859 Outer Road
Orlando, Florida 32814
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BURDOCK GROUP
Consultants

June 7, 2017

Paulette M. Gaynor, Ph.D.
Deputy Division Director
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: Notification of GRAS Status of High-Oleic Algal Oil

Dear Dr. Gaynor:

In accordance with 21 CFR §170, Subpart E – Generally Recognized As Safe (GRAS) Notice, I am submitting, as the agent of the notifier, TerraVia Holdings, Inc. (TerraVia, previously known as Solazyme, Inc.), 225 Gateway Blvd. South San Francisco, CA 94080, a notification of the conclusion of GRAS status of a high-oleic algal oil (HO algal oil) that meets the specifications of the oil previously concluded as GRAS and which was notified to the FDA on July 1, 2014 (designated as GRN 527). However, this present notification describes the production of the HO algal oil utilizing a different genetically engineered strain of *Prototheca moriformis*, and is intended to be used as an alternative to the high-oleic algal oil that was the subject of GRN 527. HO algal oil is to be used as partial replacements for conventional dietary fats or oils in the diet, such that an estimated upper consumption level of 27.47 g/day (494.83 mg/kg bw/day for eaters of this ingredient) at the 90th percentile may be reached, an increase from the GRAS determination contained in GRN 527 which estimated consumption at 12.5 g/day intake at the 90th percentile. The 90th percentile intake is typically used in the U.S. to represent a long-term or "lifetime averaged" daily intake estimate.

Please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely fashion.

Best regards,
(b) (6)

Ray A. Matulka, Ph.D.
Director of Toxicology

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000754	
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

RECEIVED
Jun 23, 2017
JUN 2? 2017
OFFICE OF FOOD ADDITIVE SAFETY

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. ____ ☐ Supplement to GRN No. ____

2. ☐ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: 5/4/2017

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: ____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
See agent	See agent

Organization *(if applicable)*: TerraVia Holdings, Inc.

Mailing Address *(number and street)*: 225 Gateway Blvd

City	State or Province	Zip Code/Postal Code	Country
South San Francisco	California	94080	United States of America

Telephone Number	Fax Number	E-Mail Address
407.802.1400		

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Ray A. Matulka, Ph.D.	Director of Toxicology

Organization *(if applicable)*: Burdock Group Consultants

Mailing Address *(number and street)*: 859 Outer Road

City	State or Province	Zip Code/Postal Code	Country
Orlando	Florida	32814	United States of America

Telephone Number	Fax Number	E-Mail Address
407.802.1400	407.802.1405	rmatulka@burdockgroup.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

ɡh-oleic algal oil

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☐ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages 51

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 527

☐ b) GRAS Affirmation Petition No. GRP

☐ c) Food Additive Petition No. FAP

☐ d) Food Master File No. FMF

☐ e) Other or Additional *(describe or enter information as above)*

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *'Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

High-oleic algal oil may be used as a partial replacement of conventional vegetable and non-vegetable oils in a variety of conventional food groups, none of which have a standard of identity, resulting in an estimated 90th percentile consumption level of 27.47 g/day by consumers of products (eater's only) of the U.S. population. The foods High-oleic algal oil will be used in are in ppm: baked goods at 20,000-70,000; baked desserts at 30,000-50,000; meal replacements at 30,000-50,000; cereals and bars at 20,000-150,000; cheese spread at 100,000; margarine and margarine-like spreads at 450,000-700,000; butter-like spreads at 700,000; vegetable oil and shorteni

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture? *(Check one)*

☒ Yes ☐ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture? *(Check one)*

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that See Agent *(name of notifier)*

has concluded that the intended use*(s)* of High-oleic algal oil *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. See Agent *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

859 Outer Road, Orlando Florida 32814
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
(b) (6) [redacted]	Ray A. Matulka, Ph.D., Director of Toxicology	06/08/2017

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert Clear GRAS Notification	1-51
	Insert Clear	
	Insert Clear	
	Insert Clear	
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	Insert Clear	

Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

1. Signed Statements and Certification

In accordance with 21 CFR §170, Subpart E – Generally Recognized As Safe (GRAS) Notice, I, Ray A. Matulka, Ph.D., am submitting a notice of the conclusion of GRAS status of the production and sale of a high-oleic algal oil (HO algal oil)[1] for the use as specified in this notification, as the agent of the notifier [TerraVia Holdings, Inc. (TerraVia, formerly known as Solazyme, Inc.)] because it has been concluded that such uses are GRAS.

Notifier:

TerraVia Holdings, Inc.
(formerly known as Solazyme, Inc.)
225 Gateway Blvd
South San Francisco, CA 94080

Agent of the Notifier:

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group
859 Outer Road
Orlando, FL 32814
Telephone: 407-802-1400
Facsimile: 407-802-1405
Email: rmatulka@burdockgroup.com

A. Name of the Notified Substance

For the purposes of this GRAS notification, the name used to describe the ingredient is:

High-Oleic Algal Oil

High-oleic algal oil is the name of the ingredient that was the subject of GRN 000527 and and as such the high-oleic algal oil that is the subject of this conclusion of GRAS status has the same specifications and uses and can therefore may be used as a substitute for the oil that was the subject of GRN 000527, and is appropriate for consumer labeling as it can be a replacement for commonly used substances with similar structure, function, and uses (*i.e.*, soybean, canola and olive oil).

B. Conditions of Use

High-oleic algal oil may be used as a partial replacement of conventional vegetable and non-vegetable dietary oils in a variety of conventional food groups (Table 1) none of which have a standard of identity,[2] resulting in an estimated 90th percentile consumption level of 27.47 g/day by the general U.S. population.

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[1] The conclusion of GRAS status specific to this notification was concluded by an Expert Panel evaluating the modification of the source organism in the production of HO algal oil as an amendment to the GRAS status for High-Oleic algal oil described in GRN 000527. The present notification incorporates the term amendment to reflect the language contained in the conclusion of GRAS status completed by the Expert Panel.

[2] All food categories designated by TerraVia have been utilized in the estimated dietary intake calculations as appropriate; however, certain categories designated by TerraVia may contain foods for which a standard of identity exists. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity. TerraVia confirms that the high-oleic algal oil will be added only

Table 1. Food groups selected for HO algal oil supplementation*

Food Category	Intended use level (ppm)
Baked goods (bread, dry mixes, rolls, bagels, biscuits, cornbread, tortillas and croutons)	20,000-70,000
Baked desserts (sweet rolls, muffins, scones, cakes, cheesecake, pie, cobbler, crisps, doughnuts, turnovers, strudel and cookies)	30,000-50,000
Meal replacement bars, drinks and protein supplements	30,000 -50,000
Cereals and bars (granola, muesli and dry cereal)	20,000-150,000
Cheese spreads	100,000
Margarine and margarine-like spreads	450,000-700,000
Butter-like spreads	700,000
Vegetable oil and shortening	700,000-1,000,000
Salad dressings and mayonnaise	500,000-700,000
Sauces, gravies and dressings	20,000-60,000
Nut spreads, nuts and seeds	50,000
Dairy and milk products (including analogs) (whipped topping, cream substitute, milk imitation and flavored)	10,000-100,000
Gelatins and puddings	30,000
Soups and broth	20,000-30,000
Meat products**	20,000-50,000
Frozen dairy desserts	30,000
Snacks (crackers, popcorn, crisp bread, salty snacks, popcorn, pretzel and chips)	145,000-350,000
Soft candy (caramels, fruit snacks, covered nuts	30,000
Confectionary (icing and marshmallows)	20,000-250,000

*The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category. ** Meat Products (mixtures), including all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation; ppm=parts *per* million.

C. Basis of GRAS Determination

Pursuant to 21 CFR §170.3, a conclusion of continued GRAS status through scientific procedures, in accordance with 170.30(a) and (b), was found for the use of high-oleic algal oil as an ingredient in food for its intended conditions of use.

D. Premarket Approval Exemption

TerraVia Holdings, Inc. (TerraVia, previously known as Solazyme, Inc.), 225 Gateway Blvd. South San Francisco, CA 94080, has concluded that high-oleic algal oil, derived from non-toxigenic classically and genetically modified strains of *Prototheca moriformis*, is generally recognized as safe (GRAS) as a food ingredient and therefore, exempt from the requirement of premarket approval of the Federal Food, Drug and Cosmetic Act and from environmental impact, under the conditions of its intended use.

E. Availability of Information

The data and information that serve as a basis for this GRAS determination are available for FDA review and copying at reasonable times at:

Burdock Group
859 Outer Road
Orlando, FL 32814

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to foods for which a standard of identity does not exist or to those foods with a standard of identity that does not specify a particular fat.

Telephone: 407-802-1400
Facsimile: 407-802-1405
Email: rmatulka@burdockgroup.com

Alternatively, a copy of the data and information that serve as a basis for this GRAS conclusion may be sent *via* electronic format or on paper to FDA upon request.

F. Freedom of Information Act Exemption

None of the information that is being provided in this notification on the conclusion of GRAS status for the continued use of HO algal oil under the conditions stated in the GRAS conclusion is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552 (*e.g.*, as trade secret or as commercial or financial information that is privileged or confidential).

G. Certification

The undersigned author of this notification of the conclusion of GRAS status of HO algal oil, utilizing information by reference from GRN 000527 and other scientific information, hereby certifies that, to the best of their knowledge, this notification is a complete, representative and balanced representation of all available information, favorable as well as unfavorable, known by the author to be pertinent to the evaluation of the safety and GRAS status of the use of HO algal oil.

Signed,

(b) (6) [redacted] Date June 8, 2017

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group
859 Outer Road
Orlando, FL 32814
Telephone: 407-802-1400
Facsimile: 407-802-1405
Email: rmatulka@burdockgroup.com

H. United States Department of Agriculture, Food Safety Inspection Service Review

Where applicable, as required by §170.270, FDA is authorized to send any trade secrets to the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) or ask FDA to exclude any trade secrets from the copy of the GRAS notice that will be sent to FSIS.

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2. Detailed Information about the Identity of the Notified Substance

A. Identity of high-oleic algal oil

High-oleic algal oil (HO algal oil) is a clear, pale to wheat yellow-colored, refined, bleached and deodorized oil with high levels of oleic acid, produced from a strain of *Prototheca moriformis* that has been modified *via* classical mutagenesis and targeted genetic modification.

The HO algal oil provided in this conclusion of GRAS status meets specifications of the algal oil described in GRN 000527, except for a change to the *p*-anisidine specification and, as shown in Table 2, the HO algal oil produced using strain *P. moriformis* S6697 meets specifications stated in GRN 000527 when evaluated under the revised *p*-anisidine specification. The *p*-anisidine specification was ≤ 2% in the GRAS that was the subject of GRN 000527 but has been amended to ≤ 10% to align with and reflect *p*-anisidine levels[3] noted in the industry for food oil quality.

Table 2. Specifications for HO algal oil.

			Batch Analysis Results of current HO algal oil (n = 3)	
Parameter	**Method**	**Specification**	**Range**	**Average**
Appearance	Visual inspection	Clear, pale yellow to wheat yellow	Conforms	Conforms
Odor	Olfactory inspection	Slight	Conforms	Conforms
Fatty acid profile	C-M-00036-000 Rev 0[a]			
Oleic acid (C18:1)		≥80 Area %	87.89 – 88.70 Area %	88.27 Area %
Linoleic acid (C18:2)		≤10 Area %	0.99 – 1.93 Area %	1.53 Area %
Alpha-Linolenic acid (C18:3 *alpha*)		≤0.8 Area %	0.16 – 0.27 Area %	0.20 Area %
Total Saturated Fat	C-M-00036-000 Rev 0[a]	≤15 Area %	7.85 – 8.38 Area %	8.06 Area %
Free Fatty Acids	AOCS Ca 5a-40	≤0.1 %	0.03 %	0.03 %
Moisture Content	C-M-00118-000Rev 1[b]	≤0.1 %	0.01 – 0.09 %	0.04 %
Unsaponifiable Matter	AOCS Ca 6a-40	≤1.0 %	0.20 – 0.70%	0.37%
Peroxide Value	AOCS Cd 8-53	≤5 meq/kg	0 – 1.18 meq/kg	0.59 meq/kg
p-Anisidine Value	ISO 6885	≤ 10 %	0.20 – 0.59 %	0.40 %
Elements by ICP				
Lead	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Arsenic	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Mercury	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Cadmium	AOCS Ca 17-01	<0.1 ppm	<0.03 ppm	<0.03 ppm
Phosphorus	AOCS Ca 20-99	≤0.5 ppm	<0.20 ppm	<0.20 ppm
Sulfur	AOCS Ca 17-01	≤3 ppm	<0.50 ppm	<0.50 ppm

[a] C-M-00036-000 Rev 0, Total Deliverable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification (internal method; APPENDIX I);
[b] C-M-00118-000 Rev 1, Karl Fischer Moisture Determination of Oil (internal method; APPENDIX II).
AOCS = American Oil Chemists' Society; ISO = International Standards Organization; meq = milliequivalents; ppm = parts *per* million.

[3] Edible marine oils evaluated under the GOED Organization suggest a maximum *p*-anisidine value of 20 (GOED, 2015), while others in the edible oil industry suggest a *p*-anisidine value of 10 as an indicator of good quality oil (Halvorsen and Blomhoff, 2011; Moigradean *et al.*, 2012; Yun and Surh, 2012; Miller, Unknown).The *p*-anisidine value is a measure of secondary oxidation, mainly of aldehydes such as 2,4-dienals and 2-alkenals, which correlate with overall oil odor intensity (Yun and Surh, 2012).

A.1. Composition of high-oleic algal oil

The fatty acid composition analysis of high-oleic algal oil (Table 3) confirms that the levels of the major fatty acids produced as discussed in this notification are within specifications described in the conclusion of GRAS status for HO algal oil as described in GRN 000527 and as such are compositionally comparable, and are not different from the levels of the fatty acids found in some commonly consumed oils. Current lot analyses of HO algal oil show that oleic acid is the predominant fatty acid (~88% TFA[4]), followed by palmitic (~3.5% TFA) and stearic fatty acids (~3.7% TFA), with low levels of linoleic (~1.53% TFA) and *alpha*-linolenic (~0.22% TFA) fatty acids. All specified fatty acid levels are within current specifications for HO algal oil provided in GRN 000527, and similar in composition to other high oleic acid-containing oils, except for the reduction in linoleic acid (Table 3).

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[4] TFA = Total fatty acid.

Table 3. Fatty acid composition (% TFA) for three lots of current HO algal oil, compared with HO algal oil data from GRN 000527 and conventional vegetable oils.

Fatty Acids (Area %)	S2532 HO algal oil Mean* (n=3)[a]	S6697 HO algal oil Lot: RBD750	S6697 HO algal oil Lot: RBD850	S6697 HO algal oil Lot: RBD854	S6697 HO algal oil Mean	Olive oil[b]	Canola oil[b]	Soybean oil[b]
C10:0 (Capric)	0.05	0.01	0.02	ND	0.02[c]	ND	ND	NR
C12:0 (Lauric)	0.14	0.02	0.03	0.03	0.03	ND	ND	ND
C14:0 (Myristic)	0.82	0.38	0.37	0.42	0.39	ND	ND	ND
C14:1	0.02	ND	ND	ND	ND	ND	ND	ND
C15:0 (Pentadecanoic)	0.05	0.01	0.01	ND	0.01[c]	NR	ND	ND
C16:0 (Palmitic)	8.11	3.87	3.02	3.72	3.54	11.29	4.30	10.46
C16:1	0.97	0.18	0.14	0.13	0.15	1.26	0.21	ND
C17:0 (Heptadecanoic)	0.06	0.04	0.04	0.05	0.04	0.02	ND	0.03
C17:1	0.10	ND	0.03	0.05	0.04[c]	0.13	ND	ND
C18:0 (Stearic)	1.55	3.73	3.99	3.27	3.67	1.95	2.09	4.43
C18:1 (Oleic)	86.66	88.70	87.89	88.22	88.27	71.27	61.77	22.55
C18:2 (Linoleic)	0.08[c]	0.99	1.68	1.93	1.53	9.76	19.01	50.95
C18:3 (α-Linolenic acid)	0.02[c]	0.17	0.16	0.27	0.22	0.76	9.14	6.79
C20:0 (Arachidic)	0.24	0.25	0.34	0.26	0.28	0.41	0.65	0.36
C20:1	0.64	0.28	1.07	0.73	0.84	0.31	1.32	0.23
C22:0 (Behenic)	0.08[c]	0.05	0.07	0.06	0.06	0.13	0.33	0.37
C22:1, *cis*-13 (Erucic)	0.01[d]	ND	ND	ND	ND	ND	ND	ND
C22:5n3	0.02[c]	ND	ND	ND	ND	ND	ND	NR
C24:0 (Lignoceric)	0.05	0.02	0.05	0.04	0.04	ND	NR	NR
Total Fatty Acids Identified	99.40	98.70	98.91	99.18	99.08	97.29	98.82	96.17

*Values stated in original GRAS determination (notified to FDA as GRN 527) for HO algal oil; n = number; ND = Analyzed, but not detected; NR = Not reported; TFA = Total fatty acid; RBD = refined, bleached, deodorized; only fatty acid values $\geq$ 0.02 are reported;

[a] n = 3, unless otherwise specified;

[b] USDA National Nutrient Database for Standard Reference, Release 26 (<http://ndb.nal.usda.gov/ndb/search/list>; site accessed January 20, 2014);

[c] n = 2

[d] n = 1

A.2. Source Organism Taxonomic Analysis

The genus *Prototheca*, which is composed of achlorophyllous,[5] eukaryotic microalgae, belongs to the Trebouxiophyceae class in phylum Chlorophyta. The more familiar *Chlorella* genus is also a member of the same class in the same phylum. Although *Prototheca* are occasionally referred to as 'colorless *Chlorella*' due their lack of chloroplasts and photosynthetic pigments and close relationship with *C. protothecoides* (aka *Auxenochlorella protothecoides*), *Prototheca* have several additional differentiating traits when compared with *Chlorella* spp.; *Prototheca* spp. are acidophilic, are thiamine auxotrophs, and cannot utilize nitrate as a sole nitrogen source, but can use a variety of hydrocarbons as sole carbon sources (Running *et al.*, 2003). Of all the heterogeneous species that comprise the *Chlorella* genus, only *C. protothecoides* (*A. protothecoides*) shares most of these distinguishing characteristics and is recognized to be particularly closely related to *Prototheca* spp. (Conte and Pore, 1973; Running *et al.*, 2003).

Prototheca are spherical or ovoid unicellular heterotrophic eukaryotes (3 – 30 µm in diameter) that have double-layered cell walls which, similar to *C. protothecoides*, contain sporopollenin.[6] These achlorophyllous green microalgae lack chloroplasts and buds (Lass-Florl and Mayr, 2007; Hillesheim and Bahrami, 2011; Mayorga *et al.*, 2012). Reproduction is by asexual endosporulation; two to 16 endospores (2 – 10 µm), symmetrically or randomly arranged in the sporangia, develop until they emerge *via* rupture of the parent sporangial cell wall (DiPersio, 2001; Lass-Florl and Mayr, 2007; Hillesheim and Bahrami, 2011; Mayorga *et al.*, 2012). In the presence of adequate nutrients, reproductive cycles with release of sporangiospores can occur every 5 - 6 hours (Lass-Florl and Mayr, 2007).

Prototheca spp. are ubiquitous in the environment. First isolated in 1892 – 1894 from the slime fluxes (*i.e.*, sap released from tree wounds) of lime, horse chestnut and elm trees in Germany (Krüger, 1894), *Prototheca* spp. have since been isolated from a wide variety of sources,[7] including environmental sources such as soil, decaying plant matter, sewage, and water, as well as from human and animal sources (*e.g.*, feces, milk, sputum, and/or lesions) (Shahan and Pore, 1991; Mayorga *et al.*, 2012).

The number of species assigned to the *Prototheca* genus has varied over the years as understanding of the phylogenetic relationships has increased. A majority of the scientific literature currently recognizes five species in the genus: *P. wickerhamii*, *P. zopfii*, *P. stagnora*, *P. ulmnea*, and *P. blaschkeae* (Roesler *et al.*, 2006; Marques *et al.*, 2008; Mayorga *et al.*, 2012). Arholdt *et al.* (2012) recognizes *P. cutis* as a sixth. Although *P. moriformis* has at times been considered a species (Pore, 1985; Roesler *et al.*, 2003; Ueno *et al.*, 2005; Jagielski and Lagneau, 2007), the assignment has generally not been accepted as valid (Pore, 1985; Roesler *et al.*, 2003; Ueno *et al.*, 2003; Ueno *et al.*, 2005; Lass-Florl and Mayr, 2007; Marques *et al.*, 2008; Pore, 2011; Mayorga *et al.*, 2012) During a recent interval that *P. moriformis* was considered a species and *P. blaschkeae* was categorized as a variant (biotype) of *P. zopfii* (Roesler *et al.*, 2006; Mayorga *et al.*, 2012), sequence analysis of the 18S rRNA gene identified *P. blaschkeae* as a species rather than a

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[5] Achlorophyllous = without photosynthetic pigments.
[6] Rare but natural oxidative carotenoid polymers that occur in a few microorganisms and plants.
[7] Of 100 randomly selected strains of *Prototheca* held in the private culture collection belonging to Professor R. Scott Pore at the West Virginia University Medical School, Charleston WV), 7 had been isolated from humans, 35 from animals, and 58 from the environment (Shahan and Pore, 1991; Running *et al.*, 2003).

biotype of *P. zopfii* and *P. moriformis* as a biotype of *P. zopfii* rather than its own species. To explain, *P. zopfii* had initially been differentiated biochemically and serologically into three variants (Roesler *et al.*, 2003; Lass-Florl and Mayr, 2007; Pore, 2011). Variant 3, which consisted primarily of strains isolated from swine farms, was reclassified as the novel species *P. blaschkeae*, a reassignment based on 18S rRNA gene sequence analysis and cellular fatty acid composition. The other two variants, although closely related, are recognized to differ strongly in their immunogenic structures and their association with disease: variant 2 consists of isolates associated with bovine protothecal mastitis and known to be opportunistically pathogenic, whereas variant 1 strains which are isolated from cow manure and barns are not associated with pathogenicity (Roesler and Hensel, 2003). Examination of the *P. zopfii* variants by 18S rRNA gene sequence analysis and cellular fatty acid composition led to the recommendation that variants 1 and 2 be reclassified as genotypes 1 and 2, respectively, while being evaluated as potential subspecies of *P. zopfii* (Roesler *et al.*, 2006; Lass-Florl and Mayr, 2007). During the study, *P. moriformis* ATCC[8] 50081 (aka RSP-1216; isolated in 1983 by R.S. Pore from cheese factory wastewater, Costa Rica[9]) was found to group closely, not with either of the genotypes, but with a central *P. zopfii* clade[10] which included *P. zopfii* var. *hydrocarbonea* ATCC 30253 (aka 48-Y; isolated by J.D. Walker in 1973 from Colgate Creek sediment, Baltimore Harbor, Chesapeake Bay, Maryland[11]) and *P. zopfii* UTEX[12] 178 (aka SAG[13] 263-1, isolated by M. W. Beijerinck before 1912 from an unknown source[14]). The authors recommended further study to determine whether this clade comprises a third subspecies (Roesler *et al.*, 2006).

Of the currently recognized species of *Prototheca*, *P. zopfii*, *P. wickerhamii*, and *P. blaschkeae* each contain strains that have been documented as opportunistic pathogens causing rare infections (*i.e.*, protothecosis) in humans and domestic and wild animals. *P. wickerhamii* is most often associated with the rare infections reported in humans. In a typical year, two to five cases of infection are reported globally; from 1964 – 2012, a total of 160 cases of human protothecosis have been reported (Todd *et al.*, 2012). Infection in humans is often associated with traumatic introduction into the skin and underlying tissues, and systemic infection is usually associated with severe immunosuppression or underlying disease (Lass-Florl and Mayr, 2007; Mayorga *et al.*, 2012). *P. zopfii* genotype 2 is most often associated with infection in animals, primarily bovine mastitis in cows and occasionally infections in dogs, but is rarely associated with disease in humans. Nearly all *P. blaschkeae* strains have been isolated from swine, although the first isolate of the species originated from a case of human onychomycosis[15] and the strains are now also associated with bovine mastitis (Roesler *et al.*, 2006; Marques *et al.*, 2008). Bovine protothecal mastitis, and indeed mastitis caused by environmental microorganism in general, is believe to be primarily a disease of poor hygiene practices and dairy management rather than due to presence or absence of the microorganism, as *Prototheca* spp. have been identified as present in the environments of dairy cattle with and without protothecal mastitis, in the environments of

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[8] ATTC = *American Type Culture Collection*

[9] <http://www.atcc.org/Products/All/50081.aspx>; site accessed January 28, 2014.

[10] Clade = a group of biological taxa (as species) that includes all descendants of one common ancestor

[11] <http://www.atcc.org/Products/All/30253.aspx>; site accessed January 28, 2014.

[12] UTEX = University of Texas Culture Collection of Algae, Austin, Texas

[13] SAG = Sammlung von Algenkulturen Göttingen, Albrecht-von-Haller-Institute for Plant Science, University of Göttingen, Göttingen, Germany

[14] <http://sagdb.uni-goettingen.de/detailedList.php?str_number=263-1>; site accessed January 28, 2014.

[15] 'Fungal' infection of the nail and/or nail bed.

dairy farms with no prior history of mastitis caused by this agent (Anderson and Walker, 1988; Pore and Shahan, 1988; Enders and Weber, 1993; Corbellini *et al.*, 2001; Janosi *et al.*, 2001).

A.3. Wild-type strain *Prototheca moriformis* Krüger

TerraVia obtained wild-type strain *P. moriformis* Krüger UTEX 1435,[16] from the University of Texas Culture Collection of Algae in Austin, Texas, where it had been deposited by W.B. Cooke in 1966. After undergoing clonal purification to ensure the culture was axenic (free of any contaminating organisms), UTEX 1435 was assigned TerraVia internal strain number 'S376'. Other deposits of this strain[17] include the ATCC 16525[18] accession held at the American Type Culture Collection (ATCC, Rockville, Maryland); Hopkins IV 7.3.2.1[18] assigned by C.B. van Niel at the Hopkins Marine Station, Stanford University (Pacific Grove, California) (Sudman and Kaplan, 1973); and the SAG 263-7[19] accession deposited by M.B. Allen held in the Sammlung von Algenkulturen Göttingen (SAG) (Albrecht-von-Haller-Institute for Plant Science, University of Göttingen, Göttingen, Germany). Believed to have been isolated prior to 1894 by W. Krüger from the sap of a lime tree near Jena, Germany and a horse chestnut tree in the botanical gardens at Halle an der Saale in Germany, the strain was most likely originally named *Prototheca zopfii* Krüger[20] (Krüger, 1894) and designated Pr-9 (*Prototheca* 9th strain) when deposited in the collection at the Microbiology Department, Delft Technical School, Delft, The Netherlands. The strain was later transferred to C.B. van Niel and deposited in the collection at Hopkins Marine Station (Stanford University, Pacific Grove, CA) where the accession was designated Hopkins IV 7.3.2.1, before being transferred by van Niel to W.B. Cooke who deposited the strain in UTEX (where the accession was designated *P. moriformis* Krüger UTEX 1435) and in the ATCC (where the accession was designated *P. moriformis* Krüger ATCC 16252). The accession deposited into the SAG by M.B. Allen (designated *P. zopfii* Krüger SAG 263-7) had also been transferred by C.B. van Niel from the Hopkins collection. *P. moriformis* UTEX 1435 is not the type strain for either *P. moriformis*[21] or *P. zopfii*.[22] Although the ATCC preserves *P. moriformis* strain ATCC 16525 under Biosafety Level (BSL) 1 conditions[18] which indicates the microorganism is not recognized to cause disease in immunocompetent adult humans, the SAG preserves identical accession SAG 263-7 under BSL 2 conditions[19] which indicates the microorganism is rarely associated with human disease and of low concern to laboratory staff.

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[16] <http://web.biosci.utexas.edu/utex/algaeDetail.aspx?algaeID=3871>; site accessed January 9, 2014.

[17] Although the CDC B-1444 accession deposited into the Center for Disease Control (CDC, Atlanta, Georgia) and the NRRL Y-6865 accession held by the Agriculture Research Service (ARS) Culture Collection (Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois) had been assigned to *P. moriformis* ATCC 16525 as identical strains (Sudman and Kaplan, 1973), they were later determined to be strains of *P. wickerhamii* (Pore, 1985).

[18] <http://www.atcc.org/Products/All/16525.aspx >; site accessed January 9, 2014.

[19] <http://sagdb.uni-goettingen.de/detailedList.php?str_number=263-7>; site accessed January 9, 2014.

[20] During this same time period, a second distinct strain of *Prototheca* was isolated from the slime flux of an elm tree at Halle an der Saale, Germany, and named *Prototheca moriformis* Krüger (Krüger, 1894).

[21] No type strain for *P. moriformis* is recognized (Pore, 1985).

[22] *P. zopfii* Krüger SAG 263-4 (aka, UTEX 327; formerly *Prototheca portoricensis* var. *trispora* Ashford, Ciferri et Dalmau), originally isolated from a human case of enteropathia by B. K. Ashford before 1930, is the *P. zopfii* type strain (Roesler *et al.*, 2003).

A.4. Modified *Prototheca moriformis*

In the original conclusion of GRAS status for HO algal oil as detailed in GRN 000527, the HO algal oil was produced from *P. moriformis* strain S2532, a classically improved and genetically modified strain originating from the wild-type *P. moriformis* Kruger strain UTEX 1435 (designated by TerraVia as S376). To summarize, S376 was subjected to classical mutagenesis, resulting in a strain with increased triglyceride production, productivity and yield of oil. This strain was then subjected to genetic engineering in which the *Saccharomyces cerevisiae*[23] sucrose invertase gene (*SUC2*) was inserted to serve as a selectable marker by conferring the ability to utilize sucrose as a carbon source. In addition, a thioesterase gene from *Carthamus tinctorius*, the safflower plant, was inserted. This gene encodes for a carrier protein that plays an essential role in oleic acid chain termination during *de novo* fatty acid synthesis. These genetic modifications resulted in strain S2532, which grows on sucrose and produces an oleic acid-rich oil (>80%).

TerraVia continued to analyze classically improved strains originating from S376 for more favorable attributes. S376 underwent a classical strain improvement regime using chemical mutagenesis to generate S1331, the source strain for S2532. S1331 then underwent further classical strain improvement, using both chemical and UV mutageneses, which resulted in a classically improved strain, S5100, which produced a higher *percentage* of oleic acid, compared to the originator strain S376.

S5100 was then transformed to disrupt a single copy of an endogenous thioesterase gene while overexpressing an endogenous *P. moriformis* synthase gene to increase the ability for fatty acid chain elongation. Fatty acid biosynthesis occurs in the plastid cellular compartment of *P. moriformis*. Here, chain elongation of fatty acid synthesis occurs *via* condensation reactions of an acyl-group with malonyl-ACP, resulting in the attachment of two carbon atoms *per* elongation cycle. An endogenous thioesterase gene in S5100 was disrupted by homologous recombination through biolistic transformation, also known as particle bombardment, resulting in ablation of the gene (Figure 1). The thioesterase is involved in terminating the fatty acid chain elongation at C16:0, without which, fatty chain elongation continues to C18:0. As part of the gene disruption cassette, two additional genes were expressed. The first is the *Saccharomyces cerevisiae SUC2* gene encoding sucrose invertase which confers the ability to utilize sucrose as a carbon source and serves as a selectable marker. The second gene is an endogenous *P. moriformis* synthase gene whose overexpression results in higher levels of synthase protein which promotes fatty acid chain elongation from C16:0 to C18:0. The C18:0 fatty acids then undergo desaturation resulting in C18:1 (oleic acid). Southern blot analysis using *SUC2* specific probes shows that the resulting strain, S5587, contains a single copy of the *SUC2* gene (Figure 2).

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[23] Typical yeast used in the production of bread, wine and other foods.

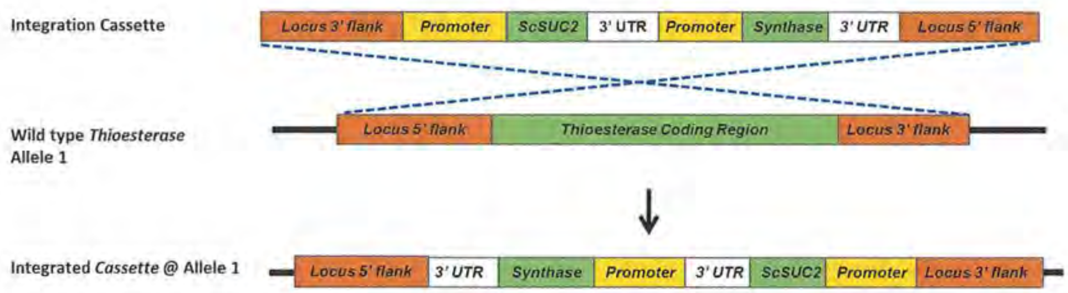

Figure 1. Schematic of the integration cassette targeting the endogenous thioesterase locus.

Strain S5587 was subsequently transformed by targeted integration at a neutral locus with a RNAi cassette expressing two genes. The first is the *Saccharomyces carlsbergensis*[24] *MEL1* gene encoding a secreted melibiase which serves as a selectable marker as well as conferring the ability to grow on melibiose. Melibiase is an enzyme that has a long history of safe commercial use in the food industry. For example, melibiase is used in the sugar refining industry for the conversion of raffinose to galactose and sucrose (Furia, 1980). The second gene is a hairpin RNAi cassette designed to reduce expression of an endogenous *P. moriformis* desaturase gene that introduces double bonds in the fatty acyl chain, leading to decreased formation of polyunsaturated fatty acids (*e.g.*, linoleic acid).

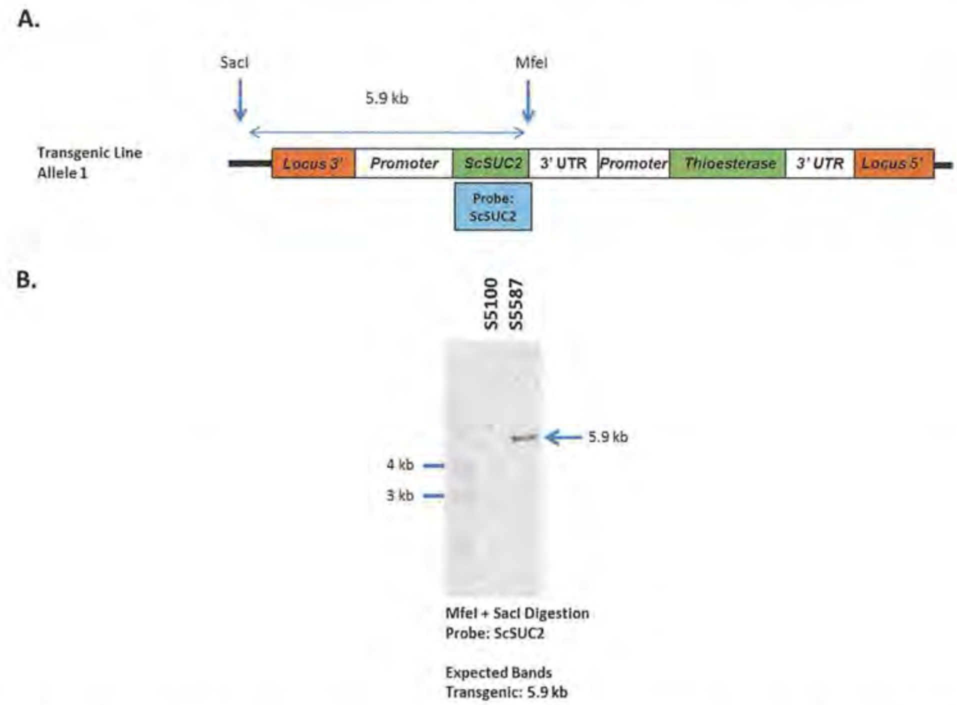

Figure 2. Southern blot analysis of DNAs isolated from S5587 and S5100, probed with a *ScSUC2* specific probe. A. The integration locus in S5586 is shown along with a *ScSUC2* specific probe and expected band size when digested with MfeI and SacI. B. Southern blot analysis of S5587 and S5100 shows an expected, single integrative band of 5.9 kb when probed with a *SUC2* specific probe.

[24] *S. carlsbergensis* is a yeast used extensively in the production of lager beer (Wendland, 2014).

The resulting strain, S6697, produces approximately 3% C16:0 (palmitic acid), 2% C18:0 (stearic acid) and 90% C18:1 (oleic acid) and is currently used in the production of HO algal oil. In summary, the genetic modifications were a reduction in the expression of an endogenous gene, an increase in expression of an endogenous gene, and insertion of two genes as selectable markers that allow for use of sucrose and melibiose as carbon sources. These genetic modifications in the strain that now produces HO algal oil are similar to the modifications in the production strain S2532 that was described in GRN 000527. The HO algal oil containing a high level of oleic acid with reduced levels of linoleic acid utilizing production strain S6697 meets the same specifications as the HO algal oil produced with S2532. There is no reason to believe that HO algal oil produced from S6697 would have a safety profile different from HO algal oil produced from S2532.

