bread, pumpernickel, toasted
378	51404500	2	Grain	Bagel, pumpernickel
379	51404510	2	Grain	Bagel, pumpernickel, toasted
380	51404550	2	Grain	Muffin, English, pumpernickel
381	51404560	2	Grain	Muffin, English, pumpernickel, toasted
382	51407010	2	Grain	Bread, black
383	51407020	2	Grain	Bread, black, toasted
384	51420000	2	Grain	Roll, rye
385	51421000	2	Grain	Roll, pumpernickel
386	51421100	2	Grain	Roll, pumpernickel, toasted
387	51501010	2	Grain	Bread, oatmeal
388	51501020	2	Grain	Bread, oatmeal, toasted
389	51501040	2	Grain	Bread, oat bran

390	51501050	2	Grain	Bread, oat bran, toasted
391	51501060	2	Grain	Bread, oat bran, reduced calorie and/or high fiber
392	51501070	2	Grain	Bread, oat bran, reduced calorie and/or high fiber, toasted
393	51501080	2	Grain	Bagel, oat bran
394	51501090	2	Grain	Bagel, oat bran, toasted
395	51502010	2	Grain	Roll, oatmeal
396	51502020	2	Grain	Roll, oatmeal, toasted
397	51502100	2	Grain	Roll, oat bran
398	51502110	2	Grain	Roll, oat bran, toasted
399	51503000	2	Grain	Muffin, English, oat bran
400	51503010	2	Grain	Muffin, English, oat bran, toasted
401	51503040	2	Grain	Muffin, English, oat bran, with raisins
402	51503050	2	Grain	Muffin, English, oat bran with raisins, toasted
403	51601010	2	Grain	Bread, multigrain, toasted
404	51601020	2	Grain	Bread, multigrain
405	51601210	2	Grain	Bread, multigrain, with raisins
406	51601220	2	Grain	Bread, multigrain, with raisins, toasted
407	51602010	2	Grain	Bread, multigrain, reduced calorie and/or high fiber
408	51602020	2	Grain	Bread, multigrain, reduced calorie and/or high fiber, toasted
409	51620000	2	Grain	Roll, multigrain
410	51620010	2	Grain	Roll, multigrain, toasted
411	51630000	2	Grain	Bagel, multigrain
412	51630010	2	Grain	Bagel, multigrain, toasted
413	51630100	2	Grain	Bagel, multigrain, with raisins
414	51630110	2	Grain	Bagel, multigrain, with raisins, toasted
415	51630200	2	Grain	Muffin, English, multigrain
416	51630210	2	Grain	Muffin, English, multigrain, toasted
417	51701010	2	Grain	Bread, cottonseed, toasted
418	51801010	2	Grain	Bread, barley
419	51801010	2	Grain	Bread, barley
420	51802010	2	Grain	Bread, triticale
421	51802020	2	Grain	Bread, triticale, toasted
422	51803010	2	Grain	Bread, buckwheat
423	51803020	2	Grain	Bread, buckwheat, toasted
424	51804010	2	Grain	Bread, soy
425	51804020	2	Grain	Bread, soy, toasted
426	51805010	2	Grain	Bread, sunflower meal
427	51805020	2	Grain	Bread, sunflower meal, toasted
428	51806010	2	Grain	Bread, rice
429	51806020	2	Grain	Bread, rice, toasted
430	51807000	2	Grain	Injera (American-style Ethiopian bread)
431	51808000	2	Grain	Bread, low gluten
432	51808010	2	Grain	Bread, low gluten, toasted

433	52101000	2	Grain	Biscuit, baking powder or buttermilk type, NS as to made from mix
434	52101000	2	Grain	Biscuit, baking powder or buttermilk type, NS as to made from mix
435	52101020	2	Grain	Biscuit dough, raw
436	52101030	2	Grain	Biscuit dough, fried
437	52101040	2	Grain	Crumpet
438	52101050	2	Grain	Crumpet, toasted
439	52101100	2	Grain	Biscuit, baking powder or buttermilk type, made from mix
440	52101100	2	Grain	Biscuit, baking powder or buttermilk type, made from mix
441	52101150	2	Grain	Biscuit, baking powder or buttermilk type, made from refrigerated
442	52101150	2	Grain	Biscuit, baking powder or buttermilk type, made from refrigerated
443	52102040	2	Grain	Biscuit, baking powder or buttermilk type, made from refrigerated
444	52102040	2	Grain	Biscuit, baking powder or buttermilk type, made from refrigerated
445	52103000	2	Grain	Biscuit, baking powder or buttermilk type, commercially baked
446	52103000	2	Grain	Biscuit, baking powder or buttermilk type, commercially baked
447	52104010	2	Grain	Biscuit, baking powder or buttermilk type, made from home recipe
448	52104010	2	Grain	Biscuit, baking powder or buttermilk type, made from home recipe
449	52104040	2	Grain	Biscuit, whole wheat
450	52104100	2	Grain	Biscuit, cheese
451	52104200	2	Grain	Biscuit, cinnamon-raisin
452	52105100	2	Grain	Scone
453	52105110	2	Grain	Scone, whole wheat
454	52105200	2	Grain	Scone, with fruit
455	52215000	2	Grain	Tortilla, NFS
456	52215100	2	Grain	Tortilla, corn
457	52215200	2	Grain	Tortilla, flour (wheat)
458	52215260	2	Grain	Tortilla, whole wheat
459	52301000	2	Grain	Muffin, NFS
460	52302010	2	Grain	Muffin, fruit and/or nuts
461	52302100	2	Grain	Muffin, fruit, fat free, cholesterol free
462	52302500	2	Grain	Muffin, chocolate chip
463	52302600	2	Grain	Muffin, chocolate
464	52303010	2	Grain	Muffin, whole wheat
465	52303500	2	Grain	Muffin, wheat
466	52303550	2	Grain	Muffin, buckwheat
467	52304010	2	Grain	Muffin, wheat bran
468	52304020	2	Grain	Muffin, wheat bran, toasted
469	52304040	2	Grain	Muffin, bran with fruit, lowfat
470	52304060	2	Grain	Muffin, bran with fruit, no fat, no cholesterol
471	52304100	2	Grain	Muffin, oatmeal
472	52304150	2	Grain	Muffin, oat bran
473	52304200	2	Grain	Muffin, oat bran with fruit and/or nuts
474	52306010	2	Grain	Muffin, plain
475	52306100	2	Grain	Muffin, plain, no wheat, sugar free

476	52306300	2	Grain	Muffin, cheese
477	52306500	2	Grain	Muffin, pumpkin
478	52306550	2	Grain	Muffin, zucchini
479	52306700	2	Grain	Muffin, carrot
480	52307020	2	Grain	Muffin, multigrain, with nuts
481	52307120	2	Grain	Muffin, multigrain, with fruit
482	52308010	2	Grain	Matzo, fritters
483	52311010	2	Grain	Popover
484	52320100	2	Grain	Toaster muffin, fruit, untoasted
485	52320110	2	Grain	Toaster muffin, fruit, toasted
486	52401000	2	Grain	Bread, Boston Brown
487	52403000	2	Grain	Bread, nut
488	52404060	2	Grain	Bread, pumpkin
489	52405010	2	Grain	Bread, fruit, without nuts
490	52405100	2	Grain	Bread, fruit and nut
491	52406010	2	Grain	Bread, whole wheat, with nuts
492	52407000	2	Grain	Bread, zucchini
493	52408000	2	Grain	Bread, Irish soda
494	53100050	2	Grain	Cake batter, raw, chocolate
495	53100070	2	Grain	Cake batter, raw, not chocolate
496	53100100	2	Grain	Cake, NS as to type, with or without icing
497	53101000	2	Grain	Cake, angel food, NS as to icing
498	53101100	2	Grain	Cake, angel food, without icing
499	53101200	2	Grain	Cake, angel food, with icing
500	53101250	2	Grain	Cake, angel food, with fruit and icing or filling
501	53101300	2	Grain	Cake, angel food, chocolate, without icing
502	53102000	2	Grain	Cake, applesauce, NS as to icing
503	53102100	2	Grain	Cake, applesauce, without icing
504	53102200	2	Grain	Cake, applesauce, with icing
505	53102300	2	Grain	Cake, applesauce, diet, without icing
506	53102500	2	Grain	Cake, banana, NS as to icing
507	53102600	2	Grain	Cake, banana, without icing
508	53102700	2	Grain	Cake, banana, with icing
509	53102800	2	Grain	Cake, black forest (chocolate-cherry)
510	53103000	2	Grain	Cake, Boston cream pie
511	53103500	2	Grain	Cake, butter, NS as to icing
512	53103550	2	Grain	Cake, butter, without icing
513	53103600	2	Grain	Cake, butter, with icing
514	53104000	2	Grain	Cake, carrot, NS as to icing
515	53104100	2	Grain	Cake, carrot, without icing
516	53104260	2	Grain	Cake, carrot, with icing
517	53104300	2	Grain	Cake, carrot, diet
518	53104400	2	Grain	Cake, coconut, with icing

519	53104500	2	Grain	Cheesecake
520	53104520	2	Grain	Cheesecake, diet
521	53104550	2	Grain	Cheesecake with fruit
522	53104570	2	Grain	Cheesecake, diet, with fruit
523	53104580	2	Grain	Cheesecake -type dessert, made with yogurt, with fruit
524	53104600	2	Grain	Cheesecake, chocolate
525	53104650	2	Grain	Cheesecake, chocolate, reduced fat
526	53104900	2	Grain	Cake, chocolate, made with mayonnaise or salad dressing, NS as to
527	53104920	2	Grain	Cake, chocolate, made with mayonnaise or salad dressing
528	53104950	2	Grain	Cake, chocolate, made with mayonnaise or salad dressing, with icing,
529	53105000	2	Grain	Cake, chocolate, devil's food, or fudge, standard-type mix
530	53105050	2	Grain	Cake, chocolate, devil s food, or fudge, made from home recipe
531	53105100	2	Grain	Cake, chocolate, devil s food, or fudge, standard-type mix
532	53105160	2	Grain	Cake, chocolate, devil s food, or fudge, without icing or filling
533	53105200	2	Grain	Cake, chocolate, devil s food, or fudge, standard-type mix
534	53105260	2	Grain	Cake, chocolate, devil s food, or fudge, with icing, coating, or filling
535	53105300	2	Grain	Cake, German chocolate, with icing and filling
536	53105500	2	Grain	Cake, chocolate, with icing, diet
537	53105600	2	Grain	Cake, chocolate, devil s food, or fudge, pudding-type mix, made by
538	53105650	2	Grain	Cake, chocolate, devils food, or fudge, pudding type mix, made by
539	53105700	2	Grain	Cake, chocolate, devil s food, or fudge, pudding type mix, made by
540	53105750	2	Grain	Cake, chocolate, devil s food, or fudge, pudding type mix, made by
541	53105900	2	Grain	Cake, chocolate, devil s food, or fudge, pudding-type mix (oil, eggs
542	53106000	2	Grain	Cake, chocolate, devil s food, or fudge, pudding-type mix (oil, eggs
543	53106050	2	Grain	Cake, chocolate, devil's food, or fudge, pudding-type mix (oil, eggs
544	53106100	2	Grain	Cake, Poor Man s (spice-type), without icing
545	53106500	2	Grain	Cake, cream, without icing or topping
546	53107000	2	Grain	Cake, cupcake, NS as to type or icing
547	53107100	2	Grain	Cake, cupcake, NS as to type, without icing
548	53107200	2	Grain	Cake, cupcake, NS as to type, with icing
549	53108000	2	Grain	Cake, cupcake, chocolate, NS as to icing
550	53108100	2	Grain	Cake, cupcake, chocolate, without icing or filling
551	53108200	2	Grain	Cake, cupcake, chocolate, with icing or filling
552	53109000	2	Grain	Cake, cupcake, not chocolate, NS as to icing
553	53109100	2	Grain	Cake, cupcake, not chocolate, without icing or filling
554	53109200	2	Grain	Cake, cupcake, not chocolate, with icing or filling
555	53109210	2	Grain	Cake, cupcake, not chocolate, with icing or filling, lowfat
556	53109250	2	Grain	Cake, cupcake, not chocolate, with fruit and cream filling
557	53109270	2	Grain	Cake, cupcake, chocolate, with or without icing, fruit filling
558	53109300	2	Grain	Cake, Dobos Torte (non-chocolate layer cake with chocolate filling
559	53110000	2	Grain	Cake, fruit cake, light or dark, holiday type cake
560	53110100	2	Grain	Cake, plum pudding
561	53111000	2	Grain	Cake, gingerbread, without icing

562	53111500	2	Grain	Cake, graham cracker, without icing
563	53112000	2	Grain	Cake, ice cream and cake roll, chocolate
564	53112100	2	Grain	Cake, ice cream and cake roll, not chocolate
565	53112150	2	Grain	Cake, frozen yogurt and cake layer, not chocolate, with icing
566	53112160	2	Grain	Cake, frozen yogurt and cake layer, chocolate, with icing
567	53112500	2	Grain	Cake, ice box with fruit and whipped cream
568	53113000	2	Grain	Cake, jelly roll
569	53113950	2	Grain	Cake, lemon, NS as to icing
570	53114000	2	Grain	Cake, lemon, without icing
571	53114100	2	Grain	Cake, lemon, with icing
572	53114150	2	Grain	Cake, lemon, lowfat, NS as to icing
573	53114200	2	Grain	Cake, lemon, lowfat, without icing
574	53114250	2	Grain	Cake, lemon, lowfat, with icing
575	53115000	2	Grain	Cake, marble, NS as to icing
576	53115100	2	Grain	Cake, marble, without icing
577	53115200	2	Grain	Cake, marble, with icing
578	53115300	2	Grain	Cake, nut, NS as to icing
579	53115310	2	Grain	Cake, nut, without icing
580	53115320	2	Grain	Cake, nut, with icing
581	53115400	2	Grain	Cake, oatmeal, without icing
582	53115410	2	Grain	Cake, oatmeal, with icing
583	53115450	2	Grain	Cake, peanut butter, with icing
584	53115500	2	Grain	Cake, pineapple, fat free, cholesterol free, without icing
585	53115600	2	Grain	Cake, poppyseed, without icing
586	53116000	2	Grain	Cake, pound, without icing
587	53116020	2	Grain	Cake, pound, with icing
588	53116270	2	Grain	Cake, pound, chocolate
589	53116280	2	Grain	Cake, pound, chocolate, fat free, cholesterol free
590	53116350	2	Grain	Cake, pound, Puerto Rican style (Ponque)
591	53116380	2	Grain	Cake, pound, fat free, cholesterol free
592	53116390	2	Grain	Cake, pound, reduced fat, cholesterol free
593	53116490	2	Grain	Cake, pumpkin, NS as to icing
594	53116500	2	Grain	Cake, pumpkin, without icing
595	53116510	2	Grain	Cake, pumpkin, with icing
596	53116550	2	Grain	Cake, raisin-nut, without icing
597	53116560	2	Grain	Cake, raisin-nut, with icing
598	53116570	2	Grain	Cake, Ravani (made with farina)
599	53116600	2	Grain	Cake, rice flour, without icing
600	53116650	2	Grain	Cake, Quezadilla, El Salvadorian style
601	53116750	2	Grain	Cake, soy flour, without icing
602	53117000	2	Grain	Cake, spice, NS as to icing
603	53117100	2	Grain	Cake, spice, without icing
604	53117200	2	Grain	Cake, spice, with icing

605	53118000	2	Grain	Cake, sponge, NS as to icing
606	53118100	2	Grain	Cake, sponge, without icing
607	53118200	2	Grain	Cake, sponge, with icing
608	53118300	2	Grain	Cake, sponge, chocolate, without icing
609	53118310	2	Grain	Cake, sponge, chocolate, with icing
610	53118350	2	Grain	Cake, sweetpotato, with icing
611	53118410	2	Grain	Cake, rum flavored, without icing (Sopa Borracha)
612	53118500	2	Grain	Cake, torte
613	53118600	2	Grain	Cake, chiffon, NS as to icing
614	53118700	2	Grain	Cake, chiffon, without icing
615	53118800	2	Grain	Cake, chiffon, with icing
616	53118900	2	Grain	Cake, chiffon, chocolate, without icing
617	53118950	2	Grain	Cake, chiffon, chocolate, with icing
618	53119000	2	Grain	Cake, upside down (all fruits)
619	53120000	2	Grain	Cake, white, standard-type mix (egg whites and water added), NS
620	53120060	2	Grain	Cake, white, made from home recipe or purchased ready-to-eat, NS
621	53120100	2	Grain	Cake, white, standard-type mix (egg whites and water added to mix)
622	53120160	2	Grain	Cake, white, without icing, made from home recipe or purchased
623	53120200	2	Grain	Cake, white, standard-type mix (egg whites and water added to mix)
624	53120260	2	Grain	Cake, white, with icing, made from home recipe or purchased
625	53120300	2	Grain	Cake, white, pudding-type mix (oil, egg whites, and water added)
626	53120330	2	Grain	Cake, white, pudding-type mix (oil, egg whites, and water added)
627	53120350	2	Grain	Cake, white, pudding-type mix (oil, egg whites, and water added)
628	53120400	2	Grain	Cake, white, eggless, lowfat
629	53120500	2	Grain	Cake, whole wheat, with fruit and nuts, without icing
630	53121000	2	Grain	Cake, yellow, standard-type mix (eggs and water added to dry mix), NS
631	53121060	2	Grain	Cake, yellow, made from home recipe or purchased ready-to-eat, NS
632	53121100	2	Grain	Cake, yellow, standard-type mix (eggs and water added to dry mix)
633	53121160	2	Grain	Cake, yellow, without icing, made from home recipe or purchased
634	53121200	2	Grain	Cake, yellow, standard-type mix (eggs and water added to dry mix)
635	53121260	2	Grain	Cake, yellow, with icing, made from home recipe or purchased
636	53121280	2	Grain	Cake, yellow, pudding-type mix (oil, eggs, and water added to dry mix)
637	53121300	2	Grain	Cake, yellow, pudding-type mix (oil, eggs, and water added to dry mix)
638	53121330	2	Grain	Cake, yellow, pudding-type mix (oil, eggs, and water added to dry mix)
639	53122070	2	Grain	Cake, shortcake, biscuit type, with whipped cream and fruit
640	53122080	2	Grain	Cake, shortcake, biscuit type, with fruit
641	53123070	2	Grain	Cake, shortcake, sponge type, with whipped cream and fruit
642	53123080	2	Grain	Cake, shortcake, sponge type, with fruit
643	53123500	2	Grain	Cake, shortcake, with whipped topping and fruit, diet
644	53124100	2	Grain	Cake, zucchini, NS as to icing
645	53124110	2	Grain	Cake, zucchini, without icing
646	53124120	2	Grain	Cake, zucchini, with icing
647	53200100	2	Grain	Cookie, batter or dough, raw, not chocolate

648	53201000	2	Grain	Cookie, NS as to type
649	53202000	2	Grain	Cookie, almond
650	53203000	2	Grain	Cookie, applesauce
651	53203050	2	Grain	Cookie, fruit, baby
652	53203100	2	Grain	Cookie, baby
653	53203500	2	Grain	Cookie, biscotti (Italian sugar cookie)
654	53204000	2	Grain	Cookie, brownie, NS as to icing
655	53204010	2	Grain	Cookie, brownie, without icing
656	53204100	2	Grain	Cookie, brownie, with icing
657	53204500	2	Grain	Cookie, brownie, with cream cheese filling, without icing
658	53204600	2	Grain	Cookie, brownie, with peanut butter fudge icing
659	53204800	2	Grain	Cookie, brownie, diet, NS as to icing
660	53204830	2	Grain	Cookie, brownie, lowfat, with icing
661	53204840	2	Grain	Cookie, brownie, lowfat, without icing
662	53204850	2	Grain	Cookie, brownie, fat free, cholesterol free, with icing
663	53204860	2	Grain	Cookie, brownie, fat free, without icing
664	53205250	2	Grain	Cookie, butterscotch, brownie
665	53205500	2	Grain	Cookie, butterscotch chip
666	53205600	2	Grain	Cookie, caramel coated, with nuts
667	53205750	2	Grain	Cookie, carob
668	53205760	2	Grain	Cookie, carob and honey brownie
669	53206000	2	Grain	Cookie, chocolate chip
670	53206010	2	Grain	Cookie, chocolate chip, with raisins
671	53206020	2	Grain	Cookie, chocolate chip, made from home recipe or purchased at a bakery
672	53206030	2	Grain	Cookie, chocolate chip, reduced fat
673	53206050	2	Grain	Cookie, rich, chocolate chip, with chocolate filling
674	53206100	2	Grain	Cookie, chocolate chip sandwich
675	53206500	2	Grain	Cookie, chocolate, made with rice cereal (no-bake)
676	53206550	2	Grain	Cookie, chocolate, made with oatmeal and coconut (no-bake)
677	53207000	2	Grain	Cookie, chocolate fudge, with/without nuts
678	53207050	2	Grain	Cookie, chocolate, with chocolate filling or coating, fat free
679	53208000	2	Grain	Cookie, chocolate-covered marshmallow
680	53208200	2	Grain	Cookie, marshmallow pie, chocolate covered
681	53209000	2	Grain	Cookie, chocolate, chocolate sandwich or chocolate-coated or striped
682	53209010	2	Grain	Cookie, chocolate-covered, sugar wafer, creme- or caramel-filled
683	53209020	2	Grain	Cookie, chocolate sandwich, reduced fat
684	53209050	2	Grain	Cookie, chocolate-covered, chocolate sandwich
685	53209100	2	Grain	Cookie, chocolate, sandwich, with extra filling
686	53209500	2	Grain	Cookie, chocolate and vanilla sandwich
687	53210000	2	Grain	Cookie, chocolate wafer
688	53210900	2	Grain	Cookie, graham cracker sandwich with chocolate and marshmallow filling
689	53210910	2	Grain	Cookie, graham cracker with marshmallow
690	53211000	2	Grain	Cookie bar, with chocolate, nuts, and graham crackers

691	53211000	2	Grain	Cookie bar, with chocolate, nuts, and graham crackers
692	53215000	2	Grain	Cookie, coconut, bars
693	53215000	2	Grain	Cookie, coconut, bars
694	53215500	2	Grain	Cookie, coconut
695	53216000	2	Grain	Cookie, coconut and nut
696	53220000	2	Grain	Cookie, fruit-filled bar
697	53220010	2	Grain	Cookie, fruit-filled bar, fat free
698	53220020	2	Grain	Cookie, date bar
699	53220020	2	Grain	Cookie, date bar
700	53220030	2	Grain	Cookie, fig bar
701	53220030	2	Grain	Cookie, fig bar
702	53220040	2	Grain	Cookie, fig bar, fat free
703	53220040	2	Grain	Cookie, fig bar, fat free
704	53222010	2	Grain	Cookie, fortune
705	53222020	2	Grain	Cookie, cone shell, ice cream type, wafer or cake
706	53222100	2	Grain	Cookie, cone shell, ice cream type, brown sugar
707	53223000	2	Grain	Cookie, gingersnaps
708	53223100	2	Grain	Cookie, granola
709	53224000	2	Grain	Cookie, ladyfinger
710	53224250	2	Grain	Cookie, lemon bar
711	53224250	2	Grain	Cookie, lemon bar
712	53225000	2	Grain	Cookie, macaroon, coconut-meringue type, no flour
713	53226000	2	Grain	Cookie, marshmallow, with coconut
714	53226500	2	Grain	Cookie, marshmallow, with rice cereal (no-bake)
715	53226550	2	Grain	Cookie, marshmallow, with rice cereal and chocolate chips
716	53226600	2	Grain	Cookie, marshmallow and peanut butter, with oat cereal
717	53227000	2	Grain	Cookie, marshmallow pies, non-chocolate coating
718	53228000	2	Grain	Cookie, meringue
719	53230000	2	Grain	Cookie, molasses
720	53231000	2	Grain	Cookie, Lebkuchen
721	53231400	2	Grain	Cookie, multigrain, high fiber
722	53233000	2	Grain	Cookie, oatmeal
723	53233010	2	Grain	Cookie, oatmeal, with raisins
724	53233020	2	Grain	Cookie, oatmeal, with fruit filling
725	53233030	2	Grain	Cookie, oatmeal, fat free, with raisins
726	53233040	2	Grain	Cookie, oatmeal, reduced fat, with raisins
727	53233050	2	Grain	Cookie, oatmeal sandwich, with creme filling
728	53233060	2	Grain	Cookie, oatmeal, with chocolate chips
729	53233080	2	Grain	Cookie, oatmeal sandwich, with peanut butter and jelly filling
730	53233100	2	Grain	Cookie, oatmeal, with chocolate and peanut butter (no-bake)
731	53233500	2	Grain	Cookie, oat bran
732	53234000	2	Grain	Cookie, peanut butter
733	53234010	2	Grain	Cookie, peanut butter, with oatmeal

734	53234100	2	Grain	Cookie, peanut butter, with chocolate
735	53234250	2	Grain	Cookie, peanut butter with rice cereal (no-bake)
736	53235000	2	Grain	Cookie, peanut
737	53235500	2	Grain	Cookie, with peanut butter filling, chocolate-coated
738	53235600	2	Grain	Cookie, Pfeffernusse
739	53236000	2	Grain	Cookie, pizzelle (Italian style wafer)
740	53236100	2	Grain	Cookie, pumpkin
741	53237000	2	Grain	Cookie, raisin
742	53237010	2	Grain	Cookie, raisin sandwich, cream-filled
743	53237500	2	Grain	Cookie, rum ball (no-bake)
744	53238000	2	Grain	Cookie, sandwich-type, not chocolate or vanilla
745	53239000	2	Grain	Cookie, shortbread
746	53239010	2	Grain	Cookie, shortbread, reduced fat
747	53239050	2	Grain	Cookie, shortbread, with chocolate filling
748	53241500	2	Grain	Cookie, butter or sugar cookie
749	53241600	2	Grain	Cookie, butter or sugar cookie, with fruit and/or nuts
750	53242000	2	Grain	Cookie, sugar wafer
751	53242250	2	Grain	Cookie, teething, baby food
752	53242500	2	Grain	Cookie, toffee bar
753	53242500	2	Grain	Cookie, toffee bar
754	53243000	2	Grain	Cookie, vanilla sandwich
755	53243050	2	Grain	Cookie, vanilla sandwich, reduced fat
756	53243100	2	Grain	Cookie, rich, all chocolate, with chocolate filling or chocolate chi
757	53244010	2	Grain	Cookie, butter or sugar, with chocolate icing or filling
758	53244020	2	Grain	Cookie, butter or sugar, iced, with icing other than chocolate
759	53245000	2	Grain	Cookie, vanilla waffle creme
760	53246000	2	Grain	Cookie, tea, Japanese
761	53247000	2	Grain	Cookie, vanilla wafer
762	53247050	2	Grain	Cookie, vanilla wafer, reduced fat
763	53247500	2	Grain	Cookie, vanilla with caramel, coconut, and chocolate coating
764	53248000	2	Grain	Cookie, whole wheat, dried fruit, nut
765	53251100	2	Grain	Cookie, rugelach
766	53260000	2	Grain	Cookie, dietetic, NFS
767	53260010	2	Grain	Cookie, dietetic, apple pastry
768	53260030	2	Grain	Cookie, dietetic, chocolate chip
769	53260050	2	Grain	Cookie, dietetic, chocolate flavored
770	53260100	2	Grain	Cookie, dietetic, fruit types
771	53260150	2	Grain	Cookie, lemon wafer, lowfat
772	53260200	2	Grain	Cookie, dietetic, oatmeal with raisins
773	53260300	2	Grain	Cookie, dietetic, sandwich type
774	53260400	2	Grain	Cookie, dietetic, sugar or plain
775	53415120	2	Grain	Fritter, apple
776	53415200	2	Grain	Fritter, banana

777	53415220	2	Grain	Fritter, berry
778	53430000	2	Grain	Crepe, dessert type, NS as to filling
779	53430100	2	Grain	Crepe, dessert type, chocolate-filled
780	53430200	2	Grain	Crepe, dessert type, fruit-filled
781	53430250	2	Grain	Crepe suzette
782	53430300	2	Grain	Crepe, dessert type, ice cream-filled
783	53510000	2	Grain	Danish pastry, plain or spice
784	53510100	2	Grain	Danish pastry, with fruit
785	53510200	2	Grain	Danish pastry, with nuts
786	53511000	2	Grain	Danish pastry, with cheese
787	53511500	2	Grain	Danish pastry, with cheese, fat free, cholesterol free
788	53520000	2	Grain	Doughnut, NS as to cake or yeast
789	53520110	2	Grain	Doughnut, cake type
790	53520120	2	Grain	Doughnut, cake type, chocolate
791	53520140	2	Grain	Doughnut, cake type, chocolate covered
792	53520150	2	Grain	Doughnut, cake type, chocolate covered, dipped in peanuts
793	53520160	2	Grain	Doughnut, cake type, chocolate, with chocolate icing
794	53520500	2	Grain	Doughnut, oriental
795	53520700	2	Grain	French cruller
796	53521100	2	Grain	Doughnut, raised or yeast, chocolate, with chocolate icing
797	53521110	2	Grain	Doughnut, raised or yeast
798	53521120	2	Grain	Doughnut, raised or yeast, chocolate
799	53521130	2	Grain	Doughnut, raised or yeast, chocolate covered
800	53521140	2	Grain	Doughnut, jelly
801	53521210	2	Grain	Doughnut, custard-filled
802	53521220	2	Grain	Doughnut, chocolate cream-filled
803	53521230	2	Grain	Doughnut, custard-filled, with icing
804	53521250	2	Grain	Doughnut, wheat
805	53521300	2	Grain	Doughnut, wheat, chocolate covered
806	53521400	2	Grain	Doughnut, eggless, carob-covered, raised or yeast
807	53540000	2	Grain	Breakfast bar, NFS
808	53540100	2	Grain	Breakfast bar, cake-like
809	53540500	2	Grain	Breakfast bar, date, with yogurt coating
810	53541100	2	Grain	Breakfast bar, diet meal type
811	53541200	2	Grain	Meal replacement bar
812	53541200	2	Grain	Meal replacement bar
813	53544450	2	Grain	PowerBar (fortified high energy bar)
815	53610000	2	Grain	Coffee cake, NFS
816	53610100	2	Grain	Coffee cake, crumb or quick-bread type
817	53610120	2	Grain	Coffee cake, crumb or quick-bread type, reduced fat, cholesterol free
818	53610150	2	Grain	Coffee cake, crumb or quick-bread type, with icing
819	53610170	2	Grain	Coffee cake, crumb or quick-bread type, with fruit
820	53610200	2	Grain	Coffee cake, crumb or quick-bread type, cheese-filled

821	53610250	2	Grain	Coffee cake, crumb or quick-bread type, custard filled
822	55101000	2	Grain	Pancakes, plain
823	55101010	2	Grain	Pancakes, reduced calorie, high fiber
824	55103000	2	Grain	Pancakes, with fruit
825	55103100	2	Grain	Pancakes, with chocolate chips
826	55105000	2	Grain	Pancakes, buckwheat
827	55105100	2	Grain	Pancakes, cornmeal
828	55105200	2	Grain	Pancakes, whole wheat
829	55105300	2	Grain	Pancakes, sour dough
830	55105400	2	Grain	Pancakes, rye
831	55201000	2	Grain	Waffle, plain
832	55202000	2	Grain	Waffle, wheat, bran, or multigrain
833	55203000	2	Grain	Waffle, fruit
834	55203500	2	Grain	Waffle, nut and honey
835	55204000	2	Grain	Waffle, cornmeal
836	55205000	2	Grain	Waffle, 100% whole wheat or 100% whole grain
837	55206000	2	Grain	Waffle, oat bran
838	55207000	2	Grain	Waffle, multi-bran
839	55211000	2	Grain	Waffle, plain, fat free
840	55211050	2	Grain	Waffle, plain, lowfat
841	55301000	2	Grain	French toast, plain
842	55301050	2	Grain	French toast sticks, plain
843	55310100	2	Grain	Bread fritters, Puerto Rican style (Torrejas, Galician fritters)
844	55401000	2	Grain	Crepe, plain
845	55701000	2	Grain	Cake made with glutinous rice
846	55702000	2	Grain	Cake or pancake made with rice flour and/or dried beans
847	55703000	2	Grain	Cake made with glutinous rice and dried beans
848	55801000	2	Grain	Funnel cake

Fruit and vegetable juices

849	61200500	10	Juice	Acerola juice
850	61201000	10	Juice	Grapefruit juice, NFS
851	61201010	10	Juice	Grapefruit juice, freshly squeezed
852	61201020	10	Juice	Grapefruit juice, unsweetened, NS as to form
853	61201220	10	Juice	Grapefruit juice, canned, bottled or in a carton, unsweetened
854	61201230	10	Juice	Grapefruit juice, canned, bottled or in a carton, with sugar
855	61201240	10	Juice	Grapefruit juice, canned, bottled, or in a carton, sweetened
856	61201620	10	Juice	Grapefruit juice, frozen, unsweetened (reconstituted with water)
857	61201630	10	Juice	Grapefruit juice, frozen, with sugar (reconstituted with water)
858	61204000	10	Juice	Lemon juice, NS as to form
859	61204010	10	Juice	Lemon juice, fresh
860	61204200	10	Juice	Lemon juice, canned or bottled
861	61204600	10	Juice	Lemon juice, frozen

862	61207000	10	Juice	Lime juice, NS as to form
863	61207010	10	Juice	Lime juice, fresh
864	61207200	10	Juice	Lime juice, canned or bottled
865	61207600	10	Juice	Lime juice, frozen
866	61210000	10	Juice	Orange juice, NFS
867	61210010	10	Juice	Orange juice, freshly squeezed
868	61210220	10	Juice	Orange juice, canned, bottled or in a carton, unsweetened
869	61210230	10	Juice	Orange juice, canned, bottled or in a carton, with sugar
870	61210250	10	Juice	Orange juice, with calcium added, canned, bottled or in a carton
871	61210620	10	Juice	Orange juice, frozen, unsweetened (reconstituted with water)
872	61210630	10	Juice	Orange juice, frozen, with sugar (reconstituted with water)
873	61210720	10	Juice	Orange juice, frozen, unsweetened, not reconstituted
874	61210730	10	Juice	Orange juice, frozen, with sugar, not reconstituted
875	61210820	10	Juice	Orange juice, frozen, with calcium added (reconstituted with water)
876	61213000	10	Juice	Tangerine juice, NFS
877	61213220	10	Juice	Tangerine juice, canned, unsweetened
878	61213230	10	Juice	Tangerine juice, canned, with sugar
879	61213620	10	Juice	Tangerine juice, frozen, unsweetened (reconstituted with water)
880	61214000	10	Juice	Grape-tangerine-lemon juice
881	61216000	10	Juice	Grapefruit and orange juice, NFS
882	61216010	10	Juice	Grapefruit and orange juice, fresh
883	61216220	10	Juice	Grapefruit and orange juice, canned, unsweetened
884	61216230	10	Juice	Grapefruit and orange juice, canned, with sugar
885	61216620	10	Juice	Grapefruit and orange juice, frozen (reconstituted with water)
886	61219000	10	Juice	Orange and banana juice
887	61219100	10	Juice	Pineapple-orange-banana juice
888	61219150	10	Juice	Orange-white grape-peach juice
889	61219650	10	Juice	Apricot-orange juice
890	61222000	10	Juice	Pineapple-grapefruit juice, NFS
891	61222200	10	Juice	Pineapple-grapefruit juice, canned, bottled or in a carton, NS as to
892	61222220	10	Juice	Pineapple-grapefruit juice, canned, bottled or in a carton, unsweetened
893	61222230	10	Juice	Pineapple-grapefruit juice, canned, bottled or in a carton, with sugar
894	61222600	10	Juice	Pineapple-grapefruit juice, frozen (reconstituted with water)
895	61225000	10	Juice	Pineapple-orange juice, NFS
896	61225200	10	Juice	Pineapple-orange juice, canned, NS as to sweetened or unsweetened
897	61225220	10	Juice	Pineapple-orange juice, canned, unsweetened
898	61225230	10	Juice	Pineapple-orange juice, canned, with sugar
899	61225600	10	Juice	Pineapple-orange juice, frozen (reconstituted with water)
900	61226000	10	Juice	Strawberry-banana-orange juice
901	74301100	10	Juice	Tomato juice
902	74301150	10	Juice	Tomato juice, low sodium
903	74302000	10	Juice	Tomato juice cocktail
904	74303000	10	Juice	Tomato and vegetable juice, mostly tomato

