DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
848	2127/2019
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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)*: _____
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
Nicole Berzins	Director Regulatory
Organization *(if applicable)*	
Myco Technology Inc.	
Mailing Address *(number and street)*	
18250 E. 40th, Ste. 50	

City	State or Province	Zip Code/Postal Code	Country
Aurora	CO	80011	United States of America

Telephone Number	Fax Number	E-Mail Address
720-547-0088		nberzins@mycotechcorp.com

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

Name of Contact Person	Position or Title
Angelica Ayala	Regulatory Affairs Specialist
Organization *(if applicable)*	
Myco Technology Inc.	
Mailing Address *(number and street)*	
18250 E. 40th, Ste. 50	

City	State or Province	Zip Code/Postal Code	Country
Aurora	Colorado	80011	United States of America

Telephone Number	Fax Number	E-Mail Address
720-897-9306 x141		aayala@mycotechcorp.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein)

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 _____

Total number of pages _____

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 _____

☐ 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.

MycoTechnology, Inc. intends to use PureTaste® as a food ingredient in multiple, specific food categories at levels ranging from 1% to 40%. The intended use levels and the food categories to which PureTaste® will be added are summarized in part 3: 170.235, in Table 7. The use levels provided are based on purity criteria of 75% protein concentrate (dry weight basis). MycoTechnology, Inc. also intends to market this protein as a supplemental protein to be used in sports nutrition applications.

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

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Nicole Berzins
(name of notifier)

has concluded that the intended use(s) of Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein)
(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. Nicole Berzins 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;
(name of notifier)

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.

18250 E 40th STE 50 AURORA, CO 80011
(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)	Nicole Berzins Director of Regulatory Affairs & Compliance	01/31/2019

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)
	NA	
	NKB 1/31/2019	

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.

GRN 848

Generally Recognized as Safe (GRAS) Conclusion for the Use of Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein) in Conventional Foods

January 31, 2019

Submitted to:

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

Submitted by:

MycoTechnology, Inc.

Table of Contents

Appendix 1: GRAS Panel Report

Part 1: 170.225 Signed Statements and Certification

In accordance with 21 CFR 170 Subpart E, MycoTechnology Inc. hereby informs the U.S. Food and Drug Administration (FDA) that PureTaste® proteinis not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) based on MycoTechnology's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.4, below.

In addition, as the responsible official of MycoTechnology Inc., Mrs. Nicole Berzins hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to MycoTechnology Inc. and pertinent to the evaluation of the safety and GRAS status of the intended use of pea and rice protein fermented by Shiitake mycelia as an ingredient for addition to food.

Signed,

(b) (6)

X

Nicole Berzins

1.1 GRAS Notice Submission

MycoTechnology Inc submits this GRAS Notification in accordance with the requirements of 21 CFR Part 170, Subpart E.

1.2 Name and Address of Notifier

MycoTechnology Inc.
3155 Chambers Rd Suite E
Aurora, CO USA 80011

1.3 Common or Usual Name of Notified Substance

The common name is **pea and rice protein fermented by Shiitake mycelia**.

The trade name of this product is PureTaste®.

1.4 Conditions of Use

Pea and rice protein fermented by Shiitake mycelia (PureTaste®) containing approximately 75% protein is intended to be added as a food ingredient, formulation aid and texturizer in foods where protein is used for nutritional purposes and in foods needing protein-source properties such as promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, increase water holding capacity and gelation, and increase of water-solubility. Intended food categories include baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pastas, milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, meal replacement/nutritional bars, and confectionary. The proposed ingredient will be used as a substitute for and/or in conjunction with other sources of protein in conventional food products and in meat and poultry applications that come under USDA jurisdiction. MycoTechnology does not intend to add PureTaste® to infant formula.

Refer to Table 7 for a summary of intended food applications with inclusion levels of PureTaste® by food category.

1.5 Statutory Basis for GRAS Status

Pea and rice protein fermented by Shiitake mycelia has been concluded to be GRAS through scientific procedures pursuant to 21 CFR Part 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR) for use as a food ingredient in certain specific categories of food where proteins are involved.

A comprehensive assessment of scientific (human and animal, quantitative and qualitative) literature and regulatory resources were consulted for this review. The safety of PureTaste® is supported, based on its intended use. Data and information were gathered from a critical and comprehensive review of the scientific literature on the safety of the GRAS substance raw

materials (pea protein and rice protein) and fermentation organism (*L. edodes*), as discussed in Part 6. In addition, the product was subjected to extensive physical and chemical analysis. Peas, rice and shiitake are an important part of the human diet in many countries and have been consumed since ancient times. Peas, rice and Shiitake are high in protein, fiber, vitamins, and minerals and are nutrient rich food products. Fermented foods have been safely consumed for hundreds of years. Based on a critical evaluation of the information presented below by qualified experts, it was concluded that the proposed use of pea and rice protein fermented by Shiitake mycelia as a food ingredient is GRAS.

1.6 Premarket Exempt Status

Pea and rice protein fermented by Shiitake mycelia is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) based on the conclusion that the notified substance is GRAS under the conditions of intended use.

1.7 Availability of Information

The data and information that serve as the basis for the conclusion that pea and rice protein fermented by Shiitake mycelia is GRAS for its intended use, will be made available to the United States (U.S.) Food and Drug Administration (FDA) upon request. At FDA's option, a complete copy of the information will be available for review and copying upon request during business hours at the offices of:

MycoTechnology Inc.
18250 East 40th Suite 50
Aurora, CO 80011

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, MycoTechnology Inc. will supply these data and information.

1.8 Freedom of Information Act, 5 U.S.C 552

It is MycoTechnology's view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

1.9 FSIS Statement

MycoTechnology authorizes FDA to forward a copy of this GRAS notice or relevant portions thereof to FSIS to permit an evaluation of the proposed use of PureTaste® in meat and poultry products that come under USDA jurisdiction.

Part 2: 170.230 Identity, method of manufacture, specifications and physical or technical effect

2.1 GRAS Material Identity

The subject of this GRAS Notice is blended pea and rice protein fermented by shiitake mycelia (herein referred to as PureTaste® protein). Fermentation with shiitake mycelia is performed to improve organoleptic qualities of the input pea and rice protein raw materials; however, the input pea and rice protein raw materials are not substantially modified following fermentation. The PureTaste® product provides a total of ≥75% protein (≥95% protein-concentrates of raw material inputs) on a dry basis. The estimated level of shiitake mycelia biomass in the final product is <0.1 wt%.