The stability of the transgenes inserted into strain S6697 was assessed by Southern blot analysis, in which cells resulting from a high cell density fermentation were grown in liquid culture with glucose as the sole carbon source (non-selective media), then plated onto media containing glucose. Individual colonies (n=48) were then transferred to a medium with melibiose as the sole carbon source. All 48 of the 48 transferred colonies exhibited growth on the melibiose-containing plate after more than 30 generations of growth in the absence of the melibiose selection, indicating 100% stability of the inserted transgenes.

Prior to insertion of the hairpin gene sequence, the oil produced from strain S5587 was analyzed for production of algal and cyanobacterial toxins by liquid chromatography with tandem mass spectrometric detection for: amnesic shellfish poisoning toxins (domoic acid), diarrrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxin-1, pectenotoxin-2, azaspiracid-1, yessotoxin, and homo-yessotoxin), paralytic shellfish poisoning toxins (gonyautoxins 1-6; decarbamoylgonyautoxins 2 and 3; saxitoxin; decarbamoylsaxitoxin; neosaxitoxin and ciguatoxins 1-4), cyanobacterial toxins (microcystin-RR, -YR, -LR, -LW, -LF, -LA, -WR, -LY and -HtyR and dm-microcystin-RR and -LR), nodularin, anatoxin and cylindrospermopsin. No toxins were detected above the limits of detection (Food GmbH Jena Analytik, 2013), consistent with the HO algal oil produced by strain S2532 described in GRN 000527. This corroborative information on the absence of algal and cyanobacterial toxins indicates that altering the expression of endogenous genes in this strain lineage does not induce toxin formation or toxin levels in the resulting oil.

S6697 strain was evaluated utilizing the Pariza and Johnson (2001) decision tree that was originally devised for the evaluation of microbial enzyme preparations to be used in food processing, but can be utilized in a general fashion in evaluating the safety of products generated from genetically engineered microorganisms. Strain S6697 meets the first aspects of the decision tree, as it is genetically modified through recombinant DNA techniques, while the expressed product (HO algal oil) has a history of safe use and is free of transferable antibiotic resistance gene DNA (no antibiotic resistance genes were used in the production of S6697). All of the introduced DNAs are well characterized, as discussed above, and are integrated into the genome in a well-defined, non-random nature.

The *P. moriformis* strain lineage is considered a safe-strain lineage, as the *P. moriformis* microalgae is not recognized in the scientific literature to be associated with pathogenicity, and the production of a refined, bleached, and deodorized oil would remove the potential for viable algal organisms in the final HO algal oil product. Pariza and Johnson (2001) concluded for enzyme preparations that "pathogenic potential is not usually an area of concern for consumer safety because enzyme preparations rarely contain viable organisms", but can still be evaluated. The same conclusion of a lack of viable organisms holds true for the production of a highly refined oil, as no viable organisms have been detected in the resulting oil when produced through the TerraVia HO algal oil production process. In addition, a corroborative study on a closely related *P. moriformis* strain (derived from the same source strain) was not pathogenic when evaluated in a study designed to determine if microbial agents would would become systemic in the body and survive or be pathogenic after oral administration (Solazyme, 2012).[25] As no viable organisms remain in the highly refined oil, this pathogenicity study is corroborative to the safety of the final HO algal oil product. Based on the data described and evaluated through the Pariza and Johnson (2001) decision tree, the genetic changes utilized do not adversely impact the safety of *P. moriformis* strain S6697 as a production strain, and the strain changes discussed above do not affect the safety or identity of the GRAS status of HO algal oil. Such process changes are necessary to improve the product for the benefit of the consumers.

B. Method of Manufacture

The source organism used to produce HO algal oil has been substantively changed (described in section A.4 of this notification), compared to the source organism detailed in GRN 000527. However, the remainder of the manufacturing process of the HO algal oil described in the conclusion of GRAS status for HO algal oil produced via strain S6697 has not changed since the original conclusion of GRAS status was finalized and detailed in GRN 000527. To summarize, the fermentation substrate used in the production of the HO algal oil has not changed since the original conclusion of GRAS status. HO algal oils are produced using processes that incorporate high temperatures and pressure. After growth of the oil-producing *P. moriformis* strain, the final broth is inactivated by heat in an evaporator, resulting in complete loss of microalgal viability. After processing through the evaporator, the inactivated broth is sent to a drum dryer and dried at high temperatures, which provides a redundant inactivation step. Following mechanical extraction, the oil itself undergoes a traditional refining, bleaching, and deodorizing (RBD) process just like other plant oils (AOCS, 2011).

Due to the RBD process, refined oils are normally free from microbial contamination and do not contain sufficient water or moisture needed for microbial growth (ICMSF, 2005). HO algal oil has less than 0.1% moisture (Table 2), similar to other refined food oils. Testing of 3 lots of HO algal oil for microbial content (Table 4) shows no microbial growth in HO algal oil produced *via* strain S6697.

Table 4. Microbial data on 3 lots of HO algal oil produced *via* strain S6697

Sample ID #	RBD 850-C	RBD 831-A	RBD 789-C
Aerobic Plate Count	<10(/g)	<10(/g)	<10(/g)
Coliforms	<3(/g)	<3(/g)	<3(/g)
E. coli	Negative(/10g)	Negative(/10g)	Negative(/10g)
Mesophilic Aerobic Spores	<1.0(/g)	<1.0(/g)	<1.0(/g)
Mold	<10(/g)	<10(/g)	<10(/g)
Pseudomonas aeruginosa	Negative(/25g)	Negative(/25g)	Negative(/25g)
Salmonella	Negative(/375g)	Negative(/375g)	Negative(/375g)
Staphylococci	Negative(/25g)	Negative(/25g)	Negative(/25g)
Yeast	<10(/g)	<10(/g)	<10(/g)

[25] Study based on the U.S. EPA Health Effects Test Guidelines, OPPTS 885.3050, Acute Oral Toxicity/Pathogenicity Study (1996) and U.S. FDA Toxicological Prinicples of the Safety Assessment of Food Ingredients, Redbook, 2000, IV.C. 3a: Short-Term Toxicity Studies with Rodents (2003).

Sample ID #	RBD 850-C	RBD 831-A	RBD 789-C

#All sample results are provided as colony forming units (CFU).

To confirm the presence or absence of algal DNA in the finished oil, HO algal oil was assayed using quantitative Real-Time quantitative polymerase chain reaction (Real-Time qPCR). Two genes present in the oil-producing *P. moriformis* strain were targeted for specific amplification and quantification: an endogenous algal gene encoding a desaturase which serves as an internal genomic control and the inserted sucrose invertase (*SUC2*) transgene.

Results showed no detection of the presence of DNA, endogenous or transgenic, in the HO algal oil or the negative controls, as evidenced by the lack of specific amplification. DNA was only detected in positive controls, where neat refined, bleached, deodorized (RBD) HO algal oil samples were spiked with genomic *P. moriformis* DNA (gDNA) in amounts ranging from 10 ng to 0.01 ng (Table 5). All samples were run in triplicate and three independent runs were conducted. This corroborative study data indicates that the RBD process removes source organism DNA from the oil, as indicated by the absence of representative endogenous and inserted DNA in the RBD HO algal oil.

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Table 5. Real-time qPCR cycle threshold for endogenous and inserted genes from *P. moriformis* genomic DNA in HO algal oil (Yu and Zhao, 2015). Samples were assayed for specific amplification with primers targeting the endogenous desaturase gene (A) or the inserted SUC2 transgene (B). The results shown are mean ± standard deviation in triplicate, for each of the three replications.

A. **Endogenous Desaturase Gene**

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev.	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	21.01	0.443	20.54	0.498	21.59	0.045
	1 ηg	24.47	0.495	25.27	0.230	25.11	0.294
	0.1 ηg	26.80	0.769	32.27	0.548	30.67	1.254
	0.01 ηg	32.47	0.743	34.74	0.640	33.20	0.944
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

B. **Inserted *SUC2* Transgene**

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	18.05	0.333	19.44	0.423	20.41	0.553
	1 ηg	23.64	0.451	24.81	0.326	23.26	0.435
	0.1 ηg	24.62	0.599	26.74	0.086	28.45	2.862
	0.01 ηg	26.78	0.402	31.65	0.076	32.95	1.323
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

NTC = negative controls containing no DNA; Cq = quantitation cycle at which fluorescence from amplification exceeds background fluorescence; ηg = nanogram.

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C. Specifications

The HO algal oil for the use as an ingredient in foods is manufactured to meet specification as indicated in Table 6.

Table 6. Specifications for HO algal oil.

Parameter	Method	Specification	Batch Analysis Results of current HO oil (n = 3)	
			Range	Average
Appearance	Visual inspection	Clear, pale yellow to wheat yellow	Conforms	Conforms
Odor	Olfactory inspection	Slight	Conforms	Conforms
Fatty acid profile	C-M-00036-000 Rev 0[a]			
Oleic acid (C18:1)		≥80 Area %	87.89 – 88.70 Area %	88.27 Area %
Linoleic acid (C18:2)		≤10 Area %	0.99 – 1.93 Area %	1.53 Area %
Alpha-Linolenic acid (C18:3 *alpha*)		≤0.8 Area %	0.16 – 0.27 Area %	0.20 Area %
Total Saturated Fat	C-M-00036-000 Rev 0[a]	≤15 Area %	7.85 – 8.38 Area %	8.06 Area %
Free Fatty Acids	AOCS Ca 5a-40	≤0.1 %	0.03 %	0.03 %
Moisture Content	C-M-00118-000Rev 1[b]	≤0.1 %	0.01 – 0.09 %	0.04 %
Unsaponifiable Matter	AOCS Ca 6a-40	≤1.0 %	0.20 – 0.70%	0.37%
Peroxide Value	AOCS Cd 8-53	≤5 meq/kg	0 – 1.18 meq/kg	0.59 meq/kg
p-Anisidine Value	ISO 6885	≤ 10 %	0.20 – 0.59 %	0.40 %
Elements by ICP				
Lead	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Arsenic	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Mercury	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Cadmium	AOCS Ca 17-01	<0.1 ppm	<0.03 ppm	<0.03 ppm
Phosphorus	AOCS Ca 20-99	≤0.5 ppm	<0.20 ppm	<0.20 ppm
Sulfur	AOCS Ca 17-01	≤3 ppm	<0.50 ppm	<0.50 ppm

[a] C-M-00036-000 Rev 0, Total Deliverable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification (internal method; APPENDIX I);
[b] C-M-00118-000 Rev 1, adapted from the Karl Fischer Moisture Determination of Oil (internal method; APPENDIX II).
AOCS = American Oil Chemists' Society; ISO = International Standards Organization; meq = milliequivalents; ppm = parts *per* million.

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3. Dietary Exposure

The intake profile (amount and frequency) by individuals in USDA's What We Eat in America (WWEIA) Continuing Survey of Food Intakes by Individuals 2011-2012 (Dwyer *et al.*, 2003) was used to calculate the estimated daily intake (EDI) of the current HO algal oil produced from a classically and genetically engineered strain of *P. moriformis*, for individuals consuming the food groups selected for the addition of HO algal oil *per* this conclusion of GRAS status (*i.e.*, "eaters only"). Through product evaluation and use assessments, TerraVia found the ability to utilize HO algal oil in additional food groups and at increased levels of use, compared to the use levels stated in GRN 000527. The food groups as defined by the FDA (21 CFR §170.3(n)) stated in the conclusion of GRAS status are provided in Table 7.

Table 7. Food groups selected for HO algal oil supplementation*

Food Category	Intended use level (ppm)
Baked goods (bread, dry mixes, rolls, bagels, biscuits, cornbread, tortillas and croutons)	20,000-70,000
Baked desserts (sweet rolls, muffins, scones, cakes, cheesecake, pie, cobbler, crisps, doughnuts, turnovers, strudel and cookies)	30,000-50,000
Meal replacement bars, drinks and protein supplements	30,000 -50,000
Cereals and bars (granola, muesli and dry cereal)	20,000-150,000
Cheese spreads	100,000
Margarine and margarine-like spreads	450,000-700,000
Butter-like spreads	700,000
Vegetable oil and shortening	700,000-1,000,000
Salad dressings and mayonnaise	500,000-700,000
Sauces, gravies and dressings	20,000-60,000
Nut spreads, nuts and seeds	50,000
Dairy and milk products (including analogs) (whipped topping, cream substitute, milk imitation and flavored)	10,000-100,000
Gelatins and puddings	30,000
Soups and broth	20,000-30,000
Meat products**	20,000-50,000
Frozen dairy desserts	30,000
Snacks (crackers, popcorn, crisp bread, salty snacks, popcorn, pretzel and chips)	145,000-350,000
Soft candy (caramels, fruit snacks, covered nuts	30,000
Confectionary (icing and marshmallows)	20,000-250,000

*The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category.
** Meat Products (mixtures), including all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.
ppm=parts *per* million

The mean and 90th percentile EDIs were calculated only for HO algal oil intake following addition of the HO algal oil to the selected food groups. The mean and 90th percentile EDIs of HO algal oil consumption is approximately 12.04 and 27.47 g/day HO algal oil, respectively (Table 8). This intake is approximately equivalent to 220.36 and 494.83 mg/kg bw/day, respectively, of the HO algal oil. The consumption of oleic acid, the major monounsaturated fatty acid (MUFA) in HO algal oil, from the addition of HO algal oil may result in a mean and 90th percentile intake of oleic acid at 10.63 and 24.17 g/day, respectively. For a 60 kg person, the oleic acid intake would be 177.17 and 402.83 mg/kg bw/day, respectively.

Table 8. Predicted intake of HO algal oil following supplementation of selected foods at the indicated levels

	Per User (g/day)	
	Mean	**90th Percentile**
Possible consumption with HO algal oil as an added ingredient to food	12.04	27.47

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4. Current, Proposed, and Self-limiting Levels of Use

As a yeast-like algal genus, *Prototheca* spp. strains were surveyed along with oleaginous yeasts to determine growth conditions that produce fatty acids appropriate for biodiesel feedstock[26] (Sitepu *et al.*, 2013). HO algal oil produced from *P. moriformis* S2532 was determined GRAS in 2014 and notified to the US FDA of the status in 2015 (GRN 527). After evaluation the GRAS determination and provided a "no objection" letter (FDA, 2015).

The intended use of HO algal oil produced by the source organism described in this notification or the source organism characterized in GRN 000527 has not changed from the use stated in the original conclusion of GRAS status as detailed in GRN 000527, which is as a partial replacement of conventional vegetable and non-vegetable dietary oils.

The quantity of the high-oleic algal oil used as a partial replacement for conventional (lower-oleic) dietary oils would be self-limiting due to potential unpalatability.

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[26] Requirements for high quality feedstocks include the efficient production of specific lipid content and profiles.

5. Experience Based on Common Use in Food Before 1958

To my knowledge, HO algal oil was not used in food prior to January 1, 1958.

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6. Basis for the Conclusion of GRAS Status

HO algal oil was determined GRAS in 2014 with a "no objection" letter from the US FDA in 2015 (GRN 000527) (FDA, 2015).

The amendment to the conclusion that the intended use of HO algal oil is GRAS is on the basis of scientific procedures, as described in the Amendment to the Dossier in Support of the Generally Recognized as Safe (GRAS) Status of High-Oleic Algal Oil from a Modified Strain of *Prototheca moriformis* as a Food Ingredient, dated 18 October 2016 (Solazyme, 2016). On the basis of the data and information described in this GRAS dossier, and other publicly available information, there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that the HO algal oil is GRAS under the intended conditions of use. The following sections describe the information that an Expert Panel utilized, along with any other publicly available information the Panel deemed necessary, to conclude that the HO algal oil, produced as indicated in the amendment, continues to meet GRAS status under the intended conditions of use.

6.1 GRAS Introduction

High-oleic algal oil (HO algal oil; ≥80% oleic acid) is isolated from a classically improved and genetically engineered strain of the *Prototheca moriformis* microalgae. HO algal oil was previously concluded to meet GRAS status for use as a source of oil in foods at an estimated 90th percentile consumption level of 12.5 g/day (Solazyme, 2014), and was notified of its GRAS status to the United States Food and Drug Administration (FDA), who responded with a "no objection" letter (FDA, 2015). The information contained in this section is a summary of the scientific evidence that supports the continued GRAS status of HO algal oil as a food ingredient for human consumption when used in additional foods or in the same foods at higher levels than were described in the original GRAS dossier notified to FDA (designated as GRN 000527), resulting in an increase in the overall consumption of HO algal oil as a food ingredient.

6.1.1. Description of the Ingredient

HO algal oil is a clear, pale to wheat yellow-colored,[27] refined, bleached and deodorized oil with high levels of oleic acid, produced from a strain of *Prototheca moriformis* that has been modified *via* classical mutagenesis and targeted genetic modification. The general descriptive characteristics of the high-oleic algal oil are presented in Table 9.

Table 9. General description of HO algal oil.

Appearance	Clear, pale to wheat-yellow oil
Packaging	44-gallon drums for most distributors; totes, bulk trucks and rail cars for large-scale purchase and use
Storage	Closed container at room temperature (22 – 27 °C)
Stability	Six months
Intended use	Partial replacement of conventional (lower-oleic acid) dietary oils
Functionality in food	Source of macronutrients consumed as a partial replacement of conventionsl (lower-oleic acid) dietary oils

[27] The HO algal oil is not added to food with the intention of acting as a color. Although the HO algal oil is pale- to wheat-yellow in bulk amounts, at the amounts added to food the color fades to near colorlessness. Under the conditions of maximum usage in foods (up to 1,000,000 ppm or 100% in vegetable oil equivalent), the color of the food to which the HO algal oil is added is not altered. HO algal oil is, therefore, exempt from the definition of a color additive [FFDCA §201(t) and 21 CFR §70.3(f)] because the ingredient is not effective as a coloring agent.

6.2. Identification of the Organism used in Ingredient Production

As described in the previous GRAS notification (GRN 000527), the genus *Prototheca*, which is composed of achlorophyllous,[28] eukaryotic microalgae, belongs to the Trebouxiophyceae class in phylum Chlorophyta. The more familiar *Chlorella* genus is also a member of the same class in the same phylum. Although *Prototheca* are occasionally referred to as 'colorless *Chlorella*' due their lack of chloroplasts and photosynthetic pigments and close relationship with *C. protothecoides* (aka, *Auxenochlorella protothecoides*), *Prototheca* have several additional differentiating traits when compared with *Chlorella* spp.; *Prototheca* spp. are acidophilic, are thiamine auxotrophs, and cannot utilize nitrate as a sole nitrogen source, but can use a variety of hydrocarbons as sole carbon sources (Running *et al.*, 2003). Of all the heterogeneous species that comprise the *Chlorella* genus, only *C. protothecoides* (*A. protothecoides*) shares most of these distinguishing characteristics and is recognized to be particularly closely related to *Prototheca* spp. (Conte and Pore, 1973; Running *et al.*, 2003).

Prototheca are spherical or ovoid unicellular heterotrophic eukaryotes (3 – 30 µm in diameter) that have double-layered cell walls which, similar to *C. protothecoides*, contain sporopollenin.[29] These achlorophyllous green microalgae lack chloroplasts and buds (Lass-Florl and Mayr, 2007; Hillesheim and Bahrami, 2011; Mayorga *et al.*, 2012). Reproduction is by asexual endosporulation; two to 16 endospores (2 – 10 µm), symmetrically or randomly arranged in the sporangia, develop until they emerge *via* rupture of the parent sporangial cell wall (DiPersio, 2001; Lass-Florl and Mayr, 2007; Hillesheim and Bahrami, 2011; Mayorga *et al.*, 2012). In the presence of adequate nutrients, reproductive cycles with release of sporangiospores can occur every 5 - 6 hours (Lass-Florl and Mayr, 2007).

Prototheca spp. are ubiquitous in the environment. First isolated in 1892 – 1894 from the slime fluxes (*i.e.*, sap released from tree wounds) of lime, horse chestnut and elm trees in Germany (Krüger, 1894), *Prototheca* spp. have since been isolated from a wide variety of sources,[30] environmental sources such as soil, decaying plant matter, sewage, and water, as well as from human and animal sources (*e.g.*, feces, milk, sputum, and/or lesions) (Shahan and Pore, 1991; Mayorga *et al.*, 2012).

The number of species assigned to the *Prototheca* genus has varied over the years as understanding of the phylogenetic relationships has increased. A majority of the scientific literature currently recognizes five species in the genus: *P. wickerhamii*, *P. zopfii*, *P. stagnora*, *P. ulmnea*, and *P. blaschkeae* (Roesler *et al.*, 2006; Marques *et al.*, 2008; Mayorga *et al.*, 2012). Arholdt *et al.* (2012) recognizes *P. cutis* as a sixth. Although *P. moriformis* has at times been considered a species (Pore, 1985; Roesler *et al.*, 2003; Ueno *et al.*, 2005; Jagielski and Lagneau, 2007), the assignment has generally not been accepted as valid (Pore, 1985; Roesler *et al.*, 2003; Ueno *et al.*, 2003; Ueno *et al.*, 2005; Lass-Florl and Mayr, 2007; Marques *et al.*, 2008; Pore, 2011; Mayorga *et al.*, 2012). During a recent interval that *P. moriformis* was considered a species and *P.*

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[28] Achlorophyllous = without photosynthetic pigments.

[29] Rare but natural oxidative carotenoid polymers that occur in a few microorganisms and plants.

[30] Of 100 randomly selected strains of *Prototheca* held in the private culture collection belonging to Professor R. Scott Pore at the West Virginia University Medical School, Charleston WV), 7 had been isolated from humans, 35 from animals, and 58 from the environment (Shahan and Pore, 1991; Running *et al.*, 2003).

blaschkeae was categorized as a variant (biotype) of *P. zopfii* (Roesler *et al.*, 2006; Mayorga *et al.*, 2012), sequence analysis of the 18S rRNA gene identified *P. blaschkeae* as a species rather than a biotype of *P. zopfii* and *P. moriformis* as a biotype of *P. zopfii* rather than its own species. To explain, *P. zopfii* had initially been differentiated biochemically and serologically into three variants (Roesler *et al.*, 2003; Lass-Florl and Mayr, 2007; Pore, 2011). Variant 3, which consisted primarily of strains isolated from swine farms, was reclassified as the novel species *P. blaschkeae*, a reassignment based on 18S rRNA gene sequence analysis and cellular fatty acid composition. The other two variants, although closely related, are recognized to differ strongly in their immunogenic structures and their association with disease: variant 2 consists of isolates associated with bovine protothecal mastitis and known to be opportunistically pathogenic, whereas variant 1 strains which are isolated from cow manure and barns are not associated with pathogenicity (Roesler and Hensel, 2003). Examination of the *P. zopfii* variants by 18S rRNA gene sequence analysis and cellular fatty acid composition led to the recommendation that variants 1 and 2 be reclassified as genotypes 1 and 2, respectively, while being evaluated as potential subspecies of *P. zopfii* (Roesler *et al.*, 2006; Lass-Florl and Mayr, 2007). During the study, *P. moriformis* ATCC[31] 50081 (aka RSP-1216; isolated in 1983 by R.S. Pore from cheese factory wastewater, Costa Rica[32]) was found to group closely, not with either of the genotypes, but with a central *P. zopfii* clade[33] which included *P. zopfii* var. *hydrocarbonea* ATCC 30253 (aka 48-Y; isolated by J.D. Walker in 1973 from Colgate Creek sediment, Baltimore Harbor, Chesapeake Bay, Maryland[34]) and *P. zopfii* UTEX[35] 178 (aka SAG[36] 263-1, isolated by M. W. Beijerinck before 1912 from an unknown source[37]). The authors recommended further study to determine whether this clade comprises a third subspecies (Roesler *et al.*, 2006).

Of the currently recognized species of *Prototheca*, *P. zopfii*, *P. wickerhamii*, and *P. blaschkeae*, each contain strains that have been documented as opportunistic pathogens causing rare infections (*i.e.*, protothecosis) in humans and domestic and wild animals. *P. wickerhamii* is most often associated with the rare infections reported in humans. In a typical year, two to five cases of infection are reported globally; from 1964 – 2012, a total of 160 cases of human protothecosis have been reported (Todd *et al.*, 2012). Infection in humans is often associated with traumatic introduction into the skin and underlying tissues, and systemic infection is usually associated with severe immunosuppression or underlying disease (Lass-Florl and Mayr, 2007; Mayorga *et al.*, 2012). *P. zopfii* genotype 2 is most often associated with infection in animals, primarily bovine mastitis in cows and occasionally infections in dogs, but is rarely associated with disease in humans. Nearly all *P. blaschkeae* strains have been isolated from swine, although the first isolate of the species originated from a case of human onychomycosis[38] and the strains are now also associated with bovine mastitis (Roesler *et al.*, 2006; Marques *et al.*, 2008). Bovine protothecal mastitis, and indeed mastitis caused by environmental microorganism in general, is believe to be primarily a disease of poor hygiene practices and dairy management rather than due

[31] ATTC = *American Type Culture Collection*
[32] <http://www.atcc.org/Products/All/50081.aspx>; site accessed January 28, 2014.
[33] Clade = a group of biological taxa (as species) that includes all descendants of one common ancestor
[34] <http://www.atcc.org/Products/All/30253.aspx>; site accessed January 28, 2014.
[35] UTEX = University of Texas Culture Collection of Algae, Austin, Texas
[36] SAG = Sammlung von Algenkulturen Göttingen, Albrecht-von-Haller-Institute for Plant Science, University of Göttingen, Göttingen, Germany
[37] <http://sagdb.uni-goettingen.de/detailedList.php?str_number=263-1>; site accessed January 28, 2014.
[38] 'Fungal' infection of the nail and/or nail bed.

to presence or absence of the microorganism, as *Prototheca* spp. have been identified as present in the environments of dairy cattle with and without protothecal mastitis, in the environments of dairy farms with no prior history of mastitis caused by this agent (Anderson and Walker, 1988; Pore and Shahan, 1988; Enders and Weber, 1993; Corbellini *et al.*, 2001; Janosi *et al.*, 2001).

6.2.1. Wild-type strain *Prototheca moriformis* Krüger

TerraVia obtained wild-type strain *P. moriformis* Krüger UTEX 1435,[39] from the University of Texas Culture Collection of Algae in Austin, Texas, where it had been deposited by W.B. Cooke in 1966. After undergoing clonal purification to ensure the culture was axenic (free of any contaminating organisms), UTEX 1435 was assigned TerraVia internal strain number 'S376'. Other deposits of this strain[40] include the ATCC 16525[41] accession held at the American Type Culture Collection (ATCC, Rockville, Maryland); Hopkins IV 7.3.2.1[18] assigned by C.B. van Niel at the Hopkins Marine Station, Stanford University (Pacific Grove, California) (Sudman and Kaplan, 1973); and the SAG 263-7[42] accession deposited by M.B. Allen held in the Sammlung von Algenkulturen Göttingen (SAG) (Albrecht-von-Haller-Institute for Plant Science, University of Göttingen, Göttingen, Germany). Believed to have been isolated prior to 1894 by W. Krüger from the sap of a lime tree near Jena, Germany and a horse chestnut tree in the botanical gardens at Halle an der Saale in Germany, the strain was most likely originally named *Prototheca zopfii* Krüger[43] (Krüger, 1894) and designated Pr-9 (*Prototheca* 9th strain) when deposited in the collection at the Microbiology Department, Delft Technical School, Delft, The Netherlands. The strain was later transferred to C.B. van Niel and deposited in the collection at Hopkins Marine Station (Stanford University, Pacific Grove, CA) where the accession was designated Hopkins IV 7.3.2.1, before being transferred by van Niel to W.B. Cooke who deposited the strain in UTEX (where the accession was designated *P. moriformis* Krüger UTEX 1435) and in the ATCC (where the accession was designated *P. moriformis* Krüger ATCC 16252). The accession deposited into the SAG by M.B. Allen (designated *P. zopfii* Krüger SAG 263-7) had also been transferred by C.B. van Niel from the Hopkins collection. *P. moriformis* UTEX 1435 is not the type strain for either *P. moriformis*[44] or *P. zopfii*.[45] Although the ATCC preserves *P. moriformis* strain ATCC 16525 under Biosafety Level (BSL) 1 conditions[18] which indicates the microorganism is not recognized to cause disease in immunocompetent adult humans, the SAG preserves identical accession SAG 263-7 under BSL 2 conditions[19] which indicates the microorganism is rarely associated with human disease and of low concern to laboratory staff.

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[39] <http://web.biosci.utexas.edu/utex/algaeDetail.aspx?algaeID=3871>; site accessed January 9, 2014.

[40] Although the CDC B-1444 accession deposited into the Center for Disease Control (CDC, Atlanta, Georgia) and the NRRL Y-6865 accession held by the Agriculture Research Service (ARS) Culture Collection (Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois) had been assigned to *P. moriformis* ATCC 16525 as identical strains (Sudman and Kaplan, 1973), they were later determined to be strains of *P. wickerhamii* (Pore, 1985).

[41] <http://www.atcc.org/Products/All/16525.aspx >; site accessed January 9, 2014.

[42] <http://sagdb.uni-goettingen.de/detailedList.php?str_number=263-7>; site accessed January 9, 2014.

[43] During this same time period, a second distinct strain of *Prototheca* was isolated from the slime flux of an elm tree at Halle an der Saale, Germany, and named *Prototheca moriformis* Krüger (Krüger, 1894).

[44] No type strain for *P. moriformis* is recognized (Pore, 1985).

[45] *P. zopfii* Krüger SAG 263-4 (aka, UTEX 327; formerly *Prototheca portoricensis* var. *trispora* Ashford, Ciferri et Dalmau), originally isolated from a human case of enteropathia by B. K. Ashford before 1930, is the *P. zopfii* type strain (Roesler *et al.*, 2003).

6.2.2. Modified *Prototheca moriformis*

In the original conclusion of GRAS status, the HO algal oil was produced from *P. moriformis* strain S2532, a classically improved and genetically modified strain originating from the wild-type *P. moriformis* Kruger strain UTEX 1435 (designated by TerraVia as S376). To summarize, S376 was subjected to classical mutagenesis, resulting in a strain with increased triglyceride production, productivity and yield of oil. This strain was then subjected to genetic engineering in which the *Saccharomyces cerevisiae*[46] sucrose invertase gene (*SUC2*) was inserted to serve as a selectable marker by conferring the ability to utilize sucrose as a carbon source. In addition, a thioesterase gene from *Carthamus tinctorius*, the safflower plant, was inserted. This gene encodes for a carrier protein that plays an essential role in oleic acid chain termination during *de novo* fatty acid synthesis. These genetic modifications resulted in strain S2532, which grows on sucrose and produces an oleic acid-rich oil (>80%).

TerraVia continued to analyze classically improved strains originating from S376 for more favorable attributes and identified a classically improved strain, S5100, which produced a higher *percentage* of oleic acid, compared to the originator strain S376. S5100 was then transformed to disrupt a single copy of an endogenous thioesterase gene while overexpressing an endogenous *P. moriformis* synthase gene to increase the ability for fatty acid chain elongation. The *S. cerevisiae SUC2* gene was also inserted to provide the ability to utilize sucrose as a carbon source and serves as a selectable marker. The resulting strain, S5587, was then transformed with a hairpin RNAi cassette to reduce expression of an endogenous desaturase gene that introduces double bonds in the fatty acyl chain, leading to decreased formation of polyunsaturated fatty acids (*e.g.*, linoleic acid). The *S. carlsbergensis*[47] *MEL1*gene was also inserted as a selectable marker to confer the ability to grow on melibiose and serves as a selectable marker. Melibiase is an enzyme that has a long history of safe commercial use in the food industry. For example, melibiase is used in the sugar refining industry for the conversion of raffinose to galactose and sucrose (Furia, 1980). The resulting *P. moriformis* strain S6697 produces approximately 3% C16:0 (palmitic acid), 2% C18:0 (stearic acid) and 90% C18:1 (oleic acid) and is to be used in the production of HO algal oil. In summary, the genetic modifications were a reduction in the expression of an endogenous gene, an increase in expression of an endogenous gene, and insertion of two genes as selectable markers that allow for use of sucrose and melibiose as carbon sources. These modifications in the strain that now produces HO algal oil are similar to the modifications used to produce S2532. The production of HO algal oil containing a high level of oleic acid with reduced levels of linoleic acid meets the same specifications as the HO algal oil produced with S2532. There is no reason to believe that HO algal oil produced from S6697 would have a safety profile different from HO algal oil produced from S2532.

The stability of the transgenes inserted into strain S6697 was assessed by Southern blot analysis, in which cells resulting from a high cell density fermentation were grown in liquid culture with glucose as the sole carbon source (non-selective media), then plated onto media containing glucose. Individual colonies (*n*=48) were then transferred to medium with melibiose as the sole carbon source. All 48 of the 48 transferred colonies exhibited growth on the melibiose-containing

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[46] Typical yeast used in the production of bread, wine and other foods.
[47] *S. carlsbergensis* is a yeast used extensively in the production of lager beer (Wendland, 2014).

plate after more than 30 generations of growth in the absence of the melibiose selection, indicating 100% stability of the inserted transgenes.

Prior to insertion of the hairpin gene sequence, the oil produced from strain S5587 was analyzed for production of algal and cyanobacterial toxins by liquid chromatography with tandem mass spectrometric detection for: amnesic shellfish poisoning toxins (domoic acid), diarrrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxin-1, pectenotoxin-2, azaspiracid-1, yessotoxin, and homo-yessotoxin), paralytic shellfish poisoning toxins (gonyautoxins 1-6; decarbamoylgonyautoxins 2 and 3; saxitoxin; decarbamoylsaxitoxin; neosaxitoxin and ciguatoxins 1-4), cyanobacterial toxins (microcystin-RR, -YR, -LR, -LW, -LF, -LA, -WR, -LY and -HtyR and dm-microcystin-RR and -LR), nodularin, anatoxin and cylindrospermopsin. No toxins were detected (Food GmbH Jena Analytik, 2013). This corroborative information indicates that altering the expression of endogenous genes in this strain lineage does not induce toxin formation or toxin levels in the resulting oil.

S6697 strain was evaluated utilizing the Pariza and Johnson (2001) decision tree that was originally devised for the evaluation of microbial enzyme preparations to be used in food processing, but can be utilized in a general fashion in evaluating the safety of products generated from genetically engineered microorganisms. Strain S6697 meets the first aspects of the decision tree, as it is genetically modified through recombinant DNA techniques, while the expressed product (HO algal oil) has a history of safe use and is free of transferable antibiotic resistance gene DNA (no antibiotic resistance genes were used in the production of S6697). All of the introduced DNAs are well characterized, as discussed above, and are integrated into the genome in a well-defined, non-random nature.

The *P. moriformis* strain lineage is considered a safe-strain lineage, as the *P. moriformis* microalgae is not recognized in the scienfitic literature to be associated with pathogenicity, and the production of a refined, bleached, and deodorized oil would remove the potential for viable algal organisms in the final HO algal oil product. Pariza and Johnson (2001) concluded for enzyme preparations that "pathogenic potential is not usually an area of concern for consumer safety because enzyme preparations rarely contain viable organisms", but can still be evaluated. The same conclusion of a lack of viable organisms holds true for the production of a highly refined oil, as no viable organisms have been detected through the TerraVia HO algal oil production process. In addition, a corroborative study on a closely related *P. moriformis* strain (derived from the same source strain) was not pathogenic when evaluated in a study designed to determine if microbial agents would would become systemic in the body and survive or be pathogenic after oral administration.[48] Based on the data described and evaluated through the Pariza and Johnson (2001) decision tree, the genetic changes utilized do not adversely impact the safety of *P. moriformis* strain S6697 as a production strain, and the strain changes discussed above do not affect the safety or identity of the GRAS HO algal oil. Such process changes are necessary to improve the product for the benefit of the consumers.

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[48] Study based on the U.S. EPA Health Effects Test Guidelines, OPPTS 885.3050, Acute Oral Toxicity/Pathogenicity Study (1996) and U.S. FDA Toxicological Prinicples of the Safety Assessment of Food Ingredients, Redbook, 2000, IV.C. 3a: Short-Term Toxicity Studies with Rodents (2003).

6.3. History of use

Microalgae, such as *Chlorella vulgaris* and *Chlorella pyrenoidosa*, have been an accepted part of the human diet for hundreds of years and wild stocks of various microalgae are harvested as current food sources in many regions of the world (Kay, 1991; Ravishankar, *et al.*,2006), There is no documentation, however, of *Prototheca* spp. being among them. Instances of consumption of *Prototheca* spp. have been reported for humans and animals, but these were unintentional (Pore *et al.*, 1983; Pore and Shahan, 1988; Jagielski and Lagneau, 2007) and neither the scientific literature nor electronic searches have revealed any documentation of *P. moriformis* strains or ingredients derived from *P. moriformis* being deliberately used in human or animal foods.

6.4. Current uses

HO algal oil was concluded to have GRAS status in 2014 when added to foods as a partial replacement for conventional vegetable oils and was notified to the US FDA of the status in 2015 (GRN 527). The FDA, after review of the conclusion of GRAS status, provided a "no objection" letter in 2015 (FDA, 2015).