905	74303100	10	Juice	Tomato and vegetable juice, mostly tomato, low sodium
906	74304000	10	Juice	Tomato juice with clam or beef juice

Salad dressings

907	83100100	2	fat/oil	Salad dressing, NFS
908	83101000	2	fat/oil	Blue or roquefort cheese dressing
909	83101500	2	fat/oil	Bacon dressing (hot)
910	83101600	2	fat/oil	Bacon and tomato dressing
911	83102000	2	fat/oil	Caesar dressing
912	83103000	2	fat/oil	Coleslaw dressing
913	83103500	2	fat/oil	Feta Cheese Dressing
914	83104000	2	fat/oil	French dressing
915	83105500	2	fat/oil	Honey mustard dressing
916	83106000	2	fat/oil	Italian dressing, made with vinegar and oil
917	83109000	2	fat/oil	Russian dressing
918	83110000	2	fat/oil	Mayonnaise-type salad dressing
919	83110010	2	fat/oil	Mayonnaise-type salad dressing, cholesterol-free
920	83111000	2	fat/oil	Boiled, cooked-type dressing
921	83112000	2	fat/oil	Green Goddess dressing
922	83112500	2	fat/oil	Creamy dressing, made with sour cream and/or buttermilk
923	83112600	2	fat/oil	Cream cheese dressing
924	83112900	2	fat/oil	Milk, vinegar, and sugar dressing
925	83112950	2	fat/oil	Poppy seed dressing
926	83112960	2	fat/oil	Peppercorn Dressing
927	83112980	2	fat/oil	Celery seed dressing
928	83112990	2	fat/oil	Sesame dressing
929	83113000	2	fat/oil	Sweet and sour dressing
930	83114000	2	fat/oil	Thousand Island dressing
931	83115000	2	fat/oil	Yogurt dressing
932	83200100	2	fat/oil	Salad dressing, low-calorie, NFS
933	83200500	2	fat/oil	Bacon and tomato dressing, low calorie
934	83201000	2	fat/oil	Blue or roquefort cheese dressing, low-calorie
935	83201050	2	fat/oil	Blue or roquefort cheese dressing, reduced calorie
936	83201200	2	fat/oil	Blue or roquefort cheese dressing, reduced calorie, fat-free
937	83201400	2	fat/oil	Coleslaw dressing, reduced calorie
938	83202000	2	fat/oil	French dressing, low-calorie
939	83202010	2	fat/oil	French dressing, reduced calorie, fat-free, cholesterol-free
940	83202020	2	fat/oil	French dressing, reduced calorie
941	83203000	2	fat/oil	Caesar dressing, low-calorie
942	83203250	2	fat/oil	Mayonnaise-type salad dressing, fat-free
943	83204050	2	fat/oil	Mayonnaise-type salad dressing, low-calorie or diet
944	83204060	2	fat/oil	Mayonnaise-type salad dressing, low-calorie or diet, cholesterol-free
945	83205000	2	fat/oil	Italian dressing, low calorie

946	83205450	2	fat/oil	Italian dressing, reduced calorie
947	83205500	2	fat/oil	Italian dressing, reduced calorie, fat-free
948	83206000	2	fat/oil	Russian dressing, low-calorie
949	83207000	2	fat/oil	Thousand Island dressing, low-calorie
950	83207100	2	fat/oil	Thousand Island dressing, reduced calorie, fat-free, cholesterol-free
951	83208000	2	fat/oil	Vinegar, sugar, and water dressing
952	83208500	2	fat/oil	Korean dressing or marinade
953	83209000	2	fat/oil	Milk, vinegar, and artificial sweetener dressing
954	83210000	2	fat/oil	Creamy dressing, made with sour cream and/or buttermilk and oil
955	83210050	2	fat/oil	Creamy dressing made with sour cream and/or buttermilk and oil
956	83210100	2	fat/oil	Creamy dressing, made with sour cream and/or buttermilk and oil
957	83210200	2	fat/oil	Creamy dressing, made with sour cream and/or buttermilk and oil
958	83210250	2	fat/oil	Creamy dressing, made with sour cream and/or buttermilk and oil
959	83220000	2	fat/oil	Salad dressing, low calorie, oil-free

Beverages

960	92510110	10	Beverage	Apple drink
961	92510120	10	Beverage	Apple-cherry drink
962	92510150	10	Beverage	Apple juice drink
963	92510170	10	Beverage	Apple-cranberry-grape juice drink
964	92510200	10	Beverage	Apple-orange-pineapple juice drink
965	92510220	10	Beverage	Apricot-pineapple juice drink
966	92510310	10	Beverage	Banana-orange drink
967	92510410	10	Beverage	Black cherry drink
968	92510610	10	Beverage	Fruit drink
969	92510630	10	Beverage	Fruit juice drink, NFS
970	92510650	10	Beverage	Tamarind drink, Puerto Rican (Refresco de tamarindo)
971	92510720	10	Beverage	Fruit punch, made with fruit juice and soda
972	92510730	10	Beverage	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
973	92510810	10	Beverage	Grapeade and grape drink
974	92510820	10	Beverage	Grape juice drink
975	92510910	10	Beverage	Grapefruit juice drink
976	92510950	10	Beverage	Guava juice drink
977	92511000	10	Beverage	Lemonade, frozen concentrate, not reconstituted
978	92511010	10	Beverage	Lemonade
979	92511020	10	Beverage	Lemon-limeade
980	92511110	10	Beverage	Limeade
981	92511190	10	Beverage	Orange juice drink
982	92511200	10	Beverage	Orange-mango juice drink
983	92511220	10	Beverage	Orange drink
984	92511230	10	Beverage	Orange-apricot juice drink
985	92511240	10	Beverage	Orange-lemon drink
986	92511250	10	Beverage	Citrus fruit juice drink

987	92511260	10	Beverage	Orange-cranberry juice drink
988	92511270	10	Beverage	Orange-peach juice drink
989	92511280	10	Beverage	Orange-grape-banana juice drink
990	92511290	10	Beverage	Papaya juice drink
991	92511310	10	Beverage	Pineapple-grapefruit juice drink
992	92511340	10	Beverage	Pineapple-orange juice drink
993	92511350	10	Beverage	Orange-raspberry juice drink
994	92511400	10	Beverage	Raspberry-flavored drink
995	92511510	10	Beverage	Strawberry-flavored drink
996	92512040	10	Beverage	Frozen daiquiri mix, frozen concentrate, not reconstituted
997	92512050	10	Beverage	Frozen daiquiri mix, from frozen concentrate, reconstituted
998	92512090	10	Beverage	Pina Colada, nonalcoholic
999	92512110	10	Beverage	Whiskey sour, nonalcoholic
1000	92520410	10	Beverage	Fruit drink, low calorie
1001	92520810	10	Beverage	Grape drink, low calorie
1002	92520910	10	Beverage	Lemonade, low calorie
1003	92530110	10	Beverage	Apple drink with vitamin C added
1004	92530210	10	Beverage	Black cherry drink with vitamin C added
1005	92530310	10	Beverage	Cherry drink with vitamin C added
1006	92530410	10	Beverage	Citrus drink with vitamin C added
1007	92530510	10	Beverage	Cranberry juice drink with vitamin C added
1008	92530520	10	Beverage	Cranberry-apple juice drink with vitamin C added
1009	92530610	10	Beverage	Fruit punch, fruit drink, or fruitade, with vitamin C added
1010	92530710	10	Beverage	Grape drink with vitamin C added
1011	92530810	10	Beverage	Grapefruit juice drink with vitamin C added
1012	92530840	10	Beverage	Guava juice drink with vitamin C added
1013	92530910	10	Beverage	Lemonade with vitamin C added
1014	92530950	10	Beverage	Vegetable and fruit juice drink, with vitamin C added
1015	92531010	10	Beverage	Orange drink and orangeade with vitamin C added
1016	92531020	10	Beverage	Orange breakfast drink, made from frozen concentrate
1017	92531030	10	Beverage	Orange breakfast drink
1018	92531110	10	Beverage	Pineapple-grapefruit juice drink with vitamin C added
1019	92531120	10	Beverage	Pineapple-orange juice drink with vitamin C added
1020	92531150	10	Beverage	Pineapple-orange-grapefruit juice drink with vitamin C added
1021	92531210	10	Beverage	Strawberry-flavored drink with vitamin C added
1022	92541010	10	Beverage	Fruit-flavored drink, made from sweetened powdered mix (fortified)
1023	92541020	10	Beverage	Lemonade-flavored drink, made from powdered mix, with sugar and vit
1024	92541040	10	Beverage	Lemonade-flavored drink, made from powdered mix, low calorie,
1025	92541100	10	Beverage	Apple cider-flavored drink, made from powdered mix, with sugar and vit
1026	92541120	10	Beverage	Apple cider-flavored drink, made from powdered mix, low calorie
1027	92542000	10	Beverage	Fruit-flavored drink, made from powdered mix, mainly sugar
1028	92544000	10	Beverage	Fruit-flavored drink, made from unsweetened powdered mix
1029	92550050	10	Beverage	Apple-white grape juice drink, low calorie, with vitamin C added

1030	92550110	10	Beverage	Cranberry juice drink, low calorie, with vitamin C added
1031	92550210	10	Beverage	Cranberry-apple juice drink, low calorie, with vitamin C added
1032	92550300	10	Beverage	Grapefruit juice drink, low calorie, with vitamin C added
1033	92550610	10	Beverage	Fruit-flavored drinks, punches, ades, low calorie, with vitamin C
1034	92551600	10	Beverage	Citrus juice drink, low calorie
1035	92551700	10	Beverage	Juice drink, low calorie
1036	92552000	10	Beverage	Fruit-flavored drink, made from powdered mix with high vitamin C
1037	92552050	10	Beverage	Orange breakfast drink, low calorie
1038	92552100	10	Beverage	Orange-cranberry juice drink, low calorie, with vitamin C added
1039	92553000	10	Beverage	Fruit-flavored thirst quencher beverage, low calorie
1040	92560000	10	Beverage	Fruit-flavored thirst quencher beverage
1041	92570100	10	Beverage	Fluid replacement, electrolyte solution
1042	92570500	10	Beverage	Fluid replacement, 5% glucose in water
1043	92582000	10	Beverage	Fruit-flavored drink, low calorie, calcium fortified
1044	92582050	10	Beverage	Fruit-flavored drink, vitamin and mineral fortified
1045	92582100	10	Beverage	Citrus juice drink, calcium fortified
1046	92582110	10	Beverage	Orange breakfast drink, calcium fortified
1047	92731000	10	Beverage	Fruit-flavored drink, non-carbonated, made from powdered mix
1048	92741000	10	Beverage	Fruit-flavored drink, non-carbonated, made from low calorie powdered
1049	92751000	10	Beverage	Root beer, noncarbonated, made from powdered mix, with sugar
1050	92900100	10	Beverage	Tang, dry concentrate
1051	92900110	10	Beverage	Fruit-flavored concentrate, dry powder, with sugar and vitamin C
1052	92900200	10	Beverage	Fruit-flavored beverage, dry concentrate, low calorie, not reconstit
1053	92900300	10	Beverage	Fruit-flavored thirst quencher beverage, dry concentrate, not recons

Submission End

000100

From: [Mejia, Luis](#)
To: [Carlson, Susan;](#)
Subject: Allergenicity Report/ Canola Proteins GRAS Notification
Date: Tuesday, May 11, 2010 10:25:37 AM
Attachments: [CANOLA ALLERGY STUDY FINAL REPORT 0808.pdf](#)

Dear Susan:

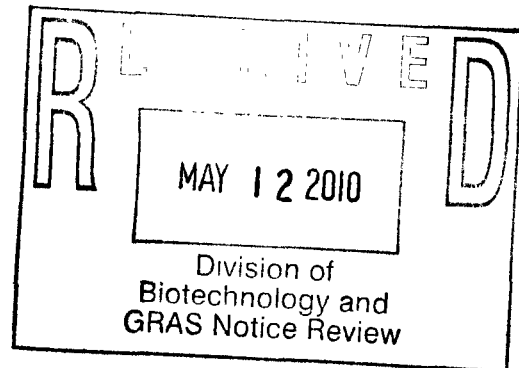
Per your request in our yesterday's conference call, find attached an electronic copy of the allergenicity report of the study of the canola proteins conducted by the University of Nebraska (Cited in the GRAS notification dossier). A hard copy will be send today by courier. Please do not hesitate to contact me if you have any questions regarding the report.

Luis

Luis A. Mejia, Ph. D.
Director, Regulatory and Scientific Affairs
Archer Daniels Midland Company
1001 North Brush College Road
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Archer Daniels Midland Company
Office of Compliance and Ethics
1001 North Brush College Road
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May 11, 2010

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

Re: Allergenicity Report - Canola Proteins GRAS Notice

Dear Dr. Carlson:

As discussed in our recent conference call, find enclosed a copy of the Allergenicity Report of the study of the canola proteins conducted by the University of Nebraska (cited in the GRAS notification dossier).

Respectfully,

Luis A. Mejia, Ph.D.
Director, Regulatory and Scientific Affairs

LAM/jm

Enc.

STUDY TITLE

**Assessment of the Potential Allergenicity and Stability in Pepsin of Canola Protein Isolates:
SuperteinTM and Puratein[®]**

AUTHORS

Richard E. Goodman
Pramod N. Siddanakoppalu

STUDY COMPLETED ON

30 April 2008

PERFORMING LABORATORY

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SUBMITTED BY

Archer Daniels Midland Company (ADM)
Decatur, Illinois

LABORATORY STUDY ID

REG-2008-1

SUMMARY

Two canola protein isolates, called Supertein™ and Puratein®, were evaluated for possible risk of food allergy. The isolates were provided by Archer Daniels Midland Company (ADM), Decatur, IL. The materials are derived from canola seed meal, following removal of oil and are being considered as possible human food sources.

Samples from five production lots and one composite (pooled) lot each for Supertein™ and Puratein® were analyzed for similarity. The protein content of the dry powdered samples was determined by LECO analysis. Dissolved samples were approximately 92% protein based on SDS-PAGE, colloidal blue-stained gel analysis. Expected protein masses for the main proteins were confirmed under both reducing and non-reducing SDS-PAGE. Protein identities of the composite samples (amino acid sequences) were confirmed by LC/MSMS of isolated bands of protein from one-dimensional SDS-PAGE gels. The amino acid sequence results of isolated Supertein™ matched multiple isomers or variants of 2S albumin storage proteins called napins. Isolated bands of Puratein® included multiple isomeric forms of the 11S globulin storage proteins called cruciferins. Based on gel staining patterns and image analysis, napins represented approximately 84% of Supertein™. The cruciferins represented approximately 74% of Puratein®. The Puratein® samples were also found to contain a small amount of detectable bands corresponding to the MW of napins and in fact some peptide fragments of napins based on highly sensitive LC-MS/MS. Similarly, the napin bands contained some fragments of cruciferins as well as a probable peptide of lipid transfer protein and at least one other prolamin.

The LC-MS/MS sequence data did not provide full-length protein sequences for any of the proteins. However, based on analysis of peptides from each band, using the Scaffold program and data supplied by Colorado State University, specific NCBI protein sequence entries of full-length or nearly full-length napins and cruciferins were matched with 95% probability of identification. Seven napins (2S albumins) and eight cruciferins (cupins) were found by the program based on peptide analysis. Each of these (15 sequences) were compared to sequences of known allergens using AllergenOnline.com, version 8.0 (with full-length and sliding 80 amino acid segment FASTA comparisons) to find the most similar allergenic proteins. As expected, the highest identity matches were to mustard allergens from *Brassica juncea* and *Sinapis alba*. The seven proteins identified as napins in Supertein™ are between 75 and 94% identical to each other and are between 50% and 95% identical to allergens listed as *Sinapis alba* or *Brassica sp.* in AllergenOnline version 8.0 based on overall FASTA alignments. The Puratein® major proteins are cruciferins (11S globulin seed storage proteins). Seven peptides matched mustard 11S globulins from *Brassicus napus* while one matched an 11S albumin from *Raphanus sativum* (another species of mustard) and based on Clustal W align with between 74% and 94% identities. All eight of these proteins matched two cruciferins (cupins) from *Sinapis alba* at from 50% to > 90% identity by full-length FASTA. The multiple cruciferin isomers were greater than 70%

identical and they closely matched the sequences of allergenic 11S storage proteins from *Sinapis alba*, or yellow mustard (sometimes referred to as white mustard).

These protein fractions were subjected to digestion in pepsin to evaluate their stability based on the protocol in Thomas et al. (2004). A number of potent food allergens have been demonstrated to be stable in this assay and it is thought to be at least partly predictive for sensitization. The pepsin was diluted to 10 units of activity per microgram of test protein. The mass ratio of pepsin to protein was approximately 3:1 under these conditions, with the specific lot of pepsin. Separate digestion assays were performed at both pH 1.2 and 2.0 to consider potential differences in stability. The specific pepsin solutions were assayed for activity in an independent test digesting a standard amount of hemoglobin. The end-point of the assay was taken as the time required to achieve approximately 90% reduction in the stained protein band in SDS-PAGE, compared to the time zero, undigested sample. The results of this study demonstrate that the Supertein™ was stable for pepsin digestion at both pH 1.2 and 2.0 up to the time interval of 60 minutes. The stability indicates a heightened risk of this protein sensitizing some individuals through dietary exposure. Whereas Puratein® protein fraction was rapidly digested in pepsin at both pH 1.2 and 2.0. No stable degradation bands were found to result from digestion however, the minor bands representing napin, within the Puratein® sample were still visible even after 60 min on pepsin degradation, mimicking the stability of the napin bands in the Supertein™ digests.

In summary, the canola proteins in Supertein™ and Puratein® share high sequence identities with allergenic mustard seed proteins from yellow and Indian mustard seeds. The identities are sufficiently high to suspect allergic cross-reactivity for those individuals with allergies to yellow or Indian mustards. The authors suggest that it would be useful to list “mustard seed protein” in the ingredient label on foods containing either or both of these protein fractions so that individuals allergic to mustard, (a relatively infrequent cause of allergy in the North America) are able to avoid a potential cause of allergic responses for them. While both Puratein® and Supertein™ also share moderately high identities (30%-40%) with seed storage allergens of some tree nuts and legumes, these would not be of additional concern as the identity matches are similar to those found between the allergens of yellow and Indian mustard and the tree nuts and there are no clear reported cases of clinical cross-reactivity between the mustards and nuts. The rapid digestion of cruciferin proteins in Puratein® indicate that it is less likely to cause new sensitizations compared to the napins in Supertein™. Even the high stability of the napins should not raise concerns as long as products containing these protein fractions are labeled so that individuals with mustard allergy or those who become sensitized can avoid the product (e.g., labeled as containing mustard proteins, or mustard-like proteins).

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

These data are the property of Archer Daniels Midland Company, and as such are considered to be confidential for all purposes. These data may not be copied or disclosed to any other parties in any form whatsoever. Submission of these data in compliance with applicable regulatory requirements does not constitute a waiver of any right to confidentiality that may exist under any other statute in any other country.

Company: Archer Daniels Midland Company

Company Agent: Luis A. Mejia, Ph.D.

Title: Director, Regulatory & Scientific Affairs

Signature: _____

Date: _____

05/13/08

STATEMENT OF GOOD LABORATORY PRACTICES

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATOR:

Richard E. Goodman

12 May 2008
Date

SUBMITTED BY:

(Title) Director, Reg. & Sci. Affairs

05/13/08
Date

SUBMITTER/SPONSOR:

Archer Daniels Midland Company (ADM)
Decatur, Illinois

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Abbreviations

aa	amino acid
A _{280 nm}	Absorbance of light at a wavelength of 280 nm
CAP	ImmunoCAP®, antigen-specific human IgE assay from Phadia
CFR	Code of Federal Regulations
ECL	Enhanced Chemiluminescence
E0	Experimental control pepsin without the test protein, time 0
E60	Experimental control pepsin without the test protein, 60 min.
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GLP	Good Laboratory Practice
Hb	Acidified 2% hemoglobin
HRP	Horseradish peroxidase
kDa	kilodalton
LOD	Lower limit of detection
mA	milliampere
mg	milligram
mL	milliliter
mM	millimolar
μL	microliter
ng	nanogram
NFDM	Nonfat dried milk
P0	Experimental control test protein without pepsin, time 0
P60	Experimental control test protein without pepsin, 60 min.
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
P/N	Product number, same as catalog number
P1/10	Experimental Control test protein 10% sample for gels
SDS	Sodium dodecyl sulfate
SGF	Simulated gastric fluid (without pepsin)
SOP	Standard operating procedure
SPT	Skin prick test
T	Time point
TCA	Trichloroacetic acid
Tris	Tris(hydroxymethyl)aminomethane
v/v	solute volume to solution volume
w/v	solute weight to solution volume

1. Introduction

This document reports the findings of a study commissioned by Archer Daniels Midland (ADM), to assess the potential food allergenicity of two protein fractions from canola seeds, “Supertein™” and “Puratein®” that are being developed as food ingredients.

2. Study Objectives

- 2.1. A literature search to review the historical food uses of plant materials from members of the mustard family and to identify studies indicating allergic risks associated with canola or rape seeds (*Brassica napus* ssp. *oleifera*), foods derived from canola seed or from food sources that are highly similar to canola.
- 2.2. Verification of partial sequence identities of the major proteins in Supertein™ and Puratein® using LC/MSMS.
- 2.3. Evaluate the potential risks of allergic cross-reactivity of using the Supertein™ and Puratein® canola protein isolate fractions in food based on bioinformatics searches to identify similarities to known allergenic proteins (Goodman, 2006).
- 2.4. Test the stability of proteins in Supertein™ and Puratein® in pepsin using a standardized assay as an indication of potential risk of food allergy (Thomas et al., 2004).

Samples of the Supertein™ and Puratein® protein fractions were supplied by ADM. This report includes results from all steps of the assessment.

3. Historical Food Use

The genus *Brassica* is in the mustard family Brassicaceae (previously called Cruciferae) which includes approximately 100 species. The family Brassicaceae includes approximately 340 genera and 3350 species including *Sinapis* sp. and *Raphanus* sp. which are vegetable crops closely related to *Brassica rapa* / *Brassica napus* / *Brassica nigra* (Johnston et al., 2005). The cultivated plant known as canola, derived from rape (*Brassica napus* L.), is included as subspecies *Brassica napus* L., spp. *oleifera*. This variety of ‘rapeseed’ mustard was derived from a complex of species in the genus *Brassica* that are cultivated and grown for a variety of distinct foods. Canola is currently the third-largest oilseed crop in the world after soybeans and palm (Canada Canola Council 2007). The seed of canola contains a high level of protein with an amino acid profile nutritionally comparable to animal muscle and arguably superior to that of soybean seed protein. Canola meal is a major by-product of the vegetable oil industry. Most of the meal is now used as animal feed. However, the high protein content (~ 40%) and nutritionally balanced amino acid profile offers great potential as primary protein source in food. Canola protein also has potentially useful functional characteristics including emulsifying, gelling, and binding properties. Traditionally, *B. napus* has been viewed as unsuitable as a

source of food due to the presence of two naturally occurring toxicants, erucic acid and glucosinolates. However, in the 1970s, intensive breeding programs in several countries produced high quality varieties that were significantly lower in these two toxicants. There is some confusion over taxonomy, but the term ‘canola’ refers to those varieties of *B. napus* that meet specific standards on the levels of erucic acid and glucosinolates. Those cultivars must yield oil low in erucic acid (below 2%) and meal low in glucosinolates (total glucosinolates of 30 µmoles/g toasted oil free meal) as defined by CODEX (1999). Those varieties are referred to as “double low” (00) varieties.

There is little direct historical use of canola in the human diet other than highly refined vegetable oil. Further, while there is a significant history of safe use of the probable parental lines that were crossed to produce canola, the uses have primarily been for oils, spices or as vegetables. In order to evaluate all aspects of the safety of using seed proteins in the human diet, we evaluated the literature of related taxa that might interbreed with canola, and that are likely to have highly identical proteins due to the close evolutionary relationships. In that regard we have considered species of the genus *Brassica* as well as the closely allied genus *Sinapis*.

B. napus (rape) probably originated in either the Mediterranean region or Northern Europe. It is thought to have originated from a cross where the maternal donor was closely related to two diploid species, *B. oleracea* and *B. rapa* (Office of the Gene Technology Regulator, Gov’t of Australia, 2002). The resulting plant is considered by some as *Brassica napus* L., *ssp. oleifera*. Rape was cultivated by ancient civilizations in Asia and the Mediterranean, possibly as early as 2000 BC in India and in Europe since the 13th century, primarily for oil used in lamps (Colton and Sykes, 1992). Rape was first grown commercially in Canada in 1942 as a lubricant for use in war ships. Other closely related species are grown for oil, spices and green vegetables in various parts of the world (*Brassica juncea* – Indian mustard, *Brassica oleracea* – kale, cauliflower, cabbage, broccoli, etc., *Brassica rapa* – field mustard, turnip, bok-choy, Chinese cabbage, includes *ssp. campestris*, *Brassica nigra* – black mustard, is a very small seeded species used as a spice in the Mediterranean area and in India) as indicated by NCBI Taxonomy.

Food use of various species of Brassicaceae includes the oil from seed of *Sinapis alba* and at least three species of the genus *Brassica* (*Brassica rapa ssp. campestris*, *Brassica juncea*, *Brassica napus*). In some cases, the oil is chemically extracted and highly refined, containing very little traceable protein. Those products are unlikely to induce allergic reactions due to minimal protein content. In other cases, the oil is derived from cold-pressing of “turnip-rape” seed, *Brassica rapa* var. *oleifera* (Hämeenlinna, brand name Virgino) and may contain modest levels of seed protein as the company description of an organic food indicates a “nutty” flavor (<http://www.organic-finland.com/co/coKankaisten.html>). Whole or ground seeds of various mustard plants are also consumed as spice or condiments, with different species commonly used in various geographies. In the European Union (EU) mustard is often a mixture of *Sinapis alba* (white mustard, also referred to as yellow mustard) and *Brassica juncea* (oriental or Chinese mustard). Finally mustard greens are consumed from some species as fresh or cooked vegetables as are turnip roots, raw or cooked.

Mustard and ingredients derived from mustard has now been listed as one of 12 allergenic ingredients that must be labeled if sold in the European Union according to the Directive 2003/89/EC (http://www.efsa.eu.int/science/nda/nda_opinions/catindex_en.html). The EFSA has indicated the Sin a 1, Bra j 1 and Sin a 2 proteins of *Sinapis alba* and *Brassica juncea* are allergens and has given some legal definition to the need to label products containing ingredients from those species (EFSA, 2007). While there are no publications demonstrating clear food allergy to proteins from canola, *Brassica napus ssp. oleifera*, the purpose of this study is to examine whether protein fractions from this species would present a potential risk to allergic subjects.

4. Major Seed Storage Proteins

Rapeseed meal is known to contain two predominant classes of seed storage proteins: 11S (or 12S) globulin (cruciferin) which represents 60% of its protein content and 2S albumin (napin), representing about 20% of the protein (Salleh et al., 2002). It also contains some minor proteins of interest, such as oleosins, thionins, trypsin inhibitors and lipid transfer protein (LTP).

Cruciferin, as a member of the 11S globulin family, shares structural features with soy glycinin. As reviewed by Berot et al. (2005), there may be as many as 10 isoforms of the cruciferin gene in *Brassica napus*, with sequence variation. Further, there are multiple gene loci that encode some of the isoforms although the gene copy number is not known. The protein products are disulfide linked acid and basic heterodimers that are associated into hexameric structure, with a basic subunit of about 36 kDa and an acidic subunit of about 23 kDa. The napins are somewhat less abundant, basic proteins of about 14 kDa which are also heterodimeric (from the same gene/translation product), and linked by a disulfide bond, separating into 10 kDa and 4 kDa peptides upon reduction (Berot et al., 2005). There may be more than one napin gene locus, however that is not clear. A minor protein variant of about 80% identity has been reported, suggesting a second gene may be present in the genome.

The two purified protein fractions from ADM were described as being predominantly composed of cruciferins (Puratein®) and napin (Supertein™). This study focuses on the dominant abundant proteins in samples provided by ADM.

5. Historical allergenicity of mustards

Allergic reactions from the ingestion of canola seed based ingredients have not been described in the scientific or clinic literature based on our keyword (canola AND allergen) searches of PubMed or on Google. However, allergies have been reported to related species as indicated by a search of the literature using the terms mustard and rape as keywords in internet searches. Since these species are closely related and the known protein structures are highly similar, the possibility of either cross-reactivity or similar reactivity is being considered. The reports of allergenicity associated with the related species are therefore described.

Although the mustard seed is frequently consumed as a spice or condiment, only a small number of articles have been published on mustard seed allergy. In North America, only two

scientific publications were found that mentioned allergy to mustard. One was a study of allergy to cashew, specifically to 2S albumin and evaluated possible cross reactivity to sesame in cashew allergic subjects (Robotham et al., 2005). That study merely indicated that two of the 40 subjects studied claimed to have food allergy to mustard, but there was no evidence of IgE or allergy to mustard or canola demonstrated for any subjects (Robotham et al., 2005). The second was a report from Mexico of a general skin prick test (SPT) screen of more than 1400 allergic subjects (age 4 to 18 years) with reported food allergy in Mexico testing for sensitivities and only mentioned a reference to mustard allergy from France (Avila Castanon *et al.*, 2002). There are however reports of allergy to mustards or related *Brassica* sp. from studies in France, Spain and Finland, which are described below.

Niinimäki and Hannuksela (1981) reported skin test data and oral challenges with various spices of selected skin test positive subjects from a population of 1120 atopic individuals and 380 controls in Finland. Curry caused positive reactions in 17% of the tested subjects, and only 0.3% of non-atopic individuals. Seventy one of those with positive skin reactions to curry were also tested with ingredients of curry including mustard, and 23 of the 71 subjects reacted to mustard. No specific proteins were identified as allergens, but this appears as one of the first reports of allergy associated with ingestion of mustard. The species of mustard was not identified. Serum from individuals allergic to natural rubber were found to contain IgE antibodies that bound to a prohevein-like protein from injured turnip root (*Brassica rapa*, Hanninen *et al.*, 1999), implicating this protein as a possible allergen. It is not clear whether this protein is found in seeds of *Brassica*, but that is possible.

More likely candidate proteins to consider as potential elicitors of food allergy to seeds have been studied. Jorro *et al.* (1995) described three cases of systemic allergic reactions to the consumption of mustard, though the proteins causing the reactions were not determined. The subjects were not tested by direct challenge, but were classified as allergic to mustard based on histories and positive responses to mustard extract by skin prick test and by positive mustard-specific IgE by CAP testing (ImmunoCAP® produced by Pharmacia, now Phadia). Caballero *et al.* (2002) reported on a clinical study of individuals who reported allergy to mustard in Spain. The study included 29 subjects from 15-58 years with 50% reporting symptoms to other foods as well as reactions to mustard (10 with local symptoms only, 19 with systemic reactions and 14 of them with reactions classified as anaphylaxis by the Caballero *et al.* 2002). Detailed histories were recorded and all reported symptoms that occurred within an hour of ingesting mustard spice or sauce. Skin prick tests (SPT) were performed and using an extract of *Brassica nigra* (black mustard) and other control extracts. Specific serum IgE test results (CAP, Pharmacia) ranged from 0.7 to 100 kU/L, with a mean of 6.3 kU/L. In a separate study in France, 7 of 30 individuals with reported allergy to mustard (spice) who were also skin prick positive to mustard seed extract were found to be allergic by double-blind placebo-controlled food challenge tests (DBPCFC, Morisset *et al.*, 2003; with comments from Rance, 2003). Skin prick tests were performed with extracts of *Brassica nigra* seeds and with extract of *Brassica juncea* flour, with high concordance. Sera from 27 subjects were screened for specific IgE using CAP tests (Phadia) for *Brassica* and *Sinapis* spp. with 21 showing statistically positive binding to "mustard" (mixed species), some with very high levels (>10 kUA/L). The seven DBPCFC positive subjects (to *Sinapis alba*) had objective symptoms of skin, airway or GI tract. The use of

DBPCFC clearly demonstrated the ability of mustard seeds to elicit a food allergic reaction in some mustard-sensitized subjects. However, there was no attempt to identify the specific proteins that bound IgE. These reports demonstrate that allergy to mustard is modestly common in some areas of Europe. A more recent study in Spain identified 38 adult patients having reported food allergy to consumption of mustard, with 10% reporting systemic reactions (Figueroa *et al.*, 2005). DBPCFC was performed with 24 of these subjects (14 were not tested due to strong historical reactions or lack of consent), with 14 having positive challenge results. The DBPCFC positive subjects had greater positive SPT wheals to mustard extract. Interestingly 97% of these subjects were also sensitized to mugwort pollen (family Asteraceae). A pool of 5 sera from this group was tested for cross-reactivity of mustard and mugwort binding to CAP material and the specific binding was partially inhibited (over a 2 log difference) by pre-incubation with the reciprocal material, compared to the solid-phased material. However, inhibition of binding to cabbage, cauliflower and broccoli using 11 individual sera from subjects allergic to all three, and using either mustard or mugwort as inhibitor showed much more similar inhibition patterns, demonstrating probable different allergens were recognized by some classes of subjects in the study, but that was not directly tested in this study (Figueroa *et al.*, 2005).

Poikonen *et al.*, (2006) reported finding 28 SPT positive turnip-rape and oilseed rape (*Brassica rapa* ssp. *oleifera* and *Brassica napus* ssp. *oleifera*, respectively) in Finland from a screen of 1887 children under 16 years of age in a general food allergy study of subjects visiting the dermatology clinic at Tampere University Hospital from 2002 to 2004. Most of the tested subjects presented with atopic dermatitis (AD) and 70% (1337) were below the age of 3 years. Of the 28 rape-positive subjects, 17 were also allergic to milk and 24 to wheat based on open food challenges. Twenty five of the 28 rape-SPT-positive children had a positive labial (17 positive) or oral challenge (4 immediate and 4 delayed reactions) to crushed rapeseed. None of the 25 control subjects (age matched children with AD to other allergens) had a positive response to rape. As discussed in the study, the finding of this relatively high percentage of AD children responding to Brassica seed was unexpected because of the relatively low level of consumption of rape products. However, as discussed above rape seed oil and turnip rape-seed oil, including cold-pressed oil, is used in Finland as a vegetable oil, in margarine and in baby food. However, the authors reported doing preliminary SPT with cold-pressed turnip rape oil using a few of the SPT positive children and not finding any positive results. The authors indicated further research is needed to evaluate the potentially important food sources clinical consequences related to products from *Brassica* sp. (Poikonen *et al.*, 2006). In an extensive clinical follow-up study the same Finish authors investigated a number of possible factors to explain the relatively high sensitization level to turnip and oilseed rape proteins. In a paired study of 64 AD – rape seed sensitized (SPT) cases and 64 rape seed non-responders (controls), there was 97% sensitization rate to mustard for cases, 2% for controls, 92% for egg in cases, 38% in controls. There was also a much higher rate of sensitization to wheat in cases (80%) compared to controls (33%). There were similarly unbalanced ratios of sensitization, but of lower magnitude, for various pollens and milk as well as roughly 10 fold higher total IgE mean concentration in cases compared to the controls. Detailed histories were also compared regarding duration of exclusive breast feeding (average 4 months in cases vs. 3 months for controls). There was no difference in incidence of AD in parents of the cases vs. controls. Asthma was of much higher (4 times) incidence in cases compared to controls and rhinitis about twice as prevalent. There was no apparent evaluation of

specific food intake to evaluate exposure to mustard or to rape seed proteins. The study was thus not able to define the likely cause of sensitization to rape or turnip rape proteins. No data was reported regarding the use of elimination diets to determine whether removal of rape and turnip rape foods from the diet would eliminate symptoms. Further, the actual incidence of allergy in Finland is not known as the subjects were preselected as individuals with AD who reported to the dermatology clinic. This study provided good evidence of challenge reactivity to rape and turnip rapeseed material, suggesting exposure of the case-subjects to unpurified dietary seed proteins from *Brassica sp.* would present a real risk of allergic reaction.