2.2 Method of Manufacture

2.2.1 Brief Production Process Description

In summary overview, the process of manufacturing PureTaste® protein involves successive fermentations of a primary culture of shiitake mycelia to build up an amount of a pure shiitake mycelial biomass, followed by a main fermentation step where the built-up shiitake mycelia biomass is combined with sterile input protein materials and allowed to ferment for 20-40 hours. At the end of the fermentation, the contents of the fermentation tank are concentrated and spray-dried. The resultant material is called PureTaste® protein (pea and rice protein fermented by shiitake mycelia). All input materials into the manufacturing process are safe and suitable for the described use in food ingredient production. A comprehensive safety assessment for the shiitake mycelia is presented in Section 6. The main fermentation step allows the shiitake mycelia biomass developed from the initial fermentations to improve the organoleptic qualities (as measured by human sensory testing; described at Section 2.5.4) of the input pea and rice protein raw materials. The input pea and rice protein raw materials are not substantially modified (see Section 2.5.3).

2.2.2 Detailed Production Process Description

In more detail, the manufacturing process consists of the following steps. The manufacturing is initiated by starting the growth of pure cultures of shiitake mycelia on agar plates developed from a confirmed shiitake spawn culture stored at -80 °C. The identity of shiitake spawn is discussed at § 2.2.4.3. The grown cultures on agar plates are used to initiate liquid cultures of shiitake mycelia in shake flasks. For the shake flask cultures, the media is an approximately 2% slurry of pea protein and rice protein concentrate, supplemented with maltodextrin, antifoam and carrot powder. Prior to culture inoculation, the media is sterilized at 121 °C for 90 minutes, and the inoculation with shiitake mycelia is carried out using sterile procedure. The inoculated shake flasks are incubated until the shiitake mycelia has achieved the desired level of growth in the shake flasks. The entirety of the volume of the shake flasks is then transferred into the first of three "seed development" bioreactors to continue to build shiitake mycelia biomass, as described in the next paragraph.

All input materials into the manufacturing process, as described, are safe and suitable for the described use in food ingredient production. A safety assessment for the shiitake mycelia is presented in Section 6.

The shiitake mycelia biomass building process is continued in the "seed development" bioreactor process using three separate fermentations in three bioreactors, each of which are larger in size. The three successive fermentations ("Fermentation 1,", "Fermentation 2," and "Fermentation 3") are carried out in these progressively larger bioreactors. Prior to inoculation, all bioreactor media is sterilized by heat treatment at 121 °C for 120 minutes and cooled down using air and water circulation, and all inoculations are carried out with sterile procedure to maintain a pure shiitake mycelia culture.

After inoculation of the first bioreactor (Fermentation 1) with the shake flask cultures, the cultures are allowed to grow for 24 to 48 hours. During all fermentations, purity of the culture and growth of the shiitake mycelia are confirmed via microscopy. At the conclusion of Fermentation 1, the entirety of the volume of the bioreactor is transferred into the second bioreactor, together with fresh media to fill to volume, to initiate Fermentation 2. Fermentation 2 is carried out for 24 to 48 hours. At the conclusion of Fermentation 2, the entirety of the volume of the second bioreactor is then transferred into the third bioreactor together with fresh media to fill to volume, to begin Fermentation 3. Fermentation 3 is carried out for 24 to 48 hours. At the conclusion of Fermentation 3, the shiitake mycelia biomass has reached a biomass level of approximately 2 g/ml. Lag phase of the shiitake mycelia is maintained between shake flasks and the seed fermentations by use of similar media, temperature, and agitation. The growth of the shiitake biomass is confirmed by pH monitoring. Change in pH is known as a lead indicator of growth of shiitake mycelia (Aminuddin et al., 2013).

In the final fermentation stage of the manufacturing process (called herein "main fermentation") for PureTaste® protein, the entirety of the volume from Fermentation 3 is inoculated into the main fermenter, for an input volume of 4% of the volume of the main fermenter. The remainder of the input is the main fermentation media. The main fermentation media differs from the media used for the seed fermentations in that the main fermentation media components are pea and rice

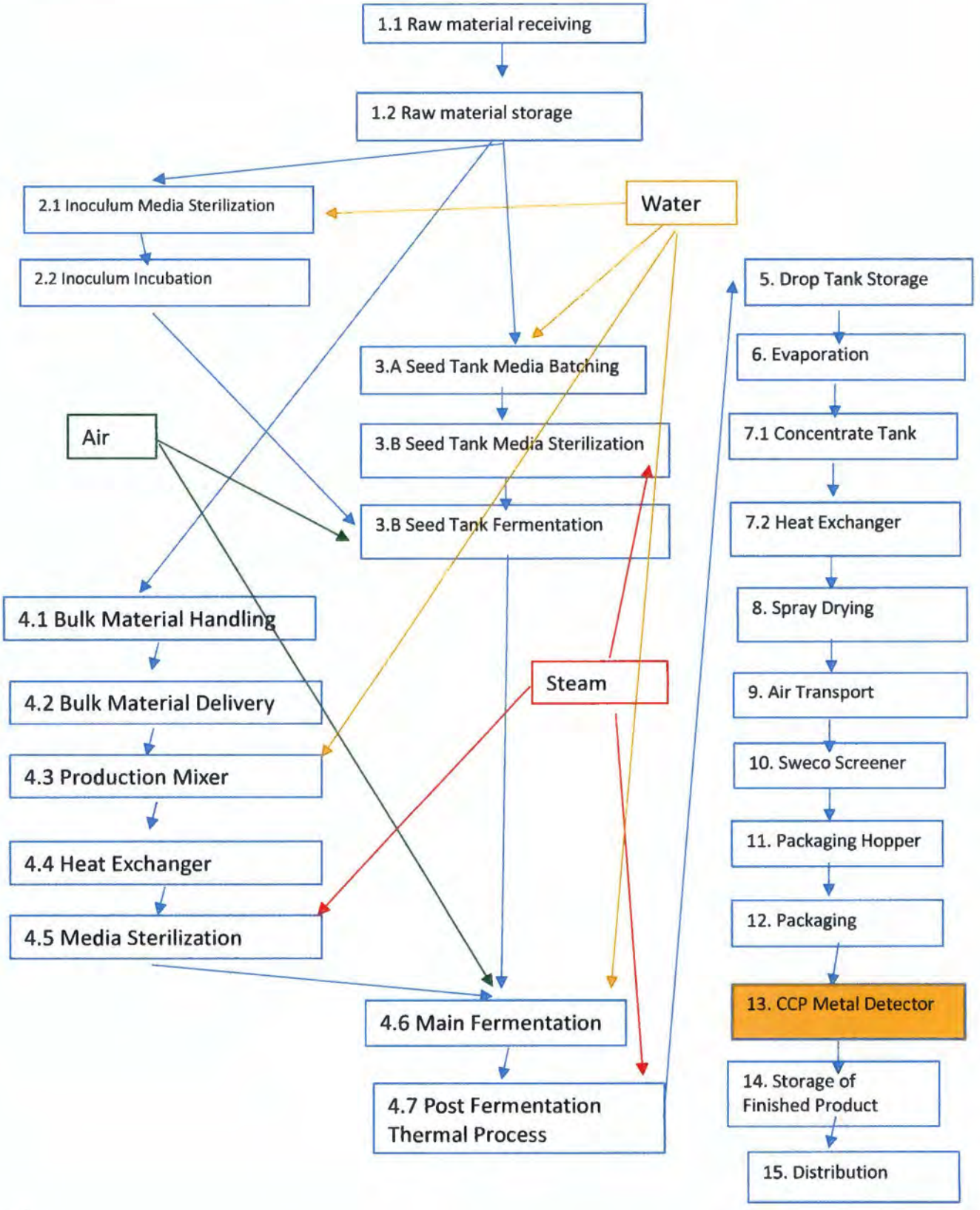
protein at significantly higher concentrations than found in the seed fermentation media. Prior to inoculation, all media is sterilized at 121 °C for 90 minutes, and all inoculations are carried out with sterile procedure. The main fermentation process is completed in 20 - 40 hours.