6.5. Proposed use or uses

The use of HO algal oil has not changed from the use stated in the previous conclusion of GRAS status, which is as a partial replacement of conventional vegetable and non-vegetable dietary oils.

6.6.Mechanisms of action

The HO algal oil will replace a portion of the dietary fats or oils in a variety of conventional foods. As a replacement source of macronutrients and energy, the lipids found in the high-oleic algal oil will be digested through the same normal physiological processes by which other plant-derived oils common to the human diet are digested and utilized.

6.7. Regulatory Status

HO algal oil was determined GRAS in 2014 and notified to the US FDA of the status in 2015 (GRN 527). FDA evaluated the GRAS determination and provided a "no objection" letter indicating that FDA had "no questions at this time" on the GRAS determination (FDA, 2015).

6.8.Description, Specifications and Manufacturing Process

6.8.1. Description and Specifications

The physical and chemical properties and specifications for HO algal oil are provided in Table 10, respectively. The specifications for the HO algal oil have not changed from the previous conclusion of GRAS status (detailed in GRN 000527), except for a change to the *p*-anisidine specification and, as shown in Table 10, the HO algal oil currently being produced using strain *P. moriformis* S6697 meets original specifications when evaluated under the revised *p*-anisidine specification. The *p*-anisidine specification was $\leq 2\%$ in the original GRAS conclusion but has been amended to $\leq 10\%$ to align with and reflect *p*-anisidine levels noted in the industry for food oil quality. Edible marine oils evaluated under the GOED Organization suggest a maximum *p*-anisidine value of 20 (GOED, 2015), while others in the edible oil industry suggest a *p*-anisidine value of 10 as an indicator of good quality oil (Halvorsen and Blomhoff, 2011; Moigradean *et al.*, 2012; Yun and Surh, 2012; Miller, Unknown). The *p*-anisidine value is a measure of secondary oxidation, mainly of aldehydes such as 2,4-dienals and 2-alkenals, which correlate with overall oil odor intensity (Yun and Surh, 2012). In addition, analysis of the fatty acids contained in the current HO algal oil (Table 3) confirms that the levels of the major fatty acids are within specifications, and are not different from the levels of the fatty acids found in some commonly consumed oils. Current lot analyses of HO algal oil show that oleic acid is the predominant fatty acid (~88% TFA[49]), followed by palmitic (~3.5% TFA) and stearic fatty acids (~3.7% TFA), with low levels of linoleic (~1.53% TFA) and *alpha*-linolenic (~0.22% TFA) fatty acids. All specified fatty acid levels are within current specifications for HO algal oil, and similar in composition to other high oleic acid-containing oils, except for the reduction in linoleic acid (Table 3), which is typically found in a variety of vegetable oils, edible seeds and nuts, and other foods and would most likely be obtained from other dietary sources.

Table 10. Specifications for HO algal oil.

			Batch Analysis Results of current HO oil (n = 3)	
Parameter	**Method**	**Specification**	**Range**	**Average**
Appearance	Visual inspection	Clear, pale yellow to wheat yellow	Conforms	Conforms
Odor	Olfactory inspection	Slight	Conforms	Conforms
Fatty acid profile	C-M-00036-000 Rev 0[a]			
Oleic acid (C18:1)		≥80 Area %	87.89 – 88.70 Area %	88.27 Area %
Linoleic acid (C18:2)		≤10 Area %	0.99 – 1.93 Area %	1.53 Area %
Alpha-Linolenic acid (C18:3 *alpha*)		≤0.8 Area %	0.16 – 0.27 Area %	0.20 Area %
Total Saturated Fat	C-M-00036-000 Rev 0[a]	≤15 Area %	7.85 – 8.38 Area %	8.06 Area %
Free Fatty Acids	AOCS Ca 5a-40	≤0.1 %	0.03 %	0.03 %
Moisture Content	C-M-00118-000Rev 1[b]	≤0.1 %	0.01 – 0.09 %	0.04 %
Unsaponifiable Matter	AOCS Ca 6a-40	≤1.0 %	0.20 – 0.70%	0.37%
Peroxide Value	AOCS Cd 8-53	≤5 meq/kg	0 – 1.18 meq/kg	0.59 meq/kg
p-Anisidine Value	ISO 6885	≤ 10 %	0.20 – 0.59 %	0.40 %
Elements by ICP				
Lead	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Arsenic	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Mercury	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Cadmium	AOCS Ca 17-01	<0.1 ppm	<0.03 ppm	<0.03 ppm
Phosphorus	AOCS Ca 20-99	≤0.5 ppm	<0.20 ppm	<0.20 ppm
Sulfur	AOCS Ca 17-01	≤3 ppm	<0.50 ppm	<0.50 ppm

[a] C-M-00036-000 Rev 0, Total Deliverable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification (internal method;
[b] C-M-00118-000 Rev 1, Karl Fischer Moisture Determination of Oil (internal method).
AOCS = American Oil Chemists' Society; ISO = International Standards Organization; meq = milliequivalents; ppm = parts *per* million.

As indicated in Table 12, the fatty acid profile of HO algal oil is similar to other vegetable oils (olive, canola and soybean oils), except that HO algal oil contains increased levels of oleic acid and decreased levels of linoleic, palmitic and α-linolenic (soybean and canola only, as α-

[49] TFA = Total fatty acid.

linolenic acid levels are comparable between HO algal oil and olive oil). However, these fatty acid levels are similar to levels found in commonly consumed oils. Content of the essential fatty acids (*i.e.*, linoleic and α-linolenic fatty acids) in HO algal oil is less than the content found in other commonly consumed oils such as soybean, canola and olive oil (Table 12). However, as shown in Table 11, there is a wide variety of food sources of linoleic and α-linolenic fatty acids that do not require these essential fatty acids to be derived strictly from a food use oil. Use of HO algal oil in the production of some of these foods (as discussed in Section 4 below) may reduce the essential fatty acid content of some of these foods, but HO algal oil is not expected to be a total replacement for all oil utilized in all marketed foods (*i.e.*, less than 100% market penetration). Other foods, such as nuts, chicken, eggs, milk and meat dishes provide substantial amounts of essential fatty acids to a varied diet, and therefore the availability of essential fatty acid consumption is not likely to be an issue for the consumer.

Table 11. Food sources of linoleic acid (PFA 18:2) and alpha-linolenic acid (PFA 18:3), listed in descending order by percentages of their contribution to intake, based on data from the National Health and Nutrition Examination Survey 2005-2006 (National Cancer Institute/Epidemiology and Genomics Research Program).

Linoleic acid*			*alpha*-Linolenic acid**		
Rank	**Food Item**	**Contribution to intake (%)**	**Rank**	**Food Item**	**Contribution to intake (%)**
1	Chicken and chicken mixed dishes	9.3	1	Salad dressing	10.5
2	Grain-based desserts	7.5	2	Chicken and chicken mixed dishes	6.4
3	Salad dressing	7.4	3	Grain-based desserts	6.1
4	Potato/corn/other chips	6.9	4	Pizza	5.8
5	Nuts/seeds and nut/seed mixed dishes	6.5	5	Yeast breads	5.0
6	Pizza	5.3	6	Mayonnaise	4.0
7	Yeast breads	4.5	7	Pasta and pasta dishes	3.5
8	Fried white potatoes	3.5	8	Quickbreads	3.4
9	Pasta and pasta dishes	3.5	9	Fried white potatoes	2.8
10	Mexican mixed dishes	3.3	10	Mexican mixed dishes	2.7
11	Mayonnaise	3.1	11	Nuts/seeds and nut/seed mixed dishes	2.7
12	Quickbreads	3.0	12	Burgers	2.6
13	Eggs and egg mixed dishes	2.8	13	Margarine	2.6
14	Popcorn	2.6	14	Regular cheese	2.6
15	Sausage, franks, bacon, and ribs	2.1	15	Dairy desserts	2.2

* Specific foods contributing at least 1% of octadecadienoic acid in descending order: other fish and fish mixed dishes, margarine, burgers, crackers, rice and rice mixed dishes, beef and beef mixed dishes, other white potatoes, beans, candy.

** Specific foods contributing at least 1% of octadecatrienoic in descending order: sausage, franks, bacon, and ribs, other white potatoes, beef and beef mixed dishes, beans, potato/corn/other chips, coleslaw, rice and rice mixed dishes, tuna and tuna mixed dishes, popcorn, and vegetable mixtures.

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Table 12. Fatty acid composition (% TFA) for three lots of current HO algal oil, compared with HO algal oil data from original GRAS and conventional vegetable oils.

Fatty Acids (Area %)	S2532 HO algal oil Mean* (n=3)[a]	S6697 HO algal oil Lot: RBD750	S6697 HO algal oil Lot: RBD850	S6697 HO algal oil Lot: RBD854	S6697 HO algal oil Mean	Olive oil [b]	Canola oil [b]	Soybean oil [b]
C10:0 (Capric)	0.05	0.01	0.02	ND	0.02[c]	ND	ND	NR
C12:0 (Lauric)	0.14	0.02	0.03	0.03	0.03	ND	ND	ND
C14:0 (Myristic)	0.82	0.38	0.37	0.42	0.39	ND	ND	ND
C14:1	0.02	ND	ND	ND	ND	ND	ND	ND
C15:0 (Pentadecanoic)	0.05	0.01	0.01	ND	0.01[c]	NR	ND	ND
C16:0 (Palmitic)	8.11	3.87	3.02	3.72	3.54	11.29	4.30	10.46
C16:1	0.97	0.18	0.14	0.13	0.15	1.26	0.21	ND
C17:0 (Heptadecanoic)	0.06	0.04	0.04	0.05	0.04	0.02	ND	0.03
C17:1	0.10	ND	0.03	0.05	0.04[c]	0.13	ND	ND
C18:0 (Stearic)	1.55	3.73	3.99	3.27	3.67	1.95	2.09	4.43
C18:1 (Oleic)	86.66	88.70	87.89	88.22	88.27	71.27	61.77	22.55
C18:2 (Linoleic)	0.08 [c]	0.99	1.68	1.93	1.53	9.76	19.01	50.95
C18:3 (α-Linolenic acid)	0.02 [c]	0.17	0.16	0.27	0.22	0.76	9.14	6.79
C20:0 (Arachidic)	0.24	0.25	0.34	0.26	0.28	0.41	0.65	0.36
C20:1	0.64	0.28	1.07	0.73	0.84	0.31	1.32	0.23
C22:0 (Behenic)	0.08 [c]	0.05	0.07	0.06	0.06	0.13	0.33	0.37
C22:1, *cis*-13 (Erucic)	0.01 [d]	ND	ND	ND	ND	ND	ND	ND
C22:5n3	0.02 [c]	ND	ND	ND	ND	ND	ND	NR
C24:0 (Lignoceric)	0.05	0.02	0.05	0.04	0.04	ND	NR	NR
Total Fatty Acids Identified	99.40	98.70	98.91	99.18	99.08	97.29	98.82	96.17

*Values stated in original GRAS determination (notified to FDA as GRN 527) for HO algal oil; n = number; ND = Analyzed, but not detected; NR = Not reported; TFA = Total fatty acid; RBD = refined, bleached, deodorized; only fatty acid values ≥ 0.02 are reported;

[a] n = 3, unless otherwise specified;

[b] USDA National Nutrient Database for Standard Reference, Release 26 (<http://ndb.nal.usda.gov/ndb/search/list>; site accessed January 20, 2014);

[c] n = 2

[d] n = 1

6.9.Manufacturing Process

The source organism used to produce HO algal oil has been substantively changed (described in section A.4 of this notification), compared to the source organism detailed in GRN 000527. However, the remainder of the manufacturing process of the HO algal oil described in the conclusion of GRAS status for HO algal oil produced *via* strain S6697 has not changed since the original conclusion of GRAS status was finalized and detailed in GRN 000527. To summarize, the fermentation substrate used in the production of the HO algal oil has not changed since the original GRAS determination. HO algal oils are produced using processes that incorporate high temperatures and pressure. After growth of the oil-producing *P. moriformis* strain, the final broth is inactivated by heat in the evaporator, resulting in complete loss of microalgae viability. After the evaporator, the inactivated broth is sent to a drum dryer and dried at high temperatures, which provides a redundant inactivation step. Following mechanical extraction, the oil itself undergoes a traditional refined, bleaching, and deodorizing (RBD) process just like other plant oils (AOCS, 2011).

Due to the RBD process, refined oils are normally free from microbial contamination and do not contain sufficient water or moisture, which is need for microbial growth (ICMSF, 2005). HO algal oil has less than 0.1% moisture (Table 10), similar to other refined food oils. Testing of 3 lots of HO algal oil for microbial content (Table 13) shows no microbial growth in HO algal oil.

Table 13. Microbial data on 3 lots of HO algal oil.

Sample ID#	RBD 850-C	RBD 831-A	RBD 789-C
Aerobic Plate Count	<10(/g)	<10(/g)	<10(/g)
Coliforms	<3(/g)	<3(/g)	<3(/g)
E. coli	Negative(/10g)	Negative(/10g)	Negative(/10g)
Mesophilic Aerobic Spores	<1.0(/g)	<1.0(/g)	<1.0(/g)
Mold	<10(/g)	<10(/g)	<10(/g)
Pseudomonas aeruginosa	Negative(/25g)	Negative(/25g)	Negative(/25g)
Salmonella	Negative(/375g)	Negative(/375g)	Negative(/375g)
Staphylococci	Negative(/25g)	Negative(/25g)	Negative(/25g)
Yeast	<10(/g)	<10(/g)	<10(/g)

#All sample results are provided as colony forming units (CFU).

To confirm the presence or absence of algal DNA in the finished oil, HO algal oil was assayed using quantitative Real-Time quantitative polymerase chain reaction (Real-Time qPCR). Two genes present in the oil-producing *P. moriformis* strain were targeted for specific amplification and quantification: an endogenous algal gene encoding a desaturase which serves as an internal genomic control and the inserted sucrose invertase (*SUC2*) transgene.

Results showed no detection of the presence of DNA, endogenous or transgenic, in the HO algal oil or the negative controls, as evidenced by the lack of specific amplification. DNA was only detected in positive controls, where neat refined, bleached, deodorized (RBD) HO algal oil samples were spiked with genomic *P. moriformis* DNA (gDNA) in amounts ranging from 10 ng to 0.01 ng (Table 14). All samples were run in triplicate and three independent runs were conducted. This study indicates that the RBD process removes source organism DNA from the oil, as indicated by the absence of representative endogenous and inserted DNA in the RBD HO algal oil.

Table 14. Real-time qPCR cycle threshold for endogenous and inserted genes from *P. moriformis* genomic DNA in HO algal oil (Yu and Zhao, 2015). Samples were assayed for specific amplification with primers targeting an endogenous desaturase gene (A) or the inserted *SUC2* transgene (B). The results shown are mean ± standard deviation in triplicate, for each of the three replications.

A. Endogenous Desaturase Gene

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev.	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	21.01	0.443	20.54	0.498	21.59	0.045
	1 ηg	24.47	0.495	25.27	0.230	25.11	0.294
	0.1 ηg	26.80	0.769	32.27	0.548	30.67	1.254
	0.01 ηg	32.47	0.743	34.74	0.640	33.20	0.944
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

B. Inserted *SUC2* Transgene

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	18.05	0.333	19.44	0.423	20.41	0.553
	1 ηg	23.64	0.451	24.81	0.326	23.26	0.435
	0.1 ηg	24.62	0.599	26.74	0.086	28.45	2.862
	0.01 ηg	26.78	0.402	31.65	0.076	32.95	1.323
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

NTC = negative controls containing no DNA.; Cq = quantitation cycle at which fluorescence from amplification exceeds background fluorescence; ηg = nanogram.

6.10. Stability

Stability studies were conducted on current HO algal oil at 5 °C, at 20 °C and for an accelerated stability study at 40 °C. During the stability evaluations, the oil was stored in 500 mL amber glass bottles. Relative humidity was not monitored or controlled because the samples used in the assay for most parameters were stored in amber glass bottles or vials with a Teflon-lined screw cap that prevented moisture transfer during the temperature treatment.

HO algal oil, with no added antioxidants, is stable for at least 18 months when stored at the controlled temperatures of 5 °C (Table 15) or 20 °C (Table 16). The HO algal oil in the absence of an antioxidant is stable for at least three months when stored at 40 °C (Table 17).

Various antioxidants were added to test stability of the oil, which included a mixture of 500 ppm mixed tocopherols and 150 ppm ascorbyl palmitate (herein referred to as Toco/AP), and 200 ppm tertiary butylhydroquinone (TBHQ). The HO algal oil is stable for at least 18 months when stored at 5 °C (Table 15) or 20 °C (Table 16), and at least 12 months when stored at 40 °C with the added antioxidants, Toco/AP and TBHQ (Table 17). *p*-Anisidine value measures rancidity and the oxidation of oil should consistently increase over time. Although some values of *p*-anisidine fluctuate, we have not observed a trend to indicate any stability problems. Stability studies on HO algal oil are ongoing.

Table 15. Stability of the high oleic algal oil with and without antioxidants stored at controlled temperature, 5 °C.

Lot	OSI Rancimat (110 °C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	60.60	0.040	0.08	0.70
6 months	62.59	0.038	0.75	0.78
9 months	61.04	0.039	1.13	1.47
12 months	60.88	0.037	0.89	0.53
18 months	59.81	0.035	1.49	1.25
TOCO/AP				
0 month	156.70	0.048	1.40	0.76
3 months	144.80	0.050	1.14	0.86
6 months	136.95	0.047	1.51	0.87
9 months	136.30	0.047	1.66	4.33
12 months	139.49	0.048	1.10	2.94
18 months	132.69	0.066	0.82	2.10
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	112.84	0.040	0.84	0.70
6 months	110.37	0.038	0.65	0.73
9 months	110.84	0.039	1.34	1.12
12 months	112.98	0.035	0.88	3.23
18 months	110.12	0.041	0.51	0.40

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

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Table 16. Stability of the high-oleic algal oil with and without antioxidants stored at controlled room temperature, 20 °C.

Lot	OSI Rancimat (110 °C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	59.21	0.040	1.02	0.70
6 months	60.92	0.039	1.35	0.77
9 months	60.14	0.041	2.85	1.13
12 months	56.42	0.040	1.37	0.66
18 months	55.41	0.042	1.77	0.99
TOCO/AP				
0 month	156.70	0.048	1.40	0.76
3 months	139.63	0.050	1.71	0.90
6 months	124.44	0.054	1.88	0.90
9 months	134.30	0.058	2.12	3.48
12 months	134.48	0.061	1.48	0.55
18 months	129.17	0.067	1.22	0.65
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	111.06	0.040	1.02	0.67
6 months	102.06	0.040	1.12	0.76
9 months	102.02	0.042	1.63	3.92
12 months	111.96	0.040	1.14	1.64
18 months	106.99	0.042	0.88	0.89

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

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Table 17. Stability of the high-oleic algal oil with and without antioxidants stored at controlled temperature, 40 °C.

Lot	OSI Rancimat (110 °C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	51.77	0.042	3.22	0.93
6 months	N/A	N/A	N/A	N/A
9 months	N/A	N/A	N/A	N/A
12 months	N/A	N/A	N/A	N/A
TOCO/AP				
0 month	156.70	0.048	1.4	0.76
3 months	138.04	0.06	1.62	0.83
6 months	134.37	0.061	1.51	0.80
9 months	130.43	0.080	1.30	2.97
12 months	N/A	N/A	N/A	N/A
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	108.68	0.040	1.42	0.71
6 months	98.56	0.046	2.00	0.72
9 months	95.97	0.052	2.18	2.43
12 months	100.85	0.067	2.10	0.74

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

6.11. Estimated Daily Intake

The intake profile (amount and frequency) by individuals in USDA's What We Eat in America (WWEIA) Continuing Survey of Food Intakes by Individuals 2011-2012 (Dwyer *et al.*, 2003) was used to calculate the estimated daily intake (EDI) of the current HO algal oil produced from a classically and genetically engineered strain of *P. moriformis*, for individuals consuming the food groups selected for the addition of HO algal oil *per* this GRAS amendment (*i.e.*, "eaters only"). The food groups as defined by the FDA (21 CFR §170.3(n)) are provided in Table 18.

As stated in the original GRAS dossier, the HO algal oil will be added only to foods for which a standard of identity does not exist. All food categories designated by TerraVia have been utilized in the calculations as appropriate; however, certain categories designated by TerraVia may include foods for which a standard of identity exists. We note that an ingredient that is lawfully added to food products may be used in a "standardized" food only if it is permitted by the applicable standard of identity. TerraVia found through market evaluation and taste evaluation that the taste profile of HO algal oil (*i.e.*, little to no discernable taste, compared with other high oleic acid-containing oils such as olive oil or canola oil) would allow for a greater replacement of other vegetable oils currently consumed in the U.S. diet. Therefore, TerraVia has expanded the use of HO algal oil to additional food products, and has increased the anticipated use levels in food categories previously stated in the original GRAS dossier.

Table 18. Food groups selected for HO algal oil supplementation.*

Food Category	Intended use level (ppm)
Baked goods (bread, dry mixes, rolls, bagels, biscuits, cornbread, tortillas and croutons)	20,000-70,000
Baked desserts (sweet rolls, muffins, scones, cakes, cheesecake, pie, cobbler, crisps, doughnuts, turnovers, strudel and cookies)	30,000-50,000
Meal replacement bars, drinks and protein supplements	30,000 -50,000
Cereals and bars (granola, muesli and dry cereal)	20,000-150,000
Cheese spreads	100,000
Margarine and margarine-like spreads	450,000-700,000
Butter-like spreads	700,000
Vegetable oil and shortening	700,000-1,000,000
Salad dressings and mayonnaise	500,000-700,000
Sauces, gravies and dressings	20,000-60,000
Nut spreads, nuts and seeds	50,000
Dairy and milk products (including analogs) (whipped topping, cream substitute, milk imitation and flavored)	10,000-100,000
Gelatins and puddings	30,000
Soups and broth	20,000-30,000
Meat products**	20,000-50,000
Frozen dairy desserts	30,000
Snacks (crackers, popcorn, crisp bread, salty snacks, popcorn, pretzel and chips)	145,000-350,000
Soft candy (caramels, fruit snacks, covered nuts	30,000
Confectionary (icing and marshmallows)	20,000-250,000

*The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category.
** Meat Products (mixtures), including all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.
ppm=parts *per* million

The mean and 90th percentile EDIs were calculated only for HO algal oil intake following addition of the HO algal oil to the selected food groups and at the levels for inclusion. Inclusion of HO algal oil into the selected foods at the maximum levels indicated would provide a mean and 90th percentile HO algal oil consumption of approximately 12.04 and 27.47 g/day HO algal oil, respectively (Table 19). This intake is approximately equivalent to 220.36 and 494.83 mg/kg bw/day, respectively, of the HO algal oil. The consumption of oleic acid, the major monounsaturated fatty acid (MUFA) in HO algal oil, from the addition of HO algal oil to foods may result in a mean and 90th percentile intake of oleic acid at 10.6 and 24.17 g/day, respectively.

Table 19. Intake of HO algal oil when added to foods.

	***Per* User (g/day)**	
	Mean	**90th Percentile**
Possible consumption with HO algal oil as an added ingredient to food	12.04	27.47

The Institute of Medicine (IOM) utilized the Continuing Survey of Food Intakes by Individuals, 1994-1996, 1998 to determine that the median MUFA intake in the US ranged from 25 – 39 g/day for men and 18 – 24 g/day for women, with the mean daily intake of MUFAs in the

U.S. in all individuals at 28.7 g (IOM, 2005). The daily value (DV) that FDA has stated for total fat intake (based on a caloric intake of 2,000 calories) is 65 g/day. Therefore, the consumption of HO algal oil at the 90th percentile intake of 27.47 g/day as a partial replacement of other oils in the diet is still well below the daily amount of total fat estimated to be consumed in the U.S., and the consumption of oleic acid from HO algal oil at the 90th percentile (24.17 g/day) is consistent with typical consumption levels of MUFA in the U.S. The 2015 – 2020 dietary guidelines for Americans (USDA, 2015) found that the typical amount of oil consumed in a 2,000 calorie healthy U.S.-style eating pattern is 27 g/day oil, which is similar to the potential consumption of HO algal oil at the 90th percentile, suggesting that other oils will be consumed in addition to HO algal oil.

6.12. Absorption, Distribution, Metabolism, and Elimination (ADME)

The lipids found in HO algal oil are expected to be digested, absorbed, metabolized and excreted through the same normal physiological processes by which other triglyercide oils (*e.g.*, olive oil, canola oil, soybean oil) common to the human diet are digested and utilized for macronutrients.

6.13. SAFETY EVALATION

As stated in the original GRAS dossier, the number of species assigned to the genus *Prototheca* has varied, as scientific analysis of the phylogenetic data has increased. *P. moriformis* species are heterogeneous in nature, previously being placed within the *P. zopfii* species (Roesler *et al.*, 2006) as a distinct biotype (genotype 1). Irrgang *et al.* (2015) states that "on the basis of biochemical, serological and phylogenetic analysis, *P. zopfii* has been divided into two genotypes, of which genotype 1 is considered to be non-pathogenic and genotype 2 is associated with bovine mastitis and human protothecosis." Other work has placed *P. moriformis* within a new clade along with some strains of *P. wickerhamii* (Ewing *et al.*, 2014). Rare cases of *P. wickerhamii* infection have been reported in immunocompromised people (Panchabhai *et al.*, 2015).

No preclinical or clinical studies, nor reports of such studies, were located since the initial GRAS determination for HO algal oil or *P. moriformis* (as described in GRN 000527) that indicate a safety concern of consuming an oil derived from a strain of *P. moriformis*. HO algal oil was previously assayed in a 90-day toxicity study in which the no-observed-adverse-effect-level (NOAEL) was 100,000 ppm, the highest dietary concentration provided in the study, which corresponded to a dietary NOAEL of 5,200 mg/kg bw/day in male rats and 6,419 mg/kg bw/day in female rats (Szabo *et al.*, 2014). This would correspond with human consumption of HO algal oil at 312 g/day (5,200 mg/kg bw/day for a 60 kg person), much greater than the estimated daily intake of HO algal oil as a partial replacement for cooking oils, with the 90th percentile level of intake estimated at 27.45 g/day (approximately 494.83 mg/kg bw/day).

A 13-week dietary study in rats was recently published that evaluated the potential of a different, stearic acid-rich fat (algal structuring fat) from a genetically related (*i.e.*, the parental *P. moriformis* strain was S376) and similarly classically and genetically engineered strain of *P. moriformis* (Matulka *et al.*, 2016). The study followed internationally-accepted protocol guidelines and provided a diet containing 0, 25,000, 50,000 and 100,000 ppm algal structuring fat (>50 Area % stearic acid, >30 Area % oleic acid and ≥2 Area % palmitic acid) to male and female Sprague Dawley rats (n=10/sex/group)[50] for 13 weeks. At the end of the study, the rats were euthanized,

[50] The control group contained 20 animals/sex.

with gross necropsies conducted on all animals and selected organs and tissues from control and high dose animals evaluated histologically.

No algal structuring fat-related mortalities occurred during the study and there were no changes in clinical signs or detailed clinical observations associated with administration of the algal structuring fat. Overall and calculated mean daily feed consumption were not different between control rats and any algal structuring fat dose group of the same sex. The overall mean daily intake of algal structuring fat in male rats fed dietary concentrations of 25,000, 50,000 and 100,000 ppm was 1285.6, 2594.3 and 5299.2 mg/kg bw/day, respectively. In female rats, the corresponding mean overall daily intake of algal structuring fat was 1606.0, 3069.7 and 6313.8 mg/kg bw/day, respectively. Mean weekly and overall body weights and calculated mean daily body weight gain for algal structuring fat dose groups were comparable to control groups for the respective sex (Figure 3).

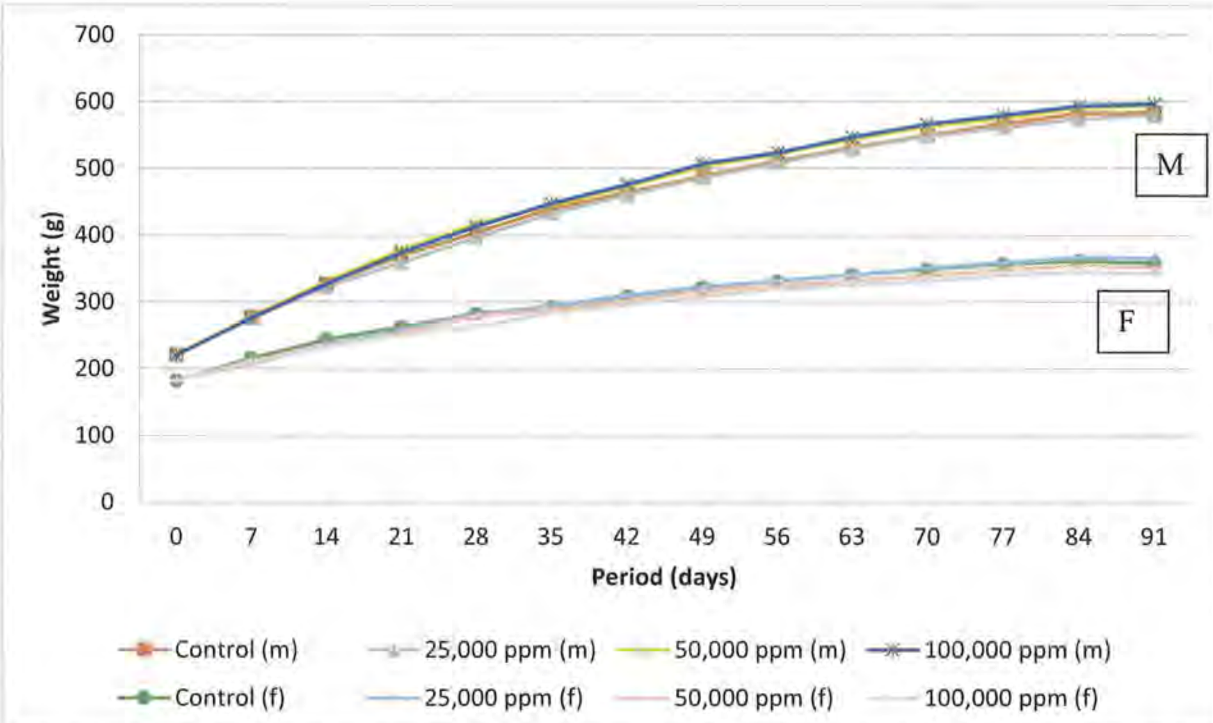

Figure 3. Mean body weights of male and female rats consuming diets containing algal structuring fat.

There were no algal structuring fat-related effects on hematology parameters for either male or female rats at any dose. A decrease ($P<0.05$) in blood urea nitrogen (BUN) was observed in the 100,000 ppm male dose group, but this clinical chemistry result was within the laboratory's historical control range and was not considered toxicologically relevant. The absolute mean organ weights and mean organ-to-body and organ-to-brain weights ratios for the male rats consuming algal structuring fat were not different from control values, and there were no absolute or relative organ weight changes in the male or female rats that were considered adverse. The liver-to-body weight ratios for the 50,000 and 100,000 ppm algal structuring fat male dose groups was significantly ($P<0.05$) decreased compared to the control group, but these changes were of small magnitude, lacked histopathological or serum chemistry correlates and were not reflected in corresponding decreases in absolute or liver-to-brain weight values. The no-observed-adverse-effect-level (NOAEL) for the algal structuring fat was 100,000 ppm for both males and females under the conditions of the study, the highest dietary concentration provided. The administered level of algal structuring fat corresponded to a dietary NOAEL of 5299.2 mg/kg bw/day in male rats and 6318.8 mg/kg bw/day in female rats (Matulka *et al.*, 2016). This study shows that different classically and genetically engineered modifications to this line of *P. moriformis* in order to produce oils with different fatty acid profiles still result in the formation of an oil that is not toxic at up to 100,000 ppm.

The safety of food ingredients can be further evaluated through the analysis of its components, especially when the ingredient is a macroingredient that cannot be administered to laboratory animals at sufficiently high enough doses for conventional risk evaluation methods (Howlett *et al.*, 2003). As stated in the original GRAS dossier (the subject of GRN 000527), the major components of HO algal oil are triglycerides (≥95%) and diglycerides (≤5.0%). Oleic acid is the main fatty acid in HO algal oil (average of 88% of the total fatty acids as stated in this amendment), with approximately 8% total saturated fat (Table 2). Unsaponifiable matter[51] for this neutral, RBD oil is ≤1.0 % and the free fatty acid content is ≤0.1%.

Vegetable oils are consumed in foods in a variety of forms, including the use of vegetable oils for the deep-fat frying of meats and vegetables (Wang *et al.*, 2016). However, research has found that the heating of vegetable and animal fats to the temperature for the frying of foods may result in the formation of α,β-unsaturated aldehydes from polyunsaturated fatty acids (PUFA), such as *alpha*-linolenic and linoleic fatty acids (Guillén and Goicoechea, 2008). *Alpha*, β-unsaturated aldehydes have been implicated in the formation of cardiovascular diseases (Grootveld *et al.*, 1998; Ng *et al.*, 2014). Although moderate consumption of used frying oils may not pose significant harm to the general population, some of the substances formed during the frying process of polyunsaturated oils may impair the nutritional value or be potentially harmful (el-Shattory *et al.*, 1991; Dobarganes and Márquez-Ruiz, 2015). The oxidation of PUFA may yield α,β-unsaturated hydroxyalkenals, such as 4-hydroxy-2-trans-nonenal (HNE). HNE is a highly reactive molecule that interacts with cellular macromolecules, forming adducts. Depending on the location, adduct formation can lead to alterations in normal cell functioning or signaling, or induce cytotoxicity and/or genotoxicity. The formation of α,β-unsaturated hydroxyalkenals have been implicated as a contributing factor in the initiation of a number of diseases (Barrera *et al.*, 2015; Csallany *et al.*, 2015). Other research indicates that α,β-unsaturated aldehydes induce oxidative stress through the reduction in cellular glutathione (GSH), which may play a role in the formation of vascular disease *via* redox-sensitive mechanisms or through direct injury to the endothelium (Lee and Park, 2013).

In an independent study, HO algal oil was evaluated for the production of α,β-unsaturated aldehydes after heating to the temperature necessary for frying (375°F) (Grootveld, 2015). HO algal oil was found to produce substantially less α,β-unsaturated hydroxyalkenals (Figure 4; this category includes HNE) and α,β-unsaturated 2,4-alkadienals (Figure 5) than other types of oils. *α*,

[51] Unsaponifiable matter is defined as all substances present in a product which, after saponification of the product by potassium hydroxide and extraction by hexane, are not volatile under the specified operating conditions. Unsaponifiable matter includes lipids of natural origin such as sterols, higher hydrocarbons and alcohols, aliphatic and terpenic alcohols, as well as any foreign organic matter extracted by the solvent and not volatile at 103 °C (*e.g.*, mineral oils) that may be present (ISO, 2000).

β-Unsaturated 2,4-alkadienals (represented by 2,4-decadienal) are possible carcinogens (EFSA, 2014). The data from this corroborative study confirm that frying of HO algal oil produces fewer α,β-unsaturated aldehydes than other vegetable oils currently used in the U.S., suggesting a decreased potential for toxicity by the use of HO algal oil instead of other types of oils. Aldehyde formation typically occurs in commonly consumed oils used in the food industry; this study only further underscores the safety-in-use of HO algal oil; this information is not pivotal to the determination of safety of the HO algal oil at the intended level of use.

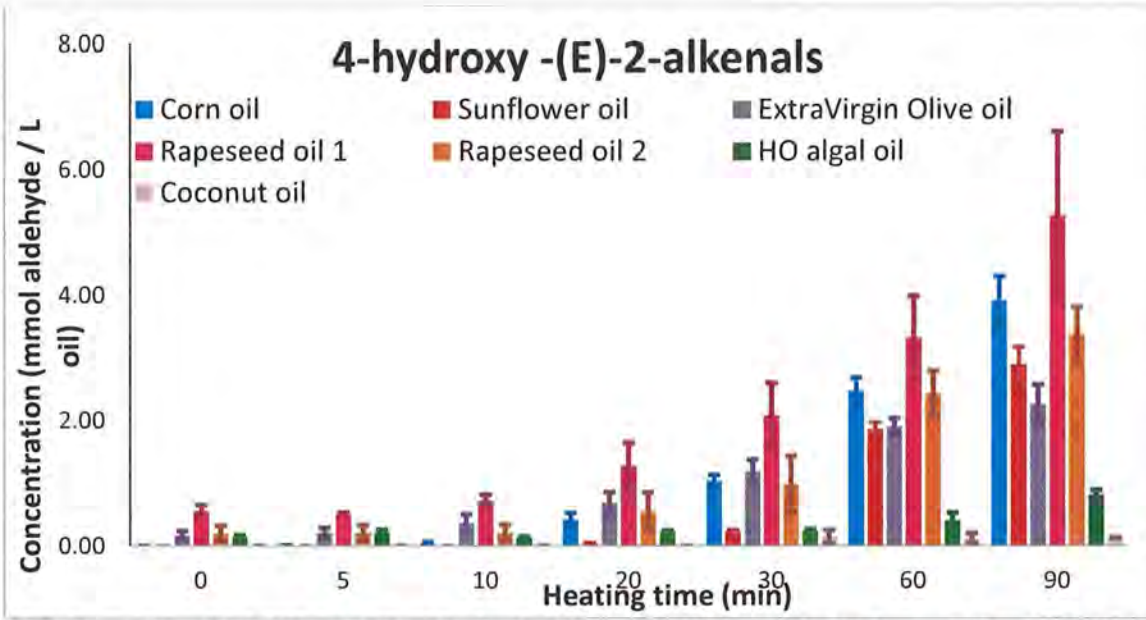

Figure 4. Formation of 4-hydroxy-2-alkenals during the heating of vegetable oils (mean ± Standard Deviation) (Grootveld, 2015).