Food allergic reactions to canola, rape or mustard seed are likely to be caused by IgE specific binding to the major seed storage proteins, similar to the most commonly reported allergens in peanuts and tree nuts. The most abundant seed storage proteins in *Brassica sp.* and *Sinapis sp.* are the 11S (or 12S) globulins and 2S albumins (Crouch and Sussex, 1981). These are discussed here.

Monsalve *et al.*, (1993) were among the first to report the protein characteristics of the allergen known as a 2S albumin of oriental mustard (*Brassica juncea*), although the clinical symptoms and histories of the 11 RAST positive subjects were not disclosed. The amino acid sequence was determined to be highly identical to the allergen from white mustard, known as Sin a 1 and also napin of *Brassica napus* (BnIII). The 2S albumins are described as being composed of a light chain peptide (37 aa) and heavy chain peptide (92 aa) joined by disulfide bonds. The complete protein is translated from a single transcript, then proteolytically cleaved after formation of the disulfide bonds (Palomares *et al.*, 2002). Monsalves *et al.* (1993) reported occupational airway allergy in one detailed case of a 48 year old individual suffering from asthma after nearly five years of exposure to rapeseed flour (*Brassica juncea* according to the authors), along with reactivity to other unrelated plant flours (Monsalve *et al.*, 1997). In this case the 2S albumin from Brassica was identified as napin (BnIII). These 2S albumins are highly similar and probably cross-reactive as well as stable to digestion and processing (Monsalve *et al.*, 1997). The 2S albumin of *Sinapis* was identified as a food allergen of mustard and the protein was expressed in recombinant form and demonstrated to be a major allergen as roughly one-half of the 21 subjects identified as allergic to mustard (*Sinapis alba*) exhibited IgE binding to this protein. The authors indicated that all subjects were diagnosed as allergic to mustard, most with oral allergy syndrome (OAS) symptoms, but also 50% with systemic symptoms, 20% with anaphylaxis (Palomares *et al.* 2005a).

More recent studies from Finland provided further proof of allergies caused by the 2S albumins of Brassicaceae (Puumalainen *et al.*, 2006). This study is a continuation of work by Poikonen *et al.*, 2005), with some shared subjects, but also new subjects. Still the allergic reactions to oilseed rape and turnip rape proteins were not proven, but in this case substantial in vitro laboratory data demonstrated coincident binding to purified 2S albumin proteins from both, which were characterized and shown to be similar in sequence to napins Sin a 1 and Bra j 1. Further, a select group of 6 subjects with specific IgE who were SPT positive to extracts of both of the rape seed sources were also tested by SPT with purified 2S albumin fractions and determined to cause significant wheals at moderate concentrations of protein. Based on N-terminal peptide sequence analysis the small subunits of the *Brassica napus* and *Brassica rapa*

were nearly identical to mustards (Sin a 1 and Bra j 1) and the large subunits were identical. Serum IgE tests from SPT positive subjects demonstrated that relatively high levels of 2S-albumin specific IgE was found for a number of subjects less than 1 year of age, the highest levels in subjects from 2 to 4 years and then lower levels in subjects from 5-9 years. Since this is not a longitudinal study, and since food consumption patterns may have changed, no significance should be given to the observation except that there are high reactions in very young children. This study provided solid evidence that the 2S albumins in these members of Brassicaceae are likely to be important allergens for those allergic to any of the related foods.

There is also some evidence that the other major seed storage protein class, 11S (or 12S) globulins (also referred to as cruciferins), are likely to be allergenic. Palomares *et al.* (2005b) tested sera from 13 Spanish subjects with reported food allergy to mustard. Subjects' symptoms were primarily oral allergy syndrome, but three had reported anaphylaxis, two reported gastrointestinal symptoms and two angioedema upon ingestion of mustard. All had positive SPT to extract of *Sinapis alba* and all but one also had pollen allergy. The IgE binding proteins of the seed were purified and analyzed by MALDI mass spectrometry and identified one of the dominant proteins as the 11S globulin, which they called Sin a 2 as an allergen. The protein mass when isolated under non-reducing conditions was 50 kDa, but upon reduction ran as two bands, one of 36 kDa with basic pI, and a second, 23 kDa band with acidic pI. Serum IgE from the majority bound to the 2S albumin (Sin a 1) of mustard extract, but 12 of the 13 also bound the 50 kDa protein. Based on inhibition of IgE binding to mustard seed extract, the 50 kDa protein accounted for between 19 and 55% of total IgE binding, while Sin a 1 accounted for from 55 to 81% of the binding for four of the patients. Together the two proteins accounted for more than 97% of the total IgE binding to mustard extract. For at least some subjects, IgE bound to both reduced fractions of the 11S protein. Sequence alignment of the 11S globulin to allergenic 11S globulins of other seed proteins including Ano o 2 of cashew, Cor a 9 of hazelnut, Ber e 2 of Brazil nut, Ara h 3 of peanut and glycinin of soybean shows conservation of structure based on cysteine alignment, but also shows substantial differences in the basic peptide area. The overall identities of the entire proteins are less than 40% identical to Sin a 2, with similarities of approximately 55%. These data suggest that there is not likely to be substantial cross-reactivity of the 11S globulins and based on literature searches that seem to be true. However, the *Sinapis alba* 11S globulin is 84% identical to the 11S globulin identified from *Brassica napus* (Rodin *et al.*, 1992), and is likely to be cross-reactive based on the high structural conservation (Aalberse, 2000). The same authors also cloned a cDNA of Sin a 2 (Palomares *et al.*, 2007) and demonstrated that the protein produced in *Escherichia coli* also bound IgE to a similar extent as protein purified from mustard, using sera from 8 mustard allergic subjects. However, in the second study the IgE from a pool of subjects did not bind to the 23 kDa peptide, but did bind to both 50 kDa and the 36 kDa peptides. This study demonstrates that the 11S globulin, cruciferin, is a likely allergen for some mustard allergic subjects based on IgE binding, but the study did not investigate SPT or challenge test with pure protein so the biological activity is not known. Based on sequence matches of protein seed storage proteins across-Brassica sp., it could be assumed that at least some of the individuals sensitized to one would exhibit IgE binding to the homologous protein in the other Brassica-related species.

In conclusion, there are reports of food allergy to seed proteins from the mustards that are scientifically credible. Most come from restricted regions of Europe that may have unusual food processing and consumption, or other environmental exposure patterns that may be unique. Mustard, rape, turnip and other *Brassica* sp. and *Sinapis* sp. shed considerable airborne pollen that might sensitize subjects in the local environment. A few animal feed industry workers have been documented to have allergic-asthma associated with exposure to rape or canola seed. And there is some evidence of individuals who may have been sensitized to mugwort pollen (*Artemisia vulgaris*), who have IgE that cross-reacts with proteins of mustard/rape. Many of the sensitized individuals may only have IgE against some proteins in canola/mustard/rape seeds and not react to oral exposure. However, some studies demonstrated clear oral reactivity in some subjects. Mustard allergy does appear to be rare in North America as there are no documented cases of allergy to mustard at this time. But no systematic search has been performed. Importantly, the European reports of allergy to mustards suggest that there may be considerable cross-reactivity of IgE to proteins from seeds of plants from the *Brassica* and *Sinapis* genera and that the 2S albumin and 11S globulin proteins are the most likely oral allergens from mustards.

6. Experimental Methods

An official international allergenicity guideline has not been formulated for the evaluation of processed food ingredients. Some developers have evaluated the potential allergenicity of new food ingredients using the Codex Alimentarius Commission guidelines for assessing the allergenicity of GM plants (2003) as a model for evaluating the potential risk. This recommends assessing the introduced protein for stability in pepsin at acidic pH as an assay to help evaluate whether the introduced protein is likely to either increase the rate of sensitization to the host crop, or increase the likelihood of eliciting an allergic response in food allergic consumers. The pepsin stability assay is one study in a weight of evidence approach intended to assess the potential allergenicity of genetically modified crops (Codex, 2003). The test method for the assessment was first described by Astwood *et al.* (1996). The assay is not meant to predict whether a given protein will always be digested in the stomach of the human consumer, but does provide a simple *in vitro* correlative assay to evaluate protein digestibility. Investigation of proteins that have been tested suggest a strong positive predictive value that food allergens causing systemic reactions are relatively stable in the assay, while non-allergenic food proteins are typically digested relatively quickly (Bannon *et al.*, 2002). Purified porcine pepsin has been used to evaluate the stability of a number of food allergens and non-allergenic proteins in a multi-laboratory study that demonstrated the rigor and reproducibility in nine laboratories (Thomas *et al.*, 2004). Porcine pepsin is an aspartic endopeptidase with broad substrate specificity. Pepsin is optimally active between pH 1.2 and 2.0, but inactive at pH 3.5 and irreversibly denatured at pH 7.0 (Collins and Fine, 1981; Crevieu-Gabriel *et al.*, 1999). The assay is performed under standard conditions of 10 units of pepsin activity per microgram of test protein. Using the more pure form of pepsin available at Sigma (P6887), that turns out to be approximately a mass ratio of 3 mg pepsin per mg of test protein. The original assay described by Astwood *et al.*, recommends performing the digestion at pH 1.2, however, the FAO/WHO (2001) recommends using two pH conditions (1.2 and 2.0). The assay is performed at 37°C and samples are removed at specific times and the activity of pepsin is quenched by neutralization with carbonate buffer and Laemmli loading buffer, then heating to more than 70°C for 3 to 5

minutes. The timed digestion samples are separated by SDS-PAGE and stained with Coomassie or colloidal blue to evaluate the extent of digestion. A review of the digestibility assay by Bannon *et al.* (2002) and by Thomas *et al.* (2004) indicates that most of the non-allergenic food proteins that have been tested digested by around 15 to 30 seconds, while major food allergens were stable, or produce pepsin-stable fragments that are visible for from eight to 60 minutes.

Assay parameters that have not been documented in some publications include verification of pepsin activity, the limit of detection of the protein in the stained gel and an objective measurement of the time of digestion. In this study we have tested the activity of the pepsin in SGF on each day of assay using a test to evaluate digestion of hemoglobin, as described by Sigma, to ensure that it is within a tolerance interval reported by Sigma for that lot of enzyme. The results of our activity assay do not exactly duplicate the labeled activity determined by Sigma, even though we use similar procedures, and we have an acceptance criterion of the Sigma certified activity, plus or minus 1,000 activity units per mg of pepsin. A second important criterion that we have included in our standard operating procedure (SOP) is an objective measured level of residual test protein (HPPD-11465 in this case) that must be reached in determining the time of digestion. We have defined the extent of digestion as 90% and determine the sample time-point when the residual is less than or equal to 10% of the amount of test protein in the initial sample. To accomplish that a dilution series of test protein is tested in the same SDS-PAGE and colloidal blue staining system as the digests are analyzed with to evaluate a limit of detection (LOD). The LOD must be lower than 10% to perform this assay. The analytical gel for the pepsin digests includes a 10% test protein sample mixed with quenched pepsin (high pH, to avoid digestion). The availability of anti-sera specific for HPPD-11465 also afforded the opportunity to verify digestion with western blots. Details and results of the study are reported here.

- 6.1. Test Substance. Five different individual lots of Supertein™ and Puratein® protein fractions were provided by ADM in addition to a sixth composite lot for both Supertein™ and Puratein® (equal amounts of each of the five individual lots, blended). Individual lots of Supertein™ were numbered 1-5 and the 6th was the composite. Similarly the individual lots of Puratein® were numbered 1-5 and the composite as number 6. The samples appeared to be homogenous spray-dried powders of light yellowish color. For this study 50 mg of each spray-dried sample was weighed and was dissolved in 5 mL of distilled water (5 mg/mL). Aliquots (1 mL each) of the dissolved samples were stored at -20°C.
- 6.2. Protein estimation of dry powdered samples by LECO analysis. The protein content of each dry powdered sample of Supertein™ and Puratein® was measured by analysis with a LECO instrument. Samples of ~ 400 mg (S1-S6, P1-P6) were submitted to Dr. Schlegel's laboratory (Dept. of Food Science & Technology, UNL) for protein estimation. Results were reported as the average percent nitrogen content in each sample (triplicate determinations). The experimental values were multiplied by a

constant conversion factor of 6.25 to estimate the percent. The percent protein was then used to correct the amount of powder used to make standard protein solutions of 5 mg/mL (e.g. based on the composite Supertein™ protein content of 85.7% shown in Table 1, a 5 mg/mL solution would require 5.83 mg of powder dissolved in 1 mL of buffer to achieve 5 mg/mL total protein).

- 6.3. Qualitative assessment of Supertein™ and Puratein® by SDS-PAGE (reducing and non-reducing) and gel staining. The protein concentration of each sample was adjusted to 5mg/mL based on results from the LECO analysis (see 6.2 above). Samples were then mixed 1:1 with Laemmli sample buffer, either with, or without 2-mercaptoethanol. Samples without Laemmli buffer were not heated prior to loading in wells of SDS-PAGE gels and electrophoresis. Samples with 2-mercaptoethanol were heated to more than 95°C for five minutes before loading in wells and separating by electrophoresis. Tris-glycine gels from Invitrogen (10-20% acrylamide or 18% acrylamide) were used according to our standard laboratory procedures. After electrophoresis, samples proteins were fixed in the gels with 7% acetic acid in 40% methanol, then stained with colloidal blue (Sigma B2025) for at least 2 hours. Gels were destained for one minute in 10, then 25% methanol until the background was clear. Images were captured using a Kodak Gel Logic 440 and analyzed with Kodak 1-D software. However, gels were stained with Coomassie blue R250 for isolating protein bands for amino acid sequence determinations by LC-MS/MS. To estimate purity of the 2S albumin (napin) and cruciferin bands of protein in the solutions of the two protein fractions samples of each were diluted and applied to non-reducing and reducing SDS-PAGE and then separated by electrophoresis (Figure 3). The image of the reducing gel was analyzed using the Kodak gel logic system and 1D software. The total pixel count (intensity) of stained bands in the H + L bands of Supertein™, Figure 3B (sample A3) were summed after background subtraction and divided by the pixel count of all bands in the lane, then multiplied by 100%. The purity of the Puratein® was estimated by the same procedure on bands in the lane marked B3 in Figure 3B. Results are shown in Figure 4.
- 6.4. Protein sequence identification of the major stained proteins in composite samples. The Supertein™ (S6) and Puratein® (P6) composite fractions were resolved in reducing SDS-PAGE (10-20%) and stained with Coomassie brilliant blue R250 (Figure 5A). The major bands in Supertein™ were labeled S6-1 (MW ~ 12 kDa based on BioRad marker M1, but ~ 9 kDa based on Invitrogen marker M2) and S6-2 (MW ~ 8 kDa based on M1, but ~ 4 kDa based on M2), were excised and submitted to Macromolecular Resources at Colorado State University for trypsin digestion and mass determination by LC-MS/MS for sequence analysis. The bands in Puratein® (Figure 5B) were labeled P6-1 (major band ~ 29 kDa), P6-2 (major band ~ 26 kDa), P6-3 (major band ~ 22 kDa),

P6-4 (minor band ~ 19 kDa), P6-5 (major band ~ 17 kDa), P6-6 (minor band ~ 12 kDa) and P6-7 (minor band ~ 8 kDa) were excised and submitted to Macromolecular Resources too. Equivalent size (MW) bands of 2 to 4 lanes were excised and pooled, then submitted to the Proteomics facility, Macromolecular Resources at Colorado State University for digestion and LC-MS/MS analysis. Samples were analyzed by Macromolecular Resources using Mascot (Matrix Science, London, UK, Version 2.2.1). Mascot was set up to search the NCBI nr_20070615 database (Viridiplantae, 375095 entries), assuming digestion enzyme was trypsin. Then data was further analyzed using Scaffold (version Scaffold-01_07_00, Proteome Software Inc., Portland, OR) to validate MS/MS based peptide and protein identifications. Peptide/protein identifications were accepted based on 95% probability criteria. Some bands were found to contain peptides that belong to distinct proteins and in other cases it was clear that a given peptide represents a protein of a different mass than the band it was isolated from. See Appendix 1 for Scaffold results from Supertein™ and Appendix 3 for results from Puratein® protein isolates and analysis.

- 6.5. Protein databases used for the sequence dependent allergenic assessment. The Allergen database (AllergenOnline.com, version 8.0, dated January 2008). Allergen specific sequence databases are very useful for improving the efficiency of the computerized sequence comparisons to identify potentially cross-reactive allergens, in contrast to searches using a more generalized sequence database such as NCBI or Swiss-Prot. A general database screen would require significantly more manual data analysis of identified matches in order to evaluate the allergenicity of the matched sequences. Now several databases of allergens are available but this study uses the Food Allergy Research and Resource Program Allergenonline at the University of Nebraska: <http://www.allergenonline.com/> database for evaluation of its study objectives as it is curated by a panel of allergy experts to remove irrelevant proteins (no proof of allergenicity). The database includes known and putative allergens that have identified from food, airway, contact and venom allergen sources. All database entries are linked to sequences in the NCBI at the National Institute of Health and have a list of relevant publications describing the sequence and the proof of allergenicity.
- 6.6. Sequence database search strategy (see Goodman, 2006 for search strategy). The website, AllergenOnline.com, provides both a full-length FASTA and a sliding 80-amino acid window FASTA search routine. The 80-mer search is accomplished as the program sequentially compares amino acids 1-80 of the query to the whole database, then amino acids 2-81, 3-82 and so-forth. The data prints from the FASTA 80mer-sliding window procedure on AllergenOnline provides the percent identity for the overall alignment of the query sequence to each matched allergen or putative allergen

along with the length of the alignment and the statistical score of significance of the alignment (*E* score). It also shows the best overall 80 amino acid segment alignment percent identity if any are over 35% identity, the number of 80-amino acid sequences with greater than 35% identity match to a given allergen and the *E* score for the 80-amino acid match. Results are reported in Appendices 2, 4 and control protein in 5.

6.6.1. FASTA3 overall search of AllergenOnline.com. As described by Goodman (2006), the best overall predictor of potential cross-reactivity would be a match of greater than 70% identity over the full-length of the query protein (in this case the napin and cruciferin proteins). AllergenOnline.com provides a straight-forward FASTA protocol, but the same search routine is included in the 80-mer search routine. High identity matches of segments of two proteins less than 26 or so amino acids are not meaningful in terms of biological cross-reactivity. Matches over the full-length are generally predictive of overall structural similarities that are more likely to lead to cross-reactivity. According to Aalberse (2000) and the empirical evidence we are aware of in the literature since then, proteins sharing lower than 50% identity over their full-length are not very likely to be cross-reactive, and those sharing less than 40% identity are rarely cross-reactive. A more conservative approach is the use of a sliding 80-mer window to identify shorter segments that might represent partially conserved allergenic peptide regions (see 6.6.2).

6.6.2. FASTA3 of AllergenOnline.com by 80-amino acid window. In order to predict potential cross-reactivity for the evaluation of proteins introduced into genetically modified crops, the Food and Agriculture Organization/World Health Organization expert panel (FAO/WHO) 2001 recommended a dual test, one looking for any match of six contiguous amino acids, the second looking for identity matches above 35% over any 80 amino acid segment of the query protein compared to any known allergen. The predictive value of a six or eight amino acid match has since then been discredited as not being predictive (Goodman, 2006). The 35% identity over any 80-amino acid segment is still very conservative and may frequently over-predict cross-reactive matches, however, detailed proof for a more appropriate criteria are lacking. It is important to note that many matches using FASTA, or BLAST, require insertion of gaps in one or both sequences to provide optimal alignments. Since the current scoring system uses percent identity, introduced gaps must be taken into account in terms of evaluating percent identities over fixed length searches. In order to do that the AllergenOnline.com protocol compensates by adjusting the percent identity. Further, since proteins having 60 amino acid matches that

are identical could well be allergenic and would calculate to greater than 35% identity over 80 amino acids, if a gap was introduced, the program compensates by simply requiring 29 or more amino acid identities over any 80 amino acid alignment. Refer to AllergenOnline.com “about” link for a full description of the protocol.

- 6.6.3. BLASTP of NCBI Entrez *allergen*. BLASTP and FASTA3 are unique computer algorithms that provide similar local alignments and results if the appropriate scoring matrices and criteria are used. The BLASTP is available on the NCBI Entrez website (<http://www.ncbi.nlm.nih.gov/BLAST/>). The current version of BLASTP search was used comparing each complete query sequence against the entire Entrez protein database, may be performed with a limit option selected to query entries for “allerg”, to align only with proteins identified as allergens. However, there are many “false” allergens that would be identified using this procedure (e.g., sequences obtained by any laboratory in the National Institute of Allergy and Infectious Disease would be called up with “allerg” as a limit. Further in a few cases, such as those of the currently recognized mustard allergens, the sequences were not identified by keywords as allergens when entered in the database. Therefore if one were to search NCBI for matches to one of the *Brassica napus* 2S albumins using BLAST with “allerg” as a key word limit, the allergens from *Sinapis alba* and *Brassica juncea* would not be found. Those sequences are in AllergenOnline.com though. Because of the finding that the most likely cross-reactive proteins were missed in a preliminary screen, the BLAST search data was not included in this report.
- 6.7. Verification of Detection System Specificity and Sensitivity for Pepsin digestion. A preliminary test was performed to ensure that the apparent molecular weight of the Supertain and Puratein® protein fractions. The range of detection of the SDS-PAGE system with colloidal blue staining and imaging system were capable of detecting the test protein over the range of the experiment (0.2-2 µg corresponds to 10% to 100% of the test protein mass). Samples of Supertain™ and Puratein® were prepared and separated in Novex 10-20% SDS-PAGE using 1 x reducing Laemmli buffer. Samples of 2.0 and 0.2 micrograms of protein were applied to appropriate wells and BIO-RAD precision plus MW markers were applied to separate lanes. Following electrophoresis, the gel was fixed and stained with Colloidal blue G 250. In addition a dilution series of both Supertain and Puratein® were prepared with sample quantities loaded in two identical gels covering the range representing 100% (2 µg) down to 1.25 % (0.025 µg). The gels were fixed, stained and the image captured using the Kodak Gel Logic 440.

6.8. Pepsin digestion assay for the Supertein™ and Puratein® protein fractions. Characterization of Supertein™ and Puratein® was reported in the earlier section of this report which was focused to study to assess the identity, concentration, purity, composition, and activity. The test substance for this study was the Supertein™ and Puratein® protein fractions supplied from ADM. There was no control substance for this study. There was no reference substance used for this study.

6.8.1. Analytical Reagents used in the digestion assay. Pepsin, porcine, Sigma Chemical Co., product #P-6887, lot 074K7717, certified as having 3,280 activity units per mg solid was used in this study. SGF with pepsin: A 35 mM NaCl solution is adjusted in pH to 1.2 or 2.0 as measured with a calibrated pH meter, using 6 N HCl, to a final normality of approximately 0.084 for pH 1.2. The quantity of powdered porcine pepsin required to achieve a final activity of 4,000 units per 1.52 mL of SGF was calculated based on the lot certified activity from Sigma. Hemoglobin from Sigma Chemical Co., product #H-2625 was used to verify the pepsin activity. Colloidal brilliant blue G-250, Sigma Chemical Co., product #B-2025 was used to stain proteins in the SDS-PAGE gels, following electrophoresis. Pepsin quenching solution was made to contain: 200 mM NaHCO₃, pH 11. 5X reducing Laemmli buffer (16.7% β-mercaptoethanol). A Precision Plus Molecular Weight marker from BIO-RAD (product #161-0374) was used to estimate the mass of stained protein bands in the gels following electrophoresis. Novex 10-20% tris-glycine polyacrylamide gels, 1mm thick, 15 wells (Invitrogen #EC61355BOX) were used to separate digestion products and intact proteins from pepsin. Running, fixing and staining buffers were made as per Standard Operating Procedures in the Goodman laboratory or as recommended by the manufacturer of the gels/stains.

6.8.2. Test System. The test system for this study was an *in vitro* digestion model using porcine pepsin in simulated gastric fluid (SGF). Standard Operating Procedures (SOPs) for preparation of the SGF, pepsin activity assay, digestion assay, SDS-PAGE, gel staining and western blotting are on record in the laboratory. The SGF preparation and digestion procedures were based on the methods described by Thomas et al. (2004). The pepsin activity assay was based on the method described by Sigma for determining the activity of pepsin.

6.8.3. Preparation of SGF plus pepsin. The simulated gastric fluid (SGF) reaction buffer is prepared by adding 122.8 mg of NaCl to 59.2 mL of distilled water. The pH of the solution is adjusted to either pH 1.2 or 2.0 using 6 N HCl and 2 N NaOH. The HCl content is approximately 0.084 N, and the salt concentration

is 35 mM NaCl. The amount of pepsin powder used to prepare SGF was calculated from the specific activity on the product label, which in this study was 3,280 units/mg solid pepsin (~ 92% pure protein) products. The amount of solid pepsin that will be dissolved in 1.52 mL of SGF is targeted to result in 10 units of activity/microgram protein. For this lot, based on the Sigma analysis, 1.22 mg of solid was added to 1.52 mL of SGF, then mixed to dissolve the enzyme.

- 6.8.4. Pepsin Activity Assay using hemoglobin as substrate. A solution of 2.5 % acidified hemoglobin (Hb) is prepared by dissolving 2.5 g of hemoglobin (Sigma # H2625) in 80 mL of distilled water, to that 20 mL of 300 mM HCl is added. To the polypropylene screw-top centrifuge tubes 5 mL of 2.5 % acidified Hb was added and all are preheated to 37°C. Then, each of the Hb tubes in turn receives 1 mL of SGF plus pepsin, is mixed, and incubated for 10 min. After the incubation time, 10 mL of 0.3 N trichloroacetic acid (Sigma 6.1 N product T0699, diluted 1:20 with distilled water) is added to stop the reaction, the tube is mixed briefly and insoluble material (undigested hemoglobin) is removed using a syringe filter (Fisher Biotech product 09-719H, 25 mm 0.45 µm PTFE) (alternatively, clarify by centrifuging at 15,000 x g for 10 minutes). Control tubes are interspersed with the Hb tubes. Control tubes (with 5 mL of Hb) receive 10 mL of 0.3 N trichloroacetic acid, then 1 mL of SGF plus pepsin and are mixed. These samples are filtered to remove insoluble material. The absorbance at 280 nm is measured on a spectrophotometer. The activity units of pepsin per mL is calculated as the mean net absorbance ($A_{280} \text{ Hb} - A_{280} \text{ controls}$) multiplied by a conversion factor of 1,000 to yield units of activity per mg of solid pepsin.
- 6.8.5. Pepsin digestion assay with test substance. A digestion tube was prepared containing 1.52 mL of pepsin/SGF reaction mixture, then this was preheated to 37°C in a water bath before adding 80 microliters of test protein (5 mg/mL) and mixing by light vortexing. At predetermined times (between time zero and 60 minutes at interval of 0, 2, 5, 10, 20, 30, and 60 min) a fixed volume (200 µL) of the digesting protein reaction mixture is withdrawn and added to sample tubes containing neutralization and denaturing reagents, which stop the digestion. These samples were then heated to ~ 95°C for 5 minutes before either running in SDS-PAGE analyses or placing in a -20°C freezer for later analysis. All samples from a single digestion are applied to wells [(13.5 µL corresponds to 2 µg of protein (100%)] of the same SDS-PAGE gel along with molecular weight markers, undigested test protein equivalent to the initial

undigested test protein sample and a 10% test protein sample and pepsin alone (to assess pepsin stainable protein bands). Samples are separated by electrophoresis, fixed in aqueous acetic acid-MeOH, stained with colloidal blue G-250, destained and then analyzed on a Kodak 1-D Image analyzer for residual protein. The stability of the protein is defined as the time required to achieve 90% digestion which is estimated based on the shortest time-digested sample with a band intensity equal to, or less than the 10% [(0.2 µg (10%)) undigested standard well (C0). Any new bands above approximately 3,000 MW that are generated as intermediate products of digestion would be noted as stable (or partially stable) intermediate proteolytic fragments and would be analyzed in addition to the test protein.

Proteins with more than 10% stainable full-length protein band remaining at 60 minutes are considered stable. Proteins reduced to < 10% stainable band at 5 to 30 minutes are considered of intermediate stability. Proteins reduced to < 10% stainable band by 2 minutes are considered labile (rapidly digested).

- 6.9. Justification for Selection of the Test System. *In vitro* digestion models are used widely to assess the digestibility of ingested substances. Previous studies have used this simple, *in vitro* assay to evaluate potential risk of food allergy, and demonstrated that digestibility is an important risk factor for food allergy, which could relate to initial sensitization, or to elicitation once the individual is sensitized (Astwood *et al.*, 1996). The FAO/WHO (2001) recommended conducting the pepsin digestion assay at pH 1.2 and pH 2.0. Results of a multi-laboratory test using a number of common stable and digestible proteins demonstrated minor differences in stability between the two pH conditions (Thomas *et al.*, 2004). While some authors have claimed a lack of predictive value for this assay (Fu *et al.*, 2002; Yagami *et al.*, 2000), Bannon *et al.* (2002) reviewed a broad range of published representative pepsin digestion studies and found a strong positive predictive value when comparing the stability of allergenic and non-allergenic dietary proteins. As defined by Codex (2003), this assay is not meant to be a stand-alone determinant in evaluating the potential allergenicity of proteins introduced into GM crops, but the results are to be judged in a weight of evidence approach by regulators Goodman *et al.* (2008).

7. Results

- 7.1. Protein profile of samples obtained at different batch preparations. The six Supertein™ and Puratein® fraction samples were separated by SDS-PAGE and stained with Colloidal blue to evaluate lot-to-lot differences on a qualitative basis. All samples were

tested under reducing and non-reducing conditions. No visible differences were noted between sample lots and the composite sample of Supertein™ (Figure 1). No visible differences were noted between sample lots and the composite sample of Puratein® (Figure 2). The dominant protein in Supertein™ appears as a single broad band at about 14 kDa in non-reducing gel (Figure 1), which separates into two fractions of approximately 9 kDa and 4 kDa under reducing conditions as reported in the literature for 2S albumins of mustard (Monsalve et al., 1997). Note that the 10 kDa band of the Precision Plus molecular weight standard from BioRad is running as a smaller apparent size than expected based on the positions of the reduced large and small subunits of 2S albumin (Supertein™) and also the 6 kDa marker protein of Invitrogen. The large and small subunits are expected to be joined by two disulfide bridges until reduced. The non-reduced Puratein® samples (Figure 2) produce similar complex patterns across the six samples. The dominant higher molecular weight band at about 48 kDa should represent intact cruciferin, which is comprised of disulfide linked acidic (alpha-) and basic (beta-) chain encoded by a single gene. After translation this is cleaved by specific proteases, but generally held together by a single disulfide bond. The moderately intense double band above 25 kDa and the triple band just below 20 kDa likely represent naturally reduced acidic and basic subunits of different cruciferin isoforms. Chemical reduction of all samples eliminated the 48 kDa band and intensified the other five bands that were visible in the non-reduced lanes (Figure 2). The Puratein® sample also seems to contain a low level of 2S albumin as there is a broad band at 14 kDa in non-reduced samples that disappears in reduced samples coincident with the appearance of one band above and one band below the 10 kDa band of Precision Plus marker protein and 6 kDa marker of Invitrogen. Based on the apparent identical results between individual sample lots and the composite samples, the composites were chosen for further testing including protein sequencing and pepsin digestion.

- 7.2. Total protein content and estimated purity of the Supertein™ and Puratein® composite samples. The total protein content over in the lyophilized sample of both Supertein™ and Puratein® protein fractions were determined by LECO analysis which is based on the total nitrogen content. The results of the protein content were expressed as a percentage over dry weight powder and have been summarized in Table 1. The Supertein™ samples are known to contain approximately 85% whereas Puratein® samples contain around 93%. All the values for the individual batched samples are given in Table 1.
- 7.3. Characteristics of Supertein™ and Puratein® protein fractions. The composite fractions S6 and P6 representing the Supertein™ and Puratein® were selected for further

characterization. Diluted samples representing 1.25, 2.5, 5 and 10 micrograms of each protein fraction were prepared and loaded in non-reducing gels (Figure 3A) and reducing gels (Figure 3B). The diluted sample staining patterns revealed a slightly more complex protein pattern than expected. The non-reducing pattern of Supertein™ (Figure 3A) showed one primary major band of approximately 14 kDa as expected. The reducing SDS-PAGE of Supertein™ indicated there are two major bands of approximately 10 kDa and 5 kDa, but possibly some minor bands within that size range. The non-reducing gel of Puratein® shows three bands which are like doublets each at 50 kDa, 30 kDa and 20 kDa. Whereas the reducing gel shows two major bands that appear as a doublet at 30 kDa and 20 kDa. Since the two dominant bands at approximately 30 kDa and three at approximately 18 kDa seemed to be constant in size and amount, while the 50 kDa bands disappeared upon reduction, it is likely that all of the Puratein® major stained bands represent different isoforms of cruciferins, and that some of the protein is already reduced in the “non-reduced” gel samples. Since the acidic and basic subunits of cruciferins are joined by a single disulfide bond, it is likely that they are reduced *in planta*, or during the processing steps leading to purification of Puratein® (Palomares et al., 2007). The 11S seed storage of some of the other allergenic plants, such as peanuts, are known to be encoded by more than one gene, with some genetic variation including minor peptide size differences (Palomares et al., 2007). The identities of the major stained protein bands were investigated further by sending samples to a proteomics core facility at Colorado State University (see section 7.5).

7.4. Densitometric estimation of napin and cruciferin purity in Supertein™ and Puratein®, respectively. The presence of major protein concentration in the reduced Supertein™ and Puratein® commercial composite fractions (S6 and P6) were analyzed by densitometry of stained gel patterns in reducing gel samples using the Kodak Gel Logic and 1D Kodak software. The results are shown in Table 2. The Supertein™ 2S albumin was taken as the sum of the 9 kDa and 4 kDa bands, which together accounted for 14 kDa protein represents approximately 84% of the total stainable protein. The 50 kDa band in Puratein® is a mixture of cruciferins and was estimated to represent approximately 74% of the stainable protein in the Puratein® sample. Puratein® also contained minor, but clearly visible bands representing the 9 kDa and 4 kDa peptides of reduced napin.