It is known from the literature that a significant change in media will induce a lag phase in an aqueous shiitake culture, specifically, the literature shows that after inoculation of shiitake mycelia into liquid media, a period of 6-10 days of culture time is required before appreciable increase is observed in mycelial biomass (Cavallazzi et al., 2005). The main fermentation phase is carried out for no longer than 40 hours under conditions known to induce lag phase in shiitake mycelia. The pH of the main fermentation does not change, indicating that no mycelial growth is taking place. From this information, MycoTechnology concludes that the shiitake mycelia culture enters lag phase upon inoculation into the main fermentation and remains in lag phase throughout the duration of the main fermentation.

The manufacturing process is continued by heat processing and spray-drying steps at the conclusion of the main fermentation. The fermentation process is terminated by heat treatment, followed by an evaporator/concentration step. A thermal deactivation step is carried out at 80 °C for 1 minute to deactivate the mycelia, followed by spray drying (air inlet 250 °C; powder outlet 75 °C) for 1 to 3 minutes. The spray dried powder is the manufacturing process final output and is designated PureTaste® protein (pea and rice protein fermented with shiitake mycelia). As discussed below, the input protein raw materials are not substantially modified after fermentation (see Section 2.5.3).

A Manufacturing Process Flow Diagram for PureTaste® protein is provided below in Figure 1.

Figure 1. Manufacturing Process Flow Diagram for PureTaste® protein



2.2.3 Quality Control Procedures

PureTaste® protein is manufactured consistent with current Good Manufacturing Practices (cGMP) as defined in 21 CFR §110 at a facility with an established Hazard Analysis and Critical Control Points (HACCP) plan. Supporting documentation is on file with MycoTechnology.

2.2.4 PureTaste® Protein Raw Materials

2.2.4.1 Rice Protein

The rice protein concentrate raw material used to produce PureTaste® protein is obtained from a commercial Supplier and meets specifications and quality criteria defined by MycoTechnology (see Table 1). MycoTechnology assures that the rice protein concentrate used to produce PureTaste® protein is appropriately similar to the rice protein material previously recognized as GRAS for use in food (e.g. GRN 609; p.000076, Food Grade Specifications). The rice protein concentrate is a free-flowing, off-white to light beige colored powder with characteristic brown rice protein flavor and a protein content of not less than 75% (dry basis). The rice protein concentrate is made after concentrating and filtering hydrolyzed rice (*Oryza sativa*) slurry which has undergone an all-natural enzymatic process. Supporting documentation is on file with MycoTechnology.

Table 1. Rice Protein Raw Material Specification

Parameter	Tolerance	Test Method
Appearance	Off white to light beige	Visual against standard
Odor	Odorless	Sensory
Protein (%)	≥75% (dry basis)	AOAC 990.03
Aerobic Plate Count	≤ 10, 000 CFU /g	AOAC 966.23
Yeast	≤ 200 CFU / g	FDA-BAM, 7th ed.
Mold	≤ 100 CFU /g	FDA-BAM, 7th ed.
Listeria	Negative / 25 g	AOAC 2016.07
Coliforms	≤ 10 CFU / g	AOAC 966.24
Salmonella	Negative / 25 g	AOAC-R1I100201
E. Coli	≤ 10 CFU / g	AOAC 966.24
Mercury	< 1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	< 0.5 ppm	ICP-MS, FDA EAM 4.7
Arsenic	< 1 ppm	ICP-MS, FDA EAM 4.7
Lead	< 1 ppm	ICP-MS, FDA EAM 4.7
Mycotoxins	< 5 ppb	HPLC AOAC 991.31(Mod.)

2.2.4.2 Pea Protein

The pea protein concentrate raw material used to produce PureTaste® protein is obtained from a commercial Supplier and meets specifications and quality criteria defined by MycoTechnology (see Table 2). MycoTechnology assures that the pea protein concentrate used to produce PureTaste® protein is appropriately similar to the pea protein material previously recognized as GRAS for use in food (e.g. GRN 608; p. 000010, Food Grade Specifications). The pea protein concentrate is a free-flowing, cream coloured powder with a protein content of not less than 75% (dry basis). The pea protein concentrate is obtained from the mechanically milled and wet fractionated portion of de-hulled yellow peas (*Pisum sativum).* Supporting documentation is on file with MycoTechnology.

Table 2. Pea Protein Raw Material Specification

Parameter	Tolerance	Test Method
Appearance	Off white	Visual against standard
Odor	Inherent Pea Odour	Sensory
Protein (%)	≥ 75 % (dry basis)	AOAC 990.03
Aerobic Plate Count	≤ 10, 000 CFU /g	AOAC 966.23
Yeast	≤ 200 CFU / g	FDA-BAM, 7th ed.
Mold	≤ 100 CFU /g	FDA-BAM, 7th ed.
Listeria	Negative / 25 g	AOAC 2016.07
Coliforms	≤ 10 CFU / g	AOAC 966.24
Salmonella	Negative / 25 g	AOAC-R1I100201
E. Coli	≤ 10 CFU / g	AOAC 966.24
Mercury	< 0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	< 0.2 ppm	ICP-MS, FDA EAM 4.7
Arsenic	< 0.5 ppm	ICP-MS, FDA EAM 4.7
Lead	< 0.1 ppm	ICP-MS, FDA EAM 4.7
Mycotoxins	< 5 ppb	HPLC AOAC 991.31(Mod.)