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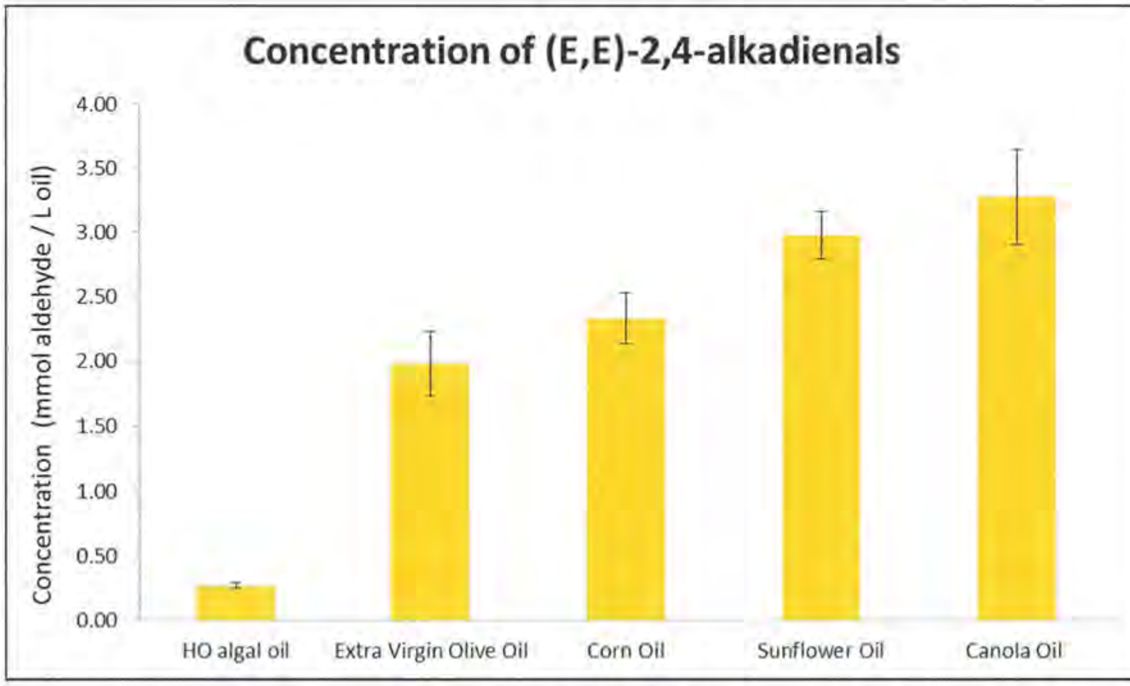

Figure 5. Concentration of (E,E)-2,4-alkadienals after Simulated Sautéing for 20 Minutes at 356°F (mean ± Standard Deviation) (Grootveld, 2015).

6.14. Observations in Humans

TerraVia has been selling HO algal oil at the retail and business-to-business levels for the past year, selling over 215,000 liters. TerraVia has not received reports of adverse event cases confirmed as a direct result of the consumption of the HO algal oil (TerraVia, 2016).

6.15. Evaluation

HO algal oil is a refined, bleached, deodorized oil composed mainly of oleic acid-rich (≥80% oleic acid) triglycerides produced from a classically improved and genetically engineered strain of *P. moriformis*. The original GRAS dossier is being amended to modify the levels of HO algal oil use in foods that were in the original GRAS dossier. Analysis of the genetic stability of the S6697 *P. moriformis* strain (evaluated in the amendment) demonstrated that the changes introduced to this genetically engineered strain were stable in the microalgae. Analysis of the oil confirms that it meets the GRAS specifications containing the revised *p*-anisidine value, and in addition, analysis showed that markers for DNA from endogenous and inserted DNA were not contained in the oil. The original GRAS dossier was notified as GRAS to the FDA and subsequently received a "no objection" letter in response.

The source organism used to produce HO algal oil has been substantively changed (described in Section 6.2.2.), compared to the source organism detailed in GRN 000527. However, the remainder of the manufacturing process of the HO algal oil described in the conclusion of GRAS status for HO algal oil produced via strain S6697 has not changed since the original conclusion of GRAS status was finalized and detailed in GRN 000527. The limit for *p*-anisidine was modified to be consistent with levels found in the oil industry. The use indicates acceptance of HO algal oil as a partial replacement for conventional vegetable oils and is being increased from 12.5 g/day to 27.47 g/day (90th percentile estimated consumption level). The new mean and 90th percentile levels are 12.04 and 27.47 g/day, respectively, representing approximately 220.36 and 494.83 mg/kg bw/day.

The HO algal oil is mainly composed of oleic, stearic and palmitic fatty acids, which are fatty acids found in commonly consumed vegetable oils. The fatty acids are provided in a triglyceride format, with few di- and mono-glycerides contained in the HO algal oil, consistent with other commonly consumed vegetable oils.

The NOAEL for HO algal oil derived from S2532 is 5,200 mg/kg bw/day in male rats and 6,419 mg/kg bw/day in female rats, the highest dose evaluated. No additional safety information that indicates a potential for toxicity from the consumption of HO algal oil has been published since the original GRAS determination that indicates a potential for toxicity from the consumption of HO algal oil. A 13-week preclinical dietary toxicology study was conducted in rats that evaluated the safety of a stearic acid-rich algal structuring fat produced using a genetically related, classically and genetically engineered strain of *P. moriformis*. The NOAEL for this algal structuring fat is 5,299 mg/kg bw/day in male rats and 6,313 mg/kg bw/day in female rats, the highest dose provided to the rats.

The anticipated upper intake 90th percentile level of HO algal oil is significantly lower than the NOAEL established in preclinical testing. In addition, no adverse events have been reported from consumer intake of HO algal oil over the past year that have confirmed HO algal oil as the causative agent.

Analysis of α,β-unsaturated aldehyde formation found that heating of HO algal oil to temperatures typical with the frying of food (375 °F) produces much lower levels of α,β-unsaturated aldehydes, implicated in a variety of cardiovascular or vascular diseases, than the common fry oils corn oil, soybean oil, and canola oil.

In summary, information available on the production, components, safety and by-products of HO algal oil continue to support the safety-in-use of HO algal oil at an anticipated upper consumption of 27.47 g/day.

The following page provides the signed Expert Panel conclusion on the amendment for the continued GRAS status of HO algal oil at the increased level of consumption.

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9. CONCLUSION

We, the Expert Panel, have individually and collectively critically evaluated information generated since the first GRAS review and summarized in this document (an amendment to the dossier in support of the generally recognized as safe (GRAS) status HO Algal Oil from a modified strain of *Prototheca moriformis* as a food ingredient and, unanimously conclude that HO algal oil, produced in accordance with current Good Manufacturing Practice (cGMP) and meeting the specifications described in this amendment is safe for use in foods at an estimated upper consumption level of 27.47 g/day (for specific foods and levels of use, refer to APPENDIX I).

We further unanimously conclude that the use of HO algal oil is Generally Recognized As Safe by scientific procedures.

It is our opinion that other experts qualified by scientific training and experience to evaluate the safety of food and food ingredients would concur with these conclusions.

10. SIGNATURES

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11/3/16
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Oct 28, 2016
Date

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Madison, Wisconsin

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11/7/16
Date

Steve Saunders, Ph.D.
Managing Director, HCTox, Inc.
Adjunct Professor of Biology, Lees-McRae College

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Solazyme. (2016) Amendment to the Dossier in Support of the Generally Recognized As Safe (GRAS) Status of High-Oleic Algal Oil from a Modified Strain Of *Prototheca moriformis* as a Food Ingredient.

TerraVia (2016) Letter discussing sales and adverse reactions. (Personal Communication)

Yu, E. and Zhao, X. (2015) RR-230 Absence of DNA in S6697 RBD oil. Real-Time qPCR cycle threshold (Table 1).

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September 13, 2017

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Re: Suitability of High Oleic Algal Oil in Food Products

Dear Dr. Green,

Recently TerraVia was requested by your office to "submit suitability studies that evaluates the use of algal oil showing equivalency of its use as a replacement and that it doesn't lead to altered taste, appearance or smell of the final product."[1] High oleic algal oil (HO Algal oil, also referred to as AlgaWise® oil) is an edible oil that has recently been concluded to be generally recognized as safe (GRAS) as a partial replacement for other high oleic acid-containing edible oils in the market. As part of the GRAS Notification process, FDA requested that we reach out to your office for a discussion on the suitability of HO Algal oil in meat-containing food products.

TerraVia's HO Algal oil has been concluded as GRAS when used as a partial replacement for other edible oils in a wide variety of food products, including products containing meat at 2% or greater (of total weight) (TerraVia, 2016).

The addition of edible oils in many different food products is typically at relatively low levels (*e.g.*, oil added to pot pies or other pie crusts, oil added to cakes or cookies) and many times the other components of the food product masks the overall flavor/acceptance of the cooking oil. The use of a plant-based oil as a fry oil is one of the harshest conditions on the oil, as it is typically heated and cooled multiple times over several days, while also providing one of the most direct abilities to assess the sensory qualities of an edible oil.

Several sensory studies have been conducted in which HO Algal oil has been compared with commercially available plant-based oils. Potato fries were utilized as the food matrix for the experimental study, as potato fries have minimal taste to mask the sensory attributes of the oil.

An experiment on the sensory profiling of fry oils utilizing potato fries was conducted by the North Carolina State University Sensory Service Center (Drake, 2014). Six different plant-based oils were evaluated in the study: canola, sunflower, Sysco® Fry-On® canola and corn oil

[1] Email sent to Dr. Ray Matulka (rmatulka@burdockgroup.com) from Ms. Valeria Green (Valeria.Green@fsis.usda.gov) and received on August 11, 2017.

blend[2], palm, HO Algal oil (containing TBHQ[3]), and HO Algal oil (containing tocopherols). Twenty 230g batches of McCain® Fresh-Style Water Blanched Fries 3/8" (SKU: MCX05157) were fried in each oil over an 8 hour heating period/day in 6.8 kg oil bench top fryers. Batches were fried for five minutes at 188°C, allowed to drain, then collected for descriptive analysis, or disposed. Descriptive analysis was conducted on both the straight oils (Time 0) and of the fries and the used oils on Days 1, 3, 5 7, 9 and then every day until failure.

The cooked potato fries and the oils were evaluated in duplicate by seven trained panelists, each with more than 120 hours of experience in the descriptive analysis of foods and flavors using the Spectrum™ descriptive analysis method (Yamagata and Sugawara, 2014). Prior to official profiling, the panelists evaluated and discussed sensory attributes of samples to generate sensory attributes. Panelists then evaluated coded samples in duplicate in separate sessions, according to appropriate sensory testing practices. Each panelist evaluated the aroma of the sample and then tasted each sample. Samples were expectorated and deionized water was used for palate cleansing.

The sensory panel that evaluated the sensory attributes of the different oils found a decreased ($P<0.05$) "oily" taste and overall aroma from the HO Algal oil, compared to palm, sunflower, canola (oily only) or the Sysco blended oil and a decreased ($P<0.05$) "toasted/sweet" flavor, when compared to sunflower oil on Day 0 of the study (Table 1). The HO Algal oil with TBHQ had increased "oily" taste on Days 1 and 3 when compared to the other oils, which was not seen with HO Algal oil with tocopherols. The HO Algal oil with tocopherols had a markedly decreased "Acrid/throat burn" taste ($P<0.05$) on Day 5, compared to the other oils, and remained low on Day 7, when compared to the sunflower, canola and Sysco blended oils, as well as having a decreased "Fatty/fryer oil" taste ($P<0.05$). The Acrid/throat burn taste of the HO Algal oils was less ($P<0.05$) than that found by the Sysco blended oil, but was similar ($P>0.05$) to the canola, sunflower and palm oils. The other noted sensory attributes of the different oils after cooking potato fries were not markedly different from one another.

Sensory evaluation of the potato fries after being cooked in the different oils found that the taste of the fried potatoes on Day 1 was not different between the different cooking oils (Table 2). On Day 3 the potatoes fried with the HO Algal oil were similar in taste with many of the other oils, except that the canola-fried potatoes had less ($P<0.05$) of an "oily" and "potato" taste than the other fries. On Day 5, the potatoes fried with HO Algal oil with tocopherols had less ($P<0.05$) of an "oily" taste, compared to the other fries and less of a "toasted" taste compared to canola-fried potato fries. Potato fries cooked in the HO Algal oil with TBHQ on Day 7 had more ($P<0.05$) of an "acrid/throat burn" taste compared to the other oils, but all other taste attributes were similar. This "acrid/throat burn" taste in fries cooked in the HO Algal oil with TBHQ remained higher ($P<0.05$) on Days 9 and 10 along with a "painty" taste, which was not as apparent with the HO Algal oil with tocopherols.

Overall, when considering the taste attributes of the HO Algal oil when compared to the other commercial oils over a 10-day use period in frying potato fries, the use of the HO Algal oils did not markedly change the overall taste profile the potato fries, and the taste of the HO Algal oil was very similar to the other commercial oils in the study.

[2] Sysco Fry-On oil product; http://www.sysco.com/fryon/files/Sysco_Fry_On.pdf; site last visited September 6, 2017.

[3] TBHQ= tertiary butylhydroquinone, a common preservative.

Table 1. Sensory attributes of different commercial oils and HO Algal oil before and after frying potato fries (Drake, 2014).

Attribute	Sysco	Canola	Sunflower	HSHO TBHQ	HSHO TOCO	Palm
Day 0						
Overall aroma	2.0a	1.6ab	2.0a	1.0b	1.3b	2.0a
Aroma comments	Oily	Green	Toasted, nutty	Sweet, toasted	Toasted, sweet	Sweet
Green	ND	1.0b	1.5a	ND	ND	ND
Oily	2.4a	1.8b	2.0ab	1.0c	1.0c	2.0a
Fatty/fryer oil	2.0	ND	ND	ND	ND	ND
Acrid/throat burn	ND	ND	ND	ND	ND	ND
Toasted/sweet	ND	1.0b	1.5a	1.0b	1.0b	ND
Sweet/carrots	ND	ND	ND	ND	ND	2.8
Day 1						
Overall aroma	3.0a	3.3a	3.0a	3.0a	3.3a	2.5b
Aroma comments	Oily, fishy	Green, fishy	Toasted, fatty	Sweet, toasted, fishy	Toasted, fatty, fishy	Sweet
Green	ND	2.8	ND	ND	ND	ND
Oily	2.0b	1.0c	2.0b	2.8a	2.0b	2.0b
Fatty/fryer oil	2.5a	0.5b	0.5b	ND	ND	ND
Fishy	1.0	1.0	ND	ND	ND	ND
Acrid/throat burn	1.3ab	ND	1.0b	1.7a	ND	ND
Toasted/sweet	ND	ND	1.5b	2.0a	2.3a	ND
Carrots/sweet	ND	ND	ND	ND	ND	2.2
Day 3						
Overall aroma	3.3a	3.3a	2.8a	3.0a	3.3a	2.8a
Aroma comments	Oily, fishy	Oily, fishy	Toasted, fatty	Sweet, toasted, fishy	Toasted, fatty, fishy	Sweet
Green	ND	ND	ND	ND	ND	ND
Oily	2.0b	2.0b	2.0b	2.8a	2.0b	2.0b
Fatty/fryer oil	3.5a	1.4d	1.8c	2.0bc	1.5cd	2.4b
Fishy	1.0a	1.0a	ND	ND	ND	ND
Painty	1.2b	1.5ab	1.0b	1.0b	ND	1.7a
Acrid/throat burn	1.9a	1.5ab	1.2b	2.2a	1.2b	2.0a
Toasted/sweet	1.0b	ND	1.3b	2.2a	2.6a	ND
Carrots/sweet	ND	ND	ND	ND	ND	2.6
Day 5						
Overall aroma	3.0a	3.3a	2.8ab	2.5b	3.3a	2.0b
Aroma comments	Oily, fatty, painty	Green, fishy	Toasted, fatty	Sweet, toasted, fishy	Toasted, fatty, fishy	Sweet
Green	ND	ND	ND	ND	ND	ND
Oily	1.0b	1.0b	1.0b	1.0b	1.0b	2.0a
Fatty/fryer oil	3.0a	3.0a	2.5a	2.6a	2.6a	2.8a
Painty	2.5a	1.8b	2.0ab	1.8b	1.7b	1.8b
Fishy	1.0	ND	ND	ND	ND	ND
Acrid/throat burn	1.5ab	1.3b	1.0b	2.0a	0.5c	1.0b
Toasted	2.8a	2.6a	2.5a	2.5a	2.3a	ND
Carrots/sweet	ND	ND	ND	ND	ND	2.5

Table 1. Sensory attributes of different commercial oils and HO Algal oil before and after frying potato fries (Drake, 2014).

Attribute	Sysco	Canola	Sunflower	HSHO TBHQ	HSHO TOCO	Palm
Flavor comments	Lingering oily/fatty	Lingering oily/fatty	Lingering oily/fatty	Lingering oily/fatty	Lingering oily/fatty	Lingering oily/fatty, carrot/ coconut
Day 7						
Overall aroma	3.0a	3.0a	3.0a	3.0a	3.3a	3.0a
Aroma comments	Oily, fatty painty	Oily painty	Toasted, painty	Toasted, burnt	Toasted, burnt	Fatty, painty coconut
Coconut	ND	2.8a	ND	ND	ND	2.5a0
Oily	1.0a	1.0a	1.0a	ND	ND	ND
Fatty/fryer oil	3.0a	3.3a	3.0a	3.0a	2.0b	3.0a
Fishy	ND	ND	ND	ND	ND	ND
Painty	2.5a	2.4ab	3.0a	2.5a	2.2b	3.0a
Acrid/throat burn	3.3a	2.5b	2.5b	3.0a	2.0c	2.0c
Toasted/burnt	ND	ND	1.5b	2.4a	2.3ab	2.8a
Carrots/sweet	ND	ND	ND	ND	ND	2.0
Day 9						
Overall aroma	2.0b	2.0b	2.0b	3.0a	2.0b	3.0a
Aroma comments	Painty, fishy, toasted	Painty, toasted	Painty, fishy	Burnt, acrid	Burnt, acrid	Painty, coconut
Coconut	ND	ND	ND	ND	ND	2.0
Toasted/burnt	ND	ND	ND	2.7a	2.8a	ND
Fatty/fryer oil	3.2a	3.2a	2.4b	2.8ab	2.8ab	3.0a
Fishy	ND	ND	ND	ND	ND	ND
Painty	2.6b	2.4b	3.5a	2.5b	2.2b	3.4a
Acrid/throat burn	3.8a	3.3ab	2.7b	3.0b	2.7b	2.8b
Cardboard	2.5ab	2.8a	2.0b	ND	ND	ND
Flavor comments	Dirty, lingering aftertaste, fail	Dirty, lingering aftertaste, fail	Dirty, lingering aftertaste, fail	Dirty, lingering aftertaste, fail	Dirty, lingering aftertaste, fail	Dirty, lingering aftertaste, fail

*Small letters after means in a row indicate significant (P<0.05) differences for that attribute with that time point; #Attributes were scored on a 0 – 15 point universal Spectrum™ intensity scale. Most flavor attributes fell between 0 and 5. ND=Not detected.

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Table 2. Sensory attributes of fries cooked in different commercial oils and HO algal oil (Drake, 2014).

Attribute	Sysco	Canola	Sunflower	HSHO TBHQ	HSHO TOCO	Palm
Day 1						
Potato	2.8a	3.2a	3.3a	3.0a	3.0a	2.8a
Oily	2.2a	2.0a	2.0a	2.0a	2.0a	2.0a
Chemical	ND	ND	ND	1.5a	1.0a	ND
Carrots	ND	ND	ND	ND	ND	2.6
Day 3						
Fry aroma	Fatty	Fried potato	Potato, fatty	Toasted, potato	Toasted, potato	Oily carrots
Potato	2.2b	3.0a	2.3b	2.3b	2.5ab	2.0b
Oily	2.3a	1.5b	2.3a	2.4a	2.0a	2.3a
Carrots	ND	ND	ND	ND	ND	2.5
Toasted	ND	ND	ND	1.3a	1.3a	ND
Fatty/fryer oil	ND	ND	ND	ND	ND	ND
Acrid/throat burn	1.0	ND	ND	ND	ND	ND
Day 5						
Potato	2.3a	2.5a	2.5a	2.0a	2.0a	2.0a
Oily	2.9a	2.5ab	2.7a	2.6a	2.0b	2.8a
Carrots	ND	ND	ND	ND	ND	3.0
Toasted	2.0ab	1.0b	1.0b	1.3ab	1.3ab	1.6a
Fatty/fryer oil	1.4ab	1.0b	1.0b	1.3ab	1.3ab	1.6a
Acrid/throat burn	1.0a	ND	ND	1.0a	1.0a	ND
Day 7						
Potato	2.4a	2.6a	2.3a	2.3a	2.2a	2.0a
Oily	2.7a	2.6a	2.4a	2.7a	2.5a	2.8a
Carrots	ND	ND	ND	ND	ND	2.8
Toasted	2.0a	2.2a	2.2a	2.4a	2.0a	2.0a
Fatty/fryer oil	1.5a	1.7a	1.5a	1.8a	1.7a	2.0a
Acrid/throat burn	1.0b	1.0b	1.5b	2.3a	1.5b	1.6b
Day 9						
Fry aroma	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	Fried burnt carrots
Potato	2.0b	2.5a	2.0b	2.0b	2.0b	1.4c
Oily	1.5a	1.6a	1.4a	1.7a	1.3a	1.0b
Carrots	ND	ND	ND	ND	ND	2.0
Toasted/burnt	2.0b	2.7a	2.4ab	2.5a	2.7a	2.0b
Fatty/fryer oil	2.7a	2.0b	2.0b	1.5c	1.9bc	2.4a
Painty	1.0b	1.0b	1.5b	2.3a	1.5b	1.6b
Acrid/throat burn	1.0c	1.0c	1.5bc	2.3a	1.8b	1.6b
Flavor comments	Sour taste, fatty	Fatty	Fatty	Toasted/burnt aftertaste	Toasted/burnt aftertaste with oily mouth coat, sour taste	Lingering fried/fatty aftertaste
Day 10						
Fry aroma	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	Oily, burnt potato	---
Potato	2.0b	2.5a	2.0b	2.0b	2.0b	---
Oily	1.7s	2.0s	1.5s	2.0s	1.5s	---
Carrots	ND	ND	ND	ND	ND	---
Toasted/burnt	2.0b	2.5a	2.5a	2.5a	2.7a	---

Fatty/fryer oil	3.0a	2.5b	2.5b	2.5b	2.5b	---
Painty	1.5c	1.5c	2.0b	3.0a	2.0b	---
Acrid/throat burn	1.0c	1.0c	1.5b	2.3a	1.8b	---
Flavor comments	Sour taste, fatty	Fatty	Fatty	Toasted/burnt aftertaste	Toasted/burnt aftertaste with oily mouth coat; sour taste	Heavy smoke; unable to fry

*Small letters after means in a row indicate significant ($P<0.05$) differences for that attribute with that time point; **Day 11 oils were smoking and fries were past acceptability with high painty and fatty sensory notes (informal tasting after single batch due to smoking oils); #Attributes were scored on a 0 – 15 point universal Spectrum™ intensity scale. Most flavor attributes fell between 0 and 5. ND=Not detected.

Additional study results were provided in which the cooking oils (high oleic canola oil, high oleic sunflower oil, high oleic Algal oil, palm and Sysco Fry-on oil) were evaluated for analysis of oxidative stability index (OSI) values, peroxide values, p-anisidine, free fatty acid analysis and color (Solazyme, 2014).

Sensory evaluation of the oils prior to frying showed that the HO Algal oil had the lowest aroma and oily taste among the tested oils (Table 1). There were no significant differences in the potato and oily flavor of the cooked potato fries in all test oils. The oxidative stability value of the HO Algal oil was higher than the other oils, and stayed consistent throughout the frying study (Figure 1). The free fatty acid values were similar for the HO Algal, canola and sunflower oils (Figure 2), while the p-anisidine values for the duration of the study were lowest for the HO Algal oil (Figure 3). This study shows HO Algal oil providing similar or better stability attributes during the food frying process.

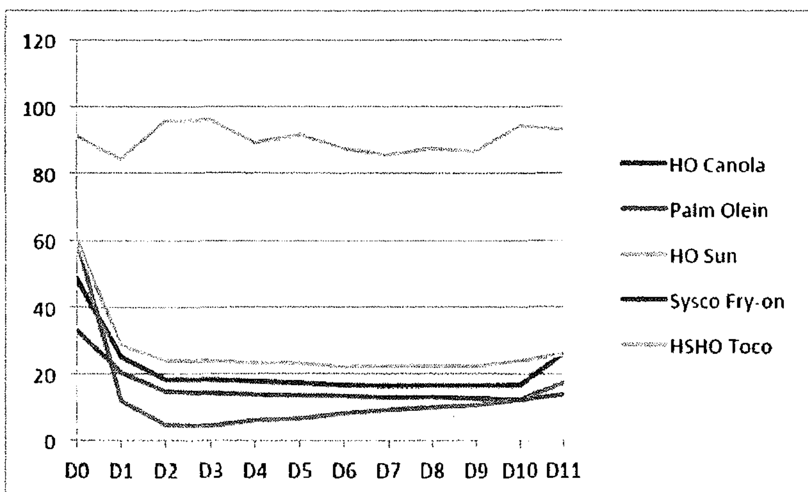

Figure 1. Oxidative Stability Index (hours) of oils used for cooking potato fries (Solazyme, 2014).

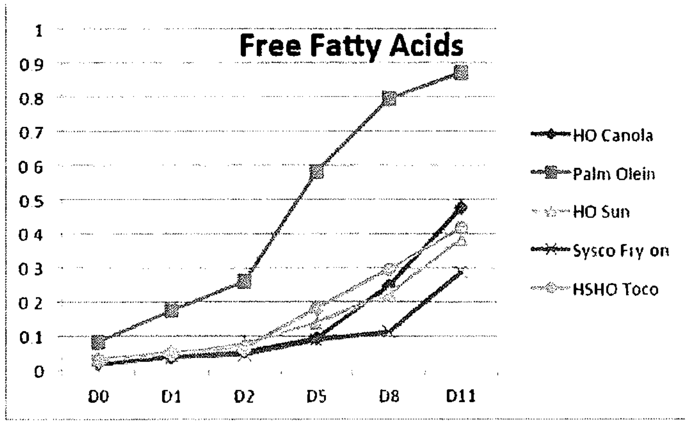

Figure 2. Free Fatty Acid values of oils used for cooking potato fries (Solazyme, 2014).

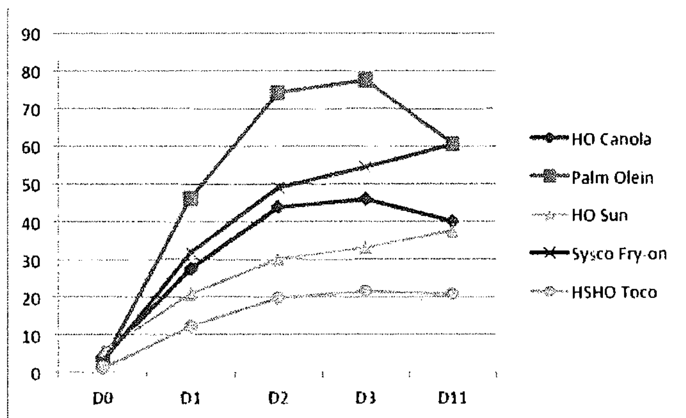

Figure 3. p-Anisidine values of oils used for cooking potato fries (Solazyme, 2014).

The trained sensory panel at North Carolina State University Sensory Service Center also provided flavor observations when comparing HO Algal oil to Mazola 100% Canola oil[4] and Napa Valley Sunflower oil,[5] the results of which are portrayed in a spider diagram (Figure 4; TerraVia, 2016a). The overall flavor impact of HO Algal oil was within the flavor parameters for both the canola oil and the sunflower oil, indicating that the HO Algal oil has the same, or a subdued flavor profile, when compared to the other two retail oils, and therefore is not expected to impart any flavors above those already seen by cooking oils that are already on the market and extensively utilized and accepted by the U.S. population.

[4] http://www.mazola.com/products/canola-oil.aspx; site last visited Sept 1, 2017.

[5] http://www.napavalleynaturals.com/Organic-Sunflower-Oil/p/NVN-SUNFLOWER&c=NapaValleyNaturals@CulinaryOils; site last visited September 1, 2017.

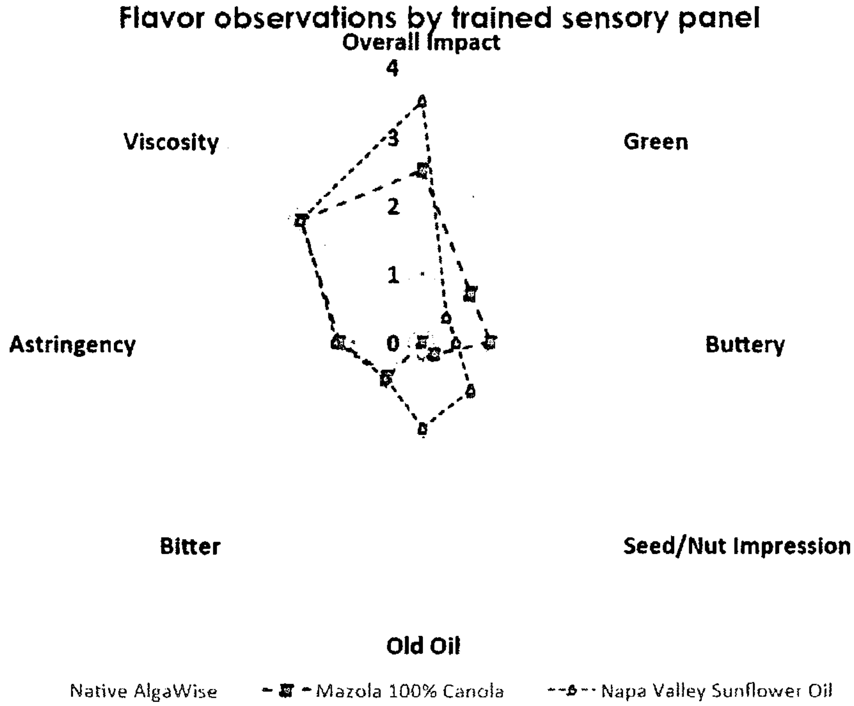

Figure 4. Flavor profile study of HO Algal oil compared to retail cooking oils (TerraVia, 2016a).

Further work conducted a comparative assessment of the HO Algal oil with other vegetable oils as a deep frying oil (Waghmare *et al.*, 2017). In this study, HO Algal oil, sunflower oil and palm oil were heated to 160 °C for 3 hours and four batches of potato fries were fried at an interval of 45 minutes/day. The process was repeated for four consecutive days with the same oils; 40 ml of each oil was retained each day and analyzed for: acid value, acidity, color, density, refractive index, viscosity, oil uptake, percentage free fatty acid, peroxide value, total polar compounds, radical scavenging activity and fatty acid profile. The cooked potato fries were evaluated for texture and sensory properties, with sensory analysis performed using a fuzzy logic method of sensory evaluation (Folorunso *et al.*, 2009). The refined sunflower and palm oils were purchased at the retail level, while the HO Algal oil (AlgaWise®) was provided by TerraVia. The potato fries were McCain® French fries purchased at the retail level.

The density[6] of the HO Algal oil was not changed during the study, while slight, nonsignificant (P>0.05) density and refractive index (RI) changes were noted in the sunflower and palm oils (Table 3). Viscosity during repetitive heating and frying cycles is typical and is dependent on the fry-related byproducts, changes in triacylglycerol molecular size and degree of saturation. The viscosity of the sunflower and palm oils significantly (P<0.05) changed during the study, while the HO Algal oil viscosity changes did not reach significance (P>0.05), compared to the oil prior to frying. Significant changes in the acid values of the three oils occurred during the frying process in this study (P<0.05), compared to their respective control samples taken prior to the fry process. However, all of the oils retained an acceptable acid value of less than 3.0 (Zhang *et al.*, 2015). Hydrolysis (as determined by peroxide value analysis) occurred in all three oils,

[6] Changes in density may be responsible for changes in heat transfer from the oil to the food (Paul and Mittal, 1996).

indicating an increase in formation of free fatty acids during the frying process, but peroxide values remained within Codex limits for refined oils (Codex, 2015). In addition, the free radical scavenging activity for all three oils decreased ($P<0.05$) over multiple fry uses, as was expected (Table 3), while the fatty acid composition of the three oils remained relatively constant over the frying study (Table 4).

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Table 3. Effect of frying cycle on physical properties, chemical properties and radical scavenging activity of deep frying oils (Waghmare *et al.*, 2017).

	Physical properties			Chemical properties					RSA	Color
Oil and frying cycle	Density (g/ml)	Refractive index	Viscosity (mPas)	AV (mg KOH/g)	Acidity (%)	% FFA (g/g)	PV (meq/kg)	TPC (g/g)	DPPH (μM/ml)	OD at 425 nm
Control sunflower oil	0.912^{a}	1.470^{a}	29.00^{a}	0.17^{a}	0.75^{a}	0.08^{a}	1.47^{a}	8.30^{a}	198.57^{c}	0.105
Sunflower oil-1	0.912^{a}	1.471^{a}	35.67^{b}	0.32^{b}	0.95^{b}	0.16^{a}	2.73^{b}	10.35^{b}	179.29^{b}	0.173
Sunflower oil-2	0.913^{a}	1.471^{a}	44.00^{c}	0.52^{c}	1.00^{b}	0.26^{b}	3.80^{c}	14.99^{c}	168.28^{b}	0.185
Sunflower oil-3	0.914^{a}	1.472^{a}	63.67^{d}	0.65^{d}	1.12^{c}	0.33^{b}	4.80^{d}	22.30^{d}	152.62^{a}	0.204
Sunflower oil-4	0.915^{a}	1.473^{a}	83.33^{e}	0.67^{d}	1.19^{d}	0.34^{b}	6.53^{e}	29.99^{e}	147.41^{a}	0.214
Control palm oil	0.890^{a}	1.464^{a}	44.33^{a}	0.15^{a}	1.07^{a}	0.08^{a}	1.53^{a}	14.75^{a}	307.70^{d}	0.408
Palm oil-1	0.893^{a}	1.464^{a}	47.33^{a}	0.43^{b}	1.07^{a}	0.22^{b}	2.87^{b}	15.99^{b}	120.16^{c}	0.425
Palm oil-2	0.898^{a}	1.464^{a}	59.67^{b}	0.58^{c}	1.19^{b}	0.29^{c}	3.93^{c}	18.37^{c}	78.13^{b}	0.437
Palm oil-3	0.905^{a}	1.465^{a}	75.67^{c}	0.71^{d}	1.53^{c}	0.36^{d}	5.13^{d}	20.46^{d}	47.99^{a}	0.460
Palm oil-4	0.914^{a}	1.465^{a}	89.00^{d}	0.88^{e}	1.91^{d}	0.44^{e}	5.93^{e}	26.75^{e}	43.64^{a}	0.468
Control algal oil	0.915^{a}	1.467^{a}	40.67^{a}	0.06^{a}	0.65^{a}	0.03^{a}	0.73^{a}	5.92^{a}	127.41^{d}	0.165
Algal oil-1	0.915^{a}	1.467^{a}	41.00^{a}	0.19^{b}	0.66^{a}	0.09^{b}	1.40^{b}	7.10^{b}	106.25^{c}	0.216
Algal oil-2	0.915^{a}	1.467^{a}	40.67^{a}	0.30^{c}	0.70^{b}	0.15^{c}	2.47^{c}	8.84^{c}	83.49^{b}	0.223
Algal oil-3	0.915^{a}	1.467^{a}	41.00^{a}	0.39^{d}	0.74^{c}	0.19^{d}	3.07_{d}	12.75^{d}	63.49^{a}	0.232
Algal oil-4	0.915^{a}	1.467^{a}	42.00^{a}	0.47^{e}	0.79^{d}	0.23^{e}	3.53^{e}	18.04^{e}	59.87^{a}	0.243

Note: AV-Acid value, FFA- Free fatty acids, PV- Peroxide value, TPC- Total polar compounds, DPPH- 1,1-Diphenyl-2-picrylhydrazyl, RSA- Radical scavenging activity, ABTS- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid. All values are averages of three determination.
Means within a column for each type of oil marked with the different letters differ significantly at $P < 0.05$.