7.5. Sequencing of major protein bands from Supertein™ (S6) and Puratein® (P6). Samples of the composite Supertein™ and Puratein® fractions were separated in reducing SDS-PAGE gels and were stained with Coomassie brilliant blue R250 stain. The Coomassie stain is less sensitive than the Colloidal blue stain and minor bands

visible in Figures 1-3 are not visible in Figure 5. Sample bands were carefully excised from the gels as indicated by the white boxes in Figure 5. These samples were placed into individual microcentrifuge tubes, marked as indicated in Figure 5 and submitted to Macromolecular Resources at Colorado State University for trypsin digestion and analysis by LC-MS/MS. The sequences of the peptides generated from each individual protein were analyzed by Mascot and by Scaffold programs at Macromolecular Resources. The data files of Scaffold are retained at FARRP and the analysis was carefully reviewed and interpreted by us. Due to sequence identity matches between closely related species of mustard, there is some ambiguity in matching a single peptide with one species, but the collection of multiple peptide identities per band, and the known source of the extract allowed assignment of protein identities with very high confidence.

The proteins identified by sequence matches of the peptides generated by trypsin digestion of isolated Coomassie stained bands (Figure 5A), to napin proteins (2S albumins) from both small (S6-2) and large (S6-1) fragments of Supertein™ are shown in Appendix 1. The full-length sequences include peptides from both subunits as the proteins are produced as translation products of one gene, and then cleaved by specific proteolysis during processing within the cells of the seeds. Five NCBI napin sequences were matched with more than 95% confidence in identity based on Scaffold prediction by peptides isolated from the large subunit (S6-1). Four of them are from *Brassica napus* (GI:1699240; GI:112746; GI:1699238; GI: 112747) and one is from *Raphanus sativus*. (GI: 169694). Two more sequences of *Brassica napus*, napin from NCBI (GI:112738; GI:108935944) were matched with peptides from the small subunit (S6-2). These isomers of napin represented in NCBI were aligned to each other by CLUSTALW (Appendix 1) and all matched the identity of at least one other napin with more than 85% identity. Further, the lowest identity match across the seven sequences was 76%. The specific peptide sequences identified by Scaffold analysis are shaded on each identified sequence within the CLUSTALW multiple sequence alignment (Appendix 1). Details of the NCBI entries are provided there. In addition to the napin peptides, the analysis of band SB-1 showed two peptide matches with greater than 95% confidence to maize lipid transfer protein (LTP), suggesting that there is probably a LTP from Brassica in the samples. Further there were single peptide matches of 80-94% confidence to three proteins from Brassica sp.; a trypsin inhibitor (GI:547742), a LTP (GI:21591782) and another napin sequence (GI:2554757), indicating other seed proteins are present in the sample that were not identified with high confidence in this protein fraction. Sequence analysis of the proteins matched with greater than 95% confidence to Supertein™ bands for identity comparison with known and putative allergens are described in section 7.6 and Appendix 2.

The Puratein® composite fraction (P6) was separated into major 7 bands by reducing 10-20% SDS-PAGE (Figure 5B) and samples indicated by number (P6-1 to P6-7) and marked by white boxes in the figure were analyzed by Macromolecular Resources. The peptides generated from the bands P6-1 to P6-5 of Puratein® matched with more than 95% certainty to seven NCBI sequences of cruciferin (11S globulin) sequences of *Brassica napus* (GI:33284900; GI:461840; GI12751302; GI:461841; GI:1345840; GI17805 and GI:86611322) and one sequence of *Raphanus sativus* (GI:21108) as described in Appendix 3. In addition, peptides matching five of the listed cruciferin sequences were also identified in the band marked P6-6 in Figure 5, which was thought to be primarily residual napin, which was also identified in P6-6. In addition, one peptide of P6-7 matched an embryonic napin precursor (GI:112747) of *Brassica napus* that was also identified in SB-1 from Supertein™, but this match was with only 78% probability. The sequences of the cruciferins identified with 95% probability were analyzed for matches to known and putative allergens as described in section 7.6 and Appendix 4.

- 7.6. Bioinformatics comparison of Supertein™ (napin) and Puratein® (cruciferin) sequences to AllergenOnline version 8.0. All of the napin and cruciferin full-length NCBI sequences identified as high probability matches in section 7.5 were used as query sequences in bioinformatics searches with allergenic sequences. The sequence of the major proteins in Supertein™ and Puratein® fractions were selected from the NCBI data base, following identification of the peptide sequences as belonging to *Brassica napus* napin and cruciferin respectively. The seven sequences of napin had the highest identity matches in both full-length alignments and best 80 amino acid window matches to *Sinapis alba*, Sin a 1 isoforms with greater than 80% identities and to *Brassica juncea*, Bra j 1 with more than 80% identity. The shorter peptide sequences of matched napin allergens produce an unusual situation with the full-length match being greater than 90% identity while the best 80mer match is only 42% (see Appendix 2 match to Bra j 1E). The lower identity is an artifact caused by the calculation (dividing by 80). These high identities strongly suggest there would be cross-reactivity between the canola 2S albumin proteins and the mustard napin proteins. The next most significant matches are approximately 35-42% identities over 80 amino acid window and less than 45% identical to full-length sequences of 2S albumins from walnut (*Juglans regia* or *Juglans nigra*, and Sesame (*Sesamum indicum*), cashew (*Anacardium occidentale*) and castor bean (*Ricinus communis*) 2S albumins. Based on a lack of documented cross-reactivity between the proteins of the recognized mustard allergens Sin a 1 and Bra j 1 and the tree nut 2S albumins, which have similar degrees of identity matching to those allergens, there is no reason to suspect cross-reactivity with the canola proteins and the non-mustard 2S albumins (see Appendix 5).

The seven *Brassica napus* and one *Raphanus sativa* sequences chosen to represent the canola 11S globulins due to peptide matches do have higher identities to allergenic tree nut and legume 11S globulins, than do the 2S albumins compared to tree nut 2S albumins. The eight 11S globulins matched two 11S globulins of *Sinapis alba* with about 55-98% identities overall alignments and 65-98% by 80 amino acid window. Those identities suggest a very high probability, almost certain cross-reactivity for canola 11S globulins for those individuals sensitized to *Sinapis alba*. There appears to be at least three distinct genes or gene clusters of the Brassicaceae 11S globulins based on CLUSTALW alignment (Appendix 3). Representatives of the clusters share similar sequence matching patterns with their most similar mustard isoforms. The FASTA alignments for the cruciferins demonstrate a wide spread of sequence identity matches to similar proteins of various allergenic tree nut, legume, buckwheat and wheat allergens depending on the cruciferin used as the query sequence that in all cases is below 50% identity in the full-length matches to the non-Brassicaceae family 11S globulins, indicating reduced likelihood of cross-reactivity compared to the within-Brassicaceae 11S globulins. Even though these cruciferins match many of the allergenic 11S seed storage proteins of tree nuts, peanut, cashew, buckwheat and wheat with greater than 35% identity over an 80 amino acid window, those matches are 10 to 30 percentage points below the matches to *Sinapis alba* 11S globulins. There are actually very few reports of cross-reactivity for any of the 11S globulins even though many are major allergens. One paper reports some in vitro cross-reactivity between sesame 11S and walnut 11S globulins for one or more patients (Wallowitz et al., 2007), however, the sesame allergic subject could consume walnut even though the proteins were cross-reactive and induced some histamine release in a cell culture assay. Another recent study evaluated structural similarities across a number of 11S globulins, focusing on reported IgE binding sites on the peanut allergen, suggests that there may be some cross-reactivity due to restricted surface structural similarities between peanut glycinin (Ara h 3), walnut (Jug r 4), hazelnut (Cor a 9) and cashew (Ana o 2) 11 S globulins, but the data are not supported by comparative IgE binding studies (Barre et al., 2007). Even though these protein homologues are approximately 60% identical over the best 80 amino acid alignment and more than 45% identical in overall alignment, there is very little evidence of cross-reactivity. Thus, the primary risk of allergic cross-reactivity presented by canola 11S globulins would clearly be with other mustards, e.g. *Sinapis alba*, due to the much higher identity and expected three-dimensional similarity.

- 7.7. Pepsin digestion assay to assess protein digestive stability. Both Supertein™ and Puratein® fractions were tested in our standardized pepsin digestion assay that was

derived from the method described by Thomas et al. (2004). Prior to performing the digestions the limit of detection of proteins in stained gels was determined (section 7.8.1) and the activity of the pepsin is verified by a hemoglobin digestion assay (section 7.8.2).

7.8.1. Verification of the limit of detection of Supertein™ and Puratein®. Stained gels of serially diluted Supertein™ (Figure 6) and Puratein® (Figure 8) were evaluated to ensure detection down to the limit of 10% residual target protein, the amount set as the level for calling the end of digestion. As the starting pepsin digestion protein quantity targeted for analysis is 2 µg per well, the minimum detection must be below 0.2 µg per well. Samples were tested down to 0.025 µg to ensure detection was below the desired limit. Specific net intensity values from the Gel Logic 440 image station (Table 2 and 3) were plotted in a regression (Figure 7 and 9) with apparent good fit based on the correlation coefficient ($r^2 = 0.989$ and 0.976 for Supertein™ and Puratein® respectively). Based on these data the limit of detection was approximately 1.25% (0.025 µg) of Supertein™ or Puratein® (100% loading (2.0 µg) used in the digestion samples), sufficiently sensitive to detect 10% residual protein.

7.8.2. Pepsin activity verification. Each time the simulated digestion fluid (SGF, pH 1.2 or 2.0, plus pepsin) was prepared for a digestion assay the activity was tested by an independent hemoglobin digestion assay to ensure the pepsin was within the activity range specified in the protocol, compared to the labeled activity claimed by Sigma Chemical (St. Louis, MO). Digestion of the test protein was accomplished within 24 hours (usually 4 hours) of preparation and of testing the activity. The activity assay we use is similar, but not identical to that used by Sigma. Our tolerance is +/- 1,000 units per mg of solid pepsin compared to the Sigma certified claim. The certified activity of the lot of pepsin from Sigma used in this study was 3,280 units per mg of solid, while the assayed value was 2,369 units per mg solid and was judged acceptable.

7.8.3. Supertein™ and Puratein® digestion results. Representative stained gels of digestion experiments with Supertein™ and Puratein® at both pH 1.2 and pH 2.0 are shown in figures 10-13. The results demonstrate that the Supertein™ protein fraction contains 2S albumin seed storage protein (napin) from *Brassica napus* at the expected positions based on characterization studies. The two Supertein™ proteins (9 kDa large subunit and 4 kDa small subunit) are both quite stable in pepsin at 1.2 and 2.0 as neither was reduced to less than 10% of the starting material based on stain density of residual bands (Figures 10-11).

However, the Puratein® (11S globulins (MW 17-30 kDa in reducing gels shown in Figures 12-13) are quite labile. They are reduced to approximately 10% residual protein by 0.5 minutes and are clearly below 10% residual protein by 2 minutes. There is however, some stable napin in the Puratein® samples as viewed in Figures 12 and 13 and as determined in the characterization study. Importantly there is no notable difference in results obtained at pH 1.2 compared to 2.0 in these experiments. The overall conclusion is that the Supertein™ proteins (predominantly napin) is stable in acidic pepsin whereas the proteins in Puratein® are susceptible to digestion by acidic protein, however there is some low level of stable napin in the Puratein® sample.

8. Conclusions

The Supertein™ and Puratein® protein fractions were analyzed to verify protein identities. Supertein™ contains slightly different isoforms of napin (2S albumins) as the dominant protein component. The Puratein® contains predominantly a number of isoforms of cruciferins (11S globulins) and also a small amount (quantity not determined) of napin.

Bioinformatic (FASTA) results demonstrate very high sequence identity matches of the napins to napins of mustards (*Brassica sp.* as well as *Sinapis alba* and *Raphanus sativa*). These matches are sufficiently high to expect allergic cross-reactivity for those individuals who are allergic to mustard. Similarly, the FASTA results using all of the identified cruciferins as query sequence showed high identity matches to cruciferins of *Sinapis alba* and *Raphanus sativa*, again suggesting a high likelihood of cross-reactivity for those allergic to mustard. The primary conclusion from the bioinformatics analysis is that individuals allergic to any of the mustards may experience allergic reactions if they consume the canola proteins. Since there are reports of systemic reactions to mustard proteins in the diet, it is reasonable to expect that products containing Supertein™ or Puratein® should be labeled in the ingredient statement as containing mustard-like proteins since many consumers might not recognize the relationship of canola and mustard.

Results from the pepsin digestion assays are quite clear. The cruciferins are rapidly digested and based on the paradigms of the Codex Alimentarius Commission guidelines for assessing the potential allergenicity of genetically modified crops, might be assumed to present a minimal risk of new sensitizations. However, the stability of the napins in pepsin should be viewed as indicating that they present some increased risk of sensitizing and a possibility of eliciting allergic reactions that is greater than proteins that are rapidly digested. They are similar in this regard to 2S albumins of Brazil nut, peanuts and some other important food crops.

9. References

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Table 1. The protein content powdered samples of Supertein™ and Puratein® was determined by LECO analysis. The measured nitrogen content was determined in triplicate samples, expressed as nitrogen content. The protein mass for the sample was calculated by multiplying the nitrogen content by the constant 6.38 to provide and adjusted based on a , expressed as a percent protein content. The amount of nitrogen by LECO analysis based on total nitrogen content.

Samples received from ADM	Sample codes (ID)	nitrogen content by LECO analysis Average ± SD	Percent protein content (%)
Supertein™			
1. Lot # C 200-E03-07	S1	13.961 ± 0.0072	87.258
2. Lot # C 200-E07-07	S2	13.836 ± 0.0609	86.477
3. Lot # C 200-D25-07	S3	13.773 ± 0.0135	86.081
4. Lot # C 200-C28-07	S4	13.412 ± 0.0345	83.816
5. Lot # C 200-F04-07	S5	13.991 ± 0.0586	87.441
6. Lot # JRRRCA-071107 (composite)	S6	13.713 ± 0.0586	85.704
Puratein®			
1. Lot # C 300-E01-07	P1	15.435 ± 0.0719	96.473
2. Lot # C 300-E14-07	P2	15.251 ± 0.0389	95.317
3. Lot # C 300-E24-07	P3	15.320 ± 0.0424	95.754
4. Lot # C 300-C28-07	P4	15.319 ± 0.0529	95.741
5. Lot # C 300-F04-07	P5	14.800 ± 0.0792	92.498
6. Lot # JRRRCB-071107 (composite)	P6	14.882 ± 0.0720	93.014

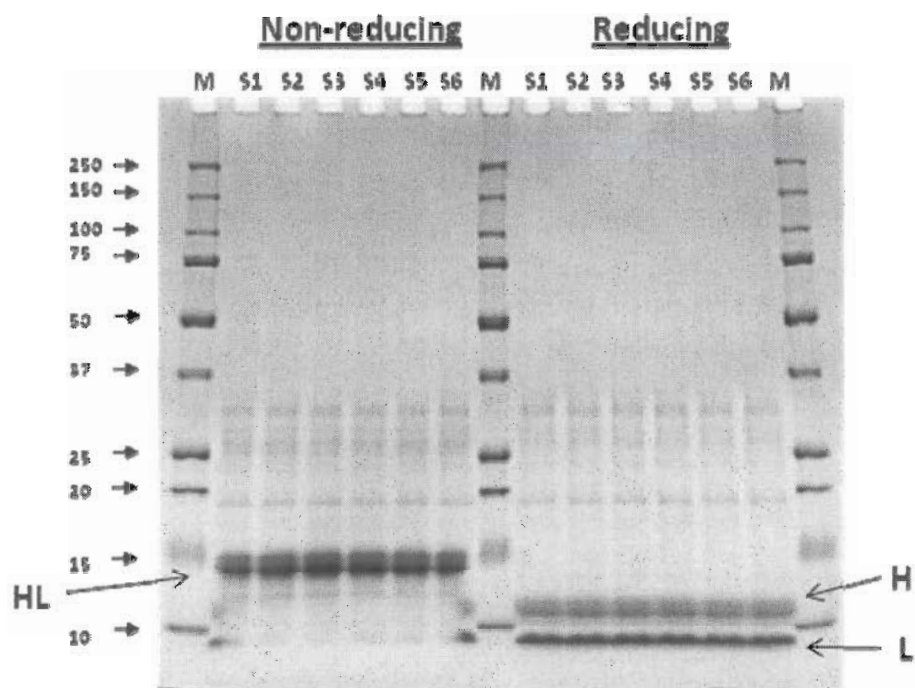


Figure 1. Non-reducing and reducing SDS-PAGE of Supertein™. Approximately 5 µg of each sample was prepared in non-reducing Laemmli buffer (no heating), or in Laemmli buffer with 2-mercaptoethanol (and heated for 5 minutes, 95°C), then separated by electrophoresis in an 18% gel before staining with Colloidal blue. Sample lanes represent: M (BioRad Precision Plus MW Marker), S1 (lot #C 200-E03-07) S2 (lot #C200-E07-07), S3 (lot #C200-D25-07), S4 (lot #C200-C28-07), S5 (lot #C200-F04-07) and S6 (lot# JRRRCA-071107-composite). The dominant bands of 2S albumin are marked as H: heavy chain of 2S albumin napin (10 kD), L: light chain of 2S albumin napin (4 kD) and HL: 2S albumin napin protein (14 kD).

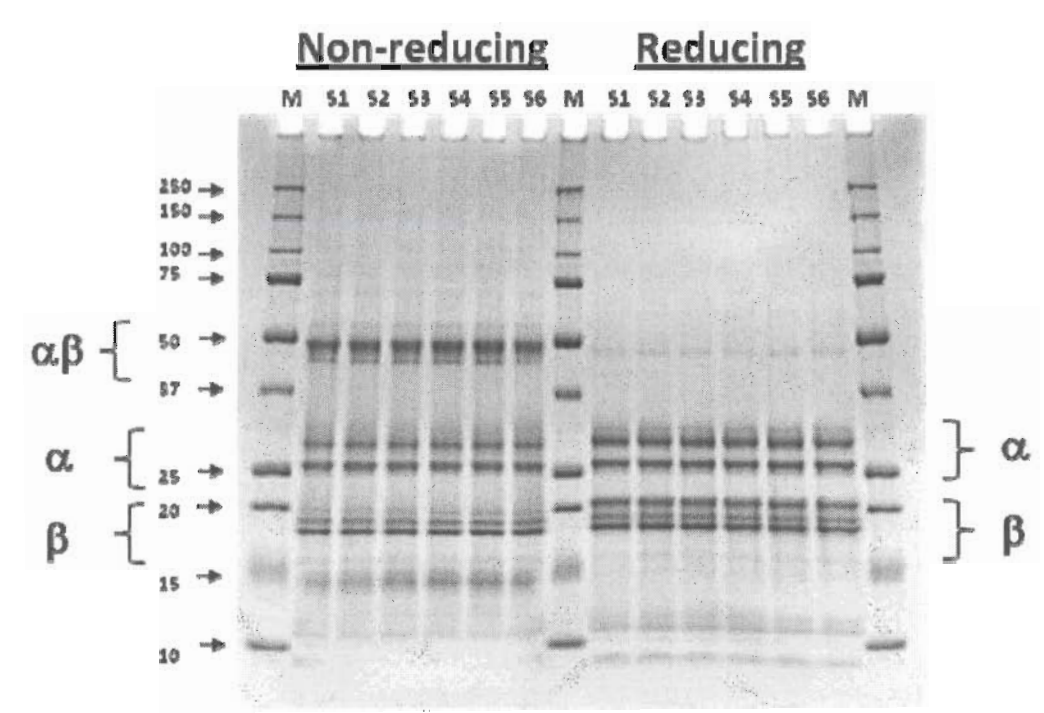


Fig 2. Non-reducing and reducing SDS-PAGE (18%) of Puratein®. Approximately 5 µg of each sample was prepared in non-reducing Laemmli buffer (no heating), or in Laemmli buffer with 2-mercaptoethanol (and heated for 5 minutes, 95°C, then separated by electrophoresis in an 18% gel before staining with Colloidal blue. Sample lanes represent: M (BioRad Precision Plus MW Marker), P1 (lot #C 300-E01-07) P2 (lot #C300-E14-07), P3 (lot #C300-E24-07), P4 (lot #C300-C28-07), P5 (lot #C300-F04-07) and P6 (lot# JRRRCB-071107-composite). No noticeable differences were found between individual batch preparations. Protein bands identified: α: acidic chain of 11 S cruciferin (30 kD), β: basic subunit of 11 S cruciferin (20 kD), αβ: cruciferin 11S (50 kD)

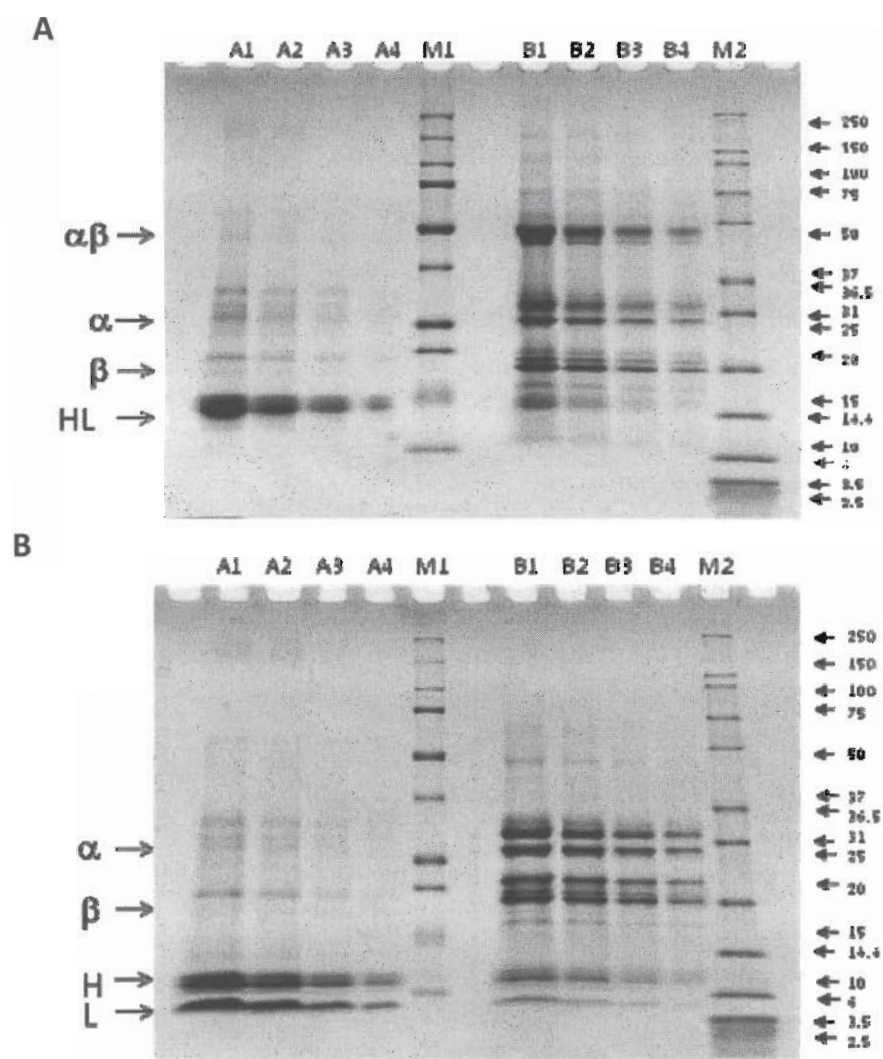


Figure 3. Non-reducing (A) and Reducing (B) SDS-PAGE of composite fractions S6 and P6. Samples were diluted in either reducing or non-reducing Laemmli buffer and separated by electrophoresis in 18% SDS-PAGE. Samples include A1: Supertein™ S6, 10 µg; A2: Supertein™ S6, 5 µg; A3: Supertein™ S6, 2.5 µg; A4: Supertein™ S6, 1.25 µg; B1: Puratein® P6, 10 µg; B2: Puratein® P6, 5 µg; B3: Puratein® P6, 2.5 µg; B4: Puratein® P6, 1.25 µg; M1: Bio-Rad Precision Plus prestained markers; M2: Mark12™ unstained marker from Invitrogen. Protein bands were identified as: cruciferins: α, acidic chain of 11 S cruciferin (20 kDa); β, basic subunit of 11 S cruciferin (30 kDa); αβ, cruciferin 11S (50 kDa) and napins: H, heavy chain of 2S albumin napin (10 kDa); L, light chain of 2S albumin napin (4 kDa); HL : 2S albumin napin protein (14 kDa).

1. Percentage of napin (2S albumin) protein in Supertein™ composite fraction

Total Supertein stained	Pixels	Napin in Supertein™ (2 bands)	Pixels
Lane total band intensity	525529.4	Napin band intensity	189578.8
Back ground intensity	326941.5	Back ground intensity	23271.9
Net band intensity	198587.9	Net Napin intensity	166306.9

Percentage of Napin (2S albumin) protein in the Supertein™ composite fraction is:

$$= \frac{166306.9}{198587.9} \times 100 = 83.74\%$$

2. Percentage of cruciferin (11S globulin) protein in Puratein® composite fraction.

Total Puratein® fraction	Pixels	Cruciferin in Puratein® (5 bands)	Pixels
Lane total band intensity	635439.9	Cruciferin band intensity	261030.6
Back ground intensity	355173.6	Background intensity	52748.5
Net band intensity	280266.3	Net cruciferin intensity	208282.1

Percentage of cruciferin (11S globulin) protein in Puratein® composite fraction is:

$$= \frac{208282.1}{280266.3} \times 100 = 74.31\%$$

Figure 4. Percent protein purity calculation of Supertein™ (S6) and Puratein® (P6) composite protein fractions. The percent purity for the complex proteins was estimated by densitometry based on stained bands at the appropriate molecular weight in with 2.5 mg of protein loaded per lane (A3 and B3 of Fgure 3A and B respectively) of reduced SDS-PAGE, stained with colloidal blue. Densitometry was performed using a Kodak Gel Logic 440 with 1D analytical software. Areas corresponding to the selected area of interest (napins or cruciferins) in each gel were scanned of the blank lanes on each gel, and the pixel count was used to subtract from the complete sample well lane scan. The specific protein scan (napin and cruciferins) were also back ground subtracted. The percent purity was estimated by dividing the specific protein pixel count by the total stained band pixel count in the lane, then multiplying the sum times 100%.

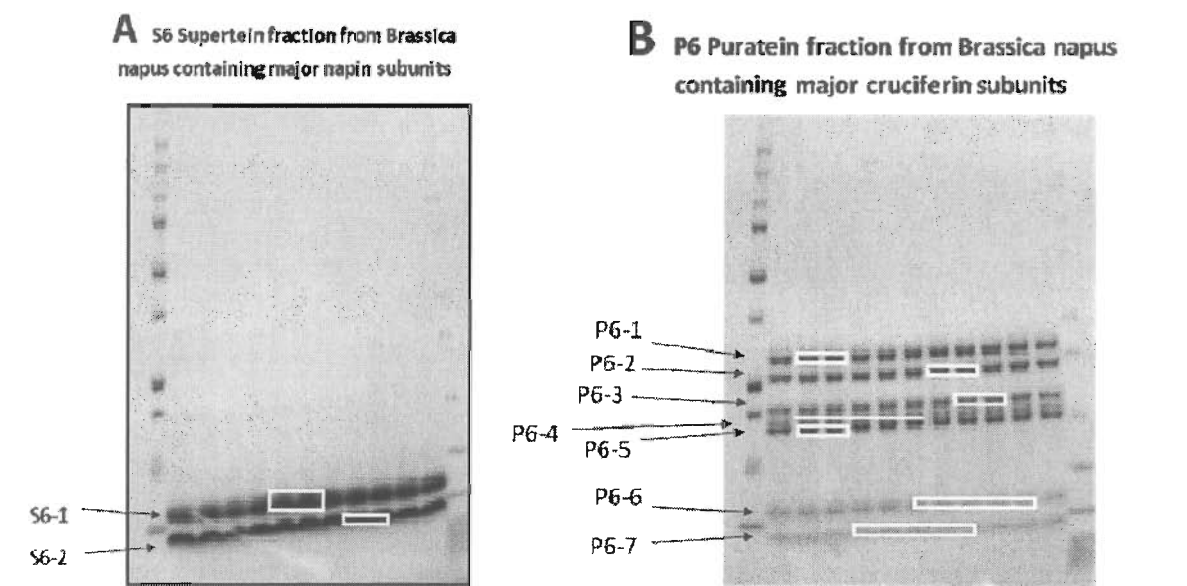


Figure 5. Protein bands excised for sequence determination of Supertein™ composite fraction (A) and Puratein® composite fraction (B). Approximately 10 mg of appropriate protein fraction were loaded following heating in reducing Laemmli buffer and electrophoresis in 10-20% polyacrylamide SDS-PAGE. Samples were stained with Coomassie Blue R250 to identify the major bands. Sample bands were excised for protein identification. Bands enclosed by the white-line box were carefully cut from the stained gel using a fresh scalpel blade for each protein using two to five wells per sample to provide sufficient mass for LC-MS/MS analysis. Sample S6-1 is intended to represent the ~ 9 kDa large subunit of napin. Sample S6-2 represents the 4 kDa small subunit. Samples P6-1 and P6-2 represent two different molecular weight bands thought to represent acidic subunits of cruciferin from *Brassica napus*. Bands P6-3, P6-4 and P6-5 are thought to represent basic subunits of cruciferin. Bands P6-6 and P6-7 are thought to represent residual napin peptides, the large and small subunits respectively. The bands indicated by white outlines were submitted to Macromolecular Resources for trypsin digestion and peptide sequencing based on mass determination by LC-MS/MS. Results are described in Appendix 1 for Supertein™ and Appendix 3 for Puratein®.

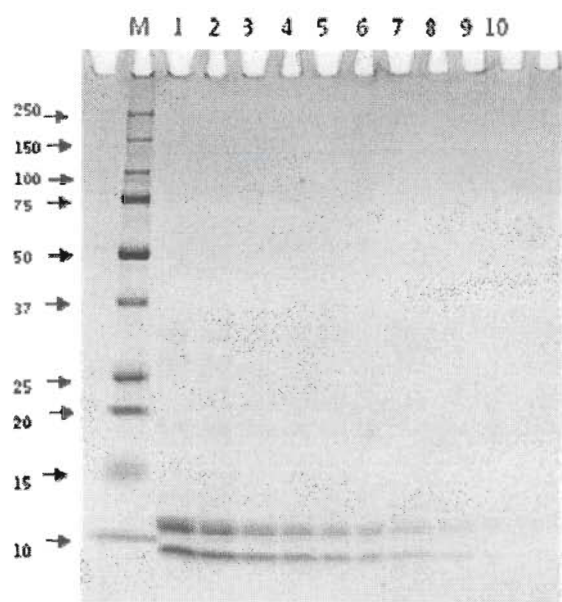


Figure 6. Colloidal blue stained SDS-PAGE for LOD of Supertein™. The Supertein™ composite sample was serially diluted a known stock and loaded in wells as indicated below. The proteins were separated by tris-glycine 10-20% SDS-PAGE. Proteins were detected by staining with Colloidal Brilliant Blue G stain after separation and the amount of stain measured by densitometry using a Kodak Gel Logic 440 and 1D software. The minimum acceptable detection is 10% of the starting digestion protein (2 µg/well).

S. No.	Sample	Protein amount (µg/well)
M	M. wt. marker	NA
1	S6 (100%)	2.00
2	S6 (75%)	1.50
3	S6 (50%)	1.00
4	S6 (40%)	0.80
5	S6 (30%)	0.60
6	S6 (20%)	0.40
7	S6 (10%)	0.20
8	S6 (5.0%)	0.10
9	S6 (2.5%)	0.05
10	S6 (1.25%)	0.025

S.No	Supertein™ (µg)	Protein (%)	Net band intensity
1.	2	100	145663
2.	1.5	75	103440
3.	1	50	64763
4.	0.8	40	53916
5.	0.6	30	34468
6.	0.4	20	28191
7.	0.2	10	20120
8.	0.1	5	12868
9.	0.05	2.5	5631
10.	0.025	1.25	1103

Table. 2. Densitometry Analysis of Supertein™. Diluted Supertein™ composite samples (left column) were separated in by SDS-PAGE (Figure 6), the gel was fixed, stained with Colloidal Brilliant Blue G and scanned with a Gel Logic 440 and the band image net intensities (pixel densities), after subtracting the minimal peripheral area backgrounds, were measured using the Kodak 1D analyzer program.

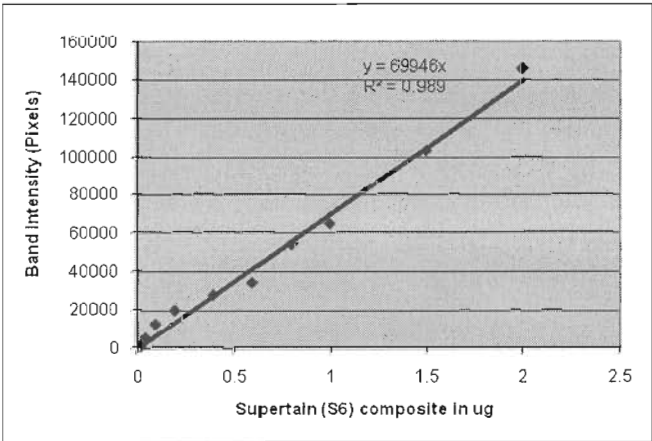


Figure 7. Linearity of Detection of Supertein™ Diluted Samples. The regression line for diluted Supertein™ fraction, as detected on Colloidal blue stained gel, is shown in the figure. The correlation coefficient (r^2) is greater than 0.989, demonstrating good linearity for estimating protein concentration based on stained band intensity.

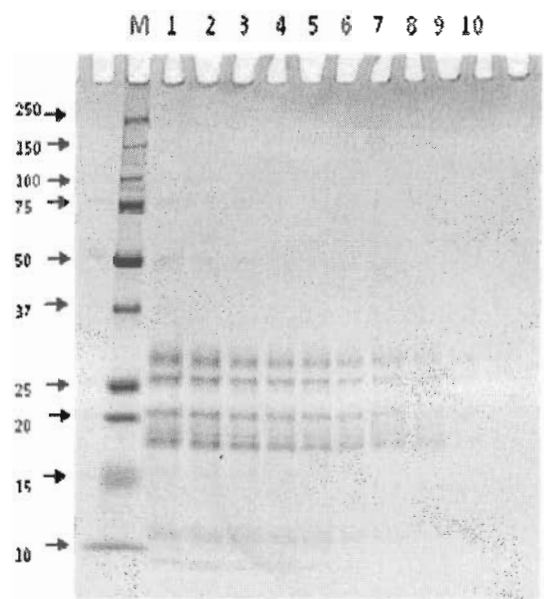


Figure 8. Colloidal blue stained SDS-PAGE for LOD of Puratein®. The Puratein® composite sample was diluted from a known stock solution and loaded in wells as indicated below. The proteins were separated by tris-glycine 10-20% SDS-PAGE. Proteins were detected by staining with Colloidal Brilliant Blue G stain after separation. The minimum acceptable detection is 10% of the starting digestion protein (2 µg/well).

S. No.	Sample	Protein amount (µg/well)
M	M. wt. marker	NA
1	P6 (100%)	2.00
2	P6 (75%)	1.50
3	P6 (50%)	1.00
4	P6 (40%)	0.80
5	P6 (30%)	0.60
6	P6 (20%)	0.40
7	P6 (10%)	0.20
8	P6 (5.0%)	0.10
9	P6 (2.5%)	0.05
10	P6 (1.25%)	0.025

S.No	Puratein® (µg)	Protein (%)	Net band intensity
1.	2	100	157211
2.	1.5	75	121718
3.	1	50	86722
4.	0.8	40	72424
5.	0.6	30	51558
6.	0.4	20	40479
7.	0.2	10	31232
8.	0.1	5	18181
9.	0.05	2.5	10268
10.	0.025	1.25	2898

Table 3. Densitometry Analysis of Puratein®. Diluted Puratein® samples (left column) were separated in by SDS-PAGE (Figure 8), the gel was fixed, stained with colloidal blue G 250 and scanned with a Gel Logic 440 and the band image net intensities (pixel densities), after subtracting the minimal peripheral area backgrounds, were measured using the Kodak 1D analyzer program.