2.2.4.3 Shiitake Mushroom Mycelia

The strain of shiitake used to produce PureTaste® protein was originally obtained from Pennsylvania State University (https://plantpath.psu.edu/facilities/mushroom/cultures-spawn). The strain was genotyped by a third-party laboratory and the cultures were identified as *L. edodes* (100% match) by internal transcribed spacer (ITS) sequencing data (28S DNA) (MycoTechnology, unpublished data on file). Based on the ITS results, MycoTechnology. concludes that the microorganism used in the manufacturing process to produce PureTaste® protein is *Lentinula edodes*. Under the conditions of use in aqueous culture *L. edodes* grows as the vegetative form (Tsivileva et al., 2005; Aminuddin et al., 2013; Aminuddin et al., 2007). This vegetative form is identified herein as "shiitake mycelia." Based on this information, MycoTechnology, Inc. concludes that the organism used in the manufacturing process for PureTaste® is the *L. edodes* vegetative form (shiitake mycelia.)

A safety assessment for the shiitake mycelia is presented in Section 6.

2.2.4.4 Additional raw materials and processing aids

All remaining raw materials and processing aids used to produce PureTaste® protein (including carrot powder, maltodextrin, and a commercial anti-foam preparation) are considered safe and suitable for the described use in food ingredient production (see Section 6.4).

2.3 GRAS Material (PureTaste® Protein) Specifications

The specifications for PureTaste® protein are summarized in Table 3. Conformance to specifications and consistency in the manufacturing process of PureTaste® protein is demonstrated by the analyses of five non-consecutive batches of commercially representative product (Table 4). Heavy metals and microbial contaminants (including mycotoxins) were confirmed to be absent or below levels of safety concern. The absence of live mycelia in the final PureTaste product is discussed below in Section 2.5.5. A safety assessment of the potential fermentation byproducts (e.g. fungal proteins and residual enzymes) in PureTaste® protein is provided in Part 6 (GRAS Narrative).

Table 3. PureTaste® Protein Specifications

Test / Parameter	Specification / Tolerance	Method
Protein, % dry weight (DW) basis	≥75.0 % (DW)	AOAC 992.15 2011.04
Moisture	≤7 %	AOAC 985.14
Aerobic plate Count	< 10,000/g	AOAC 990.12
Coliform MPN	<100 cfu/g	AOAC 991.14
Yeast and Mold	<100 cfu/g	FDA-BAM, 7th ed.
E. Coli	Negative/10 g*	AOAC 991.14
Salmonella	Negative/25g	AOAC 061203
Listeria	Negative/25g	AOAC 3014.06
Aflatoxin Bl	<5 ppb	HPLC AOAC 991.31(Mod.)
Aflatoxin B2	<5 ppb	HPLC AOAC 991.31(Mod.)
Aflatoxin Gl	<5 ppb	HPLC AOAC 991.31(Mod.)
Aflatoxin G2	<5 ppb	HPLC AOAC 991.31 (Mod.)
Aflatoxin Total	<5 ppb	HPLC AOAC 991.31(Mod.)
Arsenic	<0.1 ppm	ICP-MS
Cadmium	<0.1 ppm	ICP-MS
Lead	<0.3 ppm	ICP-MS
Mercury	<0.1 ppm	ICP-MS

* E. Coli: The standard procedure involves testing coliforms and only if coliform presence is detected, would a specific analysis for E. Coli be conducted (Not Detected in 10g as per USP dietary supplement limits for powdered herbal botanicals).

Table 4. Analytical Results for Five Non-Consecutive Batches of PureTaste® Protein

Test / Parameter	Specification / Tolerance	Batch ID: 3/26/2017	Batch ID: 4/10/2017	Batch ID: 4/24/2017	Batch ID: 4/1/2017	Batch ID: 9/26/2017
Protein, % dry weight (DW) basis	≥75.0 % (DW)	80.88	80.00	79.33	75.87	78.06
Moisture	≤7 %	5.20	6	7.80	4.50	3.9
Aerobic Plate Count	< 10,000 CFU/g	<10 CFU/g	<10 CFU/g	10 CFU/g	<10 CFU/g	<10 CFU/g
Coliform MPN	<100 CFU /g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeast	<100 CFU /g	<100 CFU/g	<100 CFU/g	<100 CFU/g	<100 CFU/g	<100 CFU/g
Mold	<100 CFU /g	<100 CFU/g	<100 CFU/g	200 CFU/g	<100 CFU/g	<100 CFU/g
E. Coli	Negative/10 g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Salmonella	Negative/25g	Negative /25g	Negative /25g	Negative /25g	Negative /25g	Negative /25g
Listeria	Negative/25g	Negative /25g	Negative /25g	Negative /25g	Negative /25g	Negative /25g
Aflatoxin Bl, B2, G1, G2 (ppb)	<5 ppb	< 5	< 5	< 5	< 5	< 5
Aflatoxin Total (ppb)	<5 ppb	< 5	< 5	< 5	< 5	< 5
Vomitoxin (Deoxynivalenol) (ppb)	NA; report only	< 10	< 10 (batch 12)	< 10	< 10	< 50
Arsenic (ppm)	<0.1 ppm	0.032	0.04	0.039	0.039	0.03
Cadmium (ppm)	<0.1 ppm	0.03	0.025	0.032	0.036	0.03
Lead (ppm)	<0.3 ppm	0.07	0.065	0.068	0.064	0.06
Mercury (ppm)	<0.1 ppm	0.014	0.012	0.012	0.025	0.02

Note: Batch ID = Date of seed fermentation start.

2.4 Technical Effect of PureTaste® Protein

PureTaste® protein (i.e. pea and rice protein fermented by Shiitake mycelia) is intended for use as a food ingredient, formulation aid and texturizer in foods where protein is used for nutritional purposes and in foods needing protein-source properties such as promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, increase water holding capacity and gelation, and increase of water-solubility. Intended food categories include baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pastas, meat and poultry products, milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, meal replacement/nutritional bars, and confectionary.

2.5 GRAS Material Characterization

2.5.1 Physical Characteristics of PureTaste® Protein

Appearance: Powder

Particle size: Mean <100μm

Color: Tan to hunter

Aroma: Cereal when concentrated

Taste: Clean taste, slight cereal

Stability: Product should be stored in a cool, dry location, and in the original sealed package away from odorous material. The protein content of this product is stable under accelerated conditions. The PureTaste® protein product has a shelf-life of 24 months from date of manufacture.