Table 4. Fatty acids composition of oil before and after frying process (Waghmare *et al.*, 2017).

Fatty acids	Frying cycle (days)	Sunflower oil (%)	Palm oil (%)	Algal oil (%)
Palmitic acid	0	10.13 ± 0.01	41.28 ± 0.55	6.03 ± 0.01
(C16:0)	4	12.27 ± 0.06	45.03 ± 0.34	7.60 ± 0.54
Stearic acid	0	4.84 ± 0.21	5.58 ± 0.27	4.97 ± 0.14
(C18:0)	4	5.18 ± 0.11	5.09 ± 0.09	3.82 ± 0.30
Oleic acid	0	26.35 ± 0.04	38.51 ± 0.12	86.60 ± 0.42
(C18:1)	4	28.78 ± 0.13	40.08 ± 0.35	85.84 ± 1.17
Linoleic acid	0	58.67 ± 0.25	14.64 ± 0.15	2.40 ± 0.26
(C18:2)	4	53.77 ± 0.04	9.81 ± 0.10	3.10 ± 0.34

All values are averages of two determination with ± standard deviation

The increase in fry time for all oils examined increased oil uptake by the fried potatoes, but there was no significant difference between the oils (Figure 5), while the overall similarity ranking of the fried potatoes cooked in the different oils indicated similar overall food product taste values (Table 5), ranging from "very good" for the potatoes fried in the HO Algal oil at the start of the study, to "fair" overall rating for the potatoes fried in the sunflower oil at the end of the study. This study shows the comparable taste qualities of HO Algal oil with commercially available sunflower and palm oils, when evaluated under harsh frying conditions (Wagmore et al., 2017).

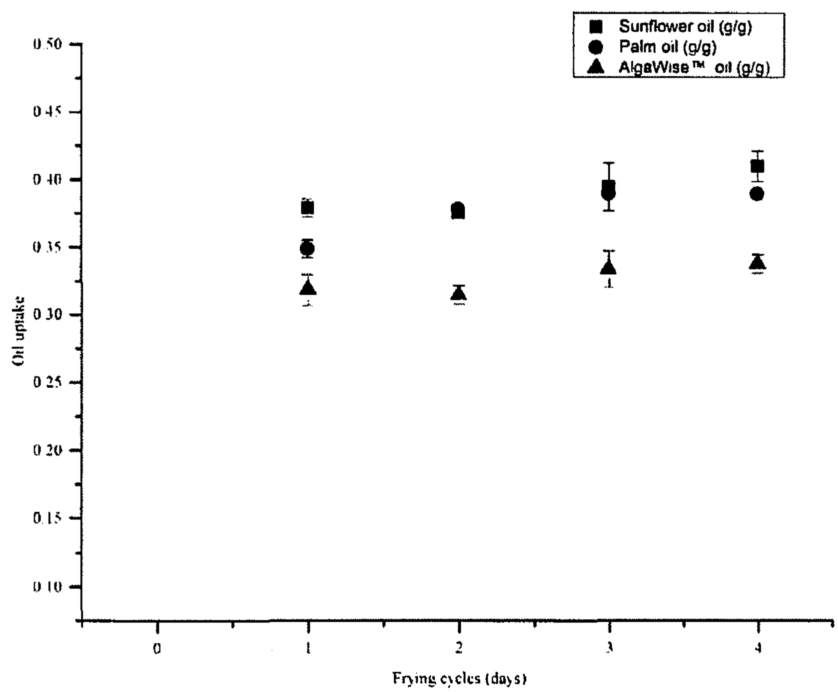

Figure 5. Effect of frying cycle on oil uptake of oils (All values are averages of three determinations with ± standard deviation).

Table 5. Similarity values of samples for overall ranking.

	Samples (Fried in sunflower oil with batch number)							
Scale factor	SO1	SO3	SO5	SO7	SO9	SO11	SO13	SO15
Not satisfactory, F1	0.00	0.00	0.00	0.02	0.01	0.03	0.14	0.22
Fair, F2	0.12	0.16	0.18	0.27	0.25	0.34	0.66	**0.81**
Satisfactory, F3	0.54	0.62	0.66	0.78	0.76	**0.85**	**0.90**	0.68
Good, F4	**0.96**	**0.96**	**0.95**	**0.92**	**0.94**	0.81	0.37	0.13
Very good, F5	0.68	0.57	0.53	0.37	0.42	0.23	0.02	0.00
Excellent, F6	0.13	0.08	0.06	0.03	0.04	0.00	0.00	0.00
	Samples (Fried in palm oil with batch number)							
Scale factor	PO1	PO3	PO5	PO7	PO9	PO11	PO13	PO15
Not satisfactory, F1	0.00	0.01	0.02	0.01	0.01	0.02	0.01	0.01
Fair, F2	0.16	0.18	0.26	0.25	0.25	0.26	0.20	0.23
Satisfactory, F3	0.62	0.66	0.79	0.77	0.77	0.77	0.70	0.73
Good, F4	**0.95**	**0.94**	**0.91**	**0.92**	**0.93**	**0.94**	**0.93**	**0.92**
Very good, F5	0.54	0.50	0.35	0.39	0.38	0.41	0.44	0.39
Excellent, F6	0.06	0.05	0.02	0.03	0.03	0.04	0.04	0.03
	Samples (Fried in algal oil with batch number)							
Scale factor	AO1	AO2	AO5	AO7	AO9	AO11	A13	A15
Not satisfactory, F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fair, F2	0.05	0.04	0.07	0.05	0.08	0.07	0.06	0.05
Satisfactory, F3	0.37	0.36	0.43	0.37	0.44	0.42	0.41	0.37
Good, F4	0.84	0.83	**0.88**	0.84	**0.89**	**0.88**	**0.87**	0.85
Very good, F5	**0.92**	**0.92**	0.85	**1.09**	0.80	0.85	0.86	**0.87**
Excellent, F6	0.31	0.30	0.25	0.47	0.20	0.26	0.26	0.25

The studies described and referenced in this letter show that HO Algal oil functions in the same manner as other commercially available cooking oils, has no adverse impact on the flavor of food products cooked in the oil, and is safe under the intended conditions of use.

Please let me know if you have any questions concerning the safety or ability of the HO Algal oil to be utilized as a partial replacement to conventional cooking oils in food.

Sincerely,

(b) (6)

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group

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FOOD ADDITIVE SAFETY

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January 19, 2018

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to 21 CFR Part 170, Subpart E, Lallemand Health Solutions (Lallemand), through me as its agent, hereby provides notice of a claim that the addition to milk-based term infant formula of three strains of probiotic bacteria (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium longum* ssp. *infantis* Rosell®-33, and *Bifidobacterium bifidum* Rosell®-71), both individually and in an 80:10:10 blend, is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Lallemand has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one copy of the GRAS monograph and one signed copy of the statement of the Expert Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the statement of the Expert Panel.

I apologize for the size of the GRAS monograph, but note that it includes information regarding three bacterial strains that have been extensively studied over many years, both alone and in combination.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5543 or jh@jheimbach.com.

Sincerely,

(b) (6)
[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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April 10, 2018

Renata Kolanos, Ph.D.
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U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Phone: 240-402-1211
Email: Renata.Kolanos@fda.hhs.gov

Dear Dr. Kolanos,

In re: GRN 000754

We received a request from you for Burdock Group to provide responses to several questions posed by your division concerning the notification of the conclusion of GRAS status for high-oleic algal oil (designated as GRN 000754). The specific questions you have provided are indicated on the next pages, numbered and in italics; we have addressed your questions as indicated by a "REPLY" statement following the numbered question.

Please let me know if you need any additional information for this notification of this conclusion of GRAS status.

It also should be noted that TerraVia Holdings, Inc. was acquired by Corbion N.V. in late 2017 and the notifier of this conclusion of GRAS status should now be considered as Corbion Biotech, Inc. (located at 1 Tower Place, Suite 600 South San Francisco, CA 94080).

Sincerely,

(b) (6) [redacted]

Ray A. Matulka, Ph.D.
Director of Toxicology
Burdock Group

1. *On page 35, the notifier states that high-oleic algal oil is stable for at least 12 months when stored at 40 °C with the added antioxidants, TOCO/AP or TBHQ. However, on page 37 in Table 17, the samples were not analyzed at 12 months with TOCO/AP. Please make the necessary correction and/or clarify the notifier's statement.*

   REPLY: The GRAS dossier states that the high-oleic algal oil is stable for at least 12 months at 40 °C using the added antioxidant TBHQ, but not TOCO/AP. Please disregard the statement in the GRAS notification that the high-oleic algal oil is stable at the stated temperature for at least 12 months at 40 °C using the added antioxidant TOCO/AP.

2. *On the page marked as 23 of 65, it is stated that the GRAS panel evaluated the information generated since the first GRAS review and summarized in the amendment to the Dossier in Support of the Generally Recognized as Safe (GRAS) Status of High-Oleic Algal Oil from a Modified Strain Prototheca moriformis as a Food Ingredient (Solazyme, 2016) to conclude that the use of high-oleic algal oil is GRAS. We note that the panel report is not included in the notice.*

   We request that the notifier provide to FDA a copy of the panel report.

   REPLY: The dossier that was assembled and evaluated by the Expert Panel did not include a "panel report" *per se*, as the dossier included the active participation of the Expert Panelists in concluding that the use of the ingredient, under the intended conditions of use, was GRAS. Therefore, we have included the GRAS dossier, attached to this letter.

   In addition, please comment on whether the notifier's GRAS panel had access to any non-public, safety-related data and information. If the notifier's GRAS panel had access to any non-public, safety-related data and information, then explain how there could be a basis for a conclusion of GRAS status if qualified experts generally do not have access to this data and information.

   REPLY: To the best of our knowledge, the GRAS Expert Panel did not have access to any non-public, safety-related data or information that was pivotal to the conclusion of safety of the HO algal oil. Our belief that the GRAS Expert Panel did not have access to additional information is based on the fact that, again to our best knowledge and belief, that we are not aware of any additional information specific to the safety of HO algal oil that was not made available to the GRAS Expert Panel in the GRAS dossier or has been published in the peer-reviewed literature.

3. *Please provide the timeframe (include the month and year) for the literature search performed prior to the submission of GRN 000754.*

   REPLY: The last literature search for this GRAS dossier prior to conclusion by the GRAS Expert Panel was conducted in June, 2016.

AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HIGH-OLEIC ALGAL OIL FROM A MODIFIED STRAIN OF *PROTOTHECA MORIFORMIS* AS A FOOD INGREDIENT

October 18, 2016

Final

Panel Members

Joseph F. Borzelleca, Ph.D.

Edward L. Carmines, Ph.D.

Eric Johnson, Ph.D.

Steve Saunders, Ph.D.

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AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HIGH-OLEIC ALGAL OIL FROM A MODIFIED STRAIN OF *PROTOTHECA MORIFORMIS* AS A FOOD INGREDIENT

TABLE OF CONTENTS

AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HIGH-OLEIC ALGAL OIL FROM A MODIFIED STRAIN OF *PROTOTHECA MORIFORMIS* AS A FOOD INGREDIENT

1. EXECUTIVE SUMMARY

On a prior occasion,[1] an independent GRAS Expert Panel[2] critically evaluated the proposed uses of a high-oleic algal oil from a genetically engineered strain of *Prototheca moriformis* (hereinafter referred to as HO algal oil) as a food ingredient. Based on scientific procedures according to the provision of the Federal Food Drug and Cosmetic Act (FD&C Act), the Expert Panel unanimously concluded that the addition of HO algal oil to food resulting in an estimated 90th percentile consumption of 12.50 g/day is Generally Recognized As Safe (GRAS).

On this occasion, TerraVia Holdings, Inc. (hereinafter referred to as TerraVia and previously known as Solazyme, Inc.) requested Burdock Group to amend the original GRAS determination to increase the use level as well as discontinue the use of HO algal oil in certain foods that were included in the original GRAS dossier and to also evaluate a process change that utilizes a genetically similar strain of *P. moriformis* to make the GRAS HO algal oil. As part of this assessment, Burdock Group: (1) updated regulatory information; (2) conducted a critical review of the relevant scientific literature since the original GRAS determination; and (3) performed an updated consumption analysis based on the 2011-2012 NHANES consumption dataset. TerraVia assures the Expert Panel that all relevant, unpublished information in its possession has been supplied to Burdock Group and has been summarized in this monograph. A comprehensive search of the scientific literature for safety information on *Prototheca moriformis*, HO algal oil and related substances was conducted by Burdock Group, for studies conducted between 2014 and June, 2016.[3] Information from pertinent studies obtained from this search is included in this document. Based on new food survey data and modified use levels, the estimated 90th percentile level of HO algal oil is 27.47 g/day, an increase from the original GRAS determination of an estimated 12.5 g/day intake at the 90th percentile.

The safety data provided in the original GRAS dossier and a critical review of the literature published since the original HO algal oil GRAS determination support a conclusion of safety of HO algal oil in conventional foods at the proposed levels of use in food. Following an independent, critical evaluation, the undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, conferred and unanimously agreed that the consumption of HO algal oil at the estimated 90th percentile of 27.47 g/day is safe.

[1] Dossier in support of the Generally Recognized As Safe (GRAS) status of High-Oleic Algal Oil from a modified strain of *Prototheca moriformis* as a food ingredient, finalized as a GRAS determination on March 31, 2014.
[2] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.
[3] Relevant literature cited in the electronic database search was reviewed. Literature not cited in the search or literature published subsequent to the search may not have been included in the review.

2. INTRODUCTION

High-oleic algal oil (HO algal oil; ≥80% oleic acid) is isolated from a classically improved and genetically engineered strain of the *Prototheca moriformis* microalgae. HO algal oil was previously determined GRAS for use as an oil source in foods at an estimated 90th percentile consumption level of 12.5 g/day (Solazyme, 2014), and was notified of its GRAS status to the United States Food and Drug Administration (FDA), who responded with a "no objection" letter (FDA, 2015). This document is a summary of the scientific evidence that supports the continued GRAS status of HO algal oil as a food ingredient for human consumption when used in additional foods or in the same foods at higher levels than were described in the original GRAS dossier, resulting in an increase in the overall consumption of HO algal oil as a food ingredient.

2.1. Description

HO algal oil is a clear, pale to wheat yellow-colored, refined, bleached and deodorized oil with high levels of oleic acid, produced from a strain of *Prototheca moriformis* that has been modified *via* classical mutagenesis and targeted genetic modification.

In the original GRAS determination, the HO algal oil was produced from *P. moriformis* strain S2532, a classically improved and genetically modified strain originating from the wild-type *P. moriformis* Kruger strain UTEX 1435 (designated by TerraVia as S376). To summarize, S376 was subjected to classical mutagenesis, resulting in a strain with increased triglyceride production, productivity and yield of oil. This strain was then subjected to genetic engineering in which the *Saccharomyces cerevisiae*[4] sucrose invertase gene (SUC2) was inserted to serve as a selectable marker by conferring the ability to utilize sucrose as a carbon source. In addition, an thioesterase gene from *Carthamus tinctorius*, the safflower plant, was inserted. This gene encodes for a carrier protein that plays an essential role in oleic acid chain termination during *de novo* fatty acid synthesis. These genetic modifications resulted in strain S2532, which grows on sucrose and produces an oleic acid-rich oil (>80%).

TerraVia continued to analyze classically improved strains originating from S376 for more favorable attributes and identified a classically improved strain (S5100) that produced a higher *percentage* of oleic acid, compared to the originator (S376) strain. S5100 was then transformed to disrupt a single copy of an endogenous gene while overexpressing the *P. moriformis* gene to increase the ability for fatty acid chain elongation. The SUC2 gene was also inserted to provide the ability to utilize sucrose as a carbon source and serve as a selectable marker. The resulting strain (strain S5587) was then transformed with a hairpin gene sequence to reduce expression of the endogenous fatty acid gene that introduces double bonds in the fatty acyl chain, leading to decreased formation of polyunsaturated fatty acids (*e.g.*, linoleic acid). The *S. carlsbergensis*[5] melibiase gene (MEL1) was also inserted as a selectable marker to confer the ability to grow on melibiose. Melibiase is an enzyme that has a long history of safe commercial use in the food industry. For example, melibiase is used in the sugar refining industry for the conversion of raffinose to galactose and sucrose (Furia, 1980). The resulting strain (S6697) produces approximately 3% C16:0 (palmitic acid), 2% C18:0 (stearic acid) and 90% C18:1 (oleic acid) and is currently used in the production of HO algal oil. In summary, the genetic modifications were a

[4] Typical yeast used in the production of bread, wine and other foods.

[5] *S. carlsbergensis* is a yeast used extensively in the production of lager beer (Wendland, 2014).

reduction in the expression of an endogenous gene, an increase in expression of an endogenous gene, and insertion of two genes as selectable markers that allow for use of sucrose and melibiose as carbon sources. These modifications in the strain that now produces HO algal oil are similar to the modifications used to produce S2532. The production of HO algal oil containing a high level of oleic acid with reduced levels of linoleic acid meet the same specifications for the HO algal oil produced with S2532. There is no reason to believe that HO algal oil produced from S6697 would have a safety profile different from HO algal oil produced from S2532.

The stability of the transgenes inserted into strain S6697 was assessed by Southern blot analysis, in which cells resulting from a high cell density fermentation were grown in liquid culture with glucose as the sole carbon source (non-selective media), then plated onto media containing glucose. Individual colonies (n=48) were then transferred to medium with melibiose as the sole carbon source. All 48 of the 48 transferred colonies exhibited growth on the melibiose-containing plate after more than 30 generations of growth in the absence of the melibiose selection, indicating 100% stability of the inserted transgenes.

Prior to insertion of the hairpin gene sequence, the oil produced from strain S5587 was analyzed for production of algal and cyanobacterial toxins by liquid chromatography with tandem mass spectrometric detection for: amnesic shellfish poisoning toxins (domoic acid), diarrrhetic shellfish poisoning toxins (okadaic acid, dinophysistoxin-1, pectenotoxin-2, azaspiracid-1, yessotoxin, and homo-yessotoxin), paralytic shellfish poisoning toxins (gonyautoxins 1-6; decarbamoylgonyautoxins 2 and 3; saxitoxin; decarbamoylsaxitoxin; neosaxitoxin and ciguatoxins 1-4), cyanobacterial toxins (microcystin-RR, -YR, -LR, -LW, -LF, -LA, -WR, -LY and -HtyR and dm-microcystin-RR and -LR), nodularin, anatoxin and cylindrospermopsin. No toxins were detected (Food GmbH Jena Analytik, 2013). This corroborative information indicates that altering the expression of endogenous genes in this strain lineage does not induce toxin formation or toxin levels in the resulting oil.

The revised S6697 strain was evaluated utilizing the Pariza and Johnson (2001) decision tree that was originally devised for the evaluation of microbial enzyme preparations to be used in food processing, but can be utilized in a general fashion in evaluating the safety of products generated from genetically engineered microorganisms. Strain S6697 meets the first aspects of the decision tree, as it is genetically modified through recombinant DNA techniques, while the expressed product (HO algal oil) has a history of safe use and is free of transferable antibiotic resistance gene DNA (no antibiotic resistance genes were used in the production of S6697). All of the introduced DNA are well characterized, as discussed above, and are integrated into the genome in a well-defined, non-random nature.

The *P. moriformis* strain lineage is considered a safe-strain lineage, as the *P. moriformis* microalgae is not recognized in the scienfitic literature to be associated with pathogenicity, and the production of a refined, bleached, and deodorized oil would remove the potential for viable algal organisms in the final HO algal oil product. Pariza and Johnson (2001) concluded for enzyme preparations that "pathogenic potential is not usually an area of concern for consumer safety because enzyme preparations rarely contain viable organisms", but can still be evaluated. The same conclusion of a lack of viable organisms holds true for the production of a highly refined oil, as no viable organisms have been detected through the TerraVia HO algal oil production process. In addition, a corroborative study on a closely related *P. moriformis* strain (derived from the same source strain) was not pathogenic when evaluated in a study designed to determine if microbial agents would would become systemic in the body and survive or be pathogenic after oral administration.[6] Based on the data described and evaluated through the Pariza and Johnson (2001) decision tree, the genetic changes utilized do not adversely impact the safety of *P. moriformis* strain S6697 as a production strain, and the strain changes discussed above do not affect the safety or identity of the GRAS HO algal oil. Such process changes are necessary to improve the product for the benefit of the consumers.

2.2.Regulatory Status

HO algal oil was determined GRAS in 2014 and notified to the US FDA of the status in 2015 (GRN 527). FDA evaluated the GRAS determination and provided a "no objection" letter indicating that FDA had "no questions at this time" on the GRAS determination (FDA, 2015).

2.3. Proposed use or uses

The use of HO algal oil has not changed from the use stated in the original GRAS, which is as a partial replacement of conventional vegetable and non-vegetable dietary oils.

3. DESCRIPTION, SPECIFICATIONS AND MANUFACTURING PROCESS

3.1. Description and Specifications

The physical and chemical properties and specifications for HO algal oil are provided in Table 1, respectively. The specifications for the HO algal oil have not changed from the original GRAS determination except for a change to the *p*-Anisidine specification and, as shown in Table 1, the HO algal oil currently being produced using strain *P. moriformis* S6697 meets original specifications when evaluated under the revised *p*-anisidine specification. The *p*-anisidine specification was $\leq 2\%$ in the original GRAS but has been amended to $\leq 10\%$ to align with and reflect *p*-anisidine levels noted in the industry for food oil quality. Edible marine oils evaluated under the GOED Organization suggest a maximum *p*-anisidine value of 20 (GOED, 2015), while others in the edible oil industry suggest a *p*-anisidine value of 10 as an indicator of good quality oil (Halvorsen and Blomhoff, 2011; Moigradean *et al.*, 2012; Yun and Surh, 2012; Miller, Unknown). The *p*-anisidine value is a measure of secondary oxidation, mainly of aldehydes such as 2,4-dienals and 2-alkenals, which correlate with overall oil odor intensity (Yun and Surh, 2012). In addition, analysis of the fatty acids contained in the current HO algal oil (Table 3) confirms that the levels of the major fatty acids are within specifications, and are not different from the levels of the fatty acids found in some commonly consumed oils. Current lot analyses of HO algal oil show that oleic acid is the predominant fatty acid (~88% TFA[7]), followed by palmitic (~3.5% TFA) and stearic fatty acids (~3.7% TFA), with low levels of linoleic (~1.53% TFA) and *alpha*-linolenic (~0.22% TFA) fatty acids. All specified fatty acid levels are within current specifications for HO algal oil, and similar in composition to other high oleic acid-containing oils, except for the reduction in linoleic acid (Table 3), which is typically found in a variety of vegetable oils, edible seeds and nuts, and other foods and would most likely be obtained from other dietary sources.

---

[6] Study based on the U.S. EPA Health Effects Test Guidelines, OPPTS 885.3050, Acute Oral Toxicity/Pathogenicity Study (1996) and U.S. FDA Toxicological Prinicples of the Safety Assessment of Food Ingredients, Redbook, 2000, IV.C. 3a: Short-Term Toxicity Studies with Rodents (2003).

[7] TFA = Total fatty acid.

Table 1. Specifications for HO algal oil.

Parameter	Method	Specification	Batch Analysis Results of current HO oil (n = 3)	
			Range	Average
Appearance	Visual inspection	Clear, pale yellow to wheat yellow	Conforms	Conforms
Odor	Olfactory inspection	Slight	Conforms	Conforms
Fatty acid profile	C-M-00036-000 Rev 0[a]			
Oleic acid (C18:1)		≥80 Area %	87.89 – 88.70 Area %	88.27 Area %
Linoleic acid (C18:2)		≤10 Area %	0.99 – 1.93 Area %	1.53 Area %
Alpha-Linolenic acid (C18:3 *alpha*)		≤0.8 Area %	0.16 – 0.27 Area %	0.20 Area %
Total Saturated Fat	C-M-00036-000 Rev 0[a]	≤15 Area %	7.85 – 8.38 Area %	8.06 Area %
Free Fatty Acids	AOCS Ca 5a-40	≤0.1 %	0.03 %	0.03 %
Moisture Content	C-M-00118-000Rev 1[b]	≤0.1 %	0.01 – 0.09 %	0.04 %
Unsaponifiable Matter	AOCS Ca 6a-40	≤1.0 %	0.20 – 0.70%	0.37%
Peroxide Value	AOCS Cd 8-53	≤5 meq/kg	0 – 1.18 meq/kg	0.59 meq/kg
p-Anisidine Value	ISO 6885	≤ 10 %	0.20 – 0.59 %	0.40 %
Elements by ICP				
Lead	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Arsenic	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Mercury	AOCS Ca 17-01	<0.2 ppm	<0.20 ppm	<0.20 ppm
Cadmium	AOCS Ca 17-01	<0.1 ppm	<0.03 ppm	<0.03 ppm
Phosphorus	AOCS Ca 20-99	≤0.5 ppm	<0.20 ppm	<0.20 ppm
Sulfur	AOCS Ca 17-01	≤3 ppm	<0.50 ppm	<0.50 ppm

[a] C-M-00036-000 Rev 0, Total Deliverable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification (internal method; APPENDIX II);
[b] C-M-00118-000 Rev 1, Karl Fischer Moisture Determination of Oil (internal method; APPENDIX III).
AOCS = American Oil Chemists' Society; ISO = International Standards Organization; meq = milliequivalents; ppm = parts *per* million.

As indicated in Table 3, the fatty acid profile of HO algal oil is similar to other vegetable oils (olive, canola and soybean oils), except that HO algal oil contains increased levels of oleic acid and decreased levels of linoleic, palmitic and α-linolenic (soybean and canola only, as α-linolenic acid levels are comparable between HO algal oil and olive oil). However, these fatty acid levels are similar to levels found in commonly consumed oils. Content of the essential fatty acids (*i.e.*, linoleic and α-linolenic fatty acids) in HO algal oil is less than the content found in other commonly consumed oils such as soybean, canola and olive oil (Table 3). However, as shown in Table 2, there is a wide variety of food sources of linoleic and α-linolenic fatty acids that do not require these essential fatty acids to be derived strictly from a food use oil. Use of HO algal oil in the production of some of these foods (as discussed in Section 4 below) may reduce the essential fatty acid content of some of these foods, but HO algal oil is not expected to be a total replacement for all oil utilized in all marketed foods (*i.e.*, less than 100% market penetration). Other foods, such as nuts, chicken, eggs, milk and meat dishes provide substantial amounts of essential fatty acids to a varied diet, and therefore the availability of essential fatty acid consumption is not likely to be an issue for the consumer.

Table 2. Food sources of linoleic acid (PFA 18:2) and alpha-linolenic acid (PFA 18:3), listed in descending order by percentages of their contribution to intake, based on data from the National Health and Nutrition Examination Survey 2005-2006 (National Cancer Institute/Epidemiology and Genomics Research Program)

Linoleic acid*			*alpha*-Linolenic acid**		
Rank	**Food Item**	**Contribution to intake (%)**	**Rank**	**Food Item**	**Contribution to intake (%)**
1	Chicken and chicken mixed dishes	9.3	1	Salad dressing	10.5
2	Grain-based desserts	7.5	2	Chicken and chicken mixed dishes	6.4
3	Salad dressing	7.4	3	Grain-based desserts	6.1
4	Potato/corn/other chips	6.9	4	Pizza	5.8
5	Nuts/seeds and nut/seed mixed dishes	6.5	5	Yeast breads	5.0
6	Pizza	5.3	6	Mayonnaise	4.0
7	Yeast breads	4.5	7	Pasta and pasta dishes	3.5
8	Fried white potatoes	3.5	8	Quickbreads	3.4
9	Pasta and pasta dishes	3.5	9	Fried white potatoes	2.8
10	Mexican mixed dishes	3.3	10	Mexican mixed dishes	2.7
11	Mayonnaise	3.1	11	Nuts/seeds and nut/seed mixed dishes	2.7
12	Quickbreads	3.0	12	Burgers	2.6
13	Eggs and egg mixed dishes	2.8	13	Margarine	2.6
14	Popcorn	2.6	14	Regular cheese	2.6
15	Sausage, franks, bacon, and ribs	2.1	15	Dairy desserts	2.2

* Specific foods contributing at least 1% of octadecadienoic acid in descending order: other fish and fish mixed dishes, margarine, burgers, crackers, rice and rice mixed dishes, beef and beef mixed dishes, other white potatoes, beans, candy.
** Specific foods contributing at least 1% of octadecatrienoic in descending order: sausage, franks, bacon, and ribs, other white potatoes, beef and beef mixed dishes, beans, potato/corn/other chips, coleslaw, rice and rice mixed dishes, tuna and tuna mixed dishes, popcorn, vegetable mixtures.

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Table 3. Fatty acid composition (% TFA) for three lots of current HO algal oil, compared with HO algal oil data from original GRAS and conventional vegetable oils.

Fatty Acids (Area %)	S2532 HO algal oil Mean* (n=3)[a]	S6697 HO algal oil Lot: RBD750	S6697 HO algal oil Lot: RBD850	S6697 HO algal oil Lot: RBD854	S6697 HO algal oil Mean	Olive oil [b]	Canola oil [b]	Soybean oil [b]
C10:0 (Capric)	0.05	0.01	0.02	ND	0.02[c]	ND	ND	NR
C12:0 (Lauric)	0.14	0.02	0.03	0.03	0.03	ND	ND	ND
C14:0 (Myristic)	0.82	0.38	0.37	0.42	0.39	ND	ND	ND
C14:1	0.02	ND	ND	ND	ND	ND	ND	ND
C15:0 (Pentadecanoic)	0.05	0.01	0.01	ND	0.01[c]	NR	ND	ND
C16:0 (Palmitic)	8.11	3.87	3.02	3.72	3.54	11.29	4.30	10.46
C16:1	0.97	0.18	0.14	0.13	0.15	1.26	0.21	ND
C17:0 (Heptadecanoic)	0.06	0.04	0.04	0.05	0.04	0.02	ND	0.03
C17:1	0.10	ND	0.03	0.05	0.04[c]	0.13	ND	ND
C18:0 (Stearic)	1.55	3.73	3.99	3.27	3.67	1.95	2.09	4.43
C18:1 (Oleic)	86.66	88.70	87.89	88.22	88.27	71.27	61.77	22.55
C18:2 (Linoleic)	0.08 [c]	0.99	1.68	1.93	1.53	9.76	19.01	50.95
C18:3 (α-Linolenic acid)	0.02 [c]	0.17	0.16	0.27	0.22	0.76	9.14	6.79
C20:0 (Arachidic)	0.24	0.25	0.34	0.26	0.28	0.41	0.65	0.36
C20:1	0.64	0.28	1.07	0.73	0.84	0.31	1.32	0.23
C22:0 (Behenic)	0.08 [c]	0.05	0.07	0.06	0.06	0.13	0.33	0.37
C22:1, *cis*-13 (Erucic)	0.01 [d]	ND	ND	ND	ND	ND	ND	ND
C22:5n3	0.02 [c]	ND	ND	ND	ND	ND	ND	NR
C24:0 (Lignoceric)	0.05	0.02	0.05	0.04	0.04	ND	NR	NR
Total Fatty Acids Identified	99.40	98.70	98.91	99.18	99.08	97.29	98.82	96.17

*Values stated in original GRAS determination (notified to FDA as GRN 527) for HO algal oil; n = number; ND = Analyzed, but not detected; NR = Not reported; TFA = Total fatty acid; RBD = refined, bleached, deodorized; only fatty acid values ≥ 0.02 are reported;
[a] n = 3, unless otherwise specified;
[b] USDA National Nutrient Database for Standard Reference, Release 26 (<http://ndb.nal.usda.gov/ndb/search/list>; site accessed January 20, 2014);
[c] n = 2
[d] n = 1

3.2. Manufacturing process

The manufacturing process of the HO algal oil has not changed since the original GRAS dossier was finalized. To summarize, the fermentation substrate used in the production of the HO oil has not changed since the original GRAS determination. HO algal oils are produced using processes that incorporate high temperatures and pressure. After growth of the oil-producing *P. moriformis* strain, the final broth is inactivated by heat in the evaporator, resulting in complete loss of microalgae viability. After the evaporator, the inactivated broth is sent to a drum dryer and dried at high temperatures, which provides a redundant inactivation step. Following mechanical extraction, the oil itself undergoes a traditional refined, bleaching, and deodorizing (RBD) process just like other plant oils (AOCS, 2011).

Due to the RBD process, refined oils are normally free from microbial contamination and do not contain sufficient water or moisture, which is need for microbial growth (ICMSF, 2005). HO algal oil has less than 0.1% moisture (Table 1), similar to other refined food oils. Testing of 3 lots of HO algal oil for microbial content (Table 4) shows no microbial growth in HO algal oil.

Table 4. Microbial data on 3 lots of HO algal oil

Sample ID#	RBD 850-C	RBD 831-A	RBD 789-C
Aerobic Plate Count	<10(/g)	<10(/g)	<10(/g)
Coliforms	<3(/g)	<3(/g)	<3(/g)
E. coli	Negative(/10g)	Negative(/10g)	Negative(/10g)
Mesophilic Aerobic Spores	<1.0(/g)	<1.0(/g)	<1.0(/g)
Mold	<10(/g)	<10(/g)	<10(/g)
Pseudomonas aeruginosa	Negative(/25g)	Negative(/25g)	Negative(/25g)
Salmonella	Negative(/375g)	Negative(/375g)	Negative(/375g)
Staphylococci	Negative(/25g)	Negative(/25g)	Negative(/25g)
Yeast	<10(/g)	<10(/g)	<10(/g)

#All sample results are provided as colony forming units (CFU).

To confirm the presence or absence of algal DNA in the finished oil, HO algal oil was assayed using quantitative real-time polymerase chain reaction (Real Time-qPCR). Two genes present in the oil-producing *P. moriformis* strain were targeted for amplification and quantification: an endogenous algal gene encoding the fatty acid desaturase which serves as an internal genomic control and the inserted sucrose invertase (*SUC2*) transgene.

Results showed no detection of the presence of DNA, endogenous or transgenic, in the HO algal oil or the negative controls, as evidenced by the lack of specific amplification. DNA was only detected in positive controls, where neat refined, bleached, deodorized (RBD) HO algal oil samples were spiked with genomic *P. moriformis* DNA (gDNA) in amounts ranging from 10 ng to 0.01 ng (Table 5). All samples were run in triplicate and three independent runs were conducted. This study indicates that the RBD process removes source organism DNA from the oil, as indicated by the absence of representative endogenous and inserted DNA in the RBD HO algal oil.

Table 5. Real-time qPCR cycle threshold for endogenous and inserted genes from *P. moriformis* genomic DNA in HO algal oil (Yu and Zhao, 2015).

A. Endogenous Gene

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev.	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	21.01	0.443	20.54	0.498	21.59	0.045
	1 ηg	24.47	0.495	25.27	0.230	25.11	0.294
	0.1 ηg	26.80	0.769	32.27	0.548	30.67	1.254
	0.01 ηg	32.47	0.743	34.74	0.640	33.20	0.944
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

B. Inserted Transgene

Sample	Amount of gDNA in PCR Reaction	*n* = 1		*n* = 2		*n* = 3	
		Cq	Cq Std. Dev	Cq	Cq Std. Dev	Cq	Cq Std. Dev
Neat RBD HO Algal Oil	10 ηg	18.05	0.333	19.44	0.423	20.41	0.553
	1 ηg	23.64	0.451	24.81	0.326	23.26	0.435
	0.1 ηg	24.62	0.599	26.74	0.086	28.45	2.862
	0.01 ηg	26.78	0.402	31.65	0.076	32.95	1.323
Neat RBD HO Algal Oil	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000
NTC	0 ηg	0.00	0.000	0.00	0.000	0.00	0.000

Positive controls are neat HO algal oil samples containing *P. moriformis* source organism genomic DNA. Negative controls (NTC) contained no DNA. Samples were assayed for specific amplification with primers targeting the endogenous *FAD2* gene (A) or the inserted *SUC2* transgene (B). The results shown are mean ± standard deviation in triplicate, for each of the three replications; Cq = quantitation cycle, the cycle in which fluorescence can be detected; ηg = nanogram.

3.3. Stability

Stability studies were conducted on current HO algal oil at 5°C, at 20°C and for an accelerated stability study at 40°C. During the stability evaluations, the oil was stored in 500 mL amber glass bottles. Relative humidity was not monitored or controlled because the samples used in the assay for most parameters were stored in amber glass bottles or vials with a Teflon-lined screw cap that prevented moisture transfer during the temperature treatment.

HO algal oil, with no added antioxidants, is stable for at least 18 months when stored at the controlled temperatures of 5°C (Table 6) or 20°C (Table 7). The HO algal oil in the absence of an antioxidant is stable for at least three months when stored at 40°C (Table 8).

Various antioxidants were added to test stability of the oil, which included a mixture of 500 ppm mixed tocopherols and 150 ppm ascorbyl palmitate (herein referred to as Toco/AP), and 200 ppm tertiary butylhydroquinone (TBHQ). The HO algal oil is stable for at least 18 months when stored at 5°C (Table 6) or 20°C (Table 7), and at least 12 months when stored at 40°C with the added antioxidants, Toco/AP and TBHQ (Table 8). *p*-Anisidine value measures rancidity and the oxidation of oil should consistently increase over time. Although some values of *p*-Anisidine fluctuate, we have not observed a trend to indicate any stability problems. Stability studies on HO algal oil are ongoing.