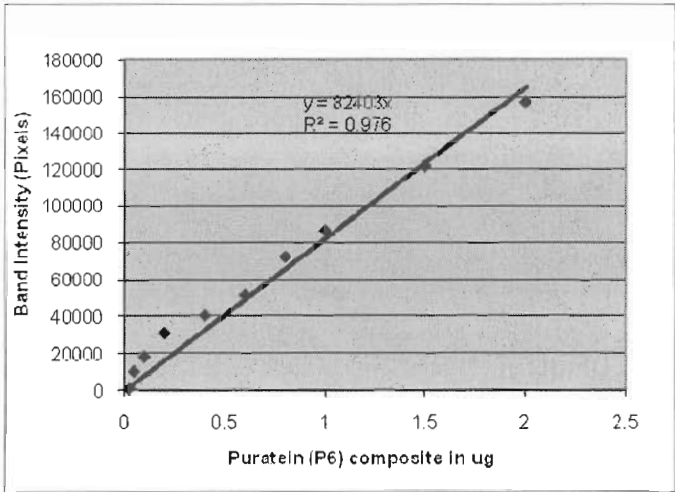


Figure 9. Linearity of Detection of Puratein® Diluted Samples. The regression line for diluted Puratein®, as detected on colloidal blue stained gel, is shown in the figure. The correlation coefficient (r^2) is greater than 0.97, demonstrating good linearity for estimating protein concentration based on stained band intensity.

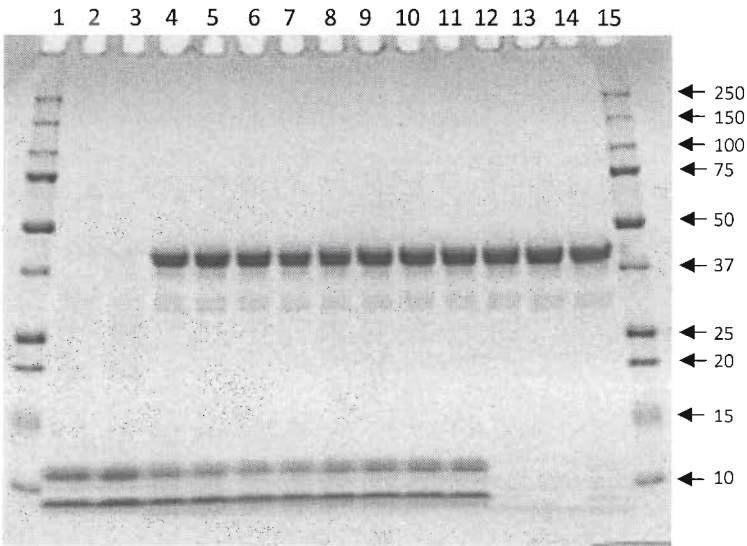


Figure 10. Pepsin Digestion Results for Supertein™ (S6) at pH 1.2. Proteins digested in at 37C and samples were removed at times indicated, quenched and heat denatured. Equal volume samples were prepared in reducing Laemmli buffer, then separated by SDS-PAGE using a 10-20% polyacrylamide gradient in a Tris-glycine buffered gel. Proteins were detected by staining with colloidal brilliant blue G250. Timed digestion and control samples of Supertein™ (S6) are loaded volumetrically to represent 2.0 µg per lane of the starting material.

Lane	Description	Incubation time
1.	Molecular weight marker	NA
2.	Experimental control without pepsin (P0)	0 min
3.	Experimental control without pepsin (P60)	60 min
4.	S6 in SGF, T=0	0 min
5.	S6 in SGF, T=1	0.5 min
6.	S6 in SGF, T=2	2 min
7.	S6 in SGF, T=3	5 min
8.	S6 in SGF, T=4	10 min
9.	S6 in SGF, T=5	20 min
10.	S6 in SGF, T=6	30 min
11.	S6 in SGF, T=7	60 min
12.	Experimental control pepsin (E0)	0 min
13.	Experimental control pepsin (E60)	60 min
14.	10% S6 with quenched pepsin (C0)	0 min
15.	Molecular weight marker	NA

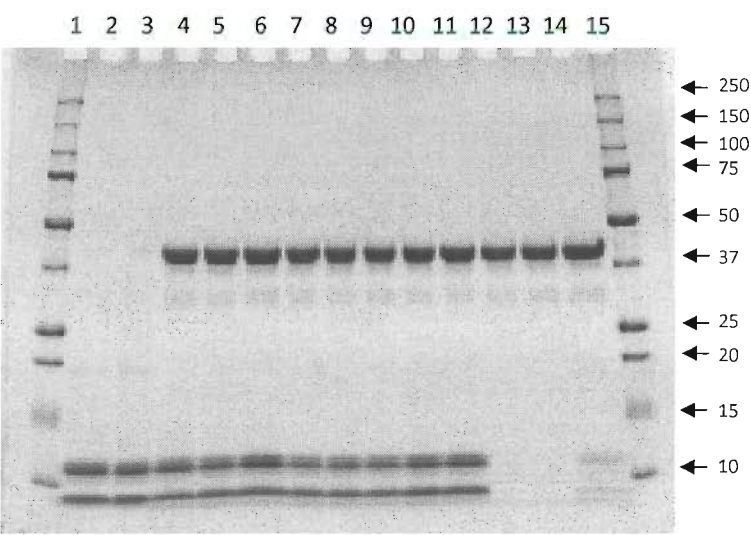


Figure 11. Pepsin Digestion Results for Supertein™ (S6) at pH 2.0. Proteins digested in at 37C and samples were removed at times indicated, quenched and heat denatured. Equal volume samples were prepared in reducing Laemmli buffer, then separated by SDS-PAGE using a 10-20% polyacrylamide gradient in a Tris-glycine buffered gel. Proteins were detected by staining with colloidal brilliant blue G250. Timed digestion and control samples of Supertein™ (S6) are loaded volumetrically to represent 2.0 µg per lane of the starting material.

Lane	Description	Incubation time
1.	Molecular weight marker	NA
2.	Experimental control without pepsin (P0)	0 min
3.	Experimental control without pepsin (P60)	60 min
4.	S6 in SGF, T=0	0 min
5.	S6 in SGF, T=1	0.5 min
6.	S6 in SGF, T=2	2 min
7.	S6 in SGF, T=3	5 min
8.	S6 in SGF, T=4	10 min
9.	S6 in SGF, T=5	20 min
10.	S6 in SGF, T=6	30 min
11.	S6 in SGF, T=7	60 min
12.	Experimental control pepsin (E0)	0 min
13.	Experimental control pepsin (E60)	60 min
14.	10% S6 with quenched pepsin (C0)	0 min
15.	Molecular weight marker	NA

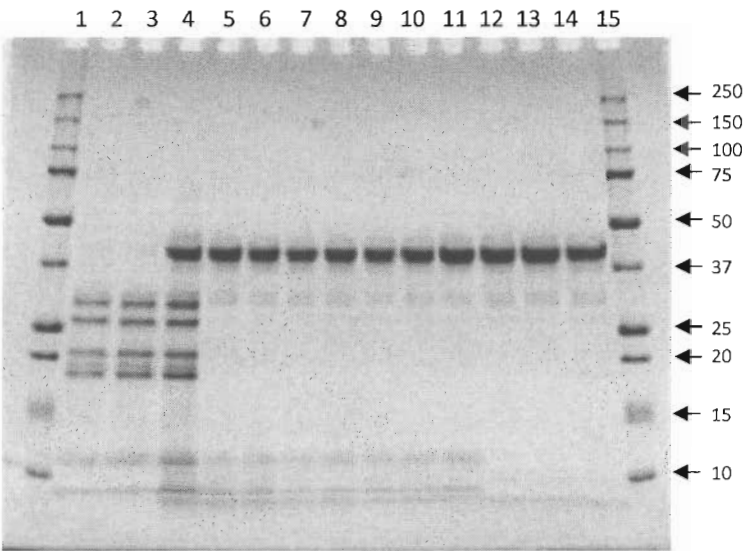


Figure 12. Pepsin Digestion Results for Puratein® (P6) at pH 1.2. Proteins digested in at 37C and samples were removed at times indicated, quenched and heat denatured. Equal volume samples were prepared in reducing Laemmli buffer, then separated by SDS-PAGE using a 10-20% polyacrylamide gradient in a Tris-glycine buffered gel. Proteins were detected by staining with colloidal brilliant blue G250. Timed digestion and control samples of Puratein® (P6) are loaded volumetrically to represent 2.0 µg per lane of the starting material.

Lane	Description	Incubation time
1.	Molecular weight marker	NA
2.	Experimental control without pepsin (P0)	0 min
3.	Experimental control without pepsin (P60)	60 min
4.	P6 in SGF, T=0	0 min
5.	P6 in SGF, T=1	0.5 min
6.	P6 in SGF, T=2	2 min
7.	P6 in SGF, T=3	5 min
8.	P6 in SGF, T=4	10 min
9.	P6 in SGF, T=5	20 min
10.	P6 in SGF, T=6	30 min
11.	P6 in SGF, T=7	60 min
12.	Experimental control pepsin (E0)	0 min
13.	Experimental control pepsin (E60)	60 min
14.	10% P6 with quenched pepsin (C0)	0 min
15.	Molecular weight marker	NA

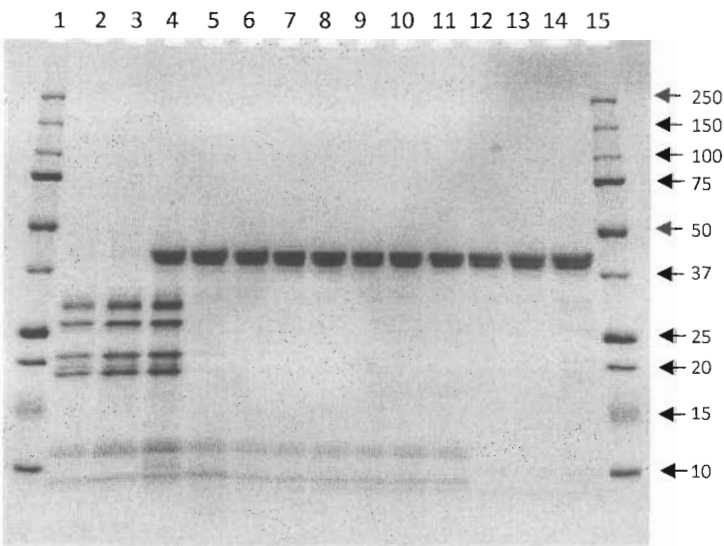


Figure 13. Pepsin Digestion Results for Puratein® (P6) at pH 2.0. Proteins digested in at 37C and samples were removed at times indicated, quenched and heat denatured. Equal volume samples were prepared in reducing Laemmli buffer, then separated by SDS-PAGE using a 10-20% polyacrylamide gradient in a Tris-glycine buffered gel. Proteins were detected by staining with colloidal brilliant blue G250. Timed digestion and control samples of Puratein® (P6) are loaded volumetrically to represent 2.0 µg per lane of the starting material.

Lane	Description	Incubation time
1.	Molecular weight marker	NA
2.	Experimental control without pepsin (P0)	0 min
3.	Experimental control without pepsin (P60)	60 min
4.	P6 in SGF, T=0	0 min
5.	P6 in SGF, T=1	0.5 min
6.	P6 in SGF, T=2	2 min
7.	P6 in SGF, T=3	5 min
8.	P6 in SGF, T=4	10 min
9.	P6 in SGF, T=5	20 min
10.	P6 in SGF, T=6	30 min
11.	P6 in SGF, T=7	60 min
12.	Experimental control pepsin (E0)	0 min
13.	Experimental control pepsin (E60)	60 min
14.	10% P6 with quenched pepsin (C0)	0 min
15.	Molecular weight marker	NA

ADM Supertein (napin) Sequences from LC-MS/MS - Appendix 1

As described in the main report, both Supertein and Puratein contain dominant protein bands, and some lower intensity stained (lower abundance) protein bands. In order to perform a meaningful bioinformatics comparison of the proteins against known allergens, it was necessary to positively identify the proteins contained in the product. As described in the body of the main report, samples of Supertein were separated by 1-D SDS-PAGE. The stained bands identified in a reducing SDS-PAGE gel (Fig. 3 of the main report) cut from an identical gel after staining with Coomassie blue R-250 (rather than colloidal blue). Dominant stained bands from multiple identical lanes were excised and sent to the Proteomics facility, Macromolecular Resources at Colorado State University in Fort Collins, CO. Each protein band sample was digested in-gel with trypsin at CSU, then analyzed by LC-MS/MS Reverse phase separation with analysis by electrospray mass spectrometry (LTQ ion trap). The Scaffold report (data not shown, but on file at FARRP, Dept. of Food Science & Technology, University of Nebraska, from the analysis was analyzed to identify the following seven protein sequences from the NCBI database that had > 95% probability of matching identified fragments for napins. The full-length of all seven sequences are shown below with GI # (NCBI identification numbers). The upper case amino acid 1-letter designations indicate sequences of peptides identified by LC-MS/MS. Lowercase amino acids were not identified in the analysis, but are known from the database. Each of the seven were analyzed using AllergenOnline.com (see appendix 2).

Proteins identified from stained band S6-1 (upper band, Figure 3) of Supertein peptides.

A few peptide fragments were identified that were too short to give a 95% probability of identification included fragments tentatively identified as lipid transfer protein (LTP), trypsin inhibitor and alpha-amylase inhibitor. Those sequences were not analyzed due to uncertainty of identification and due to the apparent low concentration in the isolated SDS-PAGE stained bands. Note that some peptide fragments were found in multiple bands as indicated in brackets.

Sequence 1, GI: 1699240 *Brassica napus* [in S6-1 AND P6-6].

PQGPQQRPLQLQCCNELHQEEPLCVCPTLKGaskavkqqrQQGQQGQQGQQLQQVISRI
YQTATHLPRVCNIPQVSICPFQKtppgy (length 99 aa).

Sequence 2, GI: 169694 *Raphanus sp.* [in S6-1 only].

siyrtvvevdeddatnpgpfriprrkefqaqhlracqqwlhkqarqsgsgpswtldgefdfeddmennpqrpplpqqccnelhqe
eplcvcptlkgaskavkqqrQQGQMGGQMGMHVISRIYQTATHLPRVCNIPQVSVCPPFQKtppgy
(length 155 aa).

Sequence 3, GI:112746 *Brassica napus* [in S6-1 AND P6-6].

manklflvsatlafflltnasiyrtvvevdeddatnpgpfripkrkefqaqhlkacqqwlhkqamqsgsgpswtldgefdfeddm
enpggpqrppllqqccnelhqeepclvcptlkgaskavkQQIQQGQQGQGLqmvsrIYQTATHLPKvckIPQV
SVCPPFQKtppgpy (length 178 aa).

Sequence 4, GI:1699238 *Brassica napus* [in S6-1 and P6-6].

pqspqqrppllqqccnelhqeepclvcptlkgaskavkqqrQQGQQGQQGQQLQQVISRIYQTATHLPKVCNIP
QVSVCPPFQKtppgpy (length 88 aa).

Sequence 5, GI: 112747 *Brassica napus* [in S6-1 and P6-7]
manklflvsatlalfflltnasvyrtvvevdeddatnpagpfripkrkefqqaqlracqqwlhkqamqpgggsgpswtldgefdfed
dvenqqqgppqrrppppqqccnelhqeepalcvcptlkgaskavrqqvrQQQGQQMQGQQMQQVISRvyqtathlprv
cnirQVSICPFQKtmpgpgfy (length 186 aa)

Proteins identified from the S6-2, lower band of Supertein (Figure 3).

Sequence 6, GI: 112738 *Brassica napus* [in S6-2]
pkcrkefqqaqlhkacqqwlhkqamqsgggpswtldgefdfeddmekqgpqrrpplhqycnelqqeepalcvcptlrgaskavk
qqiqqqeqqgkqmqmvnrIYQTATHLPKVCNIPQVSVCPFQKtmpgpsy (length 133 aa).

Sequence 7, GI:108935944, *Brassica napus*, GI# replaced with GI: 75107016 [in S6-2]
SAGPFRIPKcrKEFQQAQHLRacqqwlhkqamqsgsg (length 125 aa as full-length in GI 75107016).

CLUSTAL W alignment of the 7 identified napin sequence matches.
Pairwise Percent Identity matches of full-length NCBI entries for the seven. Note that the top identity match for sliding window of 80 amino acid segments are much higher for most matches, as demonstrated by Appendix 2.

GI numbers	1699240	169694	112746	1699238	112747	112738	75107016
1699240	100	85	82	94	85	76	91
169694	85	100	87	84	89	80	85
112746	82	87	100	86	85	92	88
1699238	94	84	86	100	87	81	97
112747	85	89	85	87	100	81	88
112738	76	80	92	81	81	100	80
75107016	91	85	88	97	88	88	100

FEATURES Location/Qualifiers
 source 1..91
 /organism="Brassica napus"
 /db_xref="taxon:3708"
 Protein 1..91
 /product="napin large chain L2C"
 /name="calmodulin antagonist/calcium-dependent protein
 kinase substrate"

ORIGIN
 1 pggpqrprpl lqqccnelhq eepclvcptl kgaskavkqg vrqqgqqqgq qgqqlqqvis
 61 riyqtathlp rvcnipqvsi cpfqkttpgp y
//

LOCUS AAA63470 155 aa linear PLN 07-MAR-1995
DEFINITION storage protein.
ACCESSION AAA63470
VERSION AAA63470.1 GI:169694
DBSOURCE locus RADNAPA accession M63841.1
KEYWORDS .
SOURCE Raphanus sativus (radish)
ORGANISM Raphanus sativus
 Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
Brassicaceae; Raphanus.

REFERENCE 1 (residues 1 to 155)
AUTHORS Raynal,M., Depigny,D., Grellet,F. and Delseny,M.
TITLE Characterization and evolution of napin-encoding genes in radish
 and related crucifers
JOURNAL Gene 99 (1), 77-86 (1991)
PUBMED 2022325
COMMENT Method: conceptual translation.
FEATURES Location/Qualifiers
 source 1..155
 /organism="Raphanus sativus"
 /db_xref="taxon:3726"
 /clone="PBG9"
 /tissue_type="immature seed"
 /dev_stage="embryogenesis"
 /tissue_lib="cDNA library in pBR322"
 Protein 1..155
 /product="storage protein"
 mat peptide 17..51
 /gene="napin"
 /product="storage protein small subunit"
 Region 31..146
 /region_name="AAI_SS"
 /note="AAI_SS: Alpha-Amylase Inhibitors (AAIs) and Seed
Storage (SS) Protein subfamily; composed of cereal-type
AAIs and SS proteins. They are mainly present in the
Seeds of a variety of plants; cd00261"
 /db_xref="CDD:72936"
 mat peptide 71..153
 /gene="napin"
 /product="storage protein large subunit"
 order(77,127,131,137,143)
 /site_type="other"
 /note="dimer interface"
 /db_xref="CDD:72936"
 Site order(88..90,97,146)
 /site_type="other"
 /note="alpha-amylase binding site"
 /db_xref="CDD:72936"
 CDS 1..155
 /gene="napin"
 /coded_by="M63841.1:1..468"

ORIGIN
 1 siyrtvvevd eddatnpgp friprrrkef qgaqlracq qwlhkqarqs gsgpswtldg

```

61 efdfeddmnen pqrpplpqgc cnelhqeepI cvcptlkgas kavkqgvrrq gqmqgqgmqh
121 visriyqtat hlprvcnipq vsvcpfqktt pgpyy
//

LOCUS      P27740              178 aa              linear      PLN 02-OCT-2007
DEFINITION Napin-B precursor (1.7S seed storage protein) [Contains: Napin-B
small chain; Napin-B large chain].
ACCESSION  P27740
VERSION    P27740.1  GI:112746
DBSOURCE   swissprot: locus 2SSB_BRANA, accession P27740;
class: standard.
created: Aug 1, 1992.
sequence updated: Aug 1, 1992.
annotation updated: Oct 2, 2007.

xrefs: X58142.1, CAA41150.1, M.5
xrefs (non-sequence databases): HSSP:P24565, InterPro:IPR003612,
InterPro:IPR000617, InterPro:IPR013771, Gene3D:G3DSA:1.10.120.10,
Pfam:PF00234, PRINTS:PR00496, ProDom:PD002498, SMART:SM00499
KEYWORDS   Seed storage protein; Signal; Storage protein.
SOURCE     Brassica napus (rape)
ORGANISM   Brassica napus
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
Brassicaceae; Brassica.
REFERENCE  1 (residues 1 to 178)
AUTHORS    Ericson,M.L., Muren,E., Gustavsson,H.O., Josefsson,L.G. and Rask,L.
TITLE      Analysis of the promoter region of napin genes from Brassica napus
            demonstrates binding of nuclear protein in vitro to a conserved
            sequence motif
JOURNAL    Eur. J. Biochem. 197 (3), 741-746 (1991)
PUBMED     2029903
REMARK     NUCLEOTIDE SEQUENCE [GENOMIC DNA].
            STRAIN=cv. Svalofs Karat 20516-K
COMMENT     On Jan 20, 2006 this sequence version replaced gi:99818.
            [FUNCTION] The small, basic, water-soluble napins are one of the
            two major kinds of storage proteins synthesized in the seed
            during its maturation.
            [SUBUNIT] The mature protein consists of a small and a large
            chain linked by disulfide bonds.
            [TISSUE SPECIFICITY] Cotyledons and the axis.
            [SIMILARITY] Belongs to the 2S seed storage albumins family.

FEATURES   Location/Qualifiers
            source          1..178
                               /organism="Brassica napus"
                               /db_xref="taxon:3708"
            gene            1..178
                               /gene="NAPB"
            Protein         1..178
                               /gene="NAPB"
                               /product="Napin-B precursor"
            Region          1..21
                               /gene="NAPB"
                               /region_name="Signal"
                               /inference="non-experimental evidence, no additional
                               details recorded"
                               /note="By similarity."
            Region          22..38
                               /gene="NAPB"
                               /region_name="Propeptide"
                               /inference="non-experimental evidence, no additional
                               details recorded"
                               /note="By similarity. /FTId=PRO_0000032123."
            Region          39..74
                               /gene="NAPB"
                               /region_name="Mature chain"
                               /inference="non-experimental evidence, no additional
                               details recorded"
```

	<div><div></div><div>/note="Napin-B small chain (By similarity). /FTId=PRO_0000032124." 47..169 /gene="NAPB" /region_name="AAI_SS" /note="AAI_SS: Alpha-Amylase Inhibitors (AAIs) and Seed Storage (SS) Protein subfamily; composed of cereal-type AAIs and SS proteins. They are mainly present in the seeds of a variety of plants; cd00261" /db_xref="CDD:72936" 75..94 /gene="NAPB" /region_name="Propeptide" /inference="non-experimental evidence, no additional details recorded" /note="By similarity. /FTId=PRO_0000032125." order(86,150,154,160,166) /gene="NAPB" /site_type="other" /note="dimer interface" /db_xref="CDD:72936" 95..178 /gene="NAPB" /region_name="Mature chain" /inference="non-experimental evidence, no additional details recorded" /note="Napin-B large chain (By similarity). /FTId=PRO_0000032126." order(113..115,122,169) /gene="NAPB" /site_type="other" /note="alpha-amylase binding site" /db_xref="CDD:72936"</div></div>
Region	
Region	
Site	
Region	
Site	
ORIGIN	<div>1 manklflvsa tlaffllltn asiyrvtvvef deddatnpag pfripkcrke fqgaqhlkac 61 qqwlhkqamq sgsgpswtld gefdfeddme npggpqqrpp llqqccnelh qeeplvcvpt 121 lkgaskavkq qiqqqqqqqg klqmvsvriyq tathlpkvck ipqvsvcpfq ktmpgpsy //</div>
LOCUS	AAB37416 88 aa linear PLN 03-DEC-1996
DEFINITION	napin large chain L2A=calmodulin antagonist/calcium-dependent protein kinase substrate [Brassica napus=kohlrabi, rapifera, seeds, Peptide, 88 aa].
ACCESSION	AAB37416
VERSION	AAB37416.1 GI:1699238
DBSOURCE	accession AAB37416.1
KEYWORDS	.
SOURCE	Brassica napus (rape)
ORGANISM	Brassica napus Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales; Brassicaceae; Brassica.
REFERENCE	1 (residues 1 to 88)
AUTHORS	Neumann,G.M., Condron,R., Thomas,I. and Polya,G.M.
TITLE	Purification and sequencing of multiple forms of Brassica napus seed napin large chains that are calmodulin antagonists and substrates for plant calcium-dependent protein kinase
JOURNAL	Biochim. Biophys. Acta 1295 (1), 34-43 (1996)
PUBMED	8679671
REMARK	GenBank staff at the National Library of Medicine created this entry [NCBI gibbsq 178581] from the original journal article.
COMMENT	Method: sequenced peptide, ordered by overlap.
FEATURES	Location/Qualifiers source 1..88 /organism="Brassica napus" /db_xref="taxon:3708" Protein 1..88 /product="napin large chain L2A"

/name="calmodulin antagonist/calcium-dependent protein
kinase substrate"

ORIGIN
1 pqspqqrppl lqqccnelhq eepalcvcptl kgaskavkqg vrrqqgggggq qlqqvisriy
61 qtathlpkvc nipqvsvcpf qktmpggs
//

LOCUS P09893 186 aa linear PLN 24-JUL-2007

DEFINITION Napin embryo-specific precursor (1.7S seed storage protein)
{Contains: Napin embryo-specific small chain; Napin embryo-specific large chain}.

ACCESSION P09893

VERSION P09893.1 GI:112747

DBSOURCE swissprot: locus 2SSE_BRANA, accession P09893;
class: standard.
created: Jul 1, 1989.
sequence updated: Jul 1, 1989.
annotation updated: Jul 24, 2007.
xrefs: J02782.1, AAA33007.1, J287506473
xrefs (non-sequence databases): HSSP:P24565, InterPro:IPR003612,
InterPro:IPR000617, InterPro:IPR013771, Gene3D:G3DSA:1.10.120.10,
Pfam:PF00234, PRINTS:PR00496, ProDom:PD002498, SMART:SM00499

KEYWORDS Seed storage protein; Signal; Storage protein.

SOURCE Brassica napus (rape)

ORGANISM Brassica napus
Eukaryota; Viridiplantae; Streptophyta; Embryophyta;Tracheophyta;
Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids
II; Brassicales; Brassicaceae; Brassica.

REFERENCE 1 (residues 1 to 186)

AUTHORS Scofield,S.R. and Crouch,M.L.

TITLE Nucleotide sequence of a member of the napin storage protein family
from Brassica napus

JOURNAL J. Biol. Chem. 262 (25), 12202-12208 (1987)

PUBMED 3040733

REMARK NUCLEOTIDE SEQUENCE [GENOMIC DNA].

COMMENT On Oct 25, 2005 this sequence version replaced gi:81689.
[FUNCTION] The small, basic, water-soluble napins are one of the during its
two major kinds of storage proteins synthesized in the seed
maturation.
[SUBUNIT] The mature protein consists of a small and a large chain
linked by disulfide bonds.
[TISSUE SPECIFICITY] Cotyledons and the axis.
[DEVELOPMENTAL STAGE] Embryo.
[SIMILARITY] Belongs to the 2S seed storage albumins family.

FEATURES Location/Qualifiers

source 1..186
/organism="Brassica napus"
/db_xref="taxon:3708"

Protein 1..186
/product="Napin embryo-specific precursor"

Region 1..21
/region_name="Signal"
/experiment="experimental evidence, no additional details
recorded"

Region 22..38
/region_name="Propeptide"
/experiment="experimental evidence, no additional details
recorded"
/note="/FTId=PRO_0000032127."

Region 39..76
/region_name="Mature chain"
/experiment="experimental evidence, no additional details
recorded"
/note="Napin embryo-specific small chain."
/FTId=PRO_0000032128."

Region 47..176
/region_name="AAI_SS"
/note="AAI_SS: Alpha-Amylase Inhibitors (AAIs) and Seed

Region

Site

Region

Site

Storage (SS) Protein subfamily; composed of cereal-type AAI

s and SS proteins. They are mainly present in the seeds of a variety of plants; cd00261"

/db_xref="CDD:72936"

77..97

/region_name="Propeptide"

/experiment="experimental evidence, no additional details recorded"

/note="/FTId=PRO_0000032129."

order(88,157,161,167,173)

/site_type="other"

/note="dimer interface"

/db_xref="CDD:72936"

98..186

/region_name="Mature chain"

/experiment="experimental evidence, no additional details recorded"

/note="Napin embryo-specific large chain."

/FTId=PRO_0000032130."

order(116..118,125,176)

/site_type="other"

/note="alpha-amylase binding site"

/db_xref="CDD:72936"

ORIGIN

1 manklflvsa tlaiffltn asvxtvvev deddatnpag pfripkcrke fqqaqhlrac

61 qqwlhkqamq pggsgpswt ldgefdfedd venqqqgpqq rppppqqccn elhqeepclv

121 cptlkgaska vrqqvrqqg qmqgqqmqq visrvyqtat hlprvcnirq vsicpfqktm

181 pgpgfy

//

LOCUS

DEFINITION

ACCESSION

VERSION

DBSOURCE

KEYWORDS

SOURCE

ORGANISM

REFERENCE

AUTHORS

TITLE

JOURNAL

PUBMED

REMARK

COMMENT

FEATURES

P01091

133 aa

linear

PLN 24-JUL-2007

Napin-1 precursor (1.7S seed storage protein) [Contains: Napin-1 small chain; Napin-1 large chain].

P01091

P01091.1

GI:112738

swissprot: locus 2SS1_BRANA, accession P01091;

class: standard.

created: Jul 21, 1986.

sequence updated: Jul 21, 1986.

annotation updated: Jul 24, 2007.

xrefs: K01544.1, AAA33005.1, L.1

xrefs (non-sequence databases): HSSP:P24565, InterPro:IPR003612, InterPro:IPR000617, InterPro:IPR013771, Gene3D:G3DSA:1.10.120.10, Pfam:PF00234, PRINTS:PR00496, ProDom:PD002498, SMART:SM00499

Seed storage protein; Storage protein.

Brassica napus (rape)

Brassica napus

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales; Brassicaceae; Brassica.

1 (residues 1 to 133)

Crouch,M.L., Tenbarga,K.M., Simon,A.E. and Ferl,R.

cdna clones for Brassica napus seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide

J. Mol. Appl. Genet. 2 (3), 273-283 (1983)

6689334

NUCLEOTIDE SEQUENCE [MRNA].

STRAIN=cv. Tower

On May 27, 2005 this sequence version replaced gi:68852.

[FUNCTION] The small, basic, water-soluble napins are one of the two major kinds of storage proteins synthesized in the seed during its maturation.

[SUBUNIT] The mature protein consists of a small and a large chain linked by disulfide bonds.

[TISSUE SPECIFICITY] Cotyledons and the axis.

[SIMILARITY] Belongs to the 2S seed storage albumins family.

Location/Qualifiers

source

Protein

Region

Region

Region

Region

Site

Site

Region

1..133
/organism="Brassica napus"
/db_xref="taxon:3708"
<1..133
/product="Napin-1 precursor"
<1..30
/region_name="Mature chain"
/experiment="experimental evidence, no additional details recorded"
/note="Napin-1 small chain. /FTId=PRO_0000032110."
3..124
/region_name="AAI_SS"
/note="AAI_SS: Alpha-Amylase Inhibitors (AAIs) and Seed Storage (SS) Protein subfamily; composed of cereal-type AAIs and SS proteins. They are mainly present in the seeds of a variety of plants; cd00261"
/db_xref="CDD:72936"
31..49
/region_name="Propeptide"
/experiment="experimental evidence, no additional details recorded"
/note="/FTId=PRO_0000032111."
50..130
/region_name="Mature chain"
/experiment="experimental evidence, no additional details recorded"
/note="Napin-1 large chain. /FTId=PRO_0000032112."
order(57,105,109,115,121)
/site_type="other"
/note="dimer interface"
/db_xref="CDD:72936"
order(68..70,77,124)
/site_type="other"
/note="alpha-amylase binding site"
/db_xref="CDD:72936"
131..133
/region_name="Propeptide"
/inference="non-experimental evidence, no additional details recorded"
/note="Potential. /FTId=PRO_0000032113."

ORIGIN

1 pkcrkefqqa qhlkacqqwl hkqamqsggg pswtldgefd feddmekqgp qqrpplhqqy
61 cnelqqeepl cvcptlrgas kavkqqiqqq eqqgqkqgmv nriyqtathl pkvcnipqvs
121 vcpfqktmpg psy
//

Replacement for GI 108935944

LOCUS P80208 125 aa linear PLN 24-JUL-2007

DEFINITION Napin-3 (Napin BnIII) (Napin nIII) (1.7S seed storage protein)
[Contains: Napin-3 small chain; Napin-3 large chain].

ACCESSION P80208

VERSION P80208.1 GI:75107016

DBSOURCE swissprot: locus 2SS3_BRANA, accession P80208;
class: standard.
created: Jun 13, 2006.
sequence updated: Mar 1, 2001.
annotation updated: Jul 24, 2007.
xrefs: [|](#) [M.1](#)
xrefs (non-sequence databases): HSSP:[P24565](#), InterPro:[IPR003612](#),
InterPro:[IPR000617](#), InterPro:[IPR013771](#), Gene3D:[G3DSA:1.10.120.10](#),
Pfam:[PF00234](#), PRINTS:[PR00496](#), ProDom:[PD002498](#), SMART:[SM00499](#)

KEYWORDS Allergen; Direct protein sequencing; Seed storage protein; Storage protein.

SOURCE Brassica napus (rape)

ORGANISM [Brassica napus](#)
[Eukaryota](#); [Viridiplantae](#); [Streptophyta](#); [Embryophyta](#); [Tracheophyta](#);
[Spermatophyta](#); [Magnoliophyta](#); [eudicotyledons](#); [core eudicotyledons](#);
[rosids](#); [eurosids II](#); [Brassicales](#); [Brassicaceae](#); [Brassica](#).

REFERENCE 1 (residues 1 to 125)
AUTHORS Monsalve,R.I., Gonzalez de la Pena,M.A., Lopez-Otin,C., Fiandor,A., Fernandez,C., Villalba,M. and Rodriguez,R.
TITLE Detection, isolation and complete amino acid sequence of an aeroallergenic protein from rapeseed flour
JOURNAL Clin. Exp. Allergy 27 (7), 833-841 (1997)
PUBMED 9249277
REMARK PROTEIN SEQUENCE, SUBUNIT, AND ALLERGENICITY.
TISSUE=Seed

REFERENCE 2 (residues 1 to 125)
AUTHORS Monsalve,R.I., Villalba,M., Lopez-Otin,C. and Rodriguez,R.
TITLE Structural analysis of the small chain of the 2S albumin, napin nIII, from rapeseed. Chemical and spectroscopic evidence of an intramolecular bond formation
JOURNAL Biochim. Biophys. Acta 1078 (2), 265-272 (1991)
PUBMED 2065094
REMARK PROTEIN SEQUENCE OF 1-37, AND SUBUNIT.
TISSUE=Seed

COMMENT On Sep 14, 2005 this sequence version replaced gi:99821.
[FUNCTION] The small, basic, water-soluble napins are one of the two major kinds of storage proteins synthesized in the seed during its maturation.
[SUBUNIT] The mature protein consists of a small and a large chain linked by disulfide bonds.
[ALLERGEN] Causes an allergic reaction in human. Binds to IgE.
[SIMILARITY] Belongs to the 2S seed storage albumins family.