2.5.2 Composition of PureTaste® Protein

The PureTaste® protein product is comprised of primarily protein (≥75%; based on 95% total protein-concentrates of raw material inputs), fat, carbohydrates and 5% adjunct material e.g., remainder of fermentation media components such as carrot powder and maltodextrin (refer to Section 2.2). The pea and rice protein are present in approximately equal amounts in the final product. The ratio of pea and rice protein may be adjusted as needed to achieve a total protein isolate content of 95%. A minimal amount of protein (<0.05%) may also be contributed by the shiitake mycelium (based on <0.1% shiitake biomass in the final PureTaste® product). Nutritional and compositional data and amino acid profile for PureTaste® protein are presented in Table 5.

Table 5. Nutritional / Compositional Data and Amino Acid Profile for PureTaste® Protein

Parameter	Results				
Batch ID (Date of seed fermentation start):	3/26/2017	4/10/2017	4/24/2017	4/1/2017	9/26/2017
Protein (% DW)	80.88	80.00	79.33	75.87	78.06
Protein (% as is)	76.67	75.19	73.14	72.46	75.02
Moisture and Volatiles (%)	5.20	6	7.80	4.50	3.9
Ash (%)	4.76	6.02	3.79	4.61	6.51
Total fat as Triglycerides (%)	9.89	9.51	9.76	10.84	10.56
Total fatty acids (%)	10.1	9.1	9.33	10.36	ND
Crude fat (%)	10.11	10.32	10.62	11.05	11.27
Carbohydrates (%) Calculated	3.48	3.28	5.51	7.59	3.30
Fiber (%)	3.80	6.00	5.70	6.20	6.51
Sucrose (%)	0.0036	0.0042	0.36	0.3	0.54
Total sugars (%)	0.0036	0.0042	0.36	<0.35	0.54
Gluten (ppm)	< 5.0	< 5.0	< 5.0	8.3	<3.0
DIAAS (Digestible Indispensable Amino Acid Score)	104.3	103.3	105.7	101	98
Amino Acid (%)	76.01	74.03	71.86	70.01	75.64
Tryptophan (%)	0.9	0.87	0.83	0.77	0.92
Cystine (%)	1.04	0.96	0.99	0.71	0.81
Methionine (%)	1.8	1.63	1.64	1.48	1.51
Alanine (%)	3.71	3.59	3.44	3.41	3.89
Arginine (%)	6.19	6.07	5.85	5.76	6.09
Aspartic Acid (%)	7.78	7.54	7.38	7.14	7.78
Glutamic Acid (%)	13.14	12.91	12.45	12.67	13.53
Glycine (%)	3.21	3.13	3.01	2.98	3.21
Histidine (%)	1.75	1.76	1.67	1.6	1.76
Isoleucine (%)	3.62	3.51	3.33	3.29	3.67
Leucine (%)	6.61	6.34	6.19	6.02	6.36
Phenylalanine (%)	4.24	4.15	4.02	3.86	4.06
Proline (%)	3.48	3.52	3.45	3.39	3.54
Serine (%)	3.85	3.77	3.73	3.59	3.67
Threonine (%)	2.83	2.78	2.72	2.61	2.76
Lysine (%)	3.89	3.78	3.76	3.53	4.18
Tyrosine (%)	3.49	3.36	3.29	3.13	3.36
Valine (%)	4.48	4.36	4.11	4.07	4.54

ND – Not Determined.

2.5.3 Similarity of PureTaste® protein to input pea and rice protein raw materials

A comparison of the amino acid profiles of rice, pea and PureTaste® protein are provided below in Table 6. The overall typical amino acid profile of PureTaste® protein (based on theoretical calculated values) generally aligns with the protein values of rice and/or of pea.

Table 6. Amino Acid Profile Comparison of PureTaste® Protein with Rice Protein and Pea Protein

	% of total amino acids		
Amino acids	**Rice [1]**	**Pea [2]**	**PureTaste® Protein[3]**
Phenyalanine	5.16	4.82	4.67
Valine	5.87	4.94	5.06
Threonine	3.67	3.72	3.46
Tryptophan	1.27	1.17	1.14
Methionine	2.25	1.07	1.55
Isoleucine	4.23	4.32	4.00
Leucine	8.28	7.50	7.39
Lysine	3.82	7.55	5.32
Histidine	2.54	2.54	2.38
Arginine	7.58	9.33	7.91
Cysteine	1.21	1.59	1.31
Glycine	4.93	4.65	4.48
Proline	4.69	4.32	4.22
Tyrosine	3.75	3.03	3.17
Aspartic acid	9.36	12.34	10.16
Alanine	5.83	4.60	4.88
Glutamic	20.38	17.88	17.91
Serine	5.18	4.60	4.58
TOTAL	**100%**	**100%**	**93.6%**

[1] Source: Rice (*Oryza sativa*) from GRAS Notice 609 (p. 000073)
[2] Source: Pea (*Pisum sativum*) from GRAS Notice 608 (p. 000008)
[3] Theoretical calculated values based on ratio of input raw materials (i.e. ~95% pea and protein concentrates).

Additional testing was performed to quantify any minor changes to PureTaste® protein relative to the input material. A minor change (<0.1% difference) in reducing sugars was observed via analysis by AOAC method 982.14, modified (MycoTechnology; unpublished data on file), which MycoTechnology concludes is due to metabolic activity of shiitake mycelia during the main fermentation step. Protein Digestibility Corrected Amino Acid Score (PDCAAS) analysis on PureTaste® protein compared with the pea and rice protein concentrate materials input into the main fermenter demonstrated that the ileal digestibility (<5% difference), which can be explained in terms of the dilution of the PureTaste® protein product with 5% other materials such as, carrot powder and maltodextrin. The amino acid composition also remained substantively unchanged (<3% difference) from the raw materials (MycoTechnology; unpublished data on file), consistent with the amino acid profile comparison shown in Table 6. In summary, the composition of the input pea and rice protein materials remain largely unchanged after fermentation with shiitake mycelia. Reported improvements in the organoleptic qualities of the pea and rice protein inputs are discussed below in Section 2.5.4.

2.5.4 *Proposed mechanism for improvement to organoleptic properties of input pea and rice protein concentrates*

Vegetable-derived protein isolates and concentrates are known to possess objectionable flavor compounds that can arise from oxidative deterioration of unsaturated fatty esters in protein-bound lipids (Rackis et al., 1979). Schindler identified several volatile organic compounds (VOC) in pea protein extracts which impart undesirable organoleptic qualities impacting their acceptance by consumers (Schindler et al., 2012; Table 1). In many cases, the VOCs associated with undesirable organoleptic qualities are below limits of detection (LOD) or limits of quantification (LOQ) by existing analytical techniques (Rackis et al., 1979; Sessa and Rackis, 1977; Buttery et al., 1988). On the other hand, human sensory (taste and smell testing) can be reliably used to detect the presence of these VOCs at levels undetectable by present analytical techniques (Yoshikawa et al., 1965).