Table 6. Stability of the high-oleic algae oil with and without antioxidants stored at controlled temperature, 5 °C

Lot	OSI Rancimat (110°C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	60.60	0.040	0.08	0.70
6 months	62.59	0.038	0.75	0.78
9 months	61.04	0.039	1.13	1.47
12 months	60.88	0.037	0.89	0.53
18 months	59.81	0.035	1.49	1.25
TOCO/AP				
0 month	156.70	0.048	1.40	0.76
3 months	144.80	0.050	1.14	0.86
6 months	136.95	0.047	1.51	0.87
9 months	136.30	0.047	1.66	4.33
12 months	139.49	0.048	1.10	2.94
18 months	132.69	0.066	0.82	2.10
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	112.84	0.040	0.84	0.70
6 months	110.37	0.038	0.65	0.73
9 months	110.84	0.039	1.34	1.12
12 months	112.98	0.035	0.88	3.23
18 months	110.12	0.041	0.51	0.40

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

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Table 7. Stability of the high-oleic algae oil with and without antioxidants stored at controlled room temperature, 20 °C

Lot	OSI Rancimat (110°C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	59.21	0.040	1.02	0.70
6 months	60.92	0.039	1.35	0.77
9 months	60.14	0.041	2.85	1.13
12 months	56.42	0.040	1.37	0.66
18 months	55.41	0.042	1.77	0.99
TOCO/AP				
0 month	156.70	0.048	1.40	0.76
3 months	139.63	0.050	1.71	0.90
6 months	124.44	0.054	1.88	0.90
9 months	134.30	0.058	2.12	3.48
12 months	134.48	0.061	1.48	0.55
18 months	129.17	0.067	1.22	0.65
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	111.06	0.040	1.02	0.67
6 months	102.06	0.040	1.12	0.76
9 months	102.02	0.042	1.63	3.92
12 months	111.96	0.040	1.14	1.64
18 months	106.99	0.042	0.88	0.89

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

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Table 8. Stability of the high-oleic algae oil with and without antioxidants stored at controlled temperature, 40°C

Lot	OSI Rancimat (110°C) (hours)	Free Fatty Acid (≤ 0.1 %)[a]	Peroxide Value (≤ 5 meq/kg)[a]	*p*-Anisidine Value (≤ 10%)[a]
Neat				
0 month	61.90	0.039	1.00	0.71
3 months	51.77	0.042	3.22	0.93
6 months	N/A	N/A	N/A	N/A
9 months	N/A	N/A	N/A	N/A
12 months	N/A	N/A	N/A	N/A
TOCO/AP				
0 month	156.70	0.048	1.4	0.76
3 months	138.04	0.06	1.62	0.83
6 months	134.37	0.061	1.51	0.80
9 months	130.43	0.080	1.30	2.97
12 months	N/A	N/A	N/A	N/A
TBHQ				
0 month	103.61	0.038	1.01	0.33
3 months	108.68	0.040	1.42	0.71
6 months	98.56	0.046	2.00	0.72
9 months	95.97	0.052	2.18	2.43
12 months	100.85	0.067	2.10	0.74

OSI = Oxidative/Oil Stability Index; NA = Not analyzed; TOCO/AP = Mixed tocopherols/Ascorbyl Palmitate; TBHQ = Tertiary butylhydroquinone; [a] Specification limit.

4. ESTIMATED DAILY INTAKE

The intake profile (amount and frequency) by individuals in USDA's What We Eat in America (WWEIA) Continuing Survey of Food Intakes by Individuals 2011-2012 (Dwyer *et al.*, 2003) was used to calculate the estimated daily intake (EDI) of the current HO algal oil produced from a classically and genetically engineered strain of *P. moriformis*, for individuals consuming the food groups selected for the addition of HO algal oil *per* this GRAS amendment (*i.e.*, "eaters only"). The food groups as defined by the FDA (21 CFR §170.3(n)) are provided in Table 9.

As stated in the original GRAS dossier, the HO algal oil will be added only to foods for which a standard of identity does not exist. All food categories designated by TerraVia have been utilized in the calculations as appropriate; however, certain categories designated by TerraVia may include foods for which a standard of identity exists. We note that an ingredient that is lawfully added to food products may be used in a "standardized" food only if it is permitted by the applicable standard of identity. TerraVia found through market evaluation and taste evaluation that the taste profile of HO algal oil (*i.e.*, little to no discernable taste, compared with other high oleic acid-containing oils such as olive oil or canola oil) would allow for a greater replacement of other vegetable oils currently consumed in the U.S. diet. Therefore, TerraVia has expanded the use of HO algal oil to additional food products, and has increased the anticipated use levels in food categories previously stated in the original GRAS dossier.

Table 9. Food groups selected for HO algal oil supplementation*

Food Category	Intended use level (ppm)
Baked goods (bread, dry mixes, rolls, bagels, biscuits, cornbread, tortillas and croutons)	20,000-70,000
Baked desserts (sweet rolls, muffins, scones, cakes, cheesecake, pie, cobbler, crisps, doughnuts, turnovers, strudel and cookies)	30,000-50,000
Meal replacement bars, drinks and protein supplements	30,000 -50,000
Cereals and bars (granola, muesli and dry cereal)	20,000-150,000
Cheese spreads	100,000
Margarine and margarine-like spreads	450,000-700,000
Butter-like spreads	700,000
Vegetable oil and shortening	700,000-1,000,000
Salad dressings and mayonnaise	500,000-700,000
Sauces, gravies and dressings	20,000-60,000
Nut spreads, nuts and seeds	50,000
Dairy and milk products (including analogs) (whipped topping, cream substitute, milk imitation and flavored)	10,000-100,000
Gelatins and puddings	30,000
Soups and broth	20,000-30,000
Meat products**	20,000-50,000
Frozen dairy desserts	30,000
Snacks (crackers, popcorn, crisp bread, salty snacks, popcorn, pretzel and chips)	145,000-350,000
Soft candy (caramels, fruit snacks, covered nuts	30,000
Confectionary (icing and marshmallows)	20,000-250,000

*The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category.
** Meat Products (mixtures), including all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.
ppm=parts *per* million

The mean and 90th percentile EDIs were calculated only for HO algal oil intake following addition of the HO algal oil to the selected food groups and at the levels for inclusion, as indicated in APPENDIX I. Inclusion of HO algal oil into the selected foods at the levels specified in APPENDIX I would provide a mean and 90th percentile HO algal oil consumption of approximately 12.04 and 27.47 g/day HO algal oil, respectively (Table 10). This intake is approximately equivalent to 220.36 and 494.83 mg/kg bw/day, respectively, of the HO algal oil. The consumption of oleic acid, the major monounsaturated fatty acid (MUFA) in HO algal oil, from the addition of HO algal oil to foods indicated in APPENDIX I may result in a mean and 90th percentile intake of oleic acid at 10.6 and 24.17 g/day, respectively.

Table 10. Intake of HO algal oil when added to foods stated in APPENDIX I.

	***Per* User (g/day)**	
	Mean	**90th Percentile**
Possible consumption with HO algal oil as an added ingredient to food	12.04	27.47

The Institute of Medicine (IOM) utilized the Continuing Survey of Food Intakes by Individuals, 1994-1996, 1998 to determine that the median MUFA intake in the US ranged from 25 – 39 g/day for men and 18 – 24 g/day for women, with the mean daily intake of MUFAs in the U.S. in all individuals at 28.7 g (IOM, 2005). The daily value (DV) that FDA has stated for total fat intake (based on a caloric intake of 2,000 calories) is 65 g/day. Therefore, the consumption of HO algal oil at the 90th percentile intake of 27.47 g/day as a partial replacement of other oils in the diet is still well below the daily amount of total fat estimated to be consumed in the U.S., and the consumption of oleic acid from HO algal oil at the 90th percentile (24.17 g/day) is consistent with typical consumption levels of MUFA in the U.S. The 2015 – 2020 dietary guidelines for Americans (USDA, 2015) found that the typical amount of oil consumed in a 2,000 calorie healthy U.S.-style eating pattern is 27 g/day oil, which is similar to the potential consumption of HO algal oil at the 90th percentile, suggesting that other oils will be consumed in addition to HO algal oil.

5. SAFETY EVALATION

As stated in the original GRAS dossier, the number of species assigned to the genus *Prototheca* has varied, as scientific analysis of the phylogenetic data has increased. *P. moriformis* species are heterogeneous in nature, previously being placed within the *P. zopfii* species (Roesler *et al.*, 2006) as a distinct biotype (genotype 1). Irrgang *et al* (2015) states that "on the basis of biochemical, serological and phylogenetic analysis, *P. zopfii* has been divided into two genotypes, of which genotype 1 is considered to be non-pathogenic and genotype 2 is associated with bovine mastitis and human protothecosis." Other work has placed *P. moriformis* within a new clade along with some strains of *P. wickerhamii* (Ewing *et al.*, 2014). Rare cases of *P. wickerhamii* infection have been reported in immunocompromised people (Panchabhai *et al.*, 2015).

No preclinical or clinical studies, nor reports of such studies, were located since the initial GRAS determination for HO algal oil or *P. moriformis* that indicate a safety concern of consuming an oil derived from a strain of *P. moriformis*. HO algal oil was previously assayed in a 90-day toxicity study in which the no-observed-adverse-effect-level (NOAEL) was 100,000 ppm, the highest dietary concentration provided in the study, which corresponded to a dietary NOAEL of 5200 mg/kg bw/day in male rats and 6419 mg/kg bw/day in female rats (Szabo *et al.*, 2014). This would correspond with consumption of HO algal oil at 312 g/day for a 60 kg human, much greater than the estimated daily intake of HO algal oil as a partial replacement for cooking oils, with the 90th percentile level of intake estimated at 27.45 g/day.

A 13-week dietary study in rats was recently published that evaluated the potential of a different, stearic acid-rich fat (algal structuring fat) from a genetically related (*i.e.*, the parental *P. moriformis* strain was S376) and similarly classically and genetically engineered strain of *P. moriformis* (Matulka *et al.*, 2016). The study followed internationally-accepted protocol guidelines and provided a diet containing 0, 25,000, 50,000 and 100,000 ppm algal structuring fat (>50 Area % stearic acid, >30 Area % oleic acid and ≥2 Area % palmitic acid) to male and female Sprague Dawley rats (n=10/sex/group)[8] for 13 weeks. At the end of the study, the rats were euthanized, with gross necropsies conducted on all animals and selected organs and tissues from control and high dose animals evaluated histologically.

[8] The control group contained 20 animals/sex.

No algal structuring fat-related mortalities occurred during the study and there were no changes in clinical signs or detailed clinical observations associated with administration of the algal structuring fat. Overall and calculated mean daily feed consumption were not different between control rats and any algal structuring fat dose group of the same sex. The overall mean daily intake of algal structuring fat in male rats fed dietary concentrations of 25,000, 50,000 and 100,000 ppm was 1285.6, 2594.3 and 5299.2 mg/kg bw/day, respectively. In female rats, the corresponding mean overall daily intake of algal structuring fat was 1606.0, 3069.7 and 6313.8 mg/kg bw/day, respectively. Mean weekly and overall body weights and calculated mean daily body weight gain for algal structuring fat dose groups were comparable to control groups for the respective sex (Figure 1).

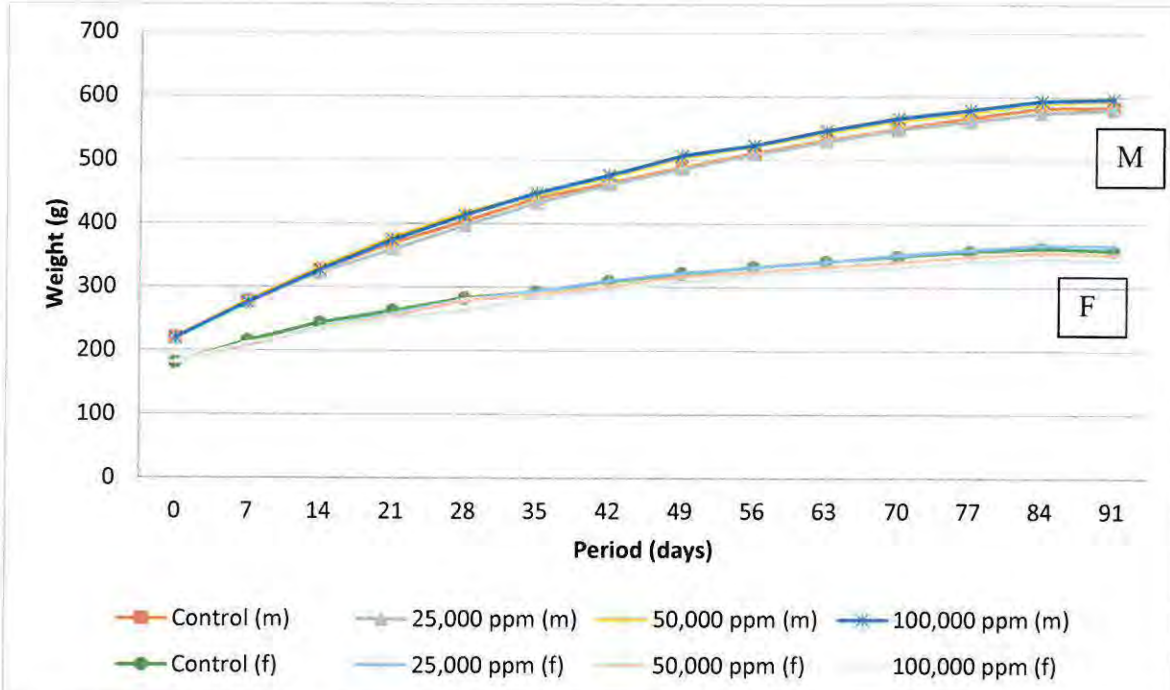

Figure 1. Mean body weights of male and female rats consuming diets containing algal structuring fat

There were no algal structuring fat-related effects on hematology parameters for either male or female rats at any dose. A decrease ($P<0.05$) in blood urea nitrogen (BUN) was observed in the 100,000 ppm male dose group, but this clinical chemistry result was within the laboratory's historical control range and was not considered toxicologically relevant. The absolute mean organ weights and mean organ-to-body and organ-to-brain weights ratios for the male rats consuming algal structuring fat were not different from control values, and there were no absolute or relative organ weight changes in the male or female rats that were considered adverse. The liver-to-body weight ratios for the 50,000 and 100,000 ppm algal structuring fat male dose groups was significantly ($P<0.05$) decreased compared to the control group, but these changes were of small magnitude, lacked histopathological or serum chemistry correlates and were not reflected in corresponding decreases in absolute or liver-to-brain weight values. The no-observed-adverse-effect-level (NOAEL) for the algal structuring fat was 100,000 ppm for both males and females under the conditions of the study, the highest dietary concentration provided. The administered level of algal structuring fat corresponded to a dietary NOAEL of 5299.2 mg/kg bw/day in male rats and 6318.8 mg/kg bw/day in female rats (Matulka *et al.*, 2016). This study shows that different classically and genetically engineered modifications to this line of *P. moriformis* in order to produce oils with different fatty acid profiles still result in the formation of an oil that is not toxic at up to 100,000 ppm.

The safety of food ingredients can be further evaluated through the analysis of its components, especially when the ingredient is a macroingredient that cannot be administered to laboratory animals at sufficiently high enough doses for conventional risk evaluation methods (Howlett *et al.*, 2003). As stated in the original GRAS dossier, the major components of HO algal oil are triglycerides (≥95%) and diglycerides (≤5.0%). Oleic acid is the main fatty acid in HO algal oil (average of 88% of the total fatty acids as stated in this amendment), with approximately 8% total saturated fat (Table 1). Unsaponifiable matter[9] for this neutral, RBD oil is ≤1.0 % and the free fatty acid content is ≤0.1%.

Vegetable oils are consumed in foods in a variety of forms, including the use of vegetable oils for the deep-fat frying of meats and vegetables (Wang *et al.*, 2016). However, research has found that the heating of vegetable and animal fats to the temperature for the frying of foods may result in the formation of α,β-unsaturated aldehydes from polyunsaturated fatty acids (PUFA), such as *alpha*-linolenic and linoleic fatty acids (Guillén and Goicoechea, 2008). *Alpha*, β-unsaturated aldehydes have been implicated in the formation of cardiovascular diseases (Grootveld *et al.*, 1998; Ng *et al.*, 2014). Although moderate consumption of used frying oils may not pose significant harm to the general population, some of the substances formed during the frying process of polyunsaturated oils may impair the nutritional value or be potentially harmful (el-Shattory *et al.*, 1991; Dobarganes and Márquez-Ruiz, 2015). The oxidation of PUFA may yield α,β-unsaturated hydroxyalkenals, such as 4-hydroxy-2-trans-nonenal (HNE). HNE is a highly reactive molecule that interacts with cellular macromolecules, forming adducts. Depending on the location, adduct formation can lead to alterations in normal cell functioning or signaling, or induce cytotoxicity and/or genotoxicity. The formation of α,β-unsaturated hydroxyalkenals have been implicated as a contributing factor in the initiation of a number of diseases (Barrera *et al.*, 2015; Csallany *et al.*, 2015). Other research indicates that α,β-unsaturated aldehydes induce oxidative stress through the reduction in cellular glutathione (GSH), which may play a role in the formation of vascular disease *via* redox-sensitive mechanisms or through direct injury to the endothelium (Lee and Park, 2013).

In an independent study, HO algal oil was evaluated for the production of α,β-unsaturated aldehydes after heating to the temperature necessary for frying (375 °F) (Grootveld, 2015). HO

[9] Unsaponifiable matter is defined as all substances present in a product which, after saponification of the product by potassium hydroxide and extraction by hexane, are not volatile under the specified operating conditions. Unsaponifiable matter includes lipids of natural origin such as sterols, higher hydrocarbons and alcohols, aliphatic and terpenic alcohols, as well as any foreign organic matter extracted by the solvent and not volatile at 103 C° (*e.g.*, mineral oils) that may be present (ISO, 2000).

algal oil was found to produce substantially less α,β-unsaturated hydroxyalkenals (Figure 2; this category includes HNE) and α,β-unsaturated 2,4-alkadienals (Figure 3) than other types of oils. *α*, β-Unsaturated 2,4-alkadienals (represented by 2,4-decadienal) are possible carcinogens (EFSA, 2014). The data confirm that frying of HO algal oil produces fewer α,β-unsaturated aldehydes than other vegetable oils currently used in the U.S., suggesting a decreased potential for toxicity by the use of HO algal oil instead of other types of oils.

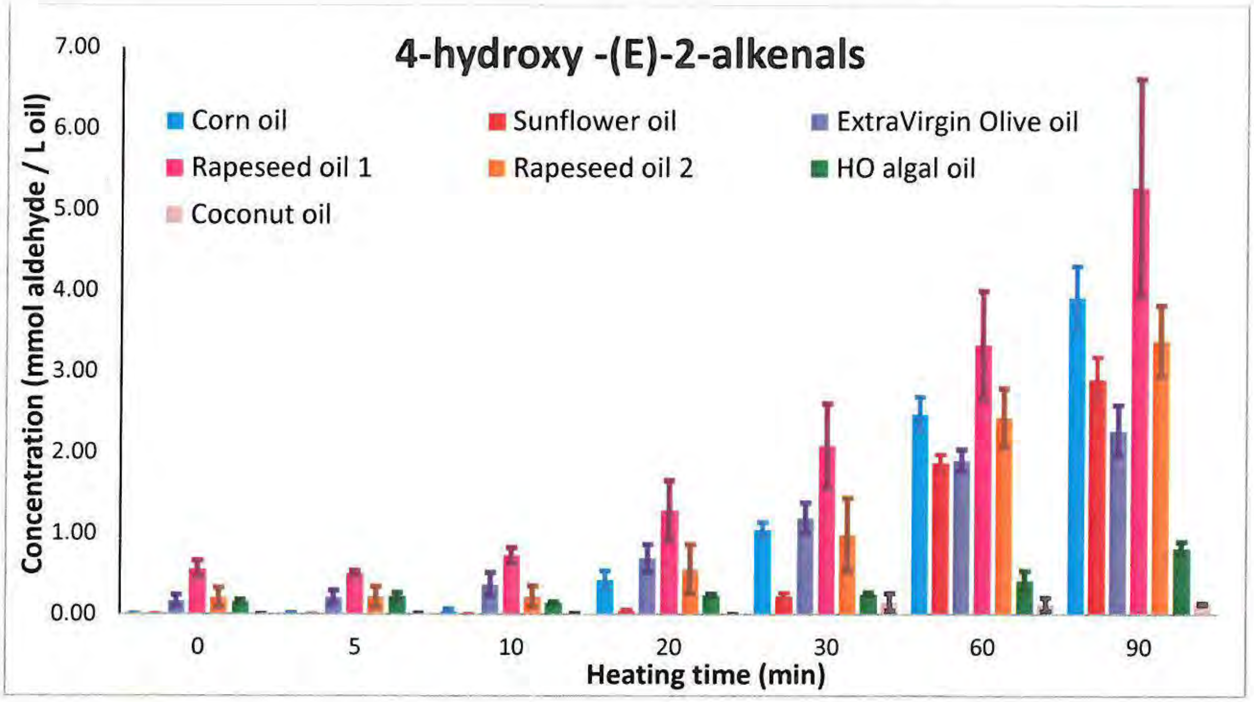

Figure 2. Formation of 4-hydroxy-2-alkenals during the heating of vegetable oils (mean ± Standard Deviation) (Grootveld, 2015).

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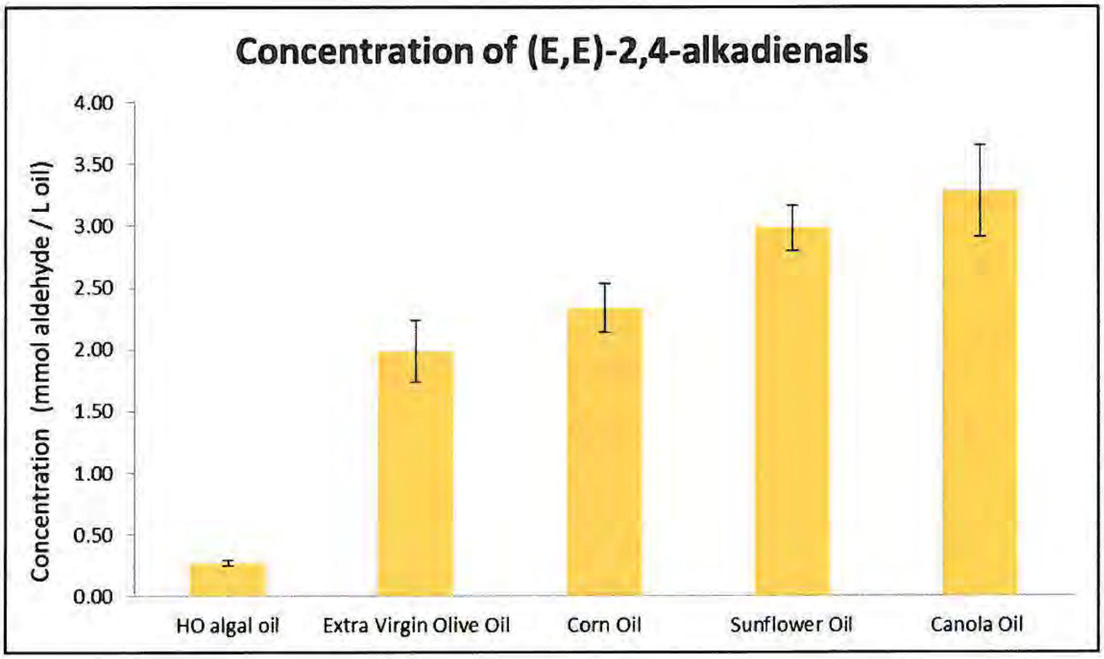

Figure 3. Concentration of (E,E)-2,4-alkadienals after Simulated Sautéing for 20 Minutes at 356°F (mean ± Standard Deviation) (Grootveld, 2015).

6. OBSERVATIONS IN HUMANS

TerraVia has been selling HO algal oil at the retail and business-to-business levels for the past year, selling over 215,000 liters. TerraVia has not received reports of adverse event cases confirmed as a direct result of the consumption of the HO algal oil (TerraVia, 2016).

7. EVALUATION

HO algal oil is a refined, bleached, deodorized oil composed mainly of oleic acid-rich (≥80% oleic acid) triglycerides produced from a classically improved and genetically engineered strain of *P. moriformis*. The original GRAS dossier is being amended to modify the levels of HO algal oil use in foods that were in the original GRAS dossier. Analysis of the genetic stability of the S6697 *P. moriformis* strain (evaluated in this amendment) demonstrated that the changes introduced to this genetically engineered strain were stable in the microalgae. Analysis of the oil confirms that it meets the GRAS specifications containing the revised *p*-anisidine value, and in addition, analysis showed that markers for DNA from endogenous and inserted DNA were not contained in the oil. The original GRAS dossier was notified as GRAS to the FDA and subsequently received a "no objection" letter in response.

The manufacturing process for HO algal oil has not changed since the original GRAS determination. The limit for *p*-anisidine was modified to be consistent with levels found in the oil industry. The use indicates acceptance of HO algal oil as a partial replacement for conventional vegetable oils and is being increased from 12.5 g/day to 27.47 g/day (90th percentile estimated consumption level). The new mean and 90th percentile levels are 12.04 and 27.47 g/day, respectively, representing 200.67 and 457.83 mg/kg bw/person/day, for a 60 kg person.

The HO algal oil is mainly composed of oleic, stearic and palmitic fatty acids, which are fatty acids found in commonly consumed vegetable oils. The fatty acids are provided in a triglyceride format, with few di- and mono-glycerides contained in the HO algal oil, consistent with other commonly consumed vegetable oils.

The NOAEL for HO algal oil derived from S2532 is 5200 mg/kg bw/day in male rats and 6419 mg/kg bw/day in female rats, the highest dose evaluated. No additional safety information that indicates a potential for toxicity from the consumption of HO algal oil has been published since the original GRAS determination that indicates a potential for toxicity from the consumption of HO algal oil. A 13-week preclinical dietary toxicology study was conducted in rats that evaluated the safety of a stearic acid-rich algal structuring fat produced using a genetically related, classically and genetically engineered strain of *P. moriformis*. The NOAEL for this algal structuring fat is 5299 mg/kg bw/day in male rats and 6313 mg/kg bw/day in female rats, the highest dose provided to the rats. The anticipated upper intake 90th percentile level of HO algal oil is significantly lower than the NOAEL established in preclinical testing. In addition, no adverse events have been reported from consumer intake of HO algal oil over the past year that have confirmed HO algal oil as the causative agent.

Analysis of α,β-unsaturated aldehyde formation found that heating of HO algal oil to temperatures typical with the frying of food (375 °F) produces much lower levels of α,β-unsaturated aldehydes, implicated in a variety of cardiovascular or vascular diseases, than the common fry oils corn oil, soybean oil, and canola oil.

In summary, information available on the production, components, safety and by-products of HO algal oil continue to support the safety-in-use of HO algal oil at an anticipated upper consumption of 27.47 g/day.

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8. CERTIFICATION

The undersigned authors of this document—a dossier in support of GRAS status determination for use of HO algal oil produced by a genetically engineered strain of *Prototheca moriformis*—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of all available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

(b) (6) [redacted]

October 19, 2016

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19 OCT 2016

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Date

9. CONCLUSION

We, the Expert Panel, have individually and collectively critically evaluated information generated since the first GRAS review and summarized in this document (an amendment to the dossier in support of the generally recognized as safe (GRAS) status HO Algal Oil from a modified strain of *Prototheca moriformis* as a food ingredient and, unanimously conclude that HO algal oil, produced in accordance with current Good Manufacturing Practice (cGMP) and meeting the specifications described in this amendment is safe for use in foods at an estimated upper consumption level of 27.47 g/day (for specific foods and levels of use, refer to APPENDIX I).

We further unanimously conclude that the use of HO algal oil is Generally Recognized As Safe by scientific procedures.

It is our opinion that other experts qualified by scientific training and experience to evaluate the safety of food and food ingredients would concur with these conclusions.

10. SIGNATURES

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APPENDIX I

Food items selected for HO algal oil supplementation with intended levels of use

Food item	**Parts *per* million (ppm)**
Milk, dry, reconstituted, NFS	10000
Milk, dry, reconstituted, whole	10000
Milk, dry, reconstituted, lowfat	10000
Milk, dry, reconstituted, nonfat	10000
Buttermilk, dry, reconstituted	10000
Milk, soy, ready-to-drink, not baby's	100000
Milk, soy, ready-to-drink, not baby's, chocolate	100000
Milk, imitation, fluid, non-soy, sweetened, flavors other than chocolate	100000
Fruit and lowfat yogurt parfait	30000
Cocoa, hot chocolate, not from dry mix, made with whole milk	50000
Hot chocolate, Puerto Rican style, made with whole milk	50000
Hot chocolate, Puerto Rican style, made with low fat milk	50000
Chocolate syrup, milk added, NS as to type of milk	50000
Chocolate syrup, whole milk added	50000
Chocolate syrup, reduced fat milk added	50000
Chocolate syrup, lowfat milk added	50000
Chocolate syrup, skim milk added	50000
Cocoa with nonfat dry milk and low calorie sweetener, mixture, water added	50000
Cocoa, whey, and low calorie sweetener, mixture, fortified, water added	50000
Cocoa and sugar mixture fortified with vitamins and minerals, milk added, NS as to type of milk, Puerto Rican style	50000
Cocoa with nonfat dry milk and low calorie sweetener, high calcium, water added	50000
Cocoa, whey, and low-calorie sweetener mixture, lowfat milk added	50000
Milk beverage with nonfat dry milk and low calorie sweetener, water added, chocolate	50000
Milk beverage with nonfat dry milk and low calorie sweetener, water added, flavors other than chocolate	50000
Milk beverage with nonfat dry milk and low calorie sweetener, high calcium, water added, chocolate	50000
Milk beverage, made with whole milk, flavors other than chocolate	50000
Milk, malted, unfortified, NS as to flavor, made with milk	100000
Milk, malted, unfortified, chocolate, made with milk	100000
Milk, malted, unfortified, natural flavor, made with milk	100000
Instant breakfast, fluid, canned	20000
Instant breakfast, powder, milk added	20000
Meal supplement or replacement, commercially prepared, ready-to-drink	50000
High calorie beverage, canned or powdered, reconstituted	50000
Meal supplement or replacement, milk-based, high protein, liquid	50000
Meal replacement or supplement, milk based, ready-to-drink	50000
Instant breakfast, powder, not reconstituted	20000
Protein supplement, milk-based, powdered, not reconstituted	50000
Meal replacement, high protein, milk based, fruit juice mixable formula, powdered, not reconstituted	50000
Meal replacement, protein type, milk-based, powdered, not reconstituted	50000
Nutrient supplement, milk-based, powdered, not reconstituted	50000
Nutrient supplement, milk-based, high protein, powdered, not reconstituted	50000

Meal replacement, protein type, milk- and soy-based, powdered, not reconstituted	50000
Cream substitute, NS as to frozen, liquid, or powdered	100000
Cream substitute, frozen	100000
Cream substitute, liquid	100000
Cream substitute, flavored, liquid	100000
Cream substitute, powdered	100000
Cream substitute, flavored, powdered	100000
Whipped topping, nondairy, NS as to canned, frozen, or made from powdered mix	100000
Whipped topping, nondairy, pressurized can	100000
Whipped topping, nondairy, frozen	100000
Whipped cream substitute, nondairy, made from powdered mix	100000
Sour cream, imitation (nondairy)	100000
Sour cream, imitation (nondairy)	100000
Ice cream, NFS	30000
Ice cream, regular, flavors other than chocolate	30000
Ice cream, regular, chocolate	30000
Ice cream, rich, flavors other than chocolate	30000
Ice cream, rich, chocolate	30000
Ice cream, rich, NS as to flavor	30000
Ice cream sandwich	30000
Ice cream cone, no topping, NS as to flavor	30000
Ice cream cone with nuts, chocolate ice cream	30000
Ice cream cone, chocolate covered or dipped, chocolate ice cream	30000
Ice cream cone, no topping, chocolate ice cream	30000
Ice cream cone, chocolate covered, with nuts, chocolate ice cream	30000
Ice cream sundae cone	30000
Ice cream soda, flavors other than chocolate	30000
Ice cream soda, chocolate	30000
Ice cream sundae, NS as to topping, with whipped cream	30000
Ice cream sundae, fruit topping, with whipped cream	30000
Ice cream sundae, prepackaged type, flavors other than chocolate	30000
Ice cream sundae, chocolate or fudge topping, with whipped cream	30000
Ice cream sundae, not fruit or chocolate topping, with whipped cream	30000
Ice cream sundae, fudge topping, with cake, with whipped cream	30000
Ice cream pie, no crust	30000
Ice cream pie, with cookie crust, fudge topping, and whipped cream	30000
Ice cream, fried	30000
Light ice cream, NS as to flavor (formerly ice milk)	30000
Light ice cream, flavors other than chocolate (formerly ice milk)	30000
Light ice cream, chocolate (formerly ice milk)	30000
Light ice cream, cone, NFS (formerly ice milk)	30000
Light ice cream, sundae, soft serve, not fruit or chocolate topping, with whipped cream (formerly ice milk)	30000
Light ice cream, sundae, soft serve, chocolate or fudge topping (without whipped cream) (formerly ice milk)	30000
Light ice cream, creamsicle or dreamsicle (formerly ice milk)	30000
Light ice cream, fudgesicle (formerly ice milk)	30000
Sherbet, all flavors	30000
Milk dessert bar, frozen, made from lowfat milk	30000
Milk dessert sandwich bar, frozen, made from lowfat milk	30000
Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk	30000
Milk dessert bar, frozen, made from lowfat milk and low calorie sweetener	30000

Baked Alaska	30000
Pudding, NFS	30000
Pudding, bread	30000
Puerto Rican bread pudding made with evaporated milk and rum (Budin de pan)	30000
Diplomat pudding, Puerto Rican style (Budin Diplomatico)	30000
Pudding, Mexican bread (Capirotada)	30000
Rice flour cream, Puerto Rican style (manjar blanco)	30000
Custard, Puerto Rican style (Maicena, Natilla)	30000
Custard	30000
Flan	30000
Pudding, rice	30000
Pudding, tapioca, made from dry mix, made with milk	30000
Pudding, tapioca, chocolate, made with milk	30000
Pudding, coconut	30000
Pudding, Indian (milk, molasses and cornmeal-based pudding)	30000
Pudding, pumpkin	30000
Puerto Rican pumpkin pudding (Flan de calabaza)	30000
Fresh corn custard, Puerto Rican style (Mazamorra, Mundo Nuevo)	30000
Pudding, flavors other than chocolate, prepared from dry mix, milk added	30000
Pudding, chocolate, prepared from dry mix, milk added	30000
Pudding, canned, chocolate, reduced fat	30000
Pudding, canned, flavors other than chocolate, reduced fat	30000
Pudding, canned, flavors other than chocolate	30000
Pudding, canned, chocolate	30000
Pudding, canned, chocolate and non-chocolate flavors combined	30000
Pudding, canned, tapioca	30000
Mousse, chocolate	30000
Mousse, not chocolate	30000
Chantilly Cream	30000
Coconut custard, Puerto Rican style (Flan de coco)	30000
Milk dessert or milk candy, Puerto Rican style (Dulce de leche)	30000
Barfi or Burfi, Indian dessert, made from milk and/or cream and/or Ricotta cheese	30000
Tiramisu	30000
White sauce, milk sauce	60000
Milk gravy, quick gravy	60000
Cheese spread, NFS	100000
Cheese spread, American or Cheddar cheese base	100000
Cheese spread, Swiss cheese base	100000
Cheese spread, cream cheese, regular	100000
Cheese spread, cream cheese, light or lite	100000
Cheese spread, pressurized can	100000
Topping from meat pizza	20000
Topping from meat and vegetable pizza	20000
Frankfurter or hot dog, breaded, baked	20000
Frankfurter or hot dog, chicken	20000
Frankfurter or hot dog, turkey	20000
Beef sausage, NFS	20000
Beef sausage, brown and serve, links, cooked	20000
Beef sausage, smoked, stick	20000
Beef sausage, smoked	20000
Beef sausage, fresh, bulk, patty or link, cooked	20000
Beef sausage with cheese, smoked	20000