FEATURES Location/Qualifiers
source 1..125
/organism="Brassica napus"
/db_xref="taxon:3708"
Protein 1..125
/product="Napin-3"
Region 1..37
/region_name="Mature chain"
/experiment="experimental evidence, no additional details recorded"
/note="Napin-3 small chain. /FTId=PRO_0000239459."
Bond bond(10,62)
/bond_type="disulfide"
/inference="non-experimental evidence, no additional details recorded"
/note="Interchain (between small and large chains) (By similarity)."
Bond bond(23,51)
/bond_type="disulfide"
/inference="non-experimental evidence, no additional details recorded"
/note="Interchain (between small and large chains) (By similarity)."
Region 38..125
/region_name="Mature chain"
/experiment="experimental evidence, no additional details recorded"
/note="Napin-3 large chain. /FTId=PRO_0000239460."
Bond bond(52,107)
/bond_type="disulfide"
/inference="non-experimental evidence, no additional details recorded"
/note="By similarity."
Bond bond(64,115)
/bond_type="disulfide"
/inference="non-experimental evidence, no additional details recorded"
/note="By similarity."

ORIGIN
1 sagpfripkc rkefqqaqhl racqqwlhkq amqsgsgpgg pqqrppllq ccnelhgeep
61 lcvcptlkga sravkqqvrq qggqggqqlq qvisriyqta thlpkvcnip qvsvcpfqkt
121 mpgps
//

ADM Supertein (napin) bioinformatics with AllergenOnline ver. 8 - Appendix 2

The seven NCBI sequences identified as napins from Supertein bands by CSU were tested using <http://AllergenOnline.com/> Version 8.0, January 2008 release date as described in the methods section.

1. GI: 1699240

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 1699240 Brassica napus [in S6-1 AND P6-6 PQGPQQRPPLLQCCNELHQEEPLCVCPTLKgaskavkqqvrQQGQQGQQGQLQQVIS RIYQTATHLPRVCNIPQVSI CPFQKttpgpy
Length	91
Number of 80 mers	12

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 407610 gb AAB27813.1 Bra j 7E large chain=all [Brassica juncea]	95.00%	12of12	1.1e-18	94.40%	90	gi 407610	GO!
2	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	91.20%	12of12	1.4e-17	90.00%	90	gi 1009438	GO!
3	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	91.20%	12of12	1.2e-17	90.00%	90	gi 1009436	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	91.20%	12of12	1.2e-17	90.00%	90	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	91.20%	12of12	1.7e-17	91.10%	90	gi 1009434	GO!
6	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	90.00%	12of12	1.9e-17	88.90%	90	gi 1009442	GO!
7	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a (Sinapis alba)	90.00%	12of12	4.7e-17	88.90%	90	gi 51338758	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	52.50%	12of12	0.0004	53.10%	81	gi 26985163	GO!
9	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicaum]	41.20%	12of12	0.14	41.00%	83	gi 13183175	GO!

AllergenOnline Database v8.0 (January, 2008)

2. GI: 169694

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 169694 Raphanus sp. [in S6-1 only] siyrtvvevdeddatnpagpfriprrrkefqqaqhlracqqwlhkqarqsgsgpswtldg efdfeddmnpqrpplpqqccnelhqeepalcvcptlkgaskavkqqvrQQGQMQQQMQH VISRIYQTATHLPRVCNIPQVSVCPFQKttpgppy
Length	155
Number of 80 mers	76

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	91.70%	76of76	7e-31	86.80%	144	gi 1009438	GO!
2	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	91.70%	76of76	1.6e-31	86.80%	144	gi 1009436	GO!
3	gi 51338758 sp P15322.2 ALL1 SINAL Allergen Sin a [Sinapis albua]	91.70%	76of76	1e-30	86.10%	144	gi 51338758	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	91.70%	76of76	2.1e-31	86.80%	144	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	91.70%	76of76	7.6e-31	86.80%	144	gi 1009434	GO!
6	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	90.50%	76of76	1e-30	86.10%	144	gi 1009442	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	87.70%	56of76	1.1e-17	87.10%	85	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	51.25%	65of76	1.3e-07	47.20%	125	gi 26985163	GO!
9	gi 407609 gb AAB27812.1 Bra j IE small chain=all [Brassica juncea]	42.50%	24of76	1.4e-06	91.90%	37	gi 407609	GO!
10	gi 31321942 gb AAM54365.1 2S albumin seed storag [Juglans nigra]	35.00%	3of76	0.012	32.00%	150	gi 31321942	GO!

3. GI: 112746

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI:112746 Brassica napus [in S6-1 AND P6-6] manklflvsatlafffltnasiyrtvvefeddatnpagpfripkcrkefqqaqhlkac qgwlhkqamqsgsgpswtldgefdfeddmenpqgpgqrppllqgccnelhqeepalcvcpt lkgaskavkQQIQQQGQQQKlqmvsrIYQTATHLPKvckIPQVSVCPPQKtmpgpsy
Length	178
Number of 80 mers	99

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	97.50%	99of99	2.8e-32	87.60%	145	gi 1009438	GO!
2	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	97.50%	99of99	5.2e-33	89.00%	145	gi 1009436	GO!
3	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a [Sinapis alba]	97.50%	99of99	3.1e-32	88.30%	145	gi 51338758	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	97.50%	99of99	6.7e-33	89.00%	145	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	97.50%	99of99	4.4e-32	87.60%	145	gi 1009434	GO!
6	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	96.30%	99of99	4e-32	86.90%	145	gi 1009442	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	84.70%	59of99	3.7e-18	85.70%	91	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	55.00%	73of99	4.4e-08	50.00%	124	gi 26985163	GO!
9	gi 407609 gb AAB27812.1 Bra j IE small chain=all [Brassica juncea]	42.50%	46of99	4.6e-07	91.90%	37	gi 407609	GO!
10	gi 112762 sp P01089.2 2SS_RICCO 2S albumin precur [Ricinus communis]	37.80%	16of99	0.14	33.30%	123	gi 112762	GO!
11	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicum]	37.50%	8of99	0.00075	32.00%	172	gi 13183175	GO!
12	gi 24473800 gb AAL91665.1 2s albumin [Anacardium [Anacardium occidentale]	35.01%	1of99	0.0027	33.10%	172	gi 24473800	GO!

4. GI: 1699238

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI:1699238 Brassica napus [in S6-1 and P6-6] pqspqqrppllqqccnelhgeepalcvcptlkgaskavkqqvrQQGQQGQQLQQVISRIY QTATHLPKVCNIPQVSVCPFPQKtmgpps
Length	88
Number of 80 mers	9

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	91.60%	9of9	1.2e-18	91.20%	91	gi 1009438	GO!
2	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	91.60%	9of9	1.6e-18	90.10%	91	gi 1009442	GO!
3	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alb]	91.60%	9of9	1.4e-18	91.20%	91	gi 1009436	GO!
4	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a [Sinapis alba]	91.60%	9of9	2.7e-18	91.20%	91	gi 51338758	GO!
5	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	91.60%	9of9	1.4e-18	91.20%	91	gi 1009440	GO!
6	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	90.40%	9of9	3.6e-18	90.10%	91	gi 1009434	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	89.20%	9of9	1.1e-18	89.00%	91	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	52.46%	9of9	2.6e-07	54.50%	77	gi 26985163	GO!
9	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicum]	36.29%	6of9	0.016	37.20%	78	gi 13183175	GO!
10	gi 24473800 gb AAL91665.1 2s albumin [Anacardium [Anacardium occidentale]	35.00%	3of9	0.0047	35.90%	78	gi 24473800	GO!

5. GI: 112747

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 112747 Brassica napus [in S6-1 and P6-7] manklflvsatlalfflltnasvyrtvvevdeddatnpagpfripkcrkefqqaqhlrac qqwlhkqamqpgggsgpswtldgefddfeddenqqggpqqrppppqccnelhqeepfcv cptlkgaskavrqqvrQQGQQMQGQQMQQVISRvyqtathlprvcnirQVSICPFQKtm pgpgfy
Length	186
Number of 80 mers	107

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	88.70 %	107of107	3.4e-29	85.00 %	147	gi 1009438	GO!
2	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	88.70 %	107of107	4e-29	84.40 %	147	gi 1009436	GO!
3	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a	88.70 %	107of107	8.7e-30	85.70 %	147	gi 51338758	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	88.70 %	107of107	5.1e-29	84.40 %	147	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	88.70 %	107of107	2.4e-29	85.70 %	147	gi 1009434	GO!
6	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	87.50 %	107of107	4.7e-29	84.40 %	147	gi 1009442	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	83.74 %	62of107	1.4e-16	83.50 %	91	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	52.50 %	79of107	0.00065	47.30 %	131	gi 26985163	GO!
9	gi 407609 gb AAB27812.1 Bra j IE small chain=all [Brassica juncea]	42.51 %	45of107	5.4e-06	87.20 %	39	gi 407609	GO!
10	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicum]	38.80 %	22of107	0.027	33.70 %	178	gi 13183175	GO!
11	gi 24473800 gb AAL91665.1 2s albumin [Anacardium [Anacardium occidentale]	37.50 %	2of107	0.0078	32.20 %	180	gi 24473800	GO!

6. GI: 112738

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 112738 Brassica napus [in S6-2] pkcrkefqqaqhlkacqqlhkqamqsgggpswtldgefddmekqgppqrrpplhqgy cnelqgeeplcvcptlrgaskavkqgiqqeqqgkqgmvnriYQTATHLPKVCNIPQVS VCPFQKtmgpsy
Length	133
Number of 80 mers	54

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	88.90%	54of54	7.2e-32	82.60%	138	gi 1009438	GO!
2	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	88.90%	54of54	3.6e-32	82.60%	138	gi 1009436	GO!
3	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a [Sinapis alba]	88.90%	54of54	2.6e-31	81.90%	138	gi 51338758	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	88.90%	54of54	4.8e-32	82.60%	138	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	88.90%	54of54	1.4e-31	81.90%	138	gi 1009434	GO!
6	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	87.70%	54of54	1.1e-31	81.90%	138	gi 1009442	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	78.80%	54of54	1.6e-18	78.30%	92	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	49.97%	47of54	3.9e-08	47.20%	123	gi 26985163	GO!
9	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicum]	35.03%	5of54	0.0021	32.00%	125	gi 13183175	GO!

AllergenOnline Database v8.0 (January, 2008)

7. GI: 75107016

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI:75107016 Brassica napus [in S6-2] SAGPFRIPKcrKEFQQAQHLRacqqwlhkqamqsgsg
Length	37
Number of 80 mers	1

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a [Sinapis alba]	45.00%	1of1	3e-18	100.00%	36	gi 51338758	GO!
2	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	45.00%	1of1	3e-18	100.00%	36	gi 1009440	GO!
3	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	45.00%	1of1	3e-18	100.00%	36	gi 1009436	GO!
4	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	43.74%	1of1	2.2e-17	97.20%	36	gi 1009438	GO!
5	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	43.74%	1of1	2.2e-17	97.20%	36	gi 1009442	GO!
6	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	43.74%	1of1	2.2e-17	97.20%	36	gi 1009434	GO!
7	gi 407609 gb AAB27812.1 Bra j IE small chain=all [Brassica juncea]	42.48%	1of1	3.8e-18	94.40%	36	gi 407609	GO!

AllergenOnline Database v8.0 (January, 2008)

SUMMARY. The bioinformatics results demonstrate clearly that there are allergenic proteins from *Sinapis alba*, *Brassica juncea* and *Brassica napus* that match one or more of the seven sequences of *Brassica napus* identified from the LC-MS/MS that are highly likely to act as cross-reactive allergens based on empirical evidence of Aalberse (2000) and others. There are also some other allergenic sequences from *Sesamum indicum* and *Anacardium occidentale* that are between 35% and 50% identical over some of the length of the protein. There is some possibility of cross-reactivity with these matched proteins; however, the primary focus would be those of much higher identity matches. All of those are in the mustard group of 2S albumins.

Sequence 3) GI:12751302 *Brassica napus* [in P6-1, P6-2, P6-3, P6-5 and P6-6]

nivrvgpfpqvrpplrqsyesekwthprgppqspqdnleeticssmrtheniddparadvkpnlgvtsvnsltlpilqyvrlsatrg
iiqgnsmlvpkYNMNANEILYCTRggarIQVVNDNGQNVLDQQVQKgglvvipqgfyvvqsqnnfewis
fkTNANAMISTLAGRtsalralplevltnayrvsle (length 196 aa).

CLUSTAL W alignment of the 8 identified cruciferin sequence matches.
Pairwise percent identity matches of full-length NCBI entries for the eight. Note that the top identity match for sliding window of 80 amino acid segments are much higher for most matches, as demonstrated by Appendix 4.

GI numbers	33284990	461840	12751302	461841	1345840	17805	86611322	21108
33284990	100	60	58	70	94	73	91	58
461840	60	100	98	53	58	56	58	93
12751302	58	98	100	52	57	56	58	94
461841	70	53	52	100	71	99	58	94
1345840	94	58	57	71	100	72	98	58
17805	73	56	56	99	72	100	72	56
86611322	91	58	58	71	98	72	100	57
21108	58	93	94	94	58	56	57	100

CLUSTAL W (1.83) multiple sequence alignment (yellow highlight indicates peptide matches that were identified by LC-MS/MS and in the Scaffold report from CSU.

gi 1345840 sp P33523.2 CRU1_BR	MARLSSLSFSLALLIFLHGSTAQQFP-----NECQLDQLNALEPSHV
gi 86611322 gb ABD14346.1	--RLSSLSFSLALLIFLHGSTAQQFP-----NECQLDQLNALEPSHV
gi 33284990 dbj BAC80213.1	-----QQFP-----NECQLDQLNALEPSHV
gi 461841 sp P33522.1 CRU4_BRA	-MGPTSLLSFFFTFLTLFHGFTAQQWP-----NECQLDQLNALEPSQI
gi 17805 emb CAA40980.1	-----
gi 461840 sp P33525.1 CRU3_BRA	MVKVPHLLVATFGVLLVLNGCLARQSLGVPPQLGNACNLNDLVDLQPTET
gi 12751302 gb AAK07609.1 AF31	MVKLAHLVATFGALLVLNGCLARQSLGVPPQIGNACNLNDLVDLQPTET
gi 21108 emb CAA42473.1	-----
gi 1345840 sp P33523.2 CRU1_BR	LKAEAGRIEVWDHHPQLRCSGVSFVRYIIESKGLYLPSPFFSTAKLSFVA
gi 86611322 gb ABD14346.1	LKAEAGRIEVWDHHPQLHCSGVSFVRYIIESKGLYLPSPFFSTAKLSFVA
gi 33284990 dbj BAC80213.1	LKAEAGRIEVWDHHPQLRCSGVSFVRYIIESKGLYLPSPFFSTAKLSFVA
gi 461841 sp P33522.1 CRU4_BRA	IKSEGGRIEVWDHHPQLRCSGFAFERFVIEPQGLYLPTFLNAGKLTFFVV
gi 17805 emb CAA40980.1	-----WDHHPQLRCSGFAFERFVIEPQGLYLPTFLNAGKLTFFVV
gi 461840 sp P33525.1 CRU3_BRA	IKSEAGRVEYWDHNNPQIRCAGVSVSRLIEQGGLYLPTFFSSPKISYVV
gi 12751302 gb AAK07609.1 AF31	IKSEAGRVEYWDHNNPQIRCAGVSVSRLIEQGGLYLPTFFSSPKISYVV
gi 21108 emb CAA42473.1	-----
gi 1345840 sp P33523.2 CRU1_BR	KGEGLMGRVVPGCAETFQDSSVFQPSGGSPSGEGQGQGQGGQGGQ-----
gi 86611322 gb ABD14346.1	KGQGLMGRVVPGCAETFQDSSVFQPGGGSPFGEQGQGQGGQGGQGGQ-----
gi 33284990 dbj BAC80213.1	KGQGLMGRVVPGCAETFQDSSVFQPGGGSPFGEQGQGQGGQGGQGGQ-----
gi 461841 sp P33522.1 CRU4_BRA	HGHALMGKVTTPGCAETFNDSPVF-----GQGQGQ-----
gi 17805 emb CAA40980.1	HGHALMGKVTTPGCAETFMDSPVF-----GQGQGQ-----
gi 461840 sp P33525.1 CRU3_BRA	QGMGISGRVVPGCAETFMDSQPMQ-----GQGQGQPWQGQGQGGQGGQ
gi 12751302 gb AAK07609.1 AF31	QGMGISGRVVPGCAETFMDSQPMQ-----GQGQGQ-----
gi 21108 emb CAA42473.1	-----
gi 1345840 sp P33523.2 CRU1_BR	-----GHQGQGQGQGQGQGQGQGSQGQGFQDMHQKVEHIRTGDTIAT
gi 86611322 gb ABD14346.1	-----GQGQGQGQGQGQ-----GQGQGQGQGFQDMHQKVEHIRTGDTIAT
gi 33284990 dbj BAC80213.1	-----GHQGQGQGQGQGQGQGQGSQGQGFQDMHQKVEHIRTGDTIAT
gi 461841 sp P33522.1 CRU4_BRA	-----QGQGQ-----QGQGQGFQDMHQKVEHLRSGDTIAT
gi 17805 emb CAA40980.1	-----QGQGQ-----QGQGQGFQDMHQKVEHLRSGDTIAT
gi 461840 sp P33525.1 CRU3_BRA	QGQGQGQGQGQGQGQGQGQGQGQGQGQGFQDMHQKVEHVRHGDIIAI
gi 12751302 gb AAK07609.1 AF31	-----QGQGQGQGQGQGQGQGLQGQGFQDMHQKVEHVRHGDVIAI
gi 21108 emb CAA42473.1	-----

gi 1345840 sp P33523.2 CRU1_BR	HPGVAQWFYNDGNQPLVIVSVLDLASHQNQLDRNPRPFYLAGNNPQGQVW
gi 86611322 gb ABD14346.1	HPGVAQWFYNDGNQPLVIVSVLDLASHQNQLDRNPRPFYLAGNNPQGQVW
gi 33284990 dbj BAC80213.1	HPGVAQWFYNNGNQPLVIVAVMDLASHQNQLDRNPRPFYLAGKNPQGQSW
gi 461841 sp P33522.1 CRU4_BRA	PPGVAQWFYNNGNEPLILVAAADIANNLNQLDRNLRFLLAGNNPQGQQW
gi 17805 emb CAA40980.1	PPGVAQWFYNNGNEPLILVAAADIANNLNQLDRNLRFLLAGNNPQGQQW
gi 461840 sp P33525.1 CRU3_BRA	TAGSSHWIYNTGDQPLVICTLLDIANYQNQLDRNPRTFRLAGNNPQGG--
gi 12751302 gb AAK07609.1 AF31	TAGSSHWIYNTGDQPLVICTLLDIANYQNQLDRNPRTFRLAGNNPQGG--
gi 21108 emb CAA42473.1	-----
gi 1345840 sp P33523.2 CRU1_BR	IEGREQQPQKNILNGFTPEVLAKAFKIDVRTAQQLQNQQDNRGNIIRVQG
gi 86611322 gb ABD14346.1	IEGREQQPQKNILNGFTPEVLAKAFKIDVRTAQQLQNQQDNRGNIIRVQG
gi 33284990 dbj BAC80213.1	LHGRGQQPQNNILNGFSPEVLAQAFKIDVRTAQQLQNQQDNRGNIIRVQG
gi 461841 sp P33522.1 CRU4_BRA	LQGRQQQKQNNIFNGFAPQILAQAFKISVETAQKQLQNQQVNRGNIIVKVQG
gi 17805 emb CAA40980.1	LQGRQQQKQNNIFNGFAPQILAQAFKISVETAQKQLQNQQVNRGNIIVKVQG
gi 461840 sp P33525.1 CRU3_BRA	-SQQQQQQQNNMLSGFDPQVLAQALKIDVRLAQELQNQQDSRGNIIVRVKG
gi 12751302 gb AAK07609.1 AF31	-SQQQQQQQNNMLSGFDPQVLAQALKIDVRLAQELQNQQDSRGNIIVRVKG
gi 21108 emb CAA42473.1	-----NIVRVKG **::**
gi 1345840 sp P33523.2 CRU1_BR	PFSVIRPPLRS-----QRPQETEVNGLEETIC SARCTDNLDDPS
gi 86611322 gb ABD14346.1	PFSVIRPPLRS-----QRPQE-EVNGLEETIC SARCTDNLDDPS
gi 33284990 dbj BAC80213.1	PFQVIRPPLKS-----QRPQETEANGLEETIC SARCTDNLDDPS
gi 461841 sp P33522.1 CRU4_BRA	QFGVIRPPLRQGGG-----QRPQE-EGNGLEETLCTMRCTENLDDPS
gi 17805 emb CAA40980.1	QFGVIRPPLRQGGG-----QRPQE-EGNGLEETLCTMRCTENLDDPS
gi 461840 sp P33525.1 CRU3_BRA	PFQVVRPPLRQPYESEQWRHPRGPPQSPQDNGLEETICSMRTHENIDDDPA
gi 12751302 gb AAK07609.1 AF31	PFQVVRPPLRQPYESEQWRHPRGPPQSPQDNGLEETICSMRTHENIDDDPA
gi 21108 emb CAA42473.1	PFQVVRPPLRQPYESEKWRHPRGPPQSPQDNGLEETICSMRTHENIDDDPA * *:*****. ** : *****: * : * :*****:
gi 1345840 sp P33523.2 CRU1_BR	NADVYKPLQGYISTLNSYDLPILRFLRLSALRGSIRQNAMVLPQWNNAN
gi 86611322 gb ABD14346.1	NADVYKPLQGYISTLNSYDLPILRFLRLSALRGSIRQNAMVLPQWNNAN
gi 33284990 dbj BAC80213.1	NADVYKPLQGYISTLNSYDLPILRVLRSLALRGSIRQNAMVLPQWNNAN
gi 461841 sp P33522.1 CRU4_BRA	SADVYKPSLGYISTLNSYNLPILRFLRLSALRGSIHNNAMVLPQWNVNAN
gi 17805 emb CAA40980.1	SADVYKPSLGYISTLNSYNLPILRFLRLSALRGSIHNNAMVLPQWNVNAN
gi 461840 sp P33525.1 CRU3_BRA	RADVYKPNLGRVTSVNSYTLPIQYIRLSATRGILOQGNAMVLPKYNMNNAN
gi 12751302 gb AAK07609.1 AF31	RADVYKPNLGRVTSVNSYTLPIQYIRLSATRGILOQGNAMVLPKYNMNNAN
gi 21108 emb CAA42473.1	RADVYKPNLGRVTSVNSLTLPIQYVRLSATRGI IQGNSMVLPKYNMNNAN *****. ** : : ** * : * * : * : *****: * * *
gi 1345840 sp P33523.2 CRU1_BR	AVLYVTDGEAHVQVVDNNGDRVFDGQVSQGGQLLSIPQGFVSVVKRATSEQF
gi 86611322 gb ABD14346.1	AVLYVTDGEAHVQVVDNNGDRVFDGQVSQGGQLLSIPQGFVSVVKRATSEQF
gi 33284990 dbj BAC80213.1	AVLYVTDGEAQIQVVDNNGDRVFDGQVSQGGQLLSIPQGFVSVVKRATSDQF
gi 461841 sp P33522.1 CRU4_BRA	AALYVTKGKAHIQNVNDNGQRVFDQEISKGQLLVVPQGFAYVVKRATSQQF
gi 17805 emb CAA40980.1	AALYVTKGKAHIQNVNDNGQRVFDQEISKGQLLVVPQGFAYVVKRATSQQF
gi 461840 sp P33525.1 CRU3_BRA	EILYCTQGGARIQVVDNNGQNVLDQQVQKGQLVVI PQGFAYVVQSHQNNF
gi 12751302 gb AAK07609.1 AF31	EILYCTQGGARIQVVDNNGQNVLDQQVQKGQLVVI PQGFAYVVQSHQNNF
gi 21108 emb CAA42473.1	EILYCTRQGARIQVVDNNGQNVLDQQVQKGQLVVI PQGFAYVVQS-QNNF * * * *:***: * *****:.* * :.:*****: :*****: * : : :.*
gi 1345840 sp P33523.2 CRU1_BR	RWIEFKTNANAQINTLAGRTSVLRGLPLEVISNGYQISLEEARRVKFNTI
gi 86611322 gb ABD14346.1	RWIEFKTNANAQINTLAGRTSVLRGLPLEVISNGYQISLEEARRVKFNTI
gi 33284990 dbj BAC80213.1	RWIEFKTNANAQINTLAGRTSVVRGLPLEVIANGYQISLEEARRVKFNTI
gi 461841 sp P33522.1 CRU4_BRA	QWIEFKSNDNAQINTLAGRTSVMRGLPLEVISNGYQISPOEARSVKFSTL
gi 17805 emb CAA40980.1	QWIEFKSNDNAQINTLAGRTSVMRGLPLEVISNGYQISPOEARSVKFSTL
gi 461840 sp P33525.1 CRU3_BRA	EWISFKTNANAMVSTLAGRTSALRALPLEVITNAFQISLEEARRIKFNTL
gi 12751302 gb AAK07609.1 AF31	EWISFKTNANAMVSTLAGRTSALRALPLEVITNAFQISLEEARRIKFNTL
gi 21108 emb CAA42473.1	EWISFKTNANAMISTLAGRTSALRALPLEVITNAYRVLSLE----- .**.***: * * :.*****.*.*****:.*.:.* :
gi 1345840 sp P33523.2 CRU1_BR	ETTLTHSSGPASYGGPRKADA
gi 86611322 gb ABD14346.1	ETTLTHSSGPASYG-----
gi 33284990 dbj BAC80213.1	ETTLTHSSGPASYGGPRKADA
gi 461841 sp P33522.1 CRU4_BRA	ETTLTQSSGPMGYGMPRVEA-
gi 17805 emb CAA40980.1	ETTLTQSSGPMGYGMPRVEA-
gi 461840 sp P33525.1 CRU3_BRA	ETTLTRARGQPQLIEEIVEA
gi 12751302 gb AAK07609.1 AF31	ETTLTRARRGQPQLIEEIVEA
gi 21108 emb CAA42473.1	-----

NCBI entries for the eight matched cruciferin sequences.

LOCUS BAC80213 467 aa linear PLN 26-JUL-2003
DEFINITION cruciferin [Brassica napus].
ACCESSION BAC80213
VERSION BAC80213.1 GI:33284990
DBSOURCE accession [AB115553.1](#)
KEYWORDS .
SOURCE Brassica napus (rape)
ORGANISM Brassica napus
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
Brassicaceae; Brassica.
REFERENCE 1
AUTHORS Tandang,M., Adachi,M. and Utsumi,S.
TITLE The effects of protein engineering of rapeseed procruciferin on
Its physicochemical and functional properties
JOURNAL Unpublished
REFERENCE 2 (residues 1 to 467)
AUTHORS Adachi,M., Tandang,M. and Utsumi,S.
TITLE Direct Submission
JOURNAL Submitted (24-JUL-2003) Mary Rose Tandang, Kyoto University,
Graduate School of Agriculture; Gokasyo, Uji, Kyoto 611-0011,
Japan (E-mail:mary@kais.kyoto-u.ac.jp, Tel:81-774-38-3762,
Fax:81-774-38-3761)
FEATURES Location/Qualifiers
source 1..467
/organism="Brassica napus"
/db_xref="taxon:3708"
/clone="cru2/3b"
/tissue_type="seed"
Protein 1..467
/product="cruciferin"
CDS 1..467
/coded_by="AB115553.1:<1..1404"
ORIGIN
1 qqfpnecql d qlnalepshv lkaeagriev wdhhapqlrc sgvsfvryii eskglylpsf
61 fstaklsfva kggqlmgrvv pgcaetfqds svfqpgggsp fgegqggqq gggqghqggg
121 gggqggqqgg gqgsqggqfr dmhgkvehir sgdtiathpg vaqwfynngn qplvivavmd
181 lashqngldr nprpfylagk npqggswlhg rgqqpqnnil ngfspevlaq afkidvrtaq
241 qlqnqqdnrg nivrvgppfg virpplksqr pqeteangle eticsarctd nlddpsnadv
301 ykpqlgyisi lnsydlpilr vlrlsalrgs irqnamvlpq wnananavly vtdgeaqlqv
361 vndngdrvfd gqvsqgqls ipqgfsvvkr atsdqfrwie fktnanaqin tlagrtsvvr
421 gipleviang yqisleearr vkfntiettl thssgpasyg gprkada
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LOCUS P33525 509 aa linear PLN 02-OCT-2007
DEFINITION Cruciferin CRU1 precursor (11S globulin) (12S storage protein)
[Contains: Cruciferin CRU1 alpha chain; Cruciferin CRU1 beta
chain].
ACCESSION P33525
VERSION P33525.1 GI:461840
DBSOURCE swissprot: locus CRU3_BRANA, accession [P33525](#);
class: standard.
created: Feb 1, 1994.
sequence updated: Feb 1, 1994.
annotation updated: Oct 2, 2007.
xrefs: [X62120.1](#), [CAA44042.1](#)
xrefs (non-sequence databases): HSSP:[P04776](#), InterPro:[IPR006045](#),
InterPro:[IPR014710](#), InterPro:[IPR006044](#), Gene3D:G3DSA:2.60.120.10,
Pfam:[PF00190](#), PRINTS:PR00439, PROSITE:PS00305
KEYWORDS Seed storage protein; Signal; Storage protein.
SOURCE Brassica napus (rape)
ORGANISM Brassica napus

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales; Brassicaceae; Brassica.

REFERENCE 1 (residues 1 to 509)
AUTHORS Rodin,J., Sjodahl,S., Josefsson,L.G. and Rask,L.
TITLE Characterization of a Brassica napus gene encoding a cruciferin subunit: estimation of sizes of cruciferin gene families
JOURNAL Plant Mol. Biol. 20 (3), 559-563 (1992)
PUBMED 1421158
REMARK NUCLEOTIDE SEQUENCE [GENOMIC DNA].
COMMENT [FUNCTION] This is a seed storage protein.
[SUBUNIT] Hexamer; each subunit is composed of an acidic and a basic chain derived from a single precursor and linked by a disulfide bond.
[SIMILARITY] Belongs to the 11S seed storage protein (globulins) family.

FEATURES Location/Qualifiers
source 1..509
/organism="Brassica napus"
/db_xref="taxon:3708"
gene 1..509
/gene="CRU1"
Protein 1..509
/gene="CRU1"
/product="Cruciferin CRU1 precursor"
Region 1..23
/gene="CRU1"
/region_name="Signal"
/inference="non-experimental evidence, no additional details recorded"
/note="By similarity."
Region 24..319
/gene="CRU1"
/region_name="Mature chain"
/experiment="experimental evidence, no additional Details recorded"
/note="Cruciferin CRU1 alpha chain. /FTId=PRO_0000032036."
Region 42..>119
/gene="CRU1"
/region_name="Cupin_1"
/note="Cupin. This family represents the conserved Barrel domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S And 7S plant seed storage proteins, and germins; Pfam00190/db_xref="CDD:84597"
Bond bond(113,326)
/gene="CRU1"
/bond_type="disulfide"
/inference="non-experimental evidence, no additional details recorded"
/note="Interchain (between alpha and beta chains) (Potential)."
Region 121..171
/gene="CRU1"
/region_name="Compositionally biased region"
/experiment="experimental evidence, no additional Details recorded"
/note="Gln/Gly-rich."
Region <173..237
/gene="CRU1"
/region_name="Cupin_1"
/note="Cupin. This family represents the conserved Barrel domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S And 7S plant seed storage proteins, and germins; pfam00190" /db_xref="CDD:84597"
Region 241..248
/gene="CRU1"
/region_name="Compositionally biased region"

Region

/experiment="experimental evidence, no additional
Details recorded"
/note="Poly-Gln."
320..509
/gene="CRU1"
/region_name="Mature chain"
/experiment="experimental evidence, no additional
Details recorded"
/note="Cruciferin CRU1 beta chain. /FTId=PRO_0000032037."
332..481
/gene="CRU1"
/region_name="Cupin_1"
/note="Cupin. This family represents the conserved
Barrel domain of the cupin superfamily (cupa is the Latin Term for a small
barrel). This family
contains 11S and 7S plant
seed storage proteins, and germins; pfam00190"
/db_xref="CDD:84597"

Region

332..481
/gene="CRU1"
/region_name="Cupin_1"
/note="Cupin. This family represents the conserved
Barrel domain of the cupin superfamily (cupa is the Latin Term for a small
barrel). This family
contains 11S and 7S plant
seed storage proteins, and germins; pfam00190"
/db_xref="CDD:84597"

ORIGIN

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1 mvkvphllva tfgvllvng clarqslgvp pqlgnacnld nldvlqptet ikseagrvey
61 wdhnnpqirc agvsrvrvi eagglylptf fsspkisyvv qgmgisgrvv pgcaetfmds
121 qpmqggqqgg pwqggqqggg qggqggqqgg qggqggqqgg qggqggqqgg qgfrdmhgkv
181 ehvrhgdiia itagssshwi ntgdqplvii clldianyqn qldrnprtfr lagnnpqggg
241 qggqggqqgm lsgfdpqvla qalkidvrla qelqngqdsr gnivrvkgpf qvvrpplrqp
301 yeseqwrhpr gppqspqng leeticsmrt heniddpara dvykpnlgvr tsvnsytlpi
361 lqyirlsatr gilggnamvl pkynmneai lyctqggari qvvdngqnv ldqqvqkgql
421 vvipqgfayv vqshqnnfaw isfktanam vstlagrtsa lralplevit nafqisleea
481 rrikfntlet tltrarggqp qlieeivea
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//

LOCUS

P33522

465 aa

linear

PLN 15-JAN-2008

DEFINITION

Cruciferin CRU4 precursor (11S globulin) (12S storage protein)
[Contains: Cruciferin CRU4 alpha chain; Cruciferin CRU4 beta
chain].

ACCESSION

P33522

VERSION

P33522.1 GI:461841

DBSOURCE

swissprot: locus CRU4_BRANA, accession [P33522](#);
class: standard.
created: Feb 1, 1994.
sequence updated: Feb 1, 1994.
annotation updated: Jan 15, 2008.
xrefs: [X57848.1](#), [CAA40978.1](#), [X57850.1](#), [CAA40980.1](#), [X57851.1](#),
[CAA40981.1](#), [S14762](#)
xrefs (non-sequence databases): HSSP:[P04776](#), GO:[0005791](#),
InterPro:[IPR006045](#), InterPro:[IPR014710](#), InterPro:[IPR006044](#),
Gene3D:[G3DSA:2.60.120.10](#), Pfam:[PF00190](#), PRINTS:[PR00439](#),
PROSITE:[PS00305](#)

KEYWORDS

Direct protein sequencing; Endoplasmic reticulum; Polymorphism;
Seed storage protein; Signal; Storage protein.

SOURCE

Brassica napus (rape)

ORGANISM

[Brassica napus](#)
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
Brassicaceae; Brassica.

REFERENCE

1 (residues 1 to 465)

AUTHORS

Sjodahl,S., Rodin,J. and Rask,L.

TITLE

Characterization of the 12S globulin complex of Brassica napus.
Evolutionary relationship to other 11-12S storage globulins

JOURNAL

Eur. J. Biochem. 196 (3), 617-621 (1991)

PUBMED

[2013284](#)

REMARK

NUCLEOTIDE SEQUENCE [MRNA].
STRAIN=cv. Svalofs Karat 20516-K; TISSUE=Seed

REFERENCE

2 (residues 1 to 465)

AUTHORS

Rodin,J., Ericson,M.L., Josefsson,L.G. and Rask,L.