Consistent with the literature, MycoTechnology's analysis of organoleptic qualities (by human sensory analysis) of the input pea protein concentrate and input rice protein concentrate used to produce PureTaste® protein found undesirable levels of burnt, vegetable, pea, cereal, rice, green, mushroom, soapy, chalky, earthy, and cardboard notes in these input materials (MycoTechnology; unpublished data on file). Sensory studies on PureTaste® protein compared to input materials and "sham" fermentation controls (e.g., a manufacturing process that is identical in all respects to the process described in § 2.2 but lacking a shiitake mycelia inoculation step) show that PureTaste® protein has improved organoleptic qualities (i.e. flavor, taste, and aroma attributes reported by trained descriptive panelists) as compared to the input materials. These results indicate that the manufacturing process described in § 2.2 remediates the undesirable organoleptic qualities associated with the control pea and rice protein starting material (MycoTechnology; unpublished data on file).

However, Quadrupole Time of Flight Liquid Chromatography Mass Spectrometry (QTOF-LCMS) analysis of PureTaste® protein showed no changes in VOC profile relative to a sham fermentation control, indicating that the organoleptic quality changes identified by human sensory testing in PureTaste® protein were not reflected in VOC analysis by conventional analytical techniques (MycoTechnology; unpublished data on file). In general, the average limit of quantification for the QTOF-LCMS with APCI data is 1-10 ppm. That MycoTechnology identified no changes to a VOC profile for PureTaste® protein relative to input protein or a sham fermentation is consistent with the literature discussed above, indicating VOCs related to sensory defects in pea and rice protein concentrates at the levels found in PureTaste® protein are below LOQ for conventional analytical techniques.

The improvement in organoleptic qualities of PureTaste® protein during the fermentation process described in § 2.2 may be due to the secretion of enzymes by the shiitake mycelia during the main fermentation step which act to modify certain VOCs known to impart unpleasant organoleptic qualities of pea protein concentrates and rice protein concentrates. As discussed above, MycoTechnology concluded that shiitake mycelia are in lag phase during the main fermentation step (see Section § 2.2.2), but the literature shows that even during lag phase,

shiitake mycelia remains metabolically active, due to adaptation of the organism to a change in media (Cavallazzi, 2005). Shiitake mycelia are known to secrete a number of secreted fungal enzymes, such as pectinases; cellulases; amylases; laccases; laminarinases; and xylanases (Mata et al., 2016). In particular, it is known that shiitake mycelia constitutively express laccases (Matjuškova et al., 2017), and expression of laccases in shiitake mycelia may be upregulated or stimulated by the presence of lignin-derived phenols and or polymeric lignin materials (Matjuškova et al., 2017; Agrawal et al., 2018). Copper-containing laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and variety of non-phenolic compounds (Agrawal et al., 2018). Laccase is currently used in the food industry for a variety of functional applications including improvement of food sensory parameters (Osma et al., 2010). For example, Schroeder et al. (2008) demonstrated that laccase treatment of apple juice degraded the levels of certain phenolic compounds, guaiacol and 2,6-dibromophenol, responsible for off-flavors in apple juice. Sensory panelists did not detect a difference between apple juices spiked with guaiacol and 2,6-dibromophenol compared to non-spiked juice after continuous enzymatic treatment with laccase (Schroeder et al., 2008). These results are corroborated by the sensory testing observations reported by MycoTechnology for PureTaste® protein.

From this information, MycoTechnology concludes that confirmed improvements to the organoleptic qualities of PureTaste® protein relative to the protein input are likely due to the action of secreted enzymes (e.g laccase) from shiitake mycelia to modify molecules that confer unpleasant organoleptic qualities to pea protein concentrates and rice protein concentrates.

2.5.5 Absence of Live Shiitake Mycelia in PureTaste® protein

As discussed above, the main fermentation phase for PureTaste® protein is carried out under conditions known to induce lag phase in shiitake mycelia (Cavallazzi et al. 2005). The pH of the main fermentation does not change, indicating that no mycelial growth is taking place. From this information, MycoTechnology concludes that the shiitake mycelia culture enters lag phase upon inoculation into the main fermentation and remains in lag phase throughout the duration of the main fermentation. The fermentation process concludes with multiple heat treatment steps followed by a thermal deactivation step at 80 °C for 1 minute which would kill any remaining live shiitake mycelia. The absence of viable yeast and mold colonies (<100 cfu; Table 4) using media that would normally support the growth of shiitake mycelia (FDA BAM, Chapter 18) also supports the conclusion that there are no active shiitake mycelia in the final PureTaste® protein product.

Part 3: 170.235 Dietary Exposure

3.1 PureTaste® Application Usage Estimates

MycoTechnology, Inc. intends to use PureTaste® as a food ingredient in multiple, specific food categories at levels ranging from 1% to 40%. The intended use levels and the food categories to which PureTaste® will be added are summarized in Table 7 below. The use levels provided are based on purity criteria of 75% protein concentrate (dry weight basis). MycoTechnology also intends to market this protein as a supplemental protein to be used in sports nutrition applications.

Table 7. PureTaste® Application Usage Estimates

Food Category	Food-Uses	Proposed Use Level (%) of PureTaste® as consumed per food category (Minimum 75 % protein)[x]
Baked Goods and Baking Mixes	Breads	4.8% - 7%
	Rolls	4.8%*
	Bagels	4.4%*
	English Muffins	4.4%*
Beverages and Beverage Bases	Non-Milk Based Meal Replacements	1.04 % - 11%
	Ready to Mix Beverage Powder	32.67g PureTaste® / 35g serving (carbohydrate base)
	Plant Based Beverage, Non-Meal Replacement	1.04 % - 3 %
Breakfast Cereals	Ready-to-Eat Breakfast Cereals	4.4% – 16%*
Dairy Product Analogs	Soy/Nut Plant Based Beverages	1.04% -5.5%
	Cashew Product	5 % (4g PureTaste® /79g serving of frozen dessert)
	Coconut Product	8 % (6.67g PureTaste® /85g serving of frozen dessert)
Fats and Oils	Salad Dressings	~ 26 %