Blood sausage	20000
Chicken and beef sausage, smoked	20000
Chorizos	20000
Head cheese	20000
Knockwurst	20000
Mortadella	20000
Polish sausage	20000
Italian sausage	20000
Sausage (not cold cut), NFS	20000
Pork sausage, fresh, bulk, patty or link, cooked	20000
Pork sausage, brown and serve, cooked	20000
Pork sausage, country style, fresh, cooked	20000
Pork sausage rice links, brown and serve, cooked	20000
Pork and beef sausage	20000
Pork and beef sausage, brown and serve, cooked	20000
Smoked sausage, pork	20000
Vienna sausage, canned	20000
Vienna sausage, chicken, canned	20000
Pickled sausage	20000
Meat spread or potted meat, NFS	20000
Ham salad spread	20000
Roast beef spread	20000
Corned beef spread	20000
Beef with tomato-based sauce (mixture)	20000
Sandwich, NFS	50000
Meat sandwich, NFS	50000
Corn dog (frankfurter or hot dog with cornbread coating)	20000
Puerto Rican sandwich (Sandwich criollo)	20000
Salami sandwich, with spread	20000
Sausage on biscuit	20000
Sausage griddle cake sandwich	20000
Sausage and cheese on English muffin	20000
Sausage balls (made with biscuit mix and cheese)	20000
Sausage sandwich	20000
Sausage and spaghetti sauce sandwich	20000
Meat spread or potted meat sandwich	20000
Hors d'oeuvres, with spread	20000
Frozen dinner, NFS	20000
Beef dinner, NFS (frozen meal)	20000
Beef with potatoes (frozen meal, large meat portion)	20000
Sirloin, chopped, dinner, NFS (frozen meal)	20000
Sirloin, chopped, with gravy, mashed potatoes, vegetable (frozen meal)	20000
Sirloin tips with gravy, potatoes, vegetable (frozen meal)	20000
Sirloin beef with gravy, potatoes, vegetable (frozen meal)	20000
Salisbury steak dinner, NFS (frozen meal)	20000
Salisbury steak with gravy, potatoes, vegetable (frozen meal)	20000
Salisbury steak with gravy, whipped potatoes, vegetable, dessert (frozen meal)	20000
Salisbury steak with gravy, potatoes, vegetable, soup or macaroni and cheese, dessert (frozen meal)	20000
Salisbury steak with gravy, potatoes, vegetable, dessert (frozen meal, large meat portion)	20000
Salisbury steak with gravy, macaroni and cheese, vegetable (frozen meal)	20000
Salisbury steak with gravy, macaroni and cheese (frozen meal)	20000

Beef, sliced, with gravy, potatoes, vegetable (frozen meal)	20000
Beef short ribs, boneless, with barbecue sauce, potatoes, vegetable (frozen meal)	20000
Meatballs, Swedish, in sauce, with noodles (frozen meal)	20000
Meatballs, Swedish, in gravy, with noodles (diet frozen meal)	20000
Corned beef hash with apple slices, vegetable (frozen meal)	20000
Salisbury steak, baked, with tomato sauce, vegetable (diet frozen meal)	20000
Beef with spaetzle or rice, vegetable (frozen meal)	20000
Meat loaf dinner, NFS (frozen meal)	20000
Meat loaf with potatoes, vegetable (frozen meal)	20000
Beef, broth, bouillon, or consomme	20000
Oxtail soup	20000
Chili beef soup	20000
Meatball soup, Mexican style (Sopa de Albondigas)	20000
Chicken, broth, bouillon, or consomme	30000
Chicken broth, without tomato, home recipe	30000
Chicken broth, with tomato, home recipe	30000
Chicken broth, bouillion, or consomme, dry, not reconstituted	30000
Mexican style chicken broth soup stock	30000
Chicken broth, canned, low sodium	30000
Fish chowder	30000
Clam chowder, NS as to Manhattan or New England style	30000
Clam chowder, Manhattan	30000
Lobster bisque	30000
Lobster gumbo	30000
Oyster stew	30000
Shrimp gumbo	30000
Meat broth, Puerto Rican style	30000
Gelatin drink, powder, unflavored, unsweetened, reconstituted	30000
Gelatin drink, powder, flavored, with low-calorie sweetener, reconstituted	30000
Gravy, poultry	60000
Gravy, beef or meat	60000
Gravy, giblet	60000
Gravy, mushroom	60000
Gravy, redeye	60000
Gravy or sauce, Chinese (soy sauce, stock or bouillon, cornstarch)	60000
Oyster-flavored sauce	60000
Mole poblano (sauce)	60000
Mole verde (sauce)	60000
Black bean sauce	60000
Soy nuts	50000
Miso sauce	60000
Miso (fermented soybean paste)	60000
Natto (fermented soybean product)	60000
Hoisin sauce	60000
Protein supplement, powdered	30000
High protein bar, candy-like, soy and milk base	50000
Nutritional supplement for people with diabetes, liquid	50000
Textured vegetable protein, dry	50000
Meal replacement or supplement, liquid, soy-base, high protein	50000
Meal replacement or supplement, liquid, soy-based	50000
Beans and franks, frozen dinner	20000
Swiss steak, with gravy, meatless	20000

Nuts, nfs	50000
Almonds, NFS	50000
Almonds, roasted	50000
Almonds, honey-roasted	50000
Brazil nuts	50000
Cashew nuts, NFS	50000
Cashew nuts, roasted (assume salted)	50000
Cashew nuts, roasted, without salt	50000
Cashew nuts, honey-roasted	50000
Macadamia nuts, roasted	50000
Mixed nuts, NFS	50000
Mixed nuts, roasted, with peanuts	50000
Mixed nuts, roasted, without peanuts	50000
Peanuts, roasted, salted	50000
Peanuts, honey-roasted	50000
Almond butter	50000
Almond paste (Marzipan paste)	50000
Cashew butter	50000
Peanut butter	50000
Peanut butter and jelly	50000
Peanut sauce	20000
Brown nut gravy, meatless	20000
Peanut butter sandwich	50000
Peanut butter and jelly sandwich	50000
Peanut butter and banana sandwich	50000
Nut mixture with dried fruit and seeds	50000
Nut mixture with seeds	50000
Pumpkin and/or squash seeds, hulled, roasted, salted	50000
Pumpkin and/or squash seeds, hulled, roasted, without salt	50000
Sunflower seeds, hulled, roasted, without salt	50000
Sunflower seeds, hulled, dry roasted	50000
Sesame sauce	20000
Sesame paste (sesame butter made from whole seeds)	50000
Sesame butter (tahini) (made from kernels)	50000
Carob chips	145000
Biscuit mix, dry	30000
Bread, white	30000
Bread, white, toasted	30000
Bread, white, made from home recipe or purchased at a bakery	30000
Bread, white, made from home recipe or purchased at a bakery, toasted	30000
Bread, Cuban	30000
Bread, Cuban, toasted	30000
Bread, French or Vienna	30000
Bread, French or Vienna, toasted	30000
Focaccia, Italian flatbread, plain	30000
Naan, Indian flatbread	30000
Bread, Italian, Grecian, Armenian	30000
Bread, Italian, Grecian, Armenian, toasted	30000
Bread, pita	30000
Bread, pita, toasted	30000
Bread, batter	30000
Bread, cheese	30000

Bread, cheese, toasted	30000
Bread, cinnamon	30000
Bread, cinnamon, toasted	30000
Bread, cornmeal and molasses	30000
Bread, cornmeal and molasses, toasted	30000
Bread, egg, Challah	30000
Bread, egg, Challah, toasted	30000
Bread, garlic	30000
Bread, garlic, toasted	30000
Bread, onion	30000
Bread, high protein	30000
Bread, high protein, toasted	30000
Bread, milk and honey	30000
Bread, milk and honey, toasted	30000
Bread, potato	30000
Bread, potato, toasted	30000
Bread, raisin	30000
Bread, raisin, toasted	30000
Bread, white, low sodium or no salt	30000
Bread, white, low sodium or no salt, toasted	30000
Bread, sour dough	30000
Bread, sour dough, toasted	30000
Bread, sweetpotato	30000
Bread, vegetable	30000
Bread, vegetable, toasted	30000
Bread, dough, fried	90000
Roll, white, soft	30000
Roll, white, soft, toasted	30000
Roll, white, hard	30000
Roll, white, hard, toasted	30000
Roll, diet	30000
Roll, egg bread	60000
Roll, egg bread, toasted	60000
Roll, cheese	30000
Roll, French or Vienna	30000
Roll, French or Vienna, toasted	30000
Roll, garlic	30000
Roll, hoagie, submarine	30000
Roll, hoagie, submarine, toasted	30000
Roll, Mexican, bolillo	30000
Roll, sour dough	30000
Roll, sweet	30000
Roll, sweet, toasted	30000
Coffee cake, yeast type	60000
Croissant	60000
Brioche	60000
Bread, Spanish coffee	60000
Bagel	30000
Bagel, toasted	30000
Bread stuffing	30000
Bread sticks, hard	30000
Bread stick, soft	30000

Bread stick, NS as to hard or soft	30000
Croutons	70000
Muffin, English	30000
Muffin, English, toasted	30000
Melba toast	20000
Anisette toast	20000
Pannetone (Italian-style sweetbread)	60000
Zwieback toast	60000
Bread, whole wheat, 100%	30000
Bread, whole wheat, 100%, toasted	30000
Bread, pita, whole wheat, 100%	30000
Muffin, English, whole wheat, 100%	30000
Muffin, English, whole wheat, 100%, toasted	30000
Bread, wheat germ	30000
Bread, wheat germ, toasted	30000
Bread, sprouted wheat	30000
Bread, sprouted wheat, toasted	30000
Bagel, whole wheat, 100%	30000
Bagel, whole wheat, 100%, toasted	30000
Roll, whole wheat, 100%	30000
Roll, whole wheat, 100%, toasted	30000
Bread, whole grain white	30000
Bread, whole grain white, toasted	30000
Bread, whole wheat, NS as to 100%	30000
Bread, whole wheat, NS as to 100%, toasted	30000
Bread, puri or poori (Indian puffed bread), whole wheat, NS as to 100%, fried	30000
Bread, wheat or cracked wheat	30000
Bread, wheat or cracked wheat, toasted	30000
Bread, pita, whole wheat, NS as to 100%	30000
Bread, pita, whole wheat, NS as to 100%, toasted	30000
Bread, pita, wheat or cracked wheat	30000
Bread, pita, wheat or cracked wheat, toasted	30000
Bagel, wheat	30000
Bagel, wheat, toasted	30000
Bagel, whole wheat, NS as to 100%	30000
Bagel, whole wheat, NS as to 100%, toasted	30000
Bagel, wheat bran	30000
Bagel, wheat bran, toasted	30000
Bread, wheat bran	30000
Bread, wheat bran, toasted	30000
Muffin, English, wheat bran	30000
Muffin, English, wheat bran, toasted	30000
Muffin, English, wheat or cracked wheat	30000
Muffin, English, wheat or cracked wheat, toasted	30000
Muffin, English, whole wheat, NS as to 100%	30000
Muffin, English, whole wheat, NS as to 100%, toasted	30000
Bread stick, hard, whole wheat, NS as to 100%	30000
Roll, wheat or cracked wheat	30000
Roll, wheat or cracked wheat, toasted	30000
Roll, whole wheat, NS as to 100%	30000
Roll, whole wheat, NS as to 100%, toasted	30000
Bread, rye	30000

Bread, rye, toasted	30000
Bread, marble rye and pumpernickel	30000
Bread, marble rye and pumpernickel, toasted	30000
Muffin, English, rye	30000
Muffin, English, rye, toasted	30000
Bread, pumpernickel	30000
Bread, pumpernickel, toasted	30000
Bagel, pumpernickel	30000
Bagel, pumpernickel, toasted	30000
Muffin, English, pumpernickel	30000
Muffin, English, pumpernickel, toasted	30000
Bread, black	30000
Roll, rye	30000
Roll, pumpernickel	30000
Roll, pumpernickel, toasted	30000
Bread, oatmeal	30000
Bread, oatmeal, toasted	30000
Bread, oat bran	30000
Bread, oat bran, toasted	30000
Bagel, oat bran	30000
Bagel, oat bran, toasted	30000
Roll, oatmeal	30000
Roll, oatmeal, toasted	30000
Roll, oat bran	30000
Roll, oat bran, toasted	30000
Muffin, English, oat bran	30000
Muffin, English, oat bran, toasted	30000
Bread, multigrain	30000
Roll, multigrain	30000
Roll, multigrain, toasted	30000
Bagel, multigrain	30000
Bagel, multigrain, toasted	30000
Muffin, English, multigrain	30000
Muffin, English, multigrain, toasted	30000
Bread, barley	30000
Bread, triticale	30000
Bread, triticale, toasted	30000
Bread, buckwheat	30000
Bread, buckwheat, toasted	30000
Bread, soy	30000
Bread, soy, toasted	30000
Bread, sunflower meal	30000
Bread, sunflower meal, toasted	30000
Bread, rice	30000
Bread, rice, toasted	30000
Injera (American-style Ethiopian bread)	30000
Bread, low gluten	30000
Bread, low gluten, toasted	30000
Biscuit, baking powder or buttermilk type, NS as to made from mix, refrigerated dough, or home recipe	30000
Biscuit dough, raw	30000
Biscuit dough, fried	70000

Crumpet	30000
Crumpet, toasted	30000
Biscuit, baking powder or buttermilk type, made from mix	50000
Biscuit, baking powder or buttermilk type, made from refrigerated dough	50000
Biscuit, whole wheat	50000
Biscuit, cheese	50000
Biscuit, cinnamon-raisin	50000
Scone	50000
Scone, whole wheat	50000
Cornbread, prepared from mix	30000
Cornbread stuffing	30000
Cornbread muffin, stick, round	30000
Corn flour patty or tart, fried	30000
Corn pone, baked	30000
Corn pone, fried	70000
Gordita/sope shell, plain, no filling, grilled, no fat added	30000
Gordita/sope shell, plain, no filling, fried in oil	70000
Hush puppy	30000
Johnnycake	30000
Spoonbread	30000
Tortilla, NFS	30000
Tortilla, corn	30000
Tortilla, flour (wheat)	30000
Tortilla, whole wheat	30000
Taco shell, corn	30000
Taco shell, flour	30000
Muffin, NFS	50000
Muffin, fruit and/or nuts	50000
Muffin, fruit and/or nut, low fat	50000
Muffin, fruit, fat free, cholesterol free	50000
Muffin, chocolate chip	50000
Muffin, chocolate	50000
Muffin, chocolate, lowfat	50000
Muffin, whole wheat	50000
Muffin, wheat	50000
Muffin, buckwheat	50000
Muffin, wheat bran	50000
Muffin, bran with fruit, lowfat	50000
Muffin, bran with fruit, no fat, no cholesterol	50000
Muffin, oatmeal	50000
Muffin, oat bran	50000
Muffin, oat bran with fruit and/or nuts	50000
Muffin, plain	50000
Muffin, cheese	50000
Muffin, pumpkin	50000
Muffin, zucchini	50000
Muffin, carrot	50000
Muffin, multigrain, with nuts	50000
Muffin, multigrain, with fruit	50000
Matzo, fritters	70000
Matzo ball	30000
Popover	30000

Toaster muffin, fruit, untoasted	30000
Toaster muffin, fruit, toasted	30000
Bread, Boston Brown	30000
Bread, nut	30000
Bread, pumpkin	30000
Bread, zucchini	30000
Bread, Irish soda	30000
Cake batter, raw, chocolate	50000
Cake batter, raw, not chocolate	50000
Cake, NS as to type, with or without icing	50000
Cake, angel food, NS as to icing	50000
Cake, angel food, without icing	50000
Cake, angel food, with icing	50000
Cake, angel food, with fruit and icing or filling	50000
Cake, angel food, chocolate, without icing	50000
Cake, applesauce, NS as to icing	50000
Cake, applesauce, without icing	50000
Cake, applesauce, with icing	50000
Cake, applesauce, diet, without icing	50000
Cake, banana, NS as to icing	50000
Cake, banana, without icing	50000
Cake, banana, with icing	50000
Cake, black forest (chocolate-cherry)	50000
Cake, Boston cream pie	50000
Cake, butter, NS as to icing	50000
Cake, butter, without icing	50000
Cake, butter, with icing	50000
Cake, carrot, NS as to icing	50000
Cake, carrot, without icing	50000
Cake, carrot, with icing	50000
Cake, carrot, diet	50000
Cheesecake	50000
Cheesecake -type dessert, made with yogurt, with fruit	50000
Cheesecake, chocolate	50000
Cake, chocolate, made with mayonnaise or salad dressing, NS as to icing	50000
Cake, chocolate, made with mayonnaise or salad dressing, without icing or filling	50000
Cake, chocolate, made with mayonnaise or salad dressing, with icing, coating, or filling	50000
Cake, chocolate, devil's food, or fudge, standard-type mix (eggs and water added to dry mix), NS as to icing	50000
Cake, chocolate, devil's food, or fudge, made from home recipe or purchased ready-to-eat, NS as to icing	50000
Cake, chocolate, devil's food, or fudge, standard-type mix (eggs and water added to dry mix), without icing or filling	50000
Cake, chocolate, devil's food, or fudge, standard-type mix (eggs and water added to dry mix), with icing, coating, or filling	50000
Cake, chocolate, devil's food, or fudge, pudding type mix, made by "cholesterol free" recipe (water, oil and egg whites added to dry mix), without icing or filling	50000
Cake, chocolate, devil's food, or fudge, pudding type mix, made by "cholesterol free" recipe (water, oil and egg whites added to dry mix), with "light" icing, coating or filling	50000
Cake, chocolate, devil's food, or fudge, pudding-type mix (oil, eggs, and water added to dry mix), NS as to icing	50000

Cake, chocolate, devil's food, or fudge, pudding-type mix (oil, eggs, and water added to dry mix), without icing or filling	50000
Cake, chocolate, devil's food, or fudge, pudding-type mix (oil, eggs, and water added to dry mix), with icing, coating, or filling	50000
Cake, Poor Man's (spice-type), without icing	50000
Cake, cream, without icing or topping	50000
Cake, cupcake, NS as to type or icing	50000
Cake, cupcake, NS as to type, without icing	50000
Cake, cupcake, NS as to type, with icing	50000
Cake, cupcake, chocolate, NS as to icing	50000
Cake, cupcake, chocolate, without icing or filling	50000
Cake, cupcake, chocolate, with icing or filling	50000
Cake, cupcake, not chocolate, NS as to icing	50000
Cake, cupcake, not chocolate, without icing or filling	50000
Cake, cupcake, not chocolate, with icing or filling	50000
Cake, cupcake, chocolate, with or without icing, fruit filling or cream filling, lowfat, cholesterol free	50000
Cake, Dobos Torte (non-chocolate layer cake with chocolate filling and icing)	50000
Cake, fruit cake, light or dark, holiday type cake	50000
Cake, plum pudding	50000
Cake, gingerbread, without icing	50000
Cake, graham cracker, without icing	50000
Cake, ice cream and cake roll, chocolate	50000
Cake, ice cream and cake roll, not chocolate	50000
Cake, ice box with fruit and whipped cream	50000
Cake, jelly roll	50000
Cake, lemon, NS as to icing	50000
Cake, lemon, without icing	50000
Cake, lemon, with icing	50000
Cake, lemon, lowfat, NS as to icing	30000
Cake, lemon, lowfat, without icing	30000
Cake, lemon, lowfat, with icing	30000
Cake, marble, NS as to icing	50000
Cake, marble, without icing	50000
Cake, marble, with icing	50000
Cake, nut, NS as to icing	50000
Cake, nut, without icing	50000
Cake, nut, with icing	50000
Cake, oatmeal, without icing	50000
Cake, oatmeal, with icing	50000
Cake, pineapple, fat free, cholesterol free, without icing	50000
Cake, poppyseed, without icing	50000
Cake, pound, without icing	50000
Cake, pound, with icing	50000
Cake, pound, chocolate	50000
Cake, pound, Puerto Rican style (Ponque)	50000
Cake, pumpkin, NS as to icing	50000
Cake, pumpkin, without icing	50000
Cake, pumpkin, with icing	50000
Cake, raisin-nut, without icing	50000
Cake, raisin-nut, with icing	50000
Cake, Ravani (made with farina)	50000

Cake, rice flour, without icing	50000
Cake, Quezadilla, El Salvadorian style	50000
Cake, soy flour, without icing	50000
Cake, spice, NS as to icing	50000
Cake, spice, without icing	50000
Cake, spice, with icing	50000
Cake, sponge, NS as to icing	50000
Cake, sponge, without icing	50000
Cake, sponge, with icing	50000
Cake, sponge, chocolate, without icing	50000
Cake, sponge, chocolate, with icing	50000
Cake, torte	50000
Cake, tres leche	50000
Cake, chiffon, NS as to icing	50000
Cake, chiffon, without icing	50000
Cake, chiffon, with icing	50000
Cake, chiffon, chocolate, without icing	50000
Cake, chiffon, chocolate, with icing	50000
Cake, upside down (all fruits)	50000
Cake, white, standard-type mix (egg whites and water added), NS as to icing	50000
Cake, white, standard-type mix (egg whites and water added to mix), without icing	50000
Cake, white, standard-type mix (egg whites and water added to mix), with icing	50000
Cake, white, pudding-type mix (oil, egg whites, and water added to dry mix), NS as to icing	50000
Cake, white, pudding-type mix (oil, egg whites, and water added to dry mix), without icing	50000
Cake, white, pudding-type mix (oil, egg whites, and water added to dry mix), with icing	50000
Cake, white, eggless, lowfat	50000
Cake, whole wheat, with fruit and nuts, without icing	50000
Cake, yellow, standard-type mix (eggs and water added to dry mix), NS as to icing	50000
Cake, yellow, standard-type mix (eggs and water added to dry mix), without icing	50000
Cake, yellow, standard-type mix (eggs and water added to dry mix), with icing	50000
Cake, yellow, pudding-type mix (oil, eggs, and water added to dry mix), without icing	50000
Cake, yellow, pudding-type mix (oil, eggs, and water added to dry mix), with icing	50000
Cake, shortcake, biscuit type, with fruit	50000
Cake, shortcake, sponge type, with fruit	50000
Cake, zucchini, NS as to icing	50000
Cake, zucchini, without icing	50000
Cake, zucchini, with icing	50000
Cookie, batter or dough, raw, not chocolate	50000
Cookie, NFS	50000
Cookie, almond	50000
Cookie, applesauce	50000
Cookie, fruit, baby	50000
Cookie, baby	50000
Cookie, biscotti (Italian sugar cookie)	50000
Cookie, brownie, NS as to icing	50000
Cookie, brownie, without icing	50000
Cookie, brownie, with icing	50000
Cookie, brownie, with cream cheese filling, without icing	50000
Cookie, brownie, lowfat, with icing	50000
Cookie, brownie, lowfat, without icing	50000
Cookie, brownie, fat free, without icing	50000
Cookie, butterscotch, brownie	50000

Cookie, butterscotch chip	50000
Cookie, caramel coated, with nuts	50000
Cookie, carob	50000
Cookie, carob and honey brownie	50000
Cookie, chocolate chip	50000
Cookie, chocolate chip, reduced fat	50000
Cookie, rich, chocolate chip, with chocolate filling	50000
Cookie, chocolate chip sandwich	50000
Cookie, chocolate, made with rice cereal	50000
Cookie, chocolate, made with oatmeal and coconut (no-bake)	50000
Cookie, chocolate fudge, with/without nuts	50000
Cookie, chocolate, with chocolate filling or coating, fat free	50000
Cookie, chocolate-covered marshmallow	50000
Cookie, marshmallow pie, chocolate covered	50000
Cookie, chocolate, chocolate sandwich or chocolate-coated or striped	50000
Cookie, chocolate-covered, sugar wafer, creme- or caramel-filled	50000
Cookie, chocolate sandwich, reduced fat	50000
Cookie, chocolate-covered, chocolate sandwich	50000
Cookie, chocolate, sandwich, with extra filling	50000
Cookie, chocolate and vanilla sandwich	50000
Cookie, chocolate wafer	50000
Cookie, graham cracker sandwich with chocolate and marshmallow filling	50000
Cookie, graham cracker with marshmallow	50000
Cookie bar, with chocolate, nuts, and graham crackers	50000
Cookie, coconut	50000
Cookie, fruit-filled bar	50000
Cookie, date bar	50000
Cookie, fig bar	50000
Cookie, fortune	50000
Cookie, cone shell, ice cream type, wafer or cake	50000
Cookie, cone shell, ice cream type, brown sugar	50000
Cookie, gingersnaps	50000
Cookie, granola	50000
Cookie, ladyfinger	50000
Cookie, lemon bar	50000
Cookie, macaroon, coconut-meringue type, no flour	50000
Cookie, marshmallow, with coconut	50000
Cookie, marshmallow, with rice cereal (no-bake)	50000
Cookie, marshmallow, with rice cereal and chocolate chips	50000
Cookie, marshmallow and peanut butter, with oat cereal (no-bake)	50000
Cookie, marshmallow pies, non-chocolate coating	50000
Cookie, meringue	50000
Cookie, molasses	50000
Cookie, Lebkuchen	50000
Cookie, multigrain, high fiber	50000
Cookie, oatmeal	50000
Cookie, oatmeal sandwich, with creme filling	50000
Cookie, oatmeal sandwich, with peanut butter and jelly filling	50000
Cookie, oatmeal, with chocolate and peanut butter (no-bake)	50000
Cookie, oat bran	50000
Cookie, peanut butter	50000
Cookie, peanut	50000

Cookie, with peanut butter filling, chocolate-coated	50000
Cookie, Pfeffernusse	50000
Cookie, pizzelle (Italian style wafer)	50000
Cookie, pumpkin	50000
Cookie, raisin sandwich, cream-filled	50000
Cookie, rum ball (no-bake)	50000
Cookie, sandwich-type, not chocolate or vanilla	50000
Cookie, shortbread	50000
Cookie, butter or sugar cookie	50000
Cookie, sugar wafer	50000
Cookie, teething, baby food	50000
Cookie, toffee bar	50000
Cookie, vanilla sandwich	50000
Cookie, rich, all chocolate, with chocolate filling or chocolate chips	50000
Cookie, butter or sugar, with icing or filling other than chocolate	50000
Cookie, vanilla waffle creme	50000
Cookie, tea, Japanese	50000
Cookie, vanilla wafer	50000
Cookie, vanilla with caramel, coconut, and chocolate coating	50000
Cookie, whole wheat, dried fruit, nut	50000
Cookie, rugelach	50000
Cookie, dietetic, chocolate chip	50000
Cookie, lemon wafer, lowfat	50000
Cookie, dietetic, oatmeal with raisins	50000
Cookie, dietetic, sandwich type	50000
Cookie, dietetic, sugar or plain	50000
Cookies, Puerto Rican (Mantecaditos polvorones)	50000
Pie, NFS	50000
Pie, fried, NFS	50000
Pie, apple, two crust	50000
Pie, apple, fried pie	50000
Pie, apricot, two crust	50000
Pie, apricot, fried pie	50000
Pie, blackberry, two crust	50000
Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry; two crust	50000
Pie, blueberry, two crust	50000
Pie, cherry, two crust	50000
Pie, cherry, fried pie	50000
Pie, lemon (not cream or meringue)	50000
Pie, lemon, fried pie	50000
Pie, mince, two crust	50000
Pie, peach, two crust	50000
Pie, peach, fried pie	50000
Pie, pear, two crust	50000
Pie, pineapple, two crust	50000
Pie, plum, two crust	50000
Pie, raisin, two crust	50000
Pie, raspberry, two crust	50000
Pie, rhubarb, two crust	50000
Pie, strawberry-rhubarb, two crust	50000
Pie, apple-sour cream	50000
Pie, cherry, made with cream cheese and sour cream	50000

Pie, banana cream	50000
Pie, buttermilk	50000
Pie, chess	50000
Pie, chocolate cream	50000
Pie, coconut cream	50000
Pie, custard	50000
Mixed fruit tart filled with custard or cream cheese	50000
Pie, lemon cream	50000
Pie, peanut butter cream	50000
Pie, pineapple cream	50000
Pie, pumpkin	50000
Pie, raspberry cream	50000
Pie, sour cream, raisin	50000
Pie, squash	50000
Pie, strawberry cream	50000
Pie, sweetpotato	50000
Pie, vanilla cream	50000
Pie, yogurt, frozen	50000
Pie, chiffon, not chocolate	50000
Pie, chiffon, chocolate	50000
Pie, black bottom	50000
Pie, lemon meringue	50000
Pie, chocolate-marshmallow	50000
Pie, pecan	50000
Pie, oatmeal	50000
Pie, pudding, flavors other than chocolate	50000
Pie, Toll house chocolate chip	50000
Pie, shoo-fly	50000
Pie, tofu with fruit	50000
Pie shell	50000
Vanilla wafer dessert base	50000
Blintz, cheese-filled	50000
Blintz, fruit-filled	50000
Cobbler, apple	50000
Cobbler, apricot	50000
Cobbler, berry	50000
Cobbler, cherry	50000
Cobbler, peach	50000
Cobbler, pear	50000
Cobbler, pineapple	50000
Cobbler, plum	50000
Cobbler, rhubarb	50000
Crisp, apple, apple dessert	50000
Fritter, apple	50000
Fritter, banana	50000
Fritter, berry	50000
Crisp, blueberry	50000
Crisp, cherry	50000
Crisp, peach	50000
Crisp, rhubarb	50000
Cream puff, eclair, custard or cream filled, NS as to icing	50000
Cream puff, eclair, custard or cream filled, not iced	50000

Cream puff, eclair, custard or cream filled, iced	50000
Cream puff, no filling or icing	50000
Air filled fritter or fried puff, without syrup, Puerto Rican style (Bunuelos de viento)	50000
Wheat flour fritter, without syrup	50000
Sopaipilla, without syrup or honey	50000
Sopaipilla with syrup or honey	50000
Crepe, dessert type, NS as to filling	50000
Crepe, dessert type, chocolate-filled	50000
Crepe, dessert type, fruit-filled	50000
Crepe suzette	50000
Crepe, dessert type, ice cream-filled	50000
Tamale, sweet	50000
Strudel, apple	50000
Strudel, berry	50000
Strudel, cherry	50000
Strudel, cheese	50000
Strudel, peach	50000
Strudel, pineapple	50000
Strudel, cheese and fruit	50000
Baklava	50000
Basbousa (semolina dessert dish)	50000
Turnover or dumpling, apple	50000
Turnover or dumpling, berry	50000
Turnover or dumpling, cherry	50000
Turnover or dumpling, lemon	50000
Turnover or dumpling, peach	50000
Turnover, guava	50000
Turnover, pumpkin	50000
Pastry, fruit-filled	50000
Pastry, Oriental, made with bean or lotus seed paste filling (baked)	50000
Pastry, Oriental, made with bean paste and salted egg yolk filling (baked)	50000
Pastry, Chinese, made with rice flour	50000
Pastry, cookie type, fried	50000
Pastry, Italian, with cheese	50000
Pastry, puff	50000
Pastry, puff, custard or cream filled, iced or not iced	50000
Cheese pastry puffs	50000
Pastry, mainly flour and water, fried	50000
Empanada, Mexican turnover, fruit-filled	50000
Empanada, Mexican turnover, pumpkin	50000
Breakfast pastry, NFS	50000
Danish pastry, plain or spice	50000
Doughnut, cake type	50000
Churros	50000
Doughnut, oriental	50000
Cruller, NFS	50000
French cruller	50000
Doughnut, chocolate, raised or yeast, with chocolate icing	300000
Doughnut, raised or yeast	300000
Doughnut, chocolate, raised or yeast	300000
Doughnut, raised or yeast, chocolate covered	300000
Doughnut, jelly	300000

Doughnut, custard-filled	300000
Doughnut, chocolate cream-filled	300000
Doughnut, wheat	300000
Doughnut, wheat, chocolate covered	300000
Breakfast tart	20000
Breakfast bar, NFS	150000
Breakfast bar, cereal crust with fruit filling, lowfat	150000
Fiber One Chewy Bar	150000
Kellogg's Nutri-Grain Cereal Bar	150000
Kellogg's Nutri-Grain Yogurt Bar	150000
Kellogg's Nutri-Grain Fruit and Nut Bar	150000
Breakfast bar, date, with yogurt coating	150000
Milk 'n Cereal bar	150000
Kellogg's Special K bar	150000
Meal replacement bar	150000
Snack bar, oatmeal	150000
Granola bar, oats, sugar, raisins, coconut	50000
Granola bar, oats, fruit and nuts, lowfat	50000
Granola bar, nonfat	50000
Granola bar, oats, reduced sugar	50000
Granola bar, peanuts, oats, sugar, wheat germ	50000
Granola bar, chocolate-coated, NFS	50000
Granola bar, with coconut, chocolate-coated	50000
Granola bar with nuts, chocolate-coated	50000
Granola bar, oats, nuts, coated with non-chocolate coating	50000
Granola bar, coated with non-chocolate coating	50000
Granola bar, high fiber, coated with non-chocolate yogurt coating	50000
Granola bar, with rice cereal	50000
Coffee cake, NFS	50000
Coffee cake, crumb or quick-bread type	50000
Crackers, NS as to sweet or nonsweet	145000
Cracker, animal	145000
Crackers, graham	145000
Crackers, graham, chocolate covered	145000
Crackers, oatmeal	145000
Crackers, Cuban	145000
Crackers, Cuca	145000
Crispbread, wheat or rye, extra crispy	145000
Crackers, matzo	145000
Crackers, milk	145000
Crackers, oat	145000
Crackers, oyster	145000
Rice cake, cracker-type	145000
Crackers, rice	145000
Puffed rice cake	145000
Popcorn cake	145000
Puffed wheat cake	145000
Rice paper	145000
Crispbread, rye, no added fat	145000
Crackers, saltine	145000
Crackers, cylindrical, peanut-butter filled	145000
Crackers, sandwich-type, NFS	145000

Cracker, sandwich-type, peanut butter filled	145000
Cracker, sandwich-type, cheese-filled	145000
Crackers, toast thins (rye, pumpernickel, white flour)	145000
Crackers, water biscuits	145000
Cracker, 100% whole wheat	145000
Crackers, whole wheat and bran	145000
Crackers, wheat	145000
Crackers, corn	145000
Salty snacks, corn or cornmeal base, nuts or nuggets, toasted	145000
Salty snacks, corn or cornmeal base, corn chips, corn-cheese chips	145000
Salty snacks, corn or cornmeal base, corn puffs and twists; corn-cheese puffs and twists	145000
Salty snacks, corn or cornmeal base, tortilla chips	145000
Salty snacks, corn or cornmeal base, corn chips, corn-cheese chips, unsalted	145000
Salty snacks, corn or cornmeal base, tortilla chips, light (baked with less oil)	145000
Salty snacks, corn or cornmeal base, with oat bran, tortilla chips	145000
Salty snacks, corn based puffs and twists, cheese puffs and twists, lowfat	145000
Salty snacks, corn or cornmeal base, tortilla chips, unsalted	145000
Salty snack mixture, mostly corn or cornmeal based, with pretzels, without nuts	145000
Salty snacks, wheat-based, high fiber	145000
Salty snacks, wheat- and corn-based chips	145000
Salty snacks, multigrain, chips	145000
Pita chips	145000
Popcorn, popped in oil, unbuttered	145000
Popcorn, popped in oil, buttered	260000
Popcorn, air-popped, buttered	260000
Popcorn, flavored	260000
Popcorn, popped in oil, lowfat, low sodium	260000
Popcorn, popped in oil, lowfat	260000
Popcorn, popped in oil, unsalted	260000
Popcorn, sugar syrup or caramel-coated	260000
Snacks, onion-flavored rings	145000
Shrimp chips (tapioca base)	145000
Pretzels, NFS	145000
Pretzels, hard	145000
Pretzels, soft	145000
Pretzel, oatbran, hard	145000
Pretzel, hard, multigrain	145000
Wheat sticks, 100% whole wheat	145000
Multigrain mixture, pretzels, cereal and/or crackers, nuts	145000
Oriental party mix, with peanuts, sesame sticks, chili rice crackers and fried green peas	145000
Multigrain mixture, bread sticks, sesame nuggets, pretzels, rye chips	145000
Yogurt chips	145000
Bagel chip	145000
Pancakes, plain	50000
Pancakes, buckwheat	50000
Pancakes, cornmeal	50000
Pancakes, whole wheat	50000
Pancakes, sour dough	50000
Pancakes, rye	50000
Waffle, plain	50000
Waffle, wheat, bran, or multigrain	50000
Waffle, cornmeal	50000