TITLE

Characterization of a cDNA clone encoding a Brassica napus 12 S
protein (cruciferin) subunit. Relationship between precursors and
mature chains

JOURNAL

J. Biol. Chem. 265 (5), 2720-2723 (1990)

PUBMED

[2303422](#)

REMARK

PROTEIN SEQUENCE OF 108-119; 139-153; 277-308; 336-360 AND 372-395.

COMMENT	On Jul 26, 2005 this sequence version replaced gi:1070654. [FUNCTION] This is a seed storage protein. [SUBUNIT] Heterohexamer; each subunit is composed of an acidic And a basic chain derived from a single precursor and linked by a disulfide bond. [SUBCELLULAR LOCATION] Rough endoplasmic reticulum. [SIMILARITY] Belongs to the 11S seed storage protein (globulins) family.
FEATURES	Location/Qualifiers
source	1..465 /organism="Brassica napus" /db_xref="taxon:3708"
gene	1..465 /gene="CRU4"
Protein	1..465 /gene="CRU4" /product="Cruciferin CRU4 precursor"
Region	1..22 /gene="CRU4" /region_name="Signal" /inference="non-experimental evidence, no additional details recorded" /note="By similarity."
Region	23..276 /gene="CRU4" /region_name="Mature chain" /experiment="experimental evidence, no additional Details recorded" /note="Cruciferin CRU4 alpha chain. /FTId=PRO_0000032038."
Region	34..196 /gene="CRU4" /region_name="Cupin_1" /note="Cupin. This family represents the conserved domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S and 7S plant barrel seed storage proteins, and germins; pfam00190/db_xref="CDD:84597"
Bond	bond(105,283) /gene="CRU4" /bond_type="disulfide" /inference="non-experimental evidence, no additional details recorded" /note="Interchain (between alpha and beta chains) (Potential)."
Region	110 /gene="CRU4" /region_name="Variant" /experiment="experimental evidence, no additional details recorded" /note="N -> M."
Region	116..134 /gene="CRU4" /region_name="Compositionally biased region" /experiment="experimental evidence, no additional recorded" /note="Gln/Gly-rich."
Region	147 /gene="CRU4" details /region_name="Variant" /experiment="experimental evidence, no additional details recorded" /note="S -> H."
Region	277..465 /gene="CRU4" /region_name="Mature chain" /experiment="experimental evidence, no additional details recorded" /note="Cruciferin CRU4 beta chain. /FTId=PRO_0000032039."
Region	289..438

Region

359

Region

374

1 mgptsllsff

61 rcsqfaferf

121 qeqggqggqg

181 lngldrnrlp

241 qqvnrngnivk

301 kpslgyistl

361 ndngqrvfdq

421 lplevisngy

ftfltlfhgf

tflnagkltf

qggqfrdmhq

fillagnpqg

vqqqfgvirp

nsynlpilrf

eiskgqlllv

qispgqarsv

taqqwpnecq

kvehlrsgdt

qgw1qgrqqq

plrqggggqg

hnnamvlpqw

tsqqfqwief

qssgpmgygm

ldqlnaleps

iatppgvaqw

kqnnifngfa

pqilaqafki

tlctmrcten

ksndnaqint

prvea

qiiakseggri

fynngnepli

svetaqklqn

lddpssadvy

tkgkahiqrn

lagrtsvmrg

evwdhhapql

dspvfqggqg

lvaaadiann

tdpssadvy

tkgkahiqrn

lagrtsvmrg

prvea

tdpssadvy

tkgkahiqrn

lagrtsvmrg

LOCUS

DEFINITION

ACCESSION

VERSION

DBSOURCE

KEYWORDS

SOURCE

ORGANISM

REFERENCE

AUTHORS

TITLE

JOURNAL

PUBMED

REMARK

COMMENT

FEATURES

P33523

Cruciferin BnC1 precursor (11S globulin) (12S storage protein)

P33523

P33523.2

swissprot: locus CRU1_BRANA, accession P33523;

Seed storage protein; Signal; Storage protein.

Brassica napus (rape)

Brassica napus

1 (residues 1 to 490)

Breen, J.P. and Crouch, M.L.

Molecular analysis of a cruciferin storage protein gene family of Brassica napus

Plant Mol. Biol. 19 (6), 1049-1055 (1992)

1511129

NUCLEOTIDE SEQUENCE [GENOMIC DNA].

On Jun 1, 1996 this sequence version replaced gi:461837.

Location/Qualifiers

490 aa

(12S storage protein)

GI:1345840

class: standard.

created: Feb 1, 1994.

sequence updated: Feb 1, 1996.

annotation updated: Jul 24, 2007.

xrefs: X59294.1, CAA41984.1

xrefs (non-sequence databases): HSSP:P04776, InterPro:IPR006045, InterPro:IPR014710, InterPro:IPR006044, Gene3D:G3DSA:2.60.120.10, Pfam:PF00190, PRINTS:PR00439, PROSITE:PS00305

Strain=cv. Tower

[FUNCTION] This is a seed storage protein.

[SUBUNIT] Hexamer; each subunit is composed of an acidic and a basic chain derived from a single precursor and linked by a disulfide bond.

[SIMILARITY] Belongs to the 11S seed storage protein (globulins) family.

source 1..490

	<div><div>/organism="Brassica napus"</div><div>/db_xref="taxon:3708"</div></div>
gene	<div><div>1..490</div><div>/gene="BnC1"</div></div>
Protein	<div><div>1..490</div><div>/gene="BnC1"</div><div>/product="Cruciferin BnC1 precursor"</div></div>
Region	<div><div>1..23</div><div>/gene="BnC1"</div><div>/region_name="Signal"</div><div>/inference="non-experimental evidence, no additional details recorded"</div><div>/note="By similarity."</div></div>
Region	<div><div>24..300</div><div>/gene="BnC1"</div><div>/region_name="Mature chain"</div><div>/experiment="experimental evidence, no additional details recorded"</div><div>/note="Cruciferin BnC1 subunit alpha."</div><div>/FTId=PRO_0000032032."</div></div>
Region	<div><div>35..>115</div><div>/gene="BnC1"</div><div>/region_name="Cupin_1"</div><div>/note="Cupin. This family represents the conserved domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S and 7S plant barrel seed storage proteins, and germins; pfam00190"</div><div>/db_xref="CDD:84597"</div></div>
Bond	<div><div>bond(106,307)</div><div>/gene="BnC1"</div><div>/bond_type="disulfide"</div><div>/inference="non-experimental evidence, no additional details recorded"</div><div>/note="Interchain (between alpha and beta chains) (Potential)."</div></div>
Region	<div><div>120..161</div><div>/gene="BnC1"</div><div>/region_name="Compositionally biased region"</div><div>/experiment="experimental evidence, no additional details recorded"</div><div>/note="Gln/Gly-rich."</div></div>
Region	<div><div><162..226</div><div>/gene="BnC1"</div><div>/region_name="Cupin_1"</div><div>/note="Cupin. This family represents the conserved barrel domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S and 7S plant seed storage proteins, and germins; pfam00190"</div><div>/db_xref="CDD:84597"</div></div>
Region	<div><div>301..490</div><div>/gene="BnC1"</div><div>/region_name="Mature chain"</div><div>/experiment="experimental evidence, no additional details recorded"</div><div>/note="Cruciferin BnC1 subunit beta."</div><div>/FTId=PRO_0000032033."</div></div>
Region	<div><div>314..462</div><div>/gene="BnC1"</div><div>/region_name="Cupin_1"</div><div>/note="Cupin. This family represents the conserved domain of the cupin superfamily (cupa is the Latin term for a small barrel). This family contains 11S and 7S plant barrel seed storage proteins, and germins; pfam00190"</div><div>/db_xref="CDD:84597"</div></div>
ORIGIN	<div><div>1 marlssllsf slalliflhg staqqfpnec qldqlnalep shvlkaeagr ievwdhhapq</div><div>61 lrcsgvsfvr yiieskglyl psffstakls fvakgeglmg rvvpngaetf qdssvfqpsg</div><div>121 gspsggggqg gggggggghq gggggggggq gggggggggq gfrdmhqkve hirtgdtiat</div><div>181 hpgvaqwfyf dgnqplvivs vldlashqng ldrnprpfyl agnnpqggvw iegregqpqk</div></div>


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241 nilngftpev lakafkidvr taqqlnqqd nrgniirvqg pfsvirpplr sqrpqetevn
301 gleeticsar ctdnlddpsn advykpqlgy istlnsydlp ilrflrlsal rgsirqnamv
361 lpqwnanana vlyvtdgeah vqvvdngdr vfdgqvsqgq llsipqgfsv vkratseqfr
421 wiefktnana qintlagrts vlrglplevi sngyqislee arrvkfntie ttlthssgpa
481 syggprkada

//

LOCUS      CAA40980                      413 aa          linear    PLN 18-APR-2005
DEFINITION cruciferin cru4 subunit [Brassica napus].
ACCESSION  CAA40980
VERSION    CAA40980.1  GI:17805
DBSOURCE   embl accession X57850.1
KEYWORDS   .
SOURCE     Brassica napus (rape)
ORGANISM   Brassica napus
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
            Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
            Brassicaceae; Brassica.
REFERENCE  1 (residues 1 to 413)
AUTHORS    Sjodahl,S., Rodin,J. and Rask,L.
TITLE      Characterization of the 12S globulin complex of Brassica napus.
            Evolutionary relationship to other 11-12S storage globulins
JOURNAL    Eur. J. Biochem. 196 (3), 617-621 (1991)
PUBMED     2013284REFERENCE  2 (residues 1 to 413)
AUTHORS    Roedin,J.
TITLE      Direct Submission
JOURNAL    Submitted (11-FEB-1991) J. Roedin, Dept of Cell Research, Swedish
            University of Agricultural Sciences, Uppsala Biomedical Center,
            Box 596, S-751 24 Uppsala, Sweden
FEATURES   Location/Qualifiers
            source                1..413
                                   /organism="Brassica napus"
                                   /strain="20516-K"
                                   /cultivar="Svaloefts Karat"
                                   /db_xref="taxon:3708"
                                   /clone="pCRU4"
                                   /haplotype="amphidiploid"
                                   /clone_lib="B.napus embryo cDNA in lambda-gt10"
                                   /dev_stage="embryo 35 days after pollination"
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                                   /product="cruciferin cru4 subunit"
            Region              2..144
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                                   /note="Cupin. This family represents the conserved
                                   barrel domain of the cupin superfamily (cupa is the
                                   for a small barrel). This family contains 11S and 7S
                                   Latin term seed storage proteins, and germins; pfam00190"
                                   plant /db_xref="CDD:84597"
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                                   domain of the cupin superfamily (cupa is the Latin term
                                   for a small barrel). This family contains 11S and 7S
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                                   /db_xref="CDD:84597"
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                                   /db_xref="GOA:P33522"
                                   /db_xref="UniProtKB/Swiss-Prot:P33522"
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61 pvfqggqqgqe qqqqggqqgg qgfrdmhqkv ehrlsgdtia tppgvaqwfy nngneplilv
121 aaadiannln qldrnlrpfl lagnnppqqgq wlqgrqqgkq nnifngfapq ilagafkiv
181 etaqklqnqq vnrgnivkvq gqfgvirppl rqqgggqqpq eegnngleetl ctmrctenld
241 dpssadvypk slgyistlns ynlpilrflr lsalrgsihn namvlpqwnv nanaalyvtk
301 gkahiqmvnd ngqrvfdgei skgqllvvpq gfavvkrats qqfqwiefks ndnaqintla
361 grtsvmrglp levisngyqi spqearsvkf stlettltqs sgpmgygmpr vea

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LOCUS ABD14346 476 aa linear PLN 12-FEB-2006
DEFINITION cruciferin-like protein [Brassica napus].
ACCESSION ABD14346
VERSION ABD14346.1 GI:86611322
DBSOURCE accession DQ328613.1
KEYWORDS .
SOURCE Brassica napus (rape)
ORGANISM Brassica napus
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons;
rosids; eurosids II; Brassicales; Brassicaceae; Brassica.
REFERENCE 1 (residues 1 to 476)
AUTHORS Wang,C., Xie,Y., Li,C., Liu,Y. and Huang,B.
TITLE Cloning and hpRNAi vector construction of cruciferin, napin,
oleosin and PEPcase in Brassica napus L.
JOURNAL Unpublished
REFERENCE 2 (residues 1 to 476)
AUTHORS Wang,C., Xie,Y. and Huang,B.
TITLE Direct Submission
JOURNAL Submitted (14-DEC-2005) Biology Department, Hubei University,
Xueyuan Road No.11, Wuhan, Hubei 430062, P.R. China
COMMENT Method: conceptual translation.
FEATURES Location/Qualifiers
source 1..476
/organism="Brassica napus"
/cultivar="huashuang No.4"
/db_xref="taxon:3708"
Protein <1..>476
/product="cruciferin-like protein"
Region 33..220
/region_name="Cupin_1"
/note="Cupin. This family represents the conserved barrel
domain of the cupin superfamily (cupa is the Latin term
for a small barrel). This family contains 11S and 7S plant
seed storage proteins, and germins; pfam00190"
/db_xref="CDD:84597"
Region 307..455
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/note="Cupin. This family represents the conserved
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Latine term for a small barrel). This family contains
11S and 7S plant seed storage proteins, and germins; pfam00190"
/db_xref="CDD:84597"
CDS 1..476
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/codon_start=2
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61 csgvsfvryi ieskglylps ffstaklsfv akqgqlmgrv vpgcaetfqd ssvfqpgggs
121 pfgegqggqg qggqggqggq gggqggqggq gggqgfrdmh qkvehirtgd tiathpgvaq
181 wfyndgnqpl vivsvldlas hqnqldrnpr pfylagnnpq gqvwiegreq qpqknlnqf
241 tpevlakafk idvrtaqqqlq nqqdnrgniv rvqgpfsvir pplrsrcrpqe evngleetic
301 sarctdnldd psnadvykpg lgyistlnsy dlpixrxfxl salrgsirqn amvlpqwnan
361 anavlyvtdg eahvqvvnnd gdrvfdgqvs qgqlsipqg fsvvkratse qfrwiefktn
421 anaqintlag rtsvlrglpl evisngyqis leearrvkfn tiettltthss gpasyg
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LOCUS CAA42473 196 aa linear PLN 14-NOV-2006
DEFINITION cruciferin [Raphanus sativus].
ACCESSION CAA42473
VERSION CAA42473.1 GI:21108
DBSOURCE embl accession X59803.1
KEYWORDS .

SOURCE Raphanus sativus (radish)
ORGANISM Raphanus sativus
 Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta;
Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales;
Brassicaceae; Raphanus.
REFERENCE 1 (residues 1 to 196)
AUTHORS Depigny-This,D., Raynal,M., Aspart,L., Delseny,M. and Grellet,F.
TITLE The cruciferin gene family in radish
JOURNAL Plant Mol. Biol. 20 (3), 467-479 (1992)
PUBMED 1421150
REFERENCE 2 (residues 1 to 196)
AUTHORS Depigny,D.M.C.
TITLE Direct Submission
JOURNAL Submitted (26-MAY-1991) D.M.C. Depigny, University of Perpignan,
 CNRS UA 565, Lab. de Physiologie Vegetale, Ave de Villeneuve, 66860
 Perpignan, Cedex, France
COMMENT Method: conceptual translation supplied by author.
FEATURES Location/Qualifiers
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 /cultivar="Rond rose a bout blanc"
 /db_xref="taxon:3726"
 /clone="pAG4"
 /dev_stage="immature seeds"
Protein 1..196
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Region 51..180
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 /note="Cupin. This family represents the conserved
 barrel domain of the cupin superfamily (cupa is the
 Latin term for a small barrel). This family contains 11S and 7S plant
 seed storage proteins, and germins; pfam00190"
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CDS 1..196
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 /db_xref="HSSP:P04776"
 /db_xref="InterPro:IPR006044"
 /db_xref="InterPro:IPR006045"
 /db_xref="InterPro:IPR011051"
 /db_xref="UniProtKB/TrEMBL:Q41165"
ORIGIN
 1 nivrvkgpfg vvrpplrqsy esekwrhprg ppqspqdnlg eeticmrth eniddparad
 61 vykpnlgrvt svnsltlpil qyvrlsatrg iiggnsmvlp kynmnaneil yctrqgariq
 121 vvndngqnlv dqgvqgqlv vipqgfayvv qsqnnfewis fktnanamis tlagrtsalr
 181 alplevltna yrvsle
//

ADM Puratein (cruciferin) sequence searches AllergenOnline ver. 8 - Appendix 4

The eight NCBI sequences identified as cruciferins that were identified as matching peptides of Puratein from protein bands by CSU were tested using <http://AllergenOnline.com/> Version 8.0, January 2008 release date as described in the methods section. Note that one sequence is from *Raphanus sativus*, a species closely related to *Brassica napus*. Based on sequence similarities, there appear to be three related cruciferin genes and the eight sequences represent variants of the three proteins.

1. GI: 33284990

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 33284990 Brassica napus [in P6-1 AND P6-2] qfqpncqlldqlnalepshvlkaeagrIEVWDHHAQLRCSGVSFVRYiieskGLYLPSF FSTAKlsfvakggglmgrvvpqcaetfqdssvfqpgggsfpgegqggqggqggghgggg qggqggqggqggqsggqgfrdmhqkvehirsgdtiathpgvaqwfyngngqplvivavmd lashqngldrnprpfylagknpqggswhgrgqgpqnnilngfspevlaqafkidivrTAQ QLQNQDNRgnivrvQGPFGVIRPPLKsgrpqeteangleeticsarctdnlddpsnadv ykpqlgyisilnsydlpilrvlrlsalrgsirqnamvlpqwnananavlyvtgdgeaigqv vndngdrvfdgqvsggqlslipqgfsvvkratsdqfrwiefktnanaqintlgrtssvr glpleviangyqisleearrvkfntiettlthssgpasyggprkda
Length	467
Number of 80 mers	388

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AA77383.1 11S globulin precursor [Sinapis alba]	75.30 %	388of388	8.9e-52	61.10 %	470	gi 62240390	GO!
2	gi 62240392 gb AA77384.1 11S globulin precursor [Sinapis alba]	71.60 %	388of388	2.9e-21	58.80 %	481	gi 62240392	GO!
3	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	63.70 %	374of388	2.4e-25	45.10 %	501	gi 18479082	GO!
4	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	60.00 %	340of388	2.8e-26	44.00 %	473	gi 25991543	GO!
5	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	57.52 %	326of388	9.8e-25	42.10 %	513	gi 56788031	GO!
6	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	53.70 %	262of388	8.4e-20	37.30 %	490	gi 18615	GO!
7	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	53.70 %	123of388	1.8e-20	48.50 %	165	gi 22353013	GO!
8	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	53.70 %	262of388	7.5e-20	37.30 %	490	gi 18635	GO!
9	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	52.50 %	345of388	1.4e-28	42.60 %	467	gi 30313867	GO!
10	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	52.50 %	211of388	7.9e-31	38.20 %	466	gi 2317674	GO!
11	gi 18639 emb CAA33217.1 glycinin	52.50 %	276of388	7.7e-	37.40 %	479	gi 18639	GO!

	subunit G3 [Glycine max]	%	8	27	%			
12	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	51.29 %	112of388	5.9e-17	43.60 %	165	gi 6979766	GO!
13	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	51.20 %	200of388	9.1e-20	36.80 %	505	gi 2317670	GO!
14	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	50.00 %	214of388	3.6e-18	31.60 %	569	gi 732706	GO!
15	gi 4895075 gb AAD32713.1 AF152003_1 major allerger [Fagopyrum esculentum]	50.00 %	227of388	1.5e-19	38.90 %	475	gi 4895075	GO!
16	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	50.00 %	224of388	4.7e-16	33.80 %	515	gi 736002	GO!
17	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	50.00 %	223of388	6.7e-18	32.10 %	570	gi 806556	GO!
18	gi 18641 emb CAA37044.1 glycinin [Glycine max]	50.00 %	221of388	8.2e-18	31.90 %	570	gi 18641	GO!
19	gi 736319 emb CAA27052.1 glutenin [Triticum aestivum]	50.00 %	74of388	4.8e-06	31.90 %	226	gi 736319	GO!
20	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	50.00 %	111of388	5.9e-16	38.30 %	188	gi 169971	GO!
21	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	48.78 %	236of388	4.2e-27	35.60 %	483	gi 18637	GO!
22	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	48.78 %	72of388	8.8e-06	31.20 %	231	gi 21743	GO!
23	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	48.78 %	236of388	4.2e-27	35.60 %	483	gi 18609	GO!
24	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	47.50 %	195of388	2e-18	35.30 %	487	gi 112380623	GO!
25	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	47.50 %	74of388	0.0016	31.20 %	231	gi 170743	GO!
26	gi 169969 gb AAA33964.1 glycinin [Glycine max]	46.30 %	198of388	2.8e-08	32.80 %	515	gi 169969	GO!
27	gi 22135348 gb AAM93157.1 trypsin inhibitor [Arachis hypogaea]	46.30 %	50of388	9.9e-07	34.30 %	204	gi 22135348	GO!
28	gi 21314465 gb AAM46958.1 AF510854_1 allergen Ara [Arachis hypogaea]	46.30 %	217of388	8.4e-17	34.00 %	520	gi 21314465	GO!
29	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	46.30 %	185of388	1.8e-16	32.40 %	531	gi 3703107	GO!
30	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	45.10 %	59of388	0.0088	29.40 %	221	gi 21751	GO!
31	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	45.03 %	62of388	0.0033	28.30 %	258	gi 22090	GO!
32	gi 21779 emb CAA26847.1 unnamed protein product [Triticum aestivum]	43.75 %	55of388	0.013	29.50 %	217	gi 21779	GO!
33	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	42.90 %	131of388	1.8e-15	31.60 %	512	gi 5712199	GO!
34	gi 170732 gb AAA34286.1 gamma-gliadin [Triticum aestivum]	38.79 %	20of388	0.15	40.30 %	77	gi 170732	GO!
35	gi 170730 gb AAA34285.1 gamma-gliadin B-I precur [Triticum aestivum]	38.79 %	20of388	0.14	40.30 %	77	gi 170730	GO!

36	gi 29539109 emb CAD87730.1 allergen Len c 1.0101 [Lens culinaris]	35.80 %	1of388	7.8	24.60 %	341	gi 29539109	GO!
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AllergenOnline Database v8.0 (January, 2008)

2. GI: 461840

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 461840 Brassica napu. {in P6-1, P6-2, P6-3, P6-5 and P6-6} mvkvphllvatfgvllvngclarqslgvppqlgnacnldnldvlgptetikseagrvey wdhnnpqircagvsrvieeqgglylptffsspkisyvvqgmgisgrvvpgcaetfmds qpmmggqqggqpwwqqggqqggqqggqqggqqggqqggqqggqqggqqggqqggfrdmhkv ehvrhgdiiaitagsshwiyntgdqplviiclldianyqnqldrnprtfrlagnnppqggs qqqqqqqqnmlsgfdpqvlaqalkidvrLAQELQNQQDSRgnivrvkgpfgvvrpplrqp yeseqwrhprgppqspqngleeticsmrtheniddparadvykpnlgrVTSVNSYTLPI LQYIRlsatrGILQGNAMVLPKYNMNEILYCTQGQARIQVVDNGQNVLDQQVQKqql vvipqgfayvvqshqnnfewisfktNANAMVSTLAGRtsalrALPLEVITNAFQISLEEA RrIKFNTLETTLTRarggqpqlieeivea
Length	509
Number of 80 mers	430

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAx77383.1 11S globulin precursor [Sinapis alba]	98.80 %	430of430	3.3e-124	91.60 %	510	gi 62240390	GO!
2	gi 62240392 gb AAx77384.1 11S globulin precursor [Sinapis alba]	97.50 %	430of430	8.2e-93	90.10 %	523	gi 62240392	GO!
3	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	63.79 %	368of430	1.9e-37	44.50 %	533	gi 56788031	GO!
4	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	60.04 %	379of430	8e-43	46.20 %	487	gi 25991543	GO!
5	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	58.80 %	394of430	2.4e-36	45.60 %	528	gi 18479082	GO!
6	gi 736319 emb CAA27052.1 glutenin [Triticum aest [Triticum aestivum]	56.26 %	73of430	1.9e-06	33.60 %	214	gi 736319	GO!
7	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	53.70 %	124of430	2.6e-20	45.70 %	175	gi 22353013	GO!
8	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	52.50 %	309of430	7.3e-35	39.70 %	506	gi 30313867	GO!
9	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	51.25 %	264of430	1.3e-16	34.40 %	546	gi 736002	GO!
10	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	51.25 %	240of430	5.5e-25	34.60 %	540	gi 2317670	GO!
11	gi 18639 emb CAA33217.1 glycinin	51.20	267of43	5.9e-	36.00	516	gi 18639	GO!

	subunit G3 [Glycine max]	%	0	23	%			
12	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	50.60 %	256of430	5.5e-17	31.70 %	580	gi 806556	GO!
13	gi 18641 emb CAA37044.1 glycinin [Glycine max]	50.60 %	239of430	4.5e-17	31.40 %	580	gi 18641	GO!
14	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	50.02 %	69of430	4.3e-06	30.60 %	222	gi 22090	GO!
15	gi 169969 gb AAA33964.1 glycinin [Glycine max]	50.00 %	242of430	1.5e-08	33.50 %	546	gi 169969	GO!
16	gi 4895075 gb AAD32713.1 AF152003_1 major allerge [Fagopyrum esculentum]	50.00 %	236of430	5.2e-26	36.80 %	486	gi 4895075	GO!
17	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	50.00 %	267of430	2.5e-27	37.10 %	517	gi 18615	GO!
18	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	50.00 %	70of430	1.6e-06	31.80 %	267	gi 21743	GO!
19	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	50.00 %	70of430	8.8e-07	31.50 %	267	gi 170743	GO!
20	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	50.00 %	213of430	4e-24	34.20 %	512	gi 2317674	GO!
21	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	50.00 %	114of430	3.5e-17	43.60 %	165	gi 6979766	GO!
22	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	50.00 %	267of430	3.6e-27	37.10 %	517	gi 18635	GO!
23	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	49.40 %	237of430	1.1e-16	31.30 %	579	gi 732706	GO!
24	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	48.78 %	71of430	2e-05	30.50 %	266	gi 21751	GO!
25	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	48.70 %	244of430	3.1e-17	35.40 %	503	gi 18637	GO!
26	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	48.70 %	244of430	3.1e-17	35.40 %	503	gi 18609	GO!
27	gi 5712199 gb AAD47382.1 glycinin [Arachis hypog [Arachis hypogaea]	47.50 %	145of430	1.1e-13	31.70 %	511	gi 5712199	GO!
28	gi 22135348 gb AAM93157.1 trypsin inhibitor [Arachis hypogaea]	47.50 %	77of430	4.3e-08	34.70 %	242	gi 22135348	GO!
29	gi 21314465 gb AAM46958.1 AF510854_1 allergen Ara [Arachis hypogaea]	47.50 %	219of430	9.1e-15	33.20 %	548	gi 21314465	GO!
30	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	47.50 %	116of430	2.9e-16	39.80 %	191	gi 169971	GO!
31	gi 21779 emb CAA26847.1 unnamed protein product [Triticum aestivum]	47.50 %	68of430	0.0011	26.60 %	418	gi 21779	GO!
32	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	46.90 %	174of430	6.7e-15	32.10 %	511	gi 3703107	GO!
33	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	46.22 %	186of430	1.2e-15	33.50 %	516	gi 112380623	GO!
34	gi 21930 emb CAA44473.1 LMW glutenin [Triticum turgidum]	43.80 %	35of430	0.0021	27.50 %	171	gi 21930	GO!
35	gi 170738 gb AAA34289.1 gamma-gliadin [Triticum aestivum]	43.79 %	34of430	0.13	29.80 %	208	gi 170738	GO!
36	gi 170732 gb AAA34286.1 gamma-	43.71	38of430	0.0085	40.50	79	gi 170732	GO!

	gliadin [Triticum aestivum]	%			%			
37	gi 170730 gb AAA34285.1 gamma-gliadin B-I precur [Triticum aestivum]	43.71 %	38of430	0.0082	40.50 %	79	gi 170730	GO!
38	gi 75219081 sp O22108 O22108_WHEAT LMM glutenin 1 [Triticum aestivum]	42.52 %	37of430	0.0054	26.60 %	173	gi 75219081	GO!
39	gi 62550933 emb CAI79052.1 putative LMW-glutenin [Triticum aestivum]	42.52 %	45of430	0.63	49.30 %	69	gi 62550933	GO!
40	gi 170736 gb AAA34288.1 gamma-gliadin [Tricium aestivum]	41.28 %	35of430	0.34	32.30 %	155	gi 170736	GO!
41	gi 897811 emb CAA24933.1 unnamed protein product [Triticum aestivum]	40.01 %	38of430	0.0069	48.40 %	64	gi 897811	GO!
42	gi 21757 emb CAA26383.1 unnamed protein product [Triticum aestivum]	39.99 %	35of430	0.025	29.80 %	178	gi 21757	GO!
43	gi 21761 emb CAA26384.1 unnamed protein product [Triticum aestivum]	38.79 %	14of430	0.013	26.80 %	179	gi 21761	GO!
44	gi 21926 emb CAA36063.1 unnamed protein product [Triticum turgidum]	38.78 %	36of430	0.0097	24.10 %	174	gi 21926	GO!
45	gi 170708 gb AAA34274.1 gamma-gliadin B precursor [Triticum aestivum]	38.77 %	32of430	0.49	29.50 %	200	gi 170708	GO!
46	gi 170724 gb AAA34282.1 pre-alpha-beta-gliadin [Triticum aestivum]	38.76 %	18of430	0.0032	28.30 %	240	gi 170724	GO!
47	gi 170702 gb AAA34272.1 gamma gliadin precursor [Triticum aestivum]	38.76 %	27of430	0.15	28.20 %	188	gi 170702	GO!
48	gi 73912496 dbj BAE20328.1 omega-5 gliadin [Triticum aestivum]	38.72 %	27of430	0.0011	29.00 %	217	gi 73912496	GO!
49	gi 1063270 dbj BAA11251.1 gamma-gliadin precursor [Triticum aestivum]	38.72 %	1of430	0.44	30.10 %	193	gi 1063270	GO!
50	gi 21673 emb CAA35238.1 unnamed protein product [Triticum aestivum]	37.52 %	30of430	0.00073	28.80 %	243	gi 21673	GO!
51	gi 170740 gb AAA34290.1 gliadin [Triticum urartu]	37.52 %	30of430	0.044	29.40 %	177	gi 170740	GO!
52	gi 75317968 sp O22116 O22116_WHEAT LMM glutenin 3 [Triticum aestivum]	36.27 %	2of430	0.034	27.90 %	208	gi 75317968	GO!
53	gi 170726 gb AAA34283.1 pre-alpha-beta-gliadin [Triticum aestivum]	36.24 %	10of430	0.4	27.50 %	204	gi 170726	GO!
54	gi 170718 gb AAA34279.1 alpha/beta-gliadin precu [Triticum aestivum]	35.04 %	3of430	0.0014	29.10 %	234	gi 170718	GO!
55	gi 21765 emb CAA26385.1 unnamed protein product [Triticum aestivum]	35.04 %	3of430	0.0017	29.10 %	234	gi 21765	GO!
56	gi 170712 gb AAA34276.1 pre-alpha-beta-gliadin [Triticum aestivum]	35.02 %	15of430	0.0066	29.00 %	183	gi 170712	GO!

3. GI: GI:12751302

17	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	50.00 %	235of410	3.1e-35	35.60 %	492	gi 2317674	GO!
18	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	50.00 %	116of410	2.1e-17	44.20 %	165	gi 6979766	GO!
19	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	50.00 %	270of410	7.2e-19	37.40 %	514	gi 18635	GO!
20	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	48.70 %	247of410	2.8e-17	36.30 %	496	gi 18637	GO!
21	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	48.70 %	247of410	2.8e-17	36.30 %	496	gi 18609	GO!
22	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	47.50 %	120of410	1.7e-16	40.30 %	191	gi 169971	GO!
23	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	46.30 %	165of410	1.3e-13	32.40 %	522	gi 5712199	GO!
24	gi 22135348 gb AAM93157.1 trypsin inhibitor [Arachis hypogaea]	46.30 %	97of410	3.4e-16	37.60 %	221	gi 22135348	GO!
25	gi 21314465 gb AAM46958.1 AF510854_1 allergen Ara [Arachis hypogaea]	46.30 %	243of410	1e-14	34.30 %	528	gi 21314465	GO!
26	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	46.30 %	191of410	7.3e-15	33.40 %	491	gi 3703107	GO!
27	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	45.05 %	200of410	1.4e-15	34.50 %	496	gi 112380623	GO!
28	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	41.25 %	27of410	0.0029	28.50 %	270	gi 21743	GO!
29	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	41.25 %	21of410	0.0067	28.90 %	270	gi 170743	GO!
30	gi 736319 emb CAA27052.1 glutenin [Triticum aestivum]	41.25 %	29of410	0.0024	29.70 %	232	gi 736319	GO!
31	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	40.70 %	22of410	0.11	30.10 %	183	gi 21751	GO!
32	gi 21765 emb CAA26385.1 unnamed protein product [Triticum aestivum]	40.00 %	13of410	0.0049	30.90 %	217	gi 21765	GO!
33	gi 21673 emb CAA35238.1 unnamed protein product [Triticum aestivum]	39.97 %	25of410	0.0044	30.90 %	223	gi 21673	GO!
34	gi 170718 gb AAA34279.1 alpha/beta-gliadin precu [Triticum aestivum]	38.79 %	13of410	0.0054	30.80 %	214	gi 170718	GO!
35	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	38.78 %	16of410	0.0081	27.30 %	227	gi 22090	GO!
36	gi 21779 emb CAA26847.1 unnamed protein product [Triticum aestivum]	37.54 %	19of410	0.19	26.60 %	252	gi 21779	GO!
37	gi 21757 emb CAA26383.1 unnamed protein product [Triticum aestivum]	37.54 %	15of410	0.026	29.20 %	161	gi 21757	GO!
38	gi 170740 gb AAA34290.1 gliadin [Triticum urartu]	36.29 %	10of410	0.031	29.20 %	161	gi 170740	GO!