Food Category	Food-Uses	Proposed Use Level (%) of PureTaste® as consumed per food category (Minimum 75 % protein)[X]
Grain Products and Pastas	Health Bars and Grain-Based Bars	20% - 33.3%
	Health Bars and Grain-Based Bars Containing Fruit and Vegetable	18-20 %*
	Pasta	4.6 – 17 %
Milk Products	Flavored Milk Drinks	1.04%*
	Milk-Based Meal Replacements	1.04% *
	Yogurt (Regular and Frozen)	1.1 - 7%
Plant Protein Products	Meat Alternatives	1 – 40%
Processed Fruits and Fruit Juices	Fruit Juice	1.04 %*
	Fruit Nectars	1.04 %*
	Fruit-Flavored Drinks	9-20%*
Processed Vegetables and Vegetable Juices	Tomato Juice	2.5 -20 %*
	Vegetable Smoothie	3.5 – 20 %*
Soups and Soup Mixes	Prepared Soups, Dry Soup Mixes, and Condensed Soups	0.96 – 3.3 %
Non Baked Goods (Bars)	Extruded Nutritional Protein Bars	36 %
Confectionary	Chocolate Dessert (Peanut Butter Cup)	7 %
Meat and Poultry†	Meatloaf, lasagna, sausage, meat patties, meat analogs	2.5 – 5%

*Values are consistent with the amount of pea protein concentrate to be added to the same product categories as supported by GRAS 608 (Pea Protein Concentrate), Table 10, and GRAS 609 (Rice Protein Concentrate), Table 9, based on minimum 80% protein. As PureTaste® would be used as a substitute protein in these categories, the use level would be considered comparable.
[X]% PureTaste® will vary due to the fact that most calculations are mass into volume assuming water. PureTaste® use levels calculated based on purity of 75%
† Subject to USDA approval.

3.2 PureTaste® Daily Consumption Calculation

PureTaste® is intended to be used as a substitute for, and/or in conjunction with, pea protein, rice protein and other protein sources in conventional food products. Target product categories include food products needing protein-source properties such as promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, increasing water holding capacity and gelation, and increase of water-solubility.

As PureTaste® is intended to be used as an alternative product to those already available at similar inclusion levels, the estimated daily intake of PureTaste® protein in the U.S. can be derived from the daily consumption estimates of other alternatives including pea and rice protein concentrates. Based on the information provided in GRAS Notice 608 for pea protein concentrate and GRAS Notice 609 for rice protein concentrate (U.S. Food & Drug Administration, 2016a,b), Table 8 below is considered representative of the Estimated Daily Intake of pea and rice protein concentrates from intended food uses in the U.S. population based on 2011-2012 NHANES Data (Centers for Disease Control and Prevention, 2012). As discussed further below, MycoTechnology concludes that the intended uses of PureTaste® protein will not result in an increase in the overall consumption of protein.

Table 8. Pea and Rice Protein Estimated Daily Intake

Population Group	Age Group (Years)	All Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	N	Mean	90th percentile
Infants and Young Children	Up to 3	5.9	12.4	83.2	683	7.1	13.4
Children	4 to 11	9.4	14.8	99.9	1347	9.4	14.8
Female Teenagers	12 to 19	10.5	16.5	98.8	526	10.6	16.5
Male Teenagers	12 to 19	11.8	18.7	98.5	508	12.0	19.7
Female Adults	20 and up	9.7	16.1	99.8	2204	9.7	16.1
Male Adults	20 and up	11.1	20.3	98.8	22067	11.2	20.5
Total Population	All Ages	10.1	17.2	98.4	7335	10.3	17.3

Source: Table 11 of GRN 608 (p.000016) for pea protein concentrate (FDA, 2016a) and Table 10 of GRN 609 (p. 000082) (FDA, 2016b).

In summary, on an "all-user" consumption basis, the resulting mean and 90th percentile intakes of rice protein concentrate by the total U.S. population from all proposed food-uses in the U.S. is estimated to be 10.3 g/person/day (181 mg/kg bw/day [GRN 608; Table 12]) and

17.3 g/person/day (388 mg/kg bw/day [GRN 608; Table 12]), respectively as determined in GRAS notice 609 and GRAS notice 608. Male adults were identified as the individual sub-population group with the highest 90th percentile consumption per day (20.5 g/person/day) and infants & young children had the lowest 90th percentile consumption per day (13.4 g/person/day). Additionally, GRAS 608 (pea protein concentrate) further estimates that the maximum pea protein concentrate anticipated from use in sports nutrition (i.e. supplemental protein) would be 30 g/ person / day.

3.2.1 PureTaste® Estimated Daily Intake

As PureTaste® is to be used as an alternative/substitute to other protein products such as rice or pea protein in similar applications and at similar inclusion levels, the mean adult daily consumption can be inferred for PureTaste® from these products. In Table 9 below, the inclusion levels and estimated daily intake are presented for PureTaste® based on the estimated intake levels for other, similar alternative protein sources. Although PureTaste® is proposed for use in a slightly broader range of food categories (Table 8), based on its proposed use as a replacement for currently available protein sources, MycoTechnology concludes that the intended uses of PureTaste® protein will not result in an increase in the overall consumption of protein.

Table 9. Inclusion Rates and Estimated Daily Intake

	PureTaste®	**Rice Protein [GRN 609] and Pea Protein [GRN 608]**
Inclusion Range in Food Category	1.1 – 40%	0.96 – 34.3%
90th Percentile (Total U.S. Population)	17.3 g/person/day	17.3 g/person/day

Based on the above information, the conservative 90th percentile estimate of the daily consumption of PureTaste® is 17.3 g/person/day.

Additionally, in GRNs 608 and 609 (pea and rice protein concentrates) it is further estimated that the maximum pea or rice protein concentrate anticipated from use in sports nutrition (i.e. supplemental protein where consumers mix their own beverages) would be 30 g/ person / day (based on estimated intakes of 5 to 15 g pea protein /serving or 15 to 25 g rice protein/serving). MycoTechnology agrees with this estimation for PureTaste® protein when used as a supplemental protein in sports nutrition applications.

3.2.2 Comparison of PureTaste® Estimated Daily Intake to Background Intake Levels for Protein

The Recommended Dietary Allowance (RDA) for protein varies from approximately 10 g/day (for infants) to 71 g/day (for pregnant and lactating women), with the RDA for adult females and males as 46 and 56 g/day, respectively (IOM, 2002/2005). The FDA daily reference value (DRV) for protein is 50 g/day for adults and children 4 or more years of age and 13 g/day for children 1 through 3 years of age (FDA, 2016). Based on previous intake estimates for pea and

rice protein (GRNs 608 and 609) presented above, the estimated 90th percentile intake of PureTaste® is similar to or less than these recommended intake levels of protein in the diet.