Waffle, 100% whole wheat or 100% whole grain	50000
Waffle, oat bran	50000
Waffle, multi-bran	50000
French toast, plain	50000
French toast sticks, plain	50000
Bread fritters, Puerto Rican style (Torrejas gallegas, Galician fritters)	50000
Crepe, plain	50000
Flour and water patty	50000
Flour and water gravy	20000
Flour and milk patty	50000
Dumpling, fried, Puerto Rican style	50000
Dumpling, plain	50000
Cake made with glutinous rice	50000
Cake or pancake made with rice flour and/or dried beans	50000
Cake made with glutinous rice and dried beans	50000
Funnel cake	50000
Cereal, cooked, NFS	20000
Cereal, cooked, instant, NS as to grain	20000
Barley, cooked, NS as to fat added in cooking	20000
Barley, cooked, fat not added in cooking	20000
Buckwheat groats, cooked, NS as to fat added in cooking	20000
Buckwheat groats, cooked, fat not added in cooking	20000
Buckwheat groats, cooked, fat added in cooking	20000
Grits, cooked, corn or hominy, NS as to regular, quick or instant, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, NS as to regular, quick, or instant, fat not added in cooking	20000
Grits, cooked, corn or hominy, regular, fat not added in cooking	20000
Grits, cooked, corn or hominy, regular, fat added in cooking	20000
Grits, cooked, corn or hominy, regular, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, NS as to regular, quick, or instant, fat added in cooking	20000
Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, fat not added in cooking	20000
Grits, cooked, corn or hominy, with cheese, NS as to regular, quick, or instant, fat added in cooking	20000
Grits, cooked, corn or hominy, with cheese, regular, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, quick, fat not added in cooking	20000
Grits, cooked, corn or hominy, quick, fat added in cooking	20000
Grits, cooked, corn or hominy, quick, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, instant, fat not added in cooking	20000
Grits, cooked, corn or hominy, instant, fat added in cooking	20000
Grits, cooked, corn or hominy, instant, NS as to fat added in cooking	20000
Grits, cooked, flavored, corn or hominy, instant, fat not added in cooking	20000
Grits, cooked, flavored, corn or hominy, instant, fat added in cooking	20000
Grits, cooked, flavored, corn or hominy, instant, NS as to fat added in cooking	20000
Grits, cooked, corn or hominy, NS as to regular, quick, or instant, NS as to fat added in cooking, made with milk	20000
Cornmeal mush, made with water	20000
Cornmeal mush, fried	20000
Cornmeal mush, made with milk	20000
Cornmeal, made with milk and sugar, Puerto Rican Style (Harina de maiz)	20000
Cornmeal dumpling	20000

Cornmeal sticks, boiled	20000
Cornmeal, lime-treated, cooked (Masa harina)	20000
Rice, frozen dessert, nondairy, flavors other than chocolate	50000
Rice dessert bar, frozen, chocolate, nondairy, chocolate covered	50000
Wheat, cream of, cooked, quick, NS as to fat added in cooking	20000
Wheat, cream of, cooked, regular, NS as to fat added in cooking	20000
Wheat, cream of, cooked, NS as to regular, quick, or instant, NS as to fat added in cooking	20000
Wheat, cream of, cooked, NS as to regular, quick, or instant, fat not added in cooking	20000
Wheat, cream of, cooked, regular, fat not added in cooking	20000
Wheat, cream of, cooked, quick, fat not added in cooking	20000
Wheat, cream of, cooked, instant, fat not added in cooking	20000
Wheat, cream of, cooked, made with milk	20000
Wheat, cream of, cooked, instant, fat added in cooking	20000
Wheat, cream of, cooked, instant, NS as to fat added in cooking	20000
Wheat, cream of, cooked, NS as to regular, quick, or instant, fat added in cooking	20000
Wheat, cream of, cooked, regular, fat added in cooking	20000
Wheat, cream of, cooked, quick, fat added in cooking	20000
Rye, cream of, cooked	20000
Nestum cereal	20000
Cereal, NFS	20000
Oat cereal, NFS	20000
Cereal, ready-to-eat, NFS	20000
Character cereals, TV or movie, General Mills	20000
Character cereals, TV or movie, Kellogg's	20000
All-Bran	20000
All-Bran with Extra Fiber	20000
Alpen	20000
Alpha-Bits	20000
Alpha-bits with marshmallows	20000
Amaranth Flakes	20000
Apple Cinnamon Cheerios	20000
Apple Cinnamon Squares Mini-Wheats, Kellogg's (formerly Apple Cinnamon Squares)	20000
Apple Jacks	20000
Banana Nut Crunch Cereal (Post)	20000
Basic 4	20000
Berry Berry Kix	20000
Berry Burst Cheerios	20000
Blueberry Morning, Post	20000
Booberry	20000
All-Bran Bran Buds, Kellogg's (formerly Bran Buds)	20000
Bran Chex	20000
Cap'n Crunch	20000
Cap'n Crunch's Christmas Crunch	20000
Cap'n Crunch's Crunch Berries	20000
Cap'n Crunch's Peanut Butter Crunch	20000
Cheerios	20000
Chex cereal, NFS	20000
Chocolate flavored frosted puffed corn cereal	20000
Chocolate Lucky Charms	20000
Cinnamon Grahams, General Mills	20000
Cinnamon Toast Crunch	20000
Cinnamon Toast Crunch Reduced Sugar	20000

Honey Nut Clusters (formerly called Clusters)	20000
Cocoa Krispies	20000
Cocoa Blasts, Quaker	20000
Cocoa Pebbles	20000
Cocoa Puffs	20000
Complete Oat Bran Flakes, Kellogg's (formerly Common Sense Oat Bran, plain)	20000
Cookie-Crisp	20000
Crunchy Corn Bran, Quaker	20000
Corn Chex	20000
Corn flakes, NFS	20000
Corn flakes, low sodium	20000
Corn flakes, Kellogg's	20000
Corn Puffs	20000
Total Corn Flakes	20000
Count Chocula	20000
Cracklin' Oat Bran	20000
Cranberry Almond Crunch, Post	20000
Crisp Crunch	20000
Crispix	20000
Crispy Brown Rice Cereal	20000
Harmony cereal, General Mills	20000
Crispy Rice	20000
Crispy Wheats'n Raisins	20000
Curves Fruit and Nut Crunch Cereal	20000
Disney cereals, Kellogg's	20000
Dora the Explorer Cereal	20000
Familia	20000
Fiber One	20000
Fiber 7 Flakes, Health Valley	20000
Bran Flakes, NFS (formerly 40% Bran Flakes, NFS)	20000
Complete Wheat Bran Flakes, Kellogg's (formerly 40% Bran Flakes)	20000
Natural Bran Flakes, Post (formerly called 40% Bran Flakes, Post)	20000
Frankenberry	20000
French Toast Crunch, General Mills	20000
Froot Loops	20000
Froot Loops Cereal Straws	20000
Frosted Cheerios	20000
Frosted Chex	20000
Frosted Mini-Wheats	20000
Frosted Wheat Bites	20000
Frosty O's	20000
Frosted rice, NFS	20000
Frosted Rice Krispies, Kellogg's	20000
Fruit & Fibre (fiber), NFS	20000
Fruit & Fibre (fiber) with dates, raisins, and walnuts	20000
Fruit Harvest cereal, Kellogg's	20000
Fruit Rings, NFS	20000
Fruit Whirls	20000
Fruity Cheerios	20000
Fruity Pebbles	20000
Golden Grahams	20000
Granola, NFS	50000

Grape-Nuts	20000
Grape-Nut Flakes	20000
Great Grains, Raisin, Date, and Pecan Whole Grain Cereal, Post	20000
Great Grains Double Pecan Whole Grain Cereal, Post	20000
Honey Bunches of Oats	20000
Honey Bunches of Oats with Almonds, Post	20000
Honeycomb, plain	20000
Honeycomb, strawberry	20000
Honey Crunch Corn Flakes, Kellogg's	20000
Honey Nut Chex	20000
Honey Nut Cheerios	20000
Honey Nut Shredded Wheat, Post	20000
Honey Smacks, Kellogg's (formerly Smacks; Honey Smacks)	20000
Jenny O's	20000
Just Right	20000
Just Right Fruit and Nut (formerly Just Right with raisins, dates, and nuts)	20000
Kaboom	20000
Kix	20000
Life (plain and cinnamon)	20000
Lucky Charms	20000
Frosted oat cereal with marshmallows	20000
Malt-O-Meal Coco-Roos	20000
Malt-O-Meal Corn Bursts	20000
Malt-O-Meal Crispy Rice	20000
Malt-O-Meal Frosted Flakes	20000
Malt-O-Meal Fruity Dyno-Bites	20000
Malt-O-Meal Honey and Nut Toasty O's	20000
Malt-O-Meal Marshmallow Mateys	20000
Malt-O-Meal Puffed Rice	20000
Malt-O-Meal Puffed Wheat	20000
Malt-O-Meal Golden Puffs (formerly Sugar Puffs)	20000
Malt-O-Meal Toasted Oat Cereal	20000
Malt-O-meal Tootie Fruities	20000
Maple Pecan Crunch Cereal, Post	20000
Marshmallow Safari, Quaker	20000
Millet, puffed	20000
Mini-Swirlz Cinnamon Bun Cereal, Kellogg's	20000
Mueslix cereal, NFS	20000
Muesli, dried fruit and nuts (formerly Muesli with raisins, dates, and almonds)	20000
Multi Bran Chex	20000
MultiGrain Cheerios	20000
Natural Muesli, Jenny's Cuisine	20000
Nu System Cuisine Toasted Grain Circles	20000
Nutty Nuggets, Ralston Purina	20000
Oat Bran Flakes, Health Valley	20000
Oatmeal Crisp, Apple Cinnamon (formerly Oatmeal Crisp with Apples)	20000
Oatmeal Crisp with Almonds	20000
Oatmeal Crisp, Raisin (formerly Oatmeal Raisin Crisp)	20000
Oh's, Honey Graham	20000
Oh's, Fruitangy, Quaker	20000
100% Bran	20000
100% Natural Cereal, plain, Quaker	20000

Sun Country 100% Natural Granola, with Almonds	20000
100 % Natural Cereal, with oats, honey and raisins, Quaker	20000
100 % Natural Wholegrain Cereal with raisins, lowfat, Quaker	20000
Optimum, Nature's Path	20000
Optimum Slim, Nature's Path	20000
Oreo O's cereal, Post	20000
Sweet Crunch, Quaker (formerly called Popeye)	20000
Sweet Puffs, Quaker	20000
Peanut Butter Toast Crunch, General Mills	20000
Product 19	20000
Quaker Oat Bran Cereal	20000
Quaker Oatmeal Squares (formerly Quaker Oat Squares)	20000
Quisp	20000
Reese's Peanut Butter Puffs cereal	20000
Rice Chex	20000
Rice Flakes, NFS	20000
Rice Krispies, Kellogg's	20000
Rice Krispies with Real Strawberries, Kellogg's	20000
Rice Krispies Treats Cereal, Kellogg's	20000
Rice, puffed	20000
Shredded Wheat'N Bran	20000
Smart Start, Kellogg's	20000
Smorz, Kellogg's	20000
Oatmeal Honey Nut Heaven, Quaker (formerly Toasted Oatmeal, Honey Nut)	20000
Corn Pops	20000
Frosted corn flakes, NFS	20000
Frosted Flakes, Kellogg's	20000
Reduced Sugar Frosted Flakes Cereal, Kellogg's	20000
Golden Crisp (Formerly called Super Golden Crisp)	20000
Toasted oat cereal	20000
Toasties, Post	20000
Malt-O-Meal Toasty O's	20000
Malt-O-Meal Apple and Cinnamon Toasty O's	20000
Total	20000
Total Cranberry Crunch	20000
Trix	20000
Trix, reduced sugar	20000
Uncle Sam Cereal (formerly Uncle Sam's Hi Fiber Cereal)	20000
Waffle Crisp, Post	20000
Weetabix Whole Wheat Cereal	20000
Wheat Chex	20000
Wheat germ, plain	20000
Wheat germ, with sugar and honey	20000
Wheat, puffed, plain	20000
Wheat, puffed, presweetened with sugar	20000
Shredded Wheat, 100%	20000
Wheaties	20000
Yogurt Burst Cheerios	20000
Oat bran, uncooked	20000
Rice bran, uncooked	20000
Whole wheat, cracked	20000
Pizza with meat, prepared from frozen, thin crust	20000

Pizza with meat, prepared from frozen, thick crust	20000
Calzone, with meat and cheese	20000
Panzerotti, with meat, vegetables, and cheese	20000
Won ton (wonton), fried, meat filled	20000
Won ton (wonton), fried, meatless	20000
Dumpling, steamed, filled with meat, poultry, or seafood	20000
Tamale casserole, Puerto Rican style (Tamales en cazuela)	20000
Meat turnover, Puerto Rican style (Pastelillo de carne; Empanadilla)	20000
Empanada, Mexican turnover, filled with meat and vegetables	20000
Crepes, filled with meat, fish, or poultry, with sauce	20000
Dumpling, meat-filled	20000
Knish, meat (pastry filled with meat)	20000
Sweet bread dough, filled with meat, steamed	20000
Quiche with meat, poultry or fish	20000
Bierock (turnover filled with ground beef and cabbage mixture)	20000
Turnover, meat-filled, no gravy	20000
Turnover, meat-filled, with gravy	20000
Turnover, meat- and cheese-filled, no gravy	20000
Turnover, meat- and bean-filled, no gravy	20000
Turnover, meat- and cheese-filled, tomato-based sauce	20000
Turnover, meat-and vegetable- filled (no potatoes, no gravy)	20000
Turnover, meat-, potato-, and vegetable-filled, no gravy	20000
Turnover, filled with egg, meat and cheese	20000
Dressing with meat and vegetables	20000
Lasagna with meat	20000
Lasagna with meat, whole wheat noodles	20000
Lasagna with meat, spinach noodles	20000
Ravioli, meat-filled, no sauce	20000
Spaghetti with tomato sauce and meatballs or spaghetti with meat sauce or spaghetti with meat sauce and meatballs	20000
Manicotti, cheese-filled, with meat sauce	20000
Stuffed shells, cheese-filled, with meat sauce	20000
Tortellini, meat-filled, with tomato sauce	20000
Tortellini, meat-filled, no sauce	20000
Chow fun noodles with meat and vegetables	20000
Lo mein, with pork	20000
Lo mein, with beef	20000
Pad Thai with meat	20000
Spaghetti with corned beef, Puerto Rican style	20000
Macaroni or noodles with cheese and beef	20000
Macaroni or noodles with cheese and pork or ham	20000
Macaroni or noodles with cheese and frankfurters or hot dogs	20000
Pasta with meat sauce	20000
Pasta with meat sauce	20000
Macaroni or noodles with beans or lentils and tomato sauce	20000
Pasta or macaroni salad with meat	20000
Rice, fried, with pork	20000
Rice, fried, with beef	20000
Paella, Valenciana style, with meat (Paella Valenciana)	20000
Rice with vienna sausage, Puerto Rican style (arroz con salchichas)	20000
Rice with Spanish sausage, Puerto Rican style	20000
Rice with beans and beef	20000

Rice with beans and pork	20000
Stuffed pepper, with meat	20000
Stuffed pepper, with rice and meat	20000
Stuffed tomato, with rice and meat	20000
Spanish rice with ground beef	20000
Wrap sandwich, filled with meat, poultry, or fish, vegetables, and rice	20000
Wrap sandwich, filled with meat, poultry, or fish, vegetables, rice, and cheese	20000
Frozen breakfast, NFS (frozen meal)	80000
Soup, NFS	30000
Noodle soup, NFS	30000
Rice soup, NFS	30000
Barley soup	30000
Barley soup, sweet, with or without nuts, Oriental Style	30000
Beef noodle soup, canned, undiluted	30000
Beef noodle soup	30000
Beef dumpling soup	30000
Beef rice soup	30000
Beef noodle soup, home recipe	30000
Chicken noodle soup	30000
Chicken noodle soup, canned, undiluted	30000
Chicken noodle soup, canned, low sodium, ready-to-serve	30000
Chicken noodle soup, home recipe	30000
Chicken noodle soup, cream of	30000
Noodle and potato soup, Puerto Rican style	30000
Chicken rice soup	30000
Chicken and rice soup, canned, undiluted	30000
Chicken or turkey rice soup, home recipe	30000
Rice and potato soup, Puerto Rican style	30000
Matzo ball soup	30000
Chicken soup with dumplings and potatoes	30000
Chicken soup with dumplings	30000
Turkey noodle soup	30000
Instant soup, NFS	30000
Instant soup, noodle	30000
Soup, mostly noodles	30000
Instant soup, rice	30000
Cherry pie filling	30000
Blueberry pie filling	30000
White potato, chips	350000
White potato, chips, restructured	350000
Potato based snacks, reduced fat, low sodium, all flavors	350000
Potato puffs, cheese-filled	145000
White potato, sticks	145000
White potato skins, chips	145000
Vegetable chips	145000
Potato pancake	50000
Norwegian Lefse, potato and flour pancake	50000
Potato pudding	30000
Potato soup, instant, made from dry mix	20000
Potato chowder	20000
Plantain chips	145000
Carrot chips, dried	145000

Tomato catsup	20000
Tomato chili sauce (catsup-type)	20000
Salsa, NFS	20000
Salsa, red, uncooked	20000
Salsa, red, cooked, not homemade	20000
Enchilada sauce, red	20000
Enchilada sauce, green	20000
Green tomato-chile sauce, raw (Salsa de tomate verde cruda)	20000
Green tomato-chile sauce, cooked (Salsa verde, NFS)	20000
Tomato sauce	20000
Tomato paste	20000
Tomato puree	20000
Spaghetti sauce, meatless	20000
Spaghetti sauce with meat, canned, no extra meat added	20000
Barbecue sauce	20000
Steak sauce, tomato-base	20000
Cocktail sauce	20000
Puerto Rican seasoning with ham	50000
Puerto Rican seasoning with ham and tomato sauce	20000
Puerto Rican seasoning without ham and tomato sauce	20000
Tomato soup, instant type, prepared with water	20000
Mushroom soup, made from dry mix	20000
Onion soup, made from dry mix	20000
Onion soup, dry mix, not reconstituted	20000
Vegetable soup, made from dry mix	20000
Vegetable soup, dry mix, not reconstituted	20000
Vegetable soup, cream of, made from dry mix, low sodium, prepared with water	20000
Potato and ham fritters, Puerto Rican style (Frituras de papa y jamon)	20000
Ripe plantain fritters, Puerto Rican style (Pionono)	20000
Ripe plantain meat pie, Puerto Rican style (Pinon)	20000
Table fat, NFS	700000
Margarine, stick, unsalted	700000
Margarine-like spread, stick, unsalted	450000
Vegetable oil-butter spread, stick, salted	450000
Butter-margarine blend, stick, unsalted	700000
Butter-vegetable oil blend	700000
Shortening, NS as to vegetable or animal	700000
Shortening, vegetable	700000
Garlic sauce	20000
Lemon-butter sauce	20000
Hollandaise sauce	20000
Bernaise sauce	20000
Orange sauce (for duck)	20000
Sandwich spread	20000
Tartar sauce	20000
Horseradish sauce	20000
Pesto sauce	20000
Tartar sauce, low calorie	20000
Honey butter	20000
Adobo fresco	50000
Vegetable oil, NFS	1000000
Corn oil	1000000

Corn and canola oil	1000000
Salad dressing, NFS, for salads	500000
Salad dressing, NFS, for sandwiches	500000
Blue or roquefort cheese dressing	500000
Bacon dressing (hot)	500000
Bacon and tomato dressing	500000
Caesar dressing	500000
Coleslaw dressing	500000
Feta Cheese Dressing	500000
French dressing	500000
Honey mustard dressing	500000
Italian dressing, made with vinegar and oil	500000
Mayonnaise, regular	700000
Mayonnaise, imitation	700000
Russian dressing	500000
Green Goddess dressing	500000
Creamy dressing, made with sour cream and/or buttermilk and oil	500000
Cream cheese dressing	500000
Milk, vinegar, and sugar dressing	500000
Poppy seed dressing	500000
Peppercorn Dressing	500000
Celery seed dressing	500000
Sesame dressing	500000
Sweet and sour dressing	500000
Thousand Island dressing	500000
Yogurt dressing	500000
Icing, chocolate	250000
Icing, white	250000
Sweet and sour sauce	20000
Fruit sauce	20000
Raisin sauce	20000
Plain dessert sauce	20000
Duck sauce	20000
Plum sauce, Oriental-style	20000
Gelatin dessert	30000
Gelatin snacks	30000
Gelatin dessert with whipped cream	30000
Danish dessert pudding	30000
Yookan (Yokan), a Japanese dessert made with bean paste and sugar	30000
Coconut cream cake, Puerto Rican style (Bien me sabe, "Tastes good to me")	30000
Pineapple custard, Puerto Rican style (Flan de pina)	30000
Haupia (coconut pudding)	30000
Almonds, chocolate covered	30000
Almonds, sugar-coated	30000
Almonds, yogurt-covered	30000
Butterscotch morsels	30000
Caramel, chocolate-flavored roll	30000
Caramel, flavor other than chocolate	30000
Caramel, with nuts	30000
Caramel candy, chocolate covered	30000
Caramel with nuts and cereal, chocolate covered	30000
Caramel with nuts, chocolate covered	30000

Caramel, all flavors, sugar free	30000
Espresso coffee beans, chocolate-covered	30000
Fruit peel, candied	30000
Date candy	30000
Soft fruit confections	30000
Fruit leather and fruit snacks candy	30000
Tamarind candy	30000
Fruit snacks candy, with high vitamin C	30000
Yogurt covered fruit snacks candy, with added vitamin C	30000
Yogurt covered fruit snacks candy rolls, with high vitamin C	30000
Gumdrops, chocolate covered	30000
Halvah, plain	30000
Halvah, chocolate covered	30000
Chocolate-flavored sprinkles	30000
Licorice	30000
Marshmallow	20000
Marshmallow, chocolate covered	20000
Marshmallow, candy-coated	20000
Marshmallow, coconut-coated	20000
Nougat, plain	30000
Nut roll, fudge or nougat, caramel and nuts	30000
Peanut bar	30000
Planters Peanut Bar	30000
Peanut brittle	30000
Peanut butter, chocolate covered	30000
Peanut butter morsels	30000
Pineapple candy, Puerto Rican style	30000
Gumdrops	30000
Sugar-coated chocolate discs	30000
Wax candy, liquid filled	30000

NS = Not specified; NFS = Not further specified

APPENDIX II

C-M-00036-000 Rev 0, Total Deliverable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

[redacted]	Solazyme, Inc.	Document Number: C-M-00036-000
Standard Operating Procedure		Revision: 0
Effective Date: FEB 09 2012		Page 1 of 7

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

Objective

This document describes the method for determining total derivable fatty acid methyl ester (FAME) content and profiles of algal oil samples by gas chromatography with flame ionization detection.

Scope

This method is applicable to oil extracted from algal biomass or other oils of interest. This may include crude oil samples, clarified and/or degummed oil, as well as refined, bleached, and deodorized algal oils.

Safety

Consult appropriate material safety data sheets (MSDS) for safe chemical handling. Always observe safe laboratory practices.

Equipment and Materials

Chemicals/Reagents:

- Methyl nonadecanoate (C19:0) with ≥ 99.5% purity (Fluka Catalog Number 74208-5g)
- GC retention time reference standard mixture: Nu-Chek Prep, Inc. GLC-411, 79, 87, 569 or other suitable fatty acid methyl ester standard mixtures
- Potassium carbonate powder (anhydrous) (Fisher Catalog Number BP365-500)
- Sodium sulfate (granular, anhydrous) (Sigma Aldrich 17876-500G)
- Toluene (certified ACS grade) (Fisher Catalog Number T324-500)
- Concentrated sulfuric acid (Fisher Catalog Number A300S-500)
- Heptane (GC grade) (EMD Chemicals OmniSolv® HX0082-1)
- Methanol (GC grade) (EMD Chemicals MX0475-1)
- De-ionized water

Equipment/Consumables:

- Class A volumetric flasks
- Analytical balance (Mettler Toledo XS64, or equivalent)
- Handystep® electronic repeating pipette (BrandTech Scientific Inc., Reference Number 705012) and/or BrandTech Scientific Inc. Transferpettor positive displacement pipette 100-500 µL (Fisher Catalog Number 13-688-252 or equivalent)
- Fisher brand disposal culture tubes s/c with marking spot (16 x 100 mm, Catalog Number 14-959-35AA)
- Kimble Chase PTFE-lined caps (Art No. 73802-15415)
- 6 mL vials (Kimble Chase, S/T short style, Art No. 60810-1940) and caps (Kimble Chase S/T closure BLPH PTFE WR, Art No. 73802-15425), and/or 20 mL I-CHEM vials with cap (Thermo Scientific Catalog Number C126-0020)
- Heating/stirring module (Pierce Reacti-therm III or equivalent)
- Sonicator with heating feature (Fisher Scientific or equivalent)
- Vortex Genie (Fisher brand or equivalent)

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

- General purpose centrifuge with suitable adaptors for the culture tubes
- Capillary GC with FID, split-splitless capable inlet, and hydrogen or helium carrier gas
- GC Column: Restek FAMEWAX 30 m, 0.32 mm ID, 0.25 µm df (Restek Catalog Number 12498), or equivalent
- 2 mL GC vials with caps (Fisher Catalog Number 03-375-27A, 03-396B)

Procedure

1. Standard Preparation

1.1. GC retention time reference standard solution

1.1.1. Prepare a standard solution of a commercially available GC reference standard mixture, or a combination of mixtures, in heptane. Different reference standards may have to be used depending on FAME's of interest. Some suggestions are listed in "Reference standard mixture" under the Chemicals/Reagents section of Equipment and Materials. Establish standard retention times for FAME peaks of interest.

1.1.1.1. Refer to Appendix A for GC retention time reference standard mixture chromatogram of Nu-Chek GLC-411 and 569, and methyl levulinate.

1.2. Methyl nonadecanoate (C19:0) internal standard solution

1.2.1. For use in quantitation of total FAME, prepare 20 mg/mL methyl nonadecanoate (C19:0) internal standard solution in toluene.

1.2.1.1. Suggested preparation: Accurately measure 1.0 g (to 0.1 mg accuracy) of methyl nonadecanoate into a 50 mL volumetric flask and bring to volume with toluene. Transfer to vials with Teflon-lined caps for storage. Minimize evaporative effects by using smallest vial allowed by the volume of internal standard. Document the exact concentration and store in refrigerator for use up to 1 week time.

1.2.2. In situations where the samples may yield significant amounts of methyl nonadecanoate, select an internal standard that the organism does not naturally produce.

2. Reagent Preparation

2.1. Prepare 1 L of 5% H_2SO_4-methanol reagent by slowly adding 50 mL of concentrated H_2SO_4 to 950 mL of pre-chilled methanol. Mix and store at room temperature.

2.2. Prepare 1 L of 6% K_2CO_3 aqueous reagent by adding 60 g of anhydrous potassium carbonate to 1 L of deionized water. Mix until completely dissolved and store at room temperature.

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

3. Sample Preparation

3.1. Weigh 20-50 mg of oil in a culture tube and record the weight.

3.2. For total FAME content analysis, accurately dispense 200 µL of internal standard solution to all the samples using a positive displacement pipette. The internal standard must be at room temperature when dispensed. Accurately add 200 µL of toluene to the samples.

3.3. For FAME profiles only, add 400 µL of toluene to the samples.

3.4. Add 2 mL of 5% H_2SO_4-methanol reagent to all samples. Cap and vortex.

3.4.1. Heat the sample using a heating/stirring module, or similar device, capable of heating the samples at 70-75 °C. Heat for at least 3.5 hours. If necessary, use a stir bar to aid in the dispersion of the sample.

3.4.2. Sonicate and vortex the samples as necessary to ensure complete sample dispersion.

3.5. When the reaction time is complete, remove tubes from the heating/stirring module and allow to cool to room temperature. Add 2 mL of heptane followed by 2 mL of 6% K_2CO_3 aqueous solution.

3.6. Shake each tube vigorously, unscrew the cap slightly to vent and mix again.

3.7. Centrifuge the culture tubes at 1000 rpm for 2 minutes to obtain two clear, distinct layers.

3.8. Add approximately 750 µL of the upper layer to GC vials containing approximately 80-120 mg of anhydrous sodium sulfate.

3.9. Set up the GC vials in the autosampler for analysis.

4. GC Parameters

4.1. ThermoScientific Trace GC Ultra

4.1.1. Inlet Parameters

PTV Inlet	
Inlet Temperature (°C)	250
Column Flow (mL/min)	1.9 (He, constant flow)
Split Flow (mL/min)	76
Split Ratio	40:1
Mode	Constant Temperature Split
Injection Volume	0.3 µL

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

4.1.2. Oven Conditions

	Rate (°C/min)	Temperature (°C)	Hold Time (min)
Initial	-----	120	2.00
Ramp 1	15.0	160	-----
Ramp 2	3.00	230	7.00

4.1.3. Detector Settings

Base Temperature (°C)	280
Air Flow (mL/min)	350
H_2 Flow (mL/min)	35
Make Up Flow (mL/min)	30 (N_2)

5. Analysis

5.1. Inject a blank (heptane), followed by the reference standard mixture as system suitability checks. Determine retention times for FAME's of interest from the standard.

5.2. Inject samples and identify FAME's based upon the retention time.

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

6. Calculations

FAME peaks in the chromatogram are quantified and expressed as:

$$Amount\ of\ FAME_X = \frac{C_{IS} \times V_{IS} \times A_{FAME_X}}{A_{IS}}$$

$$Total\ FAME\ content\ (\%w/w) = \frac{\sum Amount\ of\ FAME_{X,Y\ldots}}{M_S} \times 100$$

In addition, Relative Area % of FAME's can be expressed using the following equation:

$$Relative\ Area\ \%\ of\ FAME_X = \frac{A_{FAME_X}}{\left[\left(\sum A_{FAME_{X,Y\ldots}}\right) - A_{IS}\right]} \times 100$$

Where:

$FAME_X$	= FAME of interest
C_{IS}	=concentration of internal standard in mg/mL
V_{IS}	= volume of internal standard in mg/mL
A_{FAME_X}	= peak area of $FAME_X$
A_{IS}	= peak area of internal standard
$\sum FAME_{X,Y\ldots}$	= total amount of all FAME's analyzed
M_S	= sample amount in mg
$\sum A_{FAME_{X,Y\ldots}}$	= total peak area of all FAME's analyzed

Appendices

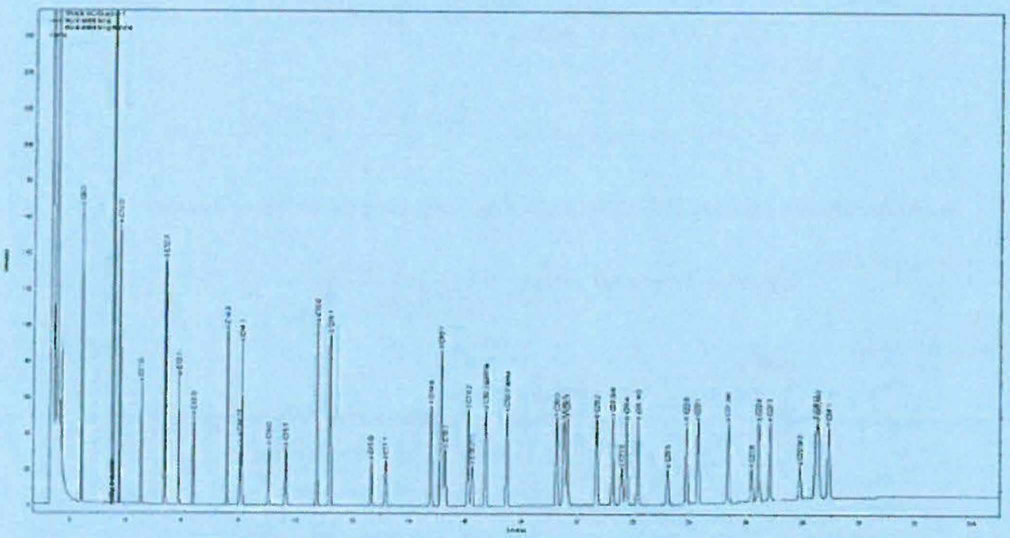
Appendix A ThermoScientific GC chromatogram of reference standard mixture (Nu-Chek GLC-411, 569, and methyl levulinate mixture, see "Procedure Section 1.1")

Appendix B ThermoScientific GC chromatogram of a typical oil sample

Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

APPENDIX A

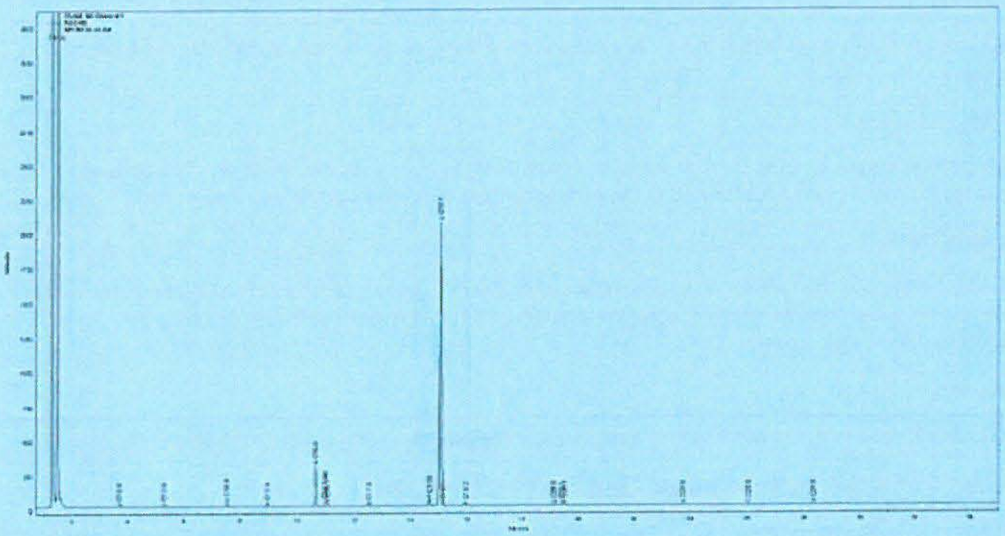
ThermoScientific GC chromatogram of reference standard mixture



Total Derivable Fatty Acid Methyl Ester Content and Profiles of Algal Oil Samples by Direct Transesterification

APPENDIX B

ThermoScientific GC chromatogram of a typical sample



APPENDIX III

C-M-00118-000 Rev 1, Karl Fischer Moisture Determination of Oil

	Solazyme, Inc.	Document Number: C-M-00118-000
Standard Operating Procedure		Revision: 1
Effective Date: OCT 11 2013		Page 1 of 2

Karl Fischer Moisture Determination of Oil

Objective

The purpose of this procedure is the determination of the water content of oils and similar non aqueous liquids.

Scope

This method is a coulometric titration of the moisture released from a sample under set conditions and a standard time. Results are reported as the ppm or percent water in a sample on a weight basis.

Responsibilities

Persons performing this method must understand and adhere to good laboratory practices, know the operation of all equipment involved, know the proper use of the operating software and properly handle the associated reagents.

References

ASTM D1533 Standard Test Method for Water in Insulating Liquids by Coulometric Karl Fischer Titration

Mettler C20/C30 Karl Fischer Coulometer Quick Guide and Memo Card

Safety

The Aquastar CombiCoulomat fritless reagent is a hazardous material and the MSDS document (00016QCL) should be reviewed before use. Assure that any waste reagent is disposed of properly.

Equipment and Materials

- Mettler Toledo C30X Compact Coulometric Karl Fischer Titrator or equivalent
- Mettler Toledo LabX Light Titration Software or equivalent
- Syringe for sample input. For most oils a 3 mL syringe is suitable.
- Needle for injection into the titration vessel (BD 405172 is a 3 inch long 20 gauge needle that works well because it assures complete transfer into the titration fluid.)
- Analytical balance capable of weighting to 0.1 mg or better
- Aquastar CombiCoulomat fritless reagent (EM1.09257.0500)

Background

This instrument choice is very versatile because of its low moisture measurement capabilities and optional accessories that automate direct measurement of moisture in solids.

Procedure

1. Select the SOLDIRECT method from the Titrator screen and start it so that water in the titration vessel can be eliminated.
2. Input the sample information including the lot number.

	Solazyme, Inc.	Document Number: C-M-00118-000
Standard Operating Procedure		Revision: 0
		Page 2 of 2

Karl Fischer Moisture Determination of Oil

3. After a low and steady drift value shows that the water in the system has been eliminated, start the drift measurement of the blank. This value is used as a blank correction by the instrument. It should be run each day or if there is a significant shift in the drift rate to accurately reflect the base moisture factors affecting the instrument.

4. While the drift/blank is running, tare a 50 mL Erlenmeyer flask on the balance. Draw up the sample into the syringe (typically about 2 mL) fitted with a needle. Wipe any oil from the outside of the needle then cover. Weigh the filled syringe system in the flask and record the weight as syringe plus sample (oil).

5. After the drift/blank measurement finishes, press the start analysis button and then inject the sample as directed.

6. Immediately weigh the empty syringe system in the tared flask and record the weight as the syringe weight. Subtract the syringe weight from the weight of syringe plus sample and record as the weight of sample. Enter this weight into the titration screen. The Titrator will display the water content on the screen.

7. Repeat steps 4 through 6 and report the average result assuming that agreement is good. If agreement is poor, repeat steps 4 through 6 an additional 2 times.

8. Record the results in your lab book and on any reporting form.

From:	Johnson,Jill Kauffman
To:	Kolanos, Renata; Gaynor, Paulette M
Cc:	Yu, Esther
Subject:	Updated Cover letter and Part 1 of GRAS Notification GRN 000754
Date:	Monday, June 18, 2018 9:11:10 AM
Attachments:	GRN 000754 Cover Letter.pdf Corbion-Part 1 of GRN000754-signed-2018JUN15.pdf

Dr. Kolanos and Dr. Gaynor,

Please find attached Corbion's updated cover letter and Part 1 of the GRAS notification signed.

If you have any questions, please do not hesitate to contact me.

Best, Jill

Jill Kauffman Johnson

Head of Global Market Development - Algae Ingredients

Corbion

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