4. GI:461841

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI:461841 Brassica napus [in P6-1, P6-2, P6-3, P6-4 and P6-6] Mgptsllsffftfltlfhgftaqwpnecqldqlnalepsqikseggrievwdhhapql rcsgfaferfviiepgglylptflnagkltfvvhghalmgkvtpgcaetfndspvfgqggg qeqggqggqggqggfrdmhgkvehlrsgdtiatppgvaqwfynngneplilvaaadiann lnqldrnlrpfllagnnpqggqqlggrqqkqnnifngfapqilaqafkisvetaqklqn qqvnrnqvkvqggqfgvirpplrqggqggqppqeeegngleetlctmrCTENLDDPSSADV KPSLGYSTLNSYNLPILRflrlsalrgsihnnamvlpqwnvnanaalyvtkgkahiqnv ndngqrVFDQEISKQQLLVVPQGFAVVKrATSQQFQWIEFKSNDNAQINTLAGRtsvmrG LPLEVISNGYQISPQEARSvkfstletltltqssgpmgygmrvea
Length	465
Number of 80 mers	386

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAX77383.1 11S globulin precursor [Sinapis alba]	70.01%	386of386	7.7e-60	55.00%	471	gi 62240390	GO!
2	gi 62240392 gb AAX77384.1 11S globulin precursor [Sinapis alba]	65.03%	386of386	2.6e-58	53.30%	482	gi 62240392	GO!
3	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	59.30%	377of386	3.5e-41	45.90%	464	gi 25991543	GO!
4	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	58.80%	386of386	5.7e-26	44.40%	502	gi 18479082	GO!
5	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	56.29%	131of386	9.8e-24	50.90%	165	gi 22353013	GO!
6	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	55.00%	293of386	5.6e-23	39.70%	448	gi 2317674	GO!
7	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	55.00%	233of386	3e-25	35.50%	507	gi 2317670	GO!
8	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	54.30%	386of386	2.2e-26	43.30%	499	gi 56788031	GO!
9	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	53.72%	116of386	2.1e-19	44.80%	165	gi 6979766	GO!
10	gi 4895075 gb AAD32713.1 AF152003_1 major allerge [Fagopyrum esculentum]	53.72%	229of386	1.9e-25	37.80%	482	gi 4895075	GO!
11	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	52.50%	262of386	1.9e-18	35.70%	502	gi 736002	GO!
12	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	52.50%	386of386	4.9e-53	43.00%	463	gi 30313867	GO!
13	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	51.25%	120of386	4.3e-18	39.60%	202	gi 169971	GO!
14	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	51.20%	226of386	4.2e-18	32.70%	544	gi 732706	GO!
15	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	51.20%	257of386	6e-21	38.10%	486	gi 18615	GO!
16	gi 18641 emb CAA37044.1 glycinin [Glycine max]	51.20%	226of386	4.2e-18	32.50%	545	gi 18641	GO!
17	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	51.20%	237of386	4.2e-18	32.70%	545	gi 806556	GO!
18	gi 18639 emb CAA33217.1 glycinin	51.20%	303of386	2.9e-	38.60%	471	gi 18639	GO!

t #		%ID	> 35%	E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAX77383.1 11S globulin precursor [Sinapis alba]	76.50 %	411of411	3.3e-55	58.90 %	501	gi 62240390	GO!
2	gi 62240392 gb AAX77384.1 11S globulin precursor [Sinapis alba]	74.10 %	411of411	1.1e-23	57.20 %	512	gi 62240392	GO!
3	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	63.70 %	383of411	1.9e-27	44.70 %	532	gi 18479082	GO!
4	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	61.30 %	367of411	3.2e-28	44.40 %	493	gi 25991543	GO!
5	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	57.52 %	351of411	2e-26	42.40 %	538	gi 56788031	GO!
6	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	55.00 %	125of411	2.4e-22	50.30 %	165	gi 22353013	GO!
7	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	55.00 %	271of411	1.7e-34	39.50 %	471	gi 2317674	GO!
8	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	53.72 %	115of411	8.6e-19	46.10 %	165	gi 6979766	GO!
9	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	53.72 %	247of411	1.4e-21	37.70 %	528	gi 2317670	GO!
10	gi 4895075 gb AAD32713.1 AF152003_1 major allerge [Fagopyrum esculentum]	53.70 %	259of411	2.3e-21	39.60 %	480	gi 4895075	GO!
11	gi 30313867 gb AAQ38859.1 11S globulin [Bertholletia excelsa]	53.70 %	364of411	1.2e-30	41.60 %	495	gi 30313867	GO!
12	gi 18639 emb CAA33217.1 glycinin subunit G3 [Glycine max]	53.70 %	277of411	3.5e-29	37.80 %	503	gi 18639	GO!
13	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	52.54 %	270of411	4.1e-21	37.60 %	511	gi 18615	GO!
14	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	52.54 %	270of411	3.7e-21	37.60 %	511	gi 18635	GO!
15	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	52.50 %	223of411	9.3e-19	31.70 %	590	gi 732706	GO!
16	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	52.50 %	256of411	1.1e-17	34.40 %	538	gi 736002	GO!
17	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	52.50 %	244of411	1e-18	31.80 %	591	gi 806556	GO!
18	gi 18641 emb CAA37044.1 glycinin [Glycine max]	52.50 %	244of411	1.4e-18	32.00 %	591	gi 18641	GO!
19	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	52.50 %	122of411	1.4e-17	40.40 %	188	gi 169971	GO!
20	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	51.29 %	230of411	2.8e-29	36.20 %	506	gi 18637	GO!
21	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	51.29 %	230of411	2.8e-29	36.20 %	506	gi 18609	GO!
22	gi 22135348 gb AAM93157.1 trypsin inhibitor [Arachis hypogaea]	49.40 %	50of411	2.6e-07	34.10 %	229	gi 22135348	GO!
23	gi 21314465 gb AAM46958.1 AF510854_1 allergen Ara [Arachis hypogaea]	49.40 %	219of411	1.4e-18	33.60 %	545	gi 21314465	GO!
24	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	49.40 %	180of411	3.1e-18	33.00 %	531	gi 3703107	GO!
25	gi 169969 gb AAA33964.1 glycinin	48.78 %	239of411	1.5e-09	33.10 %	538	gi 169969	GO!

26	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	48.70 %	214of411	2.9e-20	35.50 %	516	gi 112380623	GO!
27	gi 736319 emb CAA27052.1 glutenin [Triticum aestivum]	47.60 %	72of411	2.7e-05	30.00 %	227	gi 736319	GO!
28	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	47.50 %	72of411	5.6e-05	33.30 %	156	gi 170743	GO!
29	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	46.30 %	148of411	4.6e-17	32.30 %	538	gi 5712199	GO!
30	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	44.00 %	72of411	8.6e-05	33.30 %	156	gi 21743	GO!
31	gi 21779 emb CAA26847.1 unnamed protein product [Triticum aestivum]	42.00 %	57of411	0.024	28.60 %	199	gi 21779	GO!
32	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	41.50 %	60of411	0.00081	27.80 %	198	gi 22090	GO!
33	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	40.01 %	49of411	0.029	27.10 %	203	gi 21751	GO!
34	gi 170732 gb AAA34286.1 gamma-gliadin [Triticum aestivum]	38.79 %	30of411	0.043	40.30 %	77	gi 170732	GO!
35	gi 170730 gb AAA34285.1 gamma-gliadin B-I precur [Triticum aestivum]	38.79 %	30of411	0.041	40.30 %	77	gi 170730	GO!
36	gi 21930 emb CAA44473.1 LMW glutenin [Triticum turgidum]	37.50 %	27of411	0.1	26.10 %	180	gi 21930	GO!
37	gi 75219081 sp O22108 O22108_WHEAT LMM glutenin 1 [Triticum aestivum]	37.46 %	26of411	0.083	40.50 %	74	gi 75219081	GO!
38	gi 21926 emb CAA36063.1 unnamed protein product [Triticum turgidum]	37.46 %	25of411	0.13	40.50 %	74	gi 21926	GO!
39	gi 62550933 emb CAI79052.1 putative LMW-glutenin [Triticum aestivum]	36.24 %	9of411	2	36.70 %	79	gi 62550933	GO!

6. GI: 17805

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI: 17805 Brassica napus [in P6-3, P6-4, P6-5 and P6-6] wdhhapqlrcsgfaferfviepqglylptflnagkltfvvhghalmgkvtpgcaetfmds pvfgqggqgqegqggqggqggqgfrdmhgkvehlrsqdtiatppgvaqwfyngneplilv aaadiannlnqldrnrlrpfllagnnpqggqwlqgrqqqkqnnifngfapqilaqafkisv etaqklqnqgvnrgnivkvqggfgvirpplrqqggqgqpqeeegnleetlctmrCTENLD DPSSADVYKPSLGYISTLNSYNLPILRflrlsalrgsihnnamvlpqwnvnanaalyvtk gkahiqmvdngqrVFDQEISKGQLLVVPQGFVVKrATSQQFQWIEFKSNDNAQINTLA GRtsvmrGLPLEVISNGYQISPQEARSvkfstlettltqssgpmgygmprvea
Length	413
Number of 80 mers	334

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAx77383.1 11S globulin precursor [Sinapis alba]	70.01%	334of334	6.8e-62	55.90%	442	gi 62240390	GO!
2	gi 62240392 gb AAx77384.1 11S globulin precursor [Sinapis alba]	65.03%	334of334	2.5e-60	54.00%	452	gi 62240392	GO!
3	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	59.30%	325of334	2.5e-36	45.70%	420	gi 25991543	GO!
4	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	58.80%	334of334	5e-27	45.40%	441	gi 18479082	GO!
5	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	56.29%	131of334	1e-24	50.90%	165	gi 22353013	GO!
6	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	55.00%	241of334	6e-24	40.00%	413	gi 2317674	GO!
7	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	55.00%	185of334	2.7e-26	35.20%	477	gi 2317670	GO!
8	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	54.30%	334of334	1.8e-27	43.80%	443	gi 56788031	GO!
9	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	53.72%	116of334	3e-20	44.80%	165	gi 6979766	GO!
10	gi 4895075 gb AAD32713.1 AF152003_1 major allergen [Fagopyrum esculentum]	53.72%	177of334	1.7e-26	37.60%	447	gi 4895075	GO!
11	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	52.50%	237of334	2.8e-19	34.00%	476	gi 736002	GO!
12	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	52.50%	334of334	1.6e-51	44.00%	407	gi 30313867	GO!
13	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	51.25%	120of334	6.5e-19	39.60%	202	gi 169971	GO!
14	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	51.20%	220of334	6.2e-19	31.30%	518	gi 732706	GO!
15	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	51.20%	238of334	7.3e-22	36.90%	434	gi 18615	GO!
16	gi 18639 emb CAA33217.1 glycinin subunit G3 [Glycine max]	51.20%	283of334	3.7e-21	38.10%	423	gi 18639	GO!
17	gi 18641 emb CAA37044.1 glycinin	51.20%	226of334	6.2e-	31.20%	519	gi 18641	GO!

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAK77383.1 11S globulin precursor [Sinapis alba]	76.80 %	397of397	5.8e-34	58.30 %	501	gi 62240390	GO!
2	gi 62240392 gb AAK77384.1 11S globulin precursor [Sinapis alba]	73.80 %	397of397	9e-21	56.50 %	515	gi 62240392	GO!
3	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	63.70 %	392of397	1.1e-25	44.40 %	522	gi 18479082	GO!
4	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	61.30 %	343of397	1.2e-26	43.80 %	489	gi 25991543	GO!
5	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	57.52 %	344of397	3.7e-25	42.80 %	519	gi 56788031	GO!
6	gi 22353013 gb AAK97787.1 allergenic protein [Fagopyrum tataricum]	55.00 %	119of397	5.4e-21	49.10 %	165	gi 22353013	GO!
7	gi 4895075 gb AAD32713.1 AF152003_1 major allerge [Fagopyrum esculentum]	53.70 %	237of397	3.5e-20	38.80 %	482	gi 4895075	GO!
8	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	53.70 %	351of397	1.8e-29	41.80 %	491	gi 30313867	GO!
9	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	53.70 %	230of397	2.2e-20	36.50 %	529	gi 2317670	GO!
10	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	53.70 %	256of397	2.4e-32	39.00 %	467	gi 2317674	GO!
11	gi 736319 emb CAA27052.1 glutenin [Triticum aestivum]	52.50 %	75of397	3.7e-05	31.20 %	231	gi 736319	GO!
12	gi 18639 emb CAA33217.1 glycinin subunit G3 [Glycine max]	52.50 %	283of397	3.6e-26	37.80 %	492	gi 18639	GO!
13	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	51.29 %	112of397	1.6e-17	44.80 %	165	gi 6979766	GO!
14	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	51.20 %	219of397	2e-16	31.90 %	565	gi 732706	GO!
15	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	51.20 %	252of397	9.2e-16	34.50 %	534	gi 736002	GO!
16	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	51.20 %	231of397	3.4e-16	32.00 %	566	gi 806556	GO!
17	gi 18641 emb CAA37044.1 glycinin [Glycine max]	51.20 %	227of397	4.2e-16	32.20 %	566	gi 18641	GO!
18	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	51.20 %	119of397	1.2e-15	39.40 %	188	gi 169971	GO!
19	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	50.00 %	262of397	7.7e-20	37.30 %	507	gi 18615	GO!
20	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	50.00 %	262of397	6.9e-20	37.30 %	507	gi 18635	GO!
21	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	49.97 %	226of397	1.4e-26	36.20 %	500	gi 18637	GO!
22	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	49.97 %	226of397	1.4e-26	36.20 %	500	gi 18609	GO!
23	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	47.60 %	174of397	9.2e-17	33.40 %	509	gi 3703107	GO!
24	gi 169969 gb AAA33964.1 glycinin [Glycine max]	47.55 %	234of397	8.2e-09	33.10 %	534	gi 169969	GO!

25	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	47.55 %	234of397	6.3e-19	35.50 %	512	gi 112380623	GO!
26	gi 22135348 gb AAM93157.1 trypsin inhibitor [Arachis hypogaea]	47.50 %	60of397	1.8e-07	35.60 %	225	gi 22135348	GO!
27	gi 21314465 gb AAM46958.1 AF510854_1 allergen Ara [Arachis hypogaea]	47.50 %	231of397	3e-17	34.00 %	541	gi 21314465	GO!
28	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	46.25 %	71of397	0.00036	34.70 %	173	gi 21743	GO!
29	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	46.22 %	64of397	0.056	30.00 %	267	gi 21751	GO!
30	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	45.70 %	59of397	0.1	29.80 %	198	gi 22090	GO!
31	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	45.20 %	72of397	0.025	34.70 %	173	gi 170743	GO!
32	gi 21779 emb CAA26847.1 unnamed protein product [Triticum aestivum]	45.10 %	61of397	0.2	30.50 %	200	gi 21779	GO!
33	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	44.00 %	146of397	7.6e-16	32.40 %	527	gi 5712199	GO!

AllergenOnline Database v8.0 (January, 2008)

8. GI: 21108

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> GI:21108, Raphanus sativus [in P6-3] nivrvgpfpqvvrpplrqsyesekwrhprgppqspqdngleeticsmrtheniddparad vykphlgrvtsvnsltlpilgyvrlsatrgiiqgnsmvlpkYNNMNAEILYCTRgqarIQ VVNDNGQNVLDQQVQKqqlvvipqgfayvvqsqnnfewisfktNANAMISTLAGRTsalr alplevltnayrvsle
Length	196
Number of 80 mers	117

Hit #	Define	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 62240390 gb AAx77383.1 11S globulin precursor [Sinapis alba]	98.80%	117of117	9e-76	93.90%	196	gi 62240390	GO!
2	gi 62240392 gb AAx77384.1 11S globulin precursor [Sinapis alba]	98.80%	117of117	2.6e-75	92.90%	196	gi 62240392	GO!
3	gi 56788031 gb AAW29810.1 seed storage protein [Juglans regia]	59.30%	117of117	2.6e-35	47.60%	210	gi 56788031	GO!
4	gi 18479082 gb AAL73404.1 AF449424_1 11S globulin [Corylus avellana]	56.80%	117of117	2.3e-33	48.60%	208	gi 18479082	GO!
5	gi 22353013 gb AAK97787.1	55.60%	87of117	2.3e-	47.20%	159	gi 22353013	GO!

	allergenic protein [Fagopyrum tataricum]			31				
6	gi 25991543 gb AAN76862.1 AF453947_1 allergen Ana [Anacardium occidentale]	53.72%	117of117	5.6e-36	48.50%	200	gi 25991543	GO!
7	gi 18615 emb CAA26723.1 unnamed protein product [Glycine max]	52.50%	112of117	1.7e-28	42.90%	210	gi 18615	GO!
8	gi 30313867 gb AAO38859.1 11S globulin [Bertholletia excelsa]	52.50%	117of117	6.5e-34	47.00%	198	gi 30313867	GO!
9	gi 18635 emb CAA33215.1 glycinin subunit G1 [Glycine max]	52.50%	112of117	1.5e-28	42.90%	210	gi 18635	GO!
10	gi 4895075 gb AAD32713.1 AF152003_1 major allerge [Fagopyrum esculentum]	51.90%	108of117	1.3e-31	42.40%	184	gi 4895075	GO!
11	gi 2317674 dbj BAA21760.1 legumin-like protein [Fagopyrum esculentum]	51.90%	92of117	9.7e-31	41.40%	186	gi 2317674	GO!
12	gi 2317670 dbj BAA21758.1 legumin-like protein [Fagopyrum esculentum]	51.90%	111of117	6.3e-32	42.90%	184	gi 2317670	GO!
13	gi 18639 emb CAA33217.1 glycinin subunit G3 [Glycine max]	51.20%	96of117	6e-29	42.10%	197	gi 18639	GO!
14	gi 18637 emb CAA33216.1 glycinin subunit G2 [Glycine max]	50.00%	92of117	5.5e-26	39.40%	203	gi 18637	GO!
15	gi 18609 emb CAA26575.1 unnamed protein product [Glycine max]	50.00%	92of117	5.5e-26	39.40%	203	gi 18609	GO!
16	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	48.78%	117of117	1.9e-26	42.50%	186	gi 732706	GO!
17	gi 736002 emb CAA55977.1 Gy5 [Glycine soja]	48.78%	117of117	2e-26	42.50%	179	gi 736002	GO!
18	gi 806556 emb CAA60533.1 A5A4B3 subunit [Glycine soja]	48.78%	117of117	1.9e-26	42.50%	186	gi 806556	GO!
19	gi 18641 emb CAA37044.1 glycinin [Glycine max]	48.78%	117of117	1.9e-26	42.50%	186	gi 18641	GO!
20	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	48.78%	92of117	6.8e-28	44.20%	156	gi 6979766	GO!
21	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	48.78%	116of117	7.9e-26	41.90%	179	gi 169971	GO!
22	gi 169969 gb AAA33964.1 glycinin [Glycine max]	47.50%	116of117	3.6e-24	41.30%	179	gi 169969	GO!
23	gi 21314465 gb AAM46958.1 AF510854_1 allergen Arachis hypogaea]	46.30%	94of117	6.2e-23	37.10%	194	gi 21314465	GO!
24	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	46.30%	75of117	1.9e-21	36.10%	202	gi 5712199	GO!
25	gi 112380623 gb ABI17154.1 iso-Ara h3 [Arachis hypogaea]	45.05%	115of117	6.1e-25	38.40%	211	gi 112380623	GO!
26	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	45.05%	82of117	4.4e-23	35.70%	207	gi 3703107	GO!

AllergenOnline Database v8.0 (January, 2008)

Summary: Peptide sequences that were identified in isolated, SDS-PAGE stained Puratein bands identified eight proteins that are recognized as members of the cruciferin family of proteins, including those of allergens in *Sinapis alba*. These represent three clusters of proteins

that are highly related based on Clustal W alignment. There is enough sequence diversity to assume that slightly different FASTA alignments would differ somewhat if analyzed in against Allergenonline.com. Therefore each of the eight proteins was analyzed and indeed the patterns of sequence identity matches differed slightly between the eight individuals. It is important to note however that the closest alignments by far were between the eight query proteins and two cruciferin proteins of *Sinapis alba*, yellow (white) mustard. The highest percent identities for full-length alignments of sequences ranged from 53% to 98% over the eight query proteins, compared to the *Sinapis alba* cruciferins. There is a high likelihood that these proteins would be cross-reactive for IgE binding and that they would induce allergic reactions in individuals sensitized to the cross-reactive proteins. The best 80-amino acid sequence sliding window matches for the same proteins ranged from about 65% to 98% identity. Again, based on empirical evidence of similarly identical allergens, proteins sharing this much sequence identity are likely to elicit cross-reactions. Both *Brassica sp.* and *Sinapis sp.* are closely related evolutionarily and are considered to be mustards. Raphinus sp. is in the same subgroup of Brassicaceae as well. In many geographies the food use of oils, seeds and greens from plants in these genera is quite similar. Importantly, the next highest identity matches are to other cupin proteins in other dicotyledonous plants including tree nuts (walnut, cashew and hazelnut), legumes (peanut and soybean) and buckwheat. The identities though are markedly lower, though still significant according to Codex Alimentarius (2003) standards, ranging from 33% to 45% in overall full-length alignments and 45% to nearly 65% in the best overall 80 amino acid sliding window matches. These matches are discussed in the main report.

Control (*Sinapis alba* 2S albumin) bioinformatics with AllergenOnline ver. 8 Appendix 5

Because the *Brassica napus* sequences of Supertein and Puratein match allergens of non-Brassicaceae at lower identities, control comparisons were made to put them in context with matches between *Sinapis alba* 2S albumins and cupins to the allergen database.

1. GI: 1009438. Sin a 1.0106 *Sinapis alba* 2S albumin.

80mer Sliding Window Search Results

Database	AllergenOnline v8.0 (January, 2008)
Input Query	> sin a 1.0106 [Sinapis alba] GI:1009438 pagpfgipkcrkefqqaqhlracqqwlhkqamqsgsgpswtlddefdfeddmnpqgppqq rppllqqccnelhqeepfcvcpclkgaskavkqqrqqlgqqgqqgppqvqhvisriyqta thlpkvcnipqvsvcpfkktmpggs
Length	145
Number of 80 mers	66

Hit #	Defline	Best %ID	# Hits > 35%	Full Alignment			Links	
				E-val	%ID	length	NCBI	Details
1	gi 1009438 emb CAA62911.1 allergen sin a 1.0106 [Sinapis alba]	100.00 %	66of66	2.4e-36	100.00 %	145	gi 1009438	GO!
2	gi 1009442 emb CAA62908.1 allergen sin a 1.0108 [Sinapis alba]	100.00 %	66of66	3.4e-36	99.30%	145	gi 1009442	GO!
3	gi 51338758 sp P15322.2 ALL1_SINAL Allergen Sin a [Sinapis alba]	100.00 %	66of66	4.5e-35	97.20%	145	gi 51338758	GO!
4	gi 1009440 emb CAA62912.1 allergen sin a 1.0107 [Sinapis alba]	100.00 %	66of66	3.7e-35	97.20%	145	gi 1009440	GO!
5	gi 1009434 emb CAA62909.1 allergen sin a 1.0104 [Sinapis alba]	100.00 %	66of66	8.1e-35	95.90%	145	gi 1009434	GO!
6	gi 1009436 emb CAA62910.1 allergen sin a 1.0105 [Sinapis alba]	97.50%	66of66	1.4e-34	95.90%	145	gi 1009436	GO!
7	gi 407610 gb AAB27813.1 Bra j IE large chain=all [Brassica juncea]	88.78%	63of66	4e-19	89.00%	91	gi 407610	GO!
8	gi 26985163 gb AAN86249.1 AF448054_1 recombinant [Brassica napus]	53.72%	66of66	0.00011	50.40%	129	gi 26985163	GO!
9	gi 407609 gb AAB27812.1 Bra j IE small chain=all [Brassica juncea]	42.50%	10of66	1.7e-06	91.90%	37	gi 407609	GO!
10	gi 13183175 gb AAK15088.1 AF240005_1 2S albumin [Sesamum indicum]	37.50%	16of66	0.092	33.60%	131	gi 13183175	GO!

20	gi 3703107 gb AAC63045.1 glycinin [Arachis hypogaea]	49.40%	173of431	1e-13	32.70%	513	gi 3703107	GO!
21	gi 736002 emb CAA55977.1 Gys [Glycine soja]	48.78%	229of431	6.7e-15	32.70%	547	gi 736002	GO!
22	gi 6979766 gb AAF34634.1 AF216800_1 22kDa storage [Fagopyrum gracilipes]	48.78%	113of431	3.5e-16	43.90%	164	gi 6979766	GO!
23	gi 21743 emb CAA43331.1 high molecular weight gl [Triticum aestivum]	48.75%	67of431	2.3e-06	35.40%	229	gi 21743	GO!
24	gi 21751 emb CAA31396.1 high molecular weight gl [Triticum aestivum]	48.75%	77of431	0.00028	30.30%	297	gi 21751	GO!
25	gi 5712199 gb AAD47382.1 glycinin [Arachis hypogaea]	48.70%	162of431	1.4e-12	32.30%	539	gi 5712199	GO!
26	gi 806556 emb CAA60533.1 ASA4B3 subunit [Glycine soja]	48.10%	243of431	2.9e-15	31.00%	581	gi 806556	GO!
27	gi 22090 emb CAA43361.1 HMW glutenin subunit 1By [Triticum aestivum]	48.10%	77of431	6.4e-06	30.80%	318	gi 22090	GO!
28	gi 18641 emb CAA37044.1 glycinin [Glycine max]	48.10%	227of431	2.5e-15	30.50%	581	gi 18641	GO!
29	gi 170743 gb AAB02788.1 HMW glutenin subunit Ax2 [Triticum aestivum]	47.52%	66of431	1.4e-06	35.40%	229	gi 170743	GO!
30	gi 732706 emb CAA26478.1 unnamed protein product [Glycine max]	46.90%	221of431	4.2e-15	30.50%	580	gi 732706	GO!
31	gi 169969 gb AAA33964.1 glycinin [Glycine max]	46.30%	215of431	2.8e-08	32.00%	547	gi 169969	GO!
32	gi 169971 gb AAA33965.1 glycinin precursor [Glycine max]	46.30%	115of431	1.3e-14	38.70%	191	gi 169971	GO!
33	gi 112380623 gb AB117154.1 iso-Ara h3 [Arachis hypogaea]	46.22%	195of431	1.9e-14	32.90%	517	gi 112380623	GO!
34	gi 170732 gb AAA34286.1 gamma-gliadin [Triticum aestivum]	43.74%	42of431	0.0073	47.90%	73	gi 170732	GO!
35	gi 21930 emb CAA44473.1 LMW glutenin [Triticum turgidum]	43.74%	44of431	0.0039	29.00%	176	gi 21930	GO!
36	gi 170730 gb AAA34285.1 gamma-gliadin B-I precu [Triticum aestivum]	43.74%	42of431	0.007	47.90%	73	gi 170730	GO!
37	gi 62550933 emb CAI79052.1 putative LMW-glutenin [Triticum aestivum]	43.73%	48of431	0.14	50.70%	69	gi 62550933	GO!
38	gi 21926 emb CAA36063.1 unnamed protein product [Triticum turgidum]	42.50%	40of431	0.031	27.70%	176	gi 21926	GO!
39	gi 75317968 sp O22116 O22116_WHEAT LMW glutenin 3 [Triticum aestivum]	41.25%	18of431	0.03	42.70%	75	gi 75317968	GO!
40	gi 75219081 sp O22108 O22108_WHEAT LMW glutenin 1 [Triticum aestivum]	41.23%	36of431	0.012	27.20%	173	gi 75219081	GO!
41	gi 897811 emb CAA24933.1 unnamed protein product [Triticum aestivum]	39.98%	47of431	0.0064	48.40%	64	gi 897811	GO!
42	gi 21757 emb CAA26383.1 unnamed protein product [Triticum aestivum]	39.96%	24of431	0.063	31.30%	163	gi 21757	GO!
43	gi 473876 gb AAA37741.1 alpha-gliadin [Triticum aestivum]	39.50%	22of431	0.04	30.10%	283	gi 473876	GO!
44	gi 170722 gb AAA34281.1 pre-alpha-/beta-gliadin [Triticum aestivum]	39.50%	22of431	0.037	29.80%	188	gi 170722	GO!
45	gi 170736 gb AAA34288.1 gamma-gliadin [Triticum aestivum]	38.90%	29of431	0.48	29.60%	206	gi 170736	GO!
46	gi 21673 emb CAA35238.1 unnamed protein product [Triticum aestivum]	38.78%	30of431	0.0032	30.20%	182	gi 21673	GO!
47	gi 21761 emb CAA26784.1 unnamed protein product [Triticum aestivum]	37.80%	15of431	0.067	30.00%	180	gi 21761	GO!
48	gi 170708 gb AAA34276.1 gamma-gliadin B precursor [Triticum aestivum]	37.54%	4of431	4.8	28.60%	182	gi 170708	GO!
49	gi 170738 gb AAA34289.1 gamma-gliadin [Triticum aestivum]	37.54%	36of431	1.8	30.90%	178	gi 170738	GO!
50	gi 73912696 dbj BAE20328.1 omega-5 gliadin [Triticum aestivum]	37.54%	31of431	0.0064	30.60%	219	gi 73912696	GO!
51	gi 170740 gb AAA34290.1 gliadin [Triticum urartu]	37.50%	25of431	0.09	30.20%	192	gi 170740	GO!
52	gi 170718 gb AAA36279.1 alpha-/beta-gliadin precu [Triticum aestivum]	36.29%	28of431	0.0088	28.80%	208	gi 170718	GO!

53	gi 21765 emb CAA26385.1 unnamed protein product [Triticum aestivum]	36.29%	28of431	0.0046	28.80%	208	gi 21765	GO:
54	gi 21783 emb CAA30570.1 unnamed protein product [Triticum aestivum]	36.27%	28of431	0.035	50.90%	57	gi 21783	GO:
55	gi 170720 gb AAA34280.1 alpha/beta-gliadin precu [Triticum aestivum]	36.24%	15of431	0.047	28.70%	181	gi 170720	GO:
56	gi 21755 emb CAA25593.1 unnamed protein product [Triticum aestivum]	36.24%	15of431	0.047	28.70%	181	gi 21755	GO:
57	gi 170702 gb AAA34272.1 gamma gliadin precursor [Triticum aestivum]	36.24%	21of431	0.38	29.10%	151	gi 170702	GO:
58	gi 21773 emb CAA31685.1 unnamed protein product [Triticum aestivum]	35.03%	29of431	0.084	51.90%	54	gi 21773	GO:
59	gi 170734 gb AAA34287.1 gamma gliadin B-III [Triticum aestivum]	35.03%	29of431	0.073	51.90%	54	gi 170734	GO:
60	gi 170724 gb AAA34282.1 pre-alpha-/beta-gliadin [Triticum aestivum]	35.00%	2of431	0.0048	29.00%	176	gi 170724	GO:
61	gi 170712 gb AAA34276.1 pre-alpha-/beta-gliadin [Triticum aestivum]	35.00%	2of431	0.0081	28.40%	176	gi 170712	GO:

AllergenOnline Database v8.0 (January, 2008)

Summary: Sequence identities of the control proteins from yellow mustard (*Sinapis alba*), 2S albumin and 11S globulins, matched similar or the same allergens and with similar scores to those observed for the *Brassica napus* proteins. Based on the high identity scores between *Brassica napus* and *Sinapis alba* proteins and close evolutionary relationship between the taxa that is not surprising. However, cross-reactivity is most likely only shared between the mustards and rarely between mustards and tree nuts or legumes despite some sequence identity matches. Although the Codex Alimentarius Commission (2003) assessment for genetically modified crops suggests that there is a possibility of cross-reactivity for two proteins sharing greater than 35% identity over an 80 amino acid segment, evaluation of identities between mustards and other allergenic proteins show a tremendous drop in identities from mustards, to other allergenic organisms. For instance, the cruciferins of mustards share over 90% identity over the full-length, and 100% identity for the best 80 amino acid matches. The next highest matches are to tree nuts. The 11S albumin and these are in the range of 40% identity over the full-length and between 50 and 60% identical over the best 80 amino acid match. The sequence identity matches of 2S albumins are much lower to non-allergens, below 40% over 80 amino acids. However, a review of the literature does not demonstrate proven cross-reactivity between mustards and tree nuts. The only reference based on key word searches that might suggest otherwise is Robotham et al. (2005), which listed one subject of 40 tree nut allergic subjects as coincidentally having allergic reactions to mustard. Based on sequence identity matches it appears that the potential risk of cross-reactivity elicited by a protein from *Brassica napus* would be associated with other mustards (*Brassica sp.*, *Sinapis s.* and possibly *Raphinus sp.*) rather than to any non-mustard.

AM 

From: Mejia, Luis
To: Carlson, Susan;
cc: Empie, Mark;
Subject: RE: FSIS availability
Date: Tuesday, May 11, 2010 10:46:38 AM

Dear Susan:

I like to inform you that , mainly due to the time limitation since the data would have to be generated, we have decided to drop for now the meat category from the GRAS notification. So, there will be no need to have this conference call with FSIS any more. I greatly appreciate your help in putting this together, but it will not longer be necessary.

We will do the recalculation of the consumption data without the protein powders and meat products and will get back to you within two weeks.

Have a good day

Luis

From: Carlson, Susan [mailto:Susan.Carlson@fda.hhs.gov]
Sent: Tuesday, May 11, 2010 9:35 AM
To: Mejia, Luis
Subject: FSIS availability

Dear Luis,

I have received word from the FSIS representative that they are available for a teleconference on Wednesday, May 12 (any time except 10 to 11 a.m.) or Thursday, May 13 (any time except 1 to 3 p.m.). I have a meeting from 11:45 to 1:30 on Wednesday. So, I would say that Wednesday afternoon from 2 p.m. ET on would be good or Thursday morning (May 13). Do either of these time slots work for you? Can we use your conference call in number?

Thank you,
Susan

From: Mejia, Luis
To: Carlson, Susan;
Subject: RE: GRAS Notice 327: Powdered eggs
Date: Wednesday, May 12, 2010 6:28:02 PM

Susan:

Again, due to time constrains, we will also drop the egg substitutes category.

Thanks for your guidance.

Luis

From: Carlson, Susan [mailto:Susan.Carlson@fda.hhs.gov]
Sent: Tuesday, May 11, 2010 2:06 PM
To: Mejia, Luis
Subject: GRAS Notice 327: Powdered eggs

Dear Luis,

I'm sorry to bother you again today. I noticed that there is another use (in addition to the meat uses) that is under the regulatory purview of FSIS, namely powdered eggs. The food category is listed as 133 33102010, scrambled egg, made from powdered mixture. I asked FSIS about the type of data that they would need for this category and they replied:

However, for this submission we will need suitability data for evaluation by our TRT team and also organoleptic data such as texture and/or taste to show that the egg products containing the canola isolates are considered equivalent when compared against the control non- containing canola formulations.

If you want to pursue this egg use, or drop it along with the meat uses, please let me know. Also, just to be clear, the egg substitute products are under FDA's purview.

Thank you,
Susan



Archer Daniels Midland Company
Office of Compliance and Ethics
1001 North Brush College Road
Decatur, Illinois 62521

May 13, 2010

Susan J. Carlson, Ph.D.
Food and Drug Administration
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice No. GRN 000327 - Revised food categories and consumption data.

Dear Dr. Carlson:

According to our discussion during our teleconference on May 10, 2010 and subsequent e-mail communications regarding GRN 000327, I would like to inform you that due to time constraints to generate new data, we have decided to drop processed meat products (items 2510110 through 28355430) and egg substitute products (items 33000100 and 33102010) from the GRAS notification of the canola proteins (Puratein®/Supertein™). In accordance with our discussion on dietary supplements, we have also removed the protein powder categories (items 41430000 and 41430010). Therefore, the revised food categories are as follows:

Food Categories

Added up to*:	
Dairy Products	5%
Grain Products	2%
Fruit & Vegetable Juices/Beverages	10%
Salad Dressings	2%
Meal Replacements/Nutritional Bars	50%

* As Is Basis

May 13, 2010

Page 2

The re-calculated consumption estimates of the canola protein after eliminating the indicated above categories are now the following:

Consumption Estimates of Puratein®/Supertein™ in the U.S. Population

<u>Age Groups</u>	<u>n</u>	<u>Mean</u>	<u>90th Pctl.</u>
3-6 years	1891	25.6*	61.1
	1840	(1.3)**	(3.1)
7-12 years	3189	25.6	68.0
	3157	(0.7)	(1.9)
13-19 years	5174	31.9	89.0
	5120	(0.5)	(1.4)
≥ 20 years	12394	20.3	57.1
	12155	(0.3)	(0.8)

Based on 1999-2004 NHANES database (weighted data)

* g/person/day

** g/kg BW/day

As expected, consumption values decreased slightly and the observed differences in comparison to the original submission do not impact our safety assessment of the canola proteins.

I trust this information is sufficient to continue the GRAS assessment process of GRN 000327. Please do not hesitate to contact me again if you have questions or need more information.

Sincerely,

Luis A. Mejia, Ph.D.

Director, Regulatory & Scientific Affairs

LAM/jm

cc: M. Empie