Additionally, as discussed in GRN 608 (pea protein concentrate [FDA, 2016a]), the Reference Amount Customarily Consumed (RACC) for peas is 85 g / serving (21 CFR 101.12), with the 90th percentile daily intake estimated to be approximately double the RACC (FDA, 2006), i.e. 170 g / day. Since peas contain ~24 % protein (GRN 608; USDA NNDSF, 2018a), it can be determined that the amount of protein that would be provided by peas is 20.4 g protein per serving (mean), and 40.8 g protein per day (90th percentile). Similarly, the RACC for prepared rice is 140 g (45 g dry rice) (21 CFR 101.12), with USDA reported mean and 90th percentile intakes of 150 and 312 g/person/day, respectively (Smiciklas-Wright et al., 2002). As rice contains approximately 8% protein (GRN 609; USDA NNDSF, 2018b), the intake of protein from consumption of rice at the mean and 90th percentile in the US is estimated to be 12.0 and 25.0 g/person/day, respectively.

Since PureTaste® is intended as source of protein that will substitute for other proteins in the diet, this ingredient will not result in an overall increase in the consumption of protein in the diet. As discussed in GRN 575 for oat protein (p. 000026-27) and the associated FDA Response Letter (FDA, 2015), it is reasonable to expect that most of the U.S. population's intake of protein is expected to remain in the form of unprocessed foods including meat, poultry, fish, and legumes. Furthermore, as the 90th percentile of PureTaste® based on the intended uses in each of the proposed food categories would be 17.3 g / person / day, this is lower than the 90th percentile of dietary pea protein consumption and the recommended levels of protein intake considered safe for human consumption.

In the unlikely event that an adult consumes all intended PureTaste® food categories at the 90th percentile of intake and consumes PureTaste® as a supplemental protein in sports nutrition, the total protein intake (i.e. 47.3 g/person/day) from PureTaste® would still be similar to or less than the FDA DRV (50 g/day) or IOM RDA (56/46 g/day; males/females) for adults.

3.3 Dietary exposure to Shiitake mycelia via consumption of PureTaste® protein at estimated consumption levels

As discussed above in Section 3.1, for the Total Population All-Users Consumption dietary exposure of PureTaste® protein is estimated to be 17.3 g/day at the 90th percentile of consumption, and 10.3 g/day at the mean level of consumption (based on previous dietary exposure estimates for pea protein (GRN 608) and rice protein (GRN 609) at similar inclusion levels and applications).

The level of Shiitake mycelia present in the PureTaste® protein is determined as follows. MycoTechnology has confirmed that the Shiitake mycelia, when added as input into the main fermentation step, enters lag phase upon inoculation (see Method of Manufacture §2.2). Lack of additional biomass accumulation of the Shiitake mycelia during the main fermentation is confirmed by microscopy and pH monitoring. The input Shiitake mycelia into the final

fermentation step is <0.1 wt.%. Thus, MycoTechnology concludes that the relative amount of Shiitake mycelia in the PureTaste® protein matches the relative amount of the input Shiitake mycelia, or <0.1 wt.%.

At <0.1% w/w Shiitake mycelia in PureTaste® protein, for an estimated Total Population All-Users Consumption at the 90th percentile level of estimated daily consumption of PureTaste® protein of 17.3 g, MycoTechnology concludes that the corresponding level of Shiitake mycelia dietary exposure is 17 mg per person per day. At <0.1% w/w Shiitake mycelia in PureTaste® protein, for an estimated Total Population All-Users Consumption mean level of estimated daily consumption of PureTaste® protein per person of 10.3 g, MycoTechnology concludes that the corresponding level of Shiitake mycelia dietary exposure is 10 mg per person per day. At a mean body weight of 60 kg this would result in an intake of approximately 0.28 mg/kg bw/day at the 90th percentile level of estimated daily consumption of PureTaste® protein or 0.17 mg/kg bw/day at the mean level of estimated daily consumption for the Total Population.

The safety assessment of the consumption of Shiitake mycelia at estimated dietary exposures in PureTaste® protein is presented below in Section 6.3.3.

3.4 Dietary Exposure Conclusions

The PureTaste® product will be used in a number of food products. Most of the population's protein intake is derived from, and will continue to be derived from, unprocessed foods, including meat, poultry, fish, and legumes. PureTaste® will be added to products as a competitive meat alternative ingredient on the market in a similar manner to other products on the market such as pea and rice protein. Thus, the addition of PureTaste® protein ingredients will simply serve as a replacement for these other competitive protein sources and will not increase overall consumer exposure to protein. In addition, the conservative estimate of PureTaste® protein intake is below current FDA and IOM recommendations for protein in the diet further supporting a conclusion of safety.

As discussed below in Section 6.3.3, consumption of Shiitake mycelia at estimated dietary exposures in PureTaste® protein is also considered to be safe.

Part 4: 170.240 Self Limiting Levels of Use

The use of the PureTaste® as a food ingredient is limited by the level that can technically be added to a given food without jeopardizing its quality and consumer acceptability. The self-limiting level of use is independent of safety (toxicity, allergenic, etc.) concerns.

Part 5: 170.245 Experience Based on Common Use in Food Before 1958

The statutory basis for the GRAS conclusion for PureTaste® is based on scientific procedures; therefore, information regarding experience based on common use of the notified substance in food prior 1958 is not applicable. The historical consumption of the GRAS material raw ingredients (pea and rice protein) and fermentation organism (Shiitake mushroom) is discussed below in § 170.250 Part 6 (GRAS Narrative) as supporting information.

Part 6: 170.250 GRAS Narrative and Safety Rationale

PureTaste® is composed of pea and rice protein fermented with *Lentinula edodes* (Shiitake) mycelium. Based on method of the manufacture (§2.2) and demonstrated confirmation that the input raw materials are not substantially modified following fermentation (§2.5.3), the safety of the GRAS material can be established through the safety of the ingredients used to produce PureTaste®.

6.1 Safety of Pea Protein Raw Material

Pea protein concentrate (containing ≥80% protein) was previously determined to be GRAS for use in applications similar to that of PureTaste® at similar use rates (GRN 581 [U.S. Food & Drug Administration, 2016a]; GRN 608 [U.S. Food & Drug Administration, 2016b]; GRN 788 [U.S. Food & Drug Administration, 2018]). Since PureTaste® comprises ≥95% total protein concentrates as raw material inputs (approximately equal parts pea and rice protein delivering ≥75% protein in final product; ratio may be adjusted), the exposure to pea protein from the proposed uses of PureTaste® is concluded to be safe as the use of this ingredient is supported by GRAS Notices 581, 608 and 788.

The mean and 90th percentile estimated daily intake of peas was estimated to be 85 and 170 g/person/day (or 20.4 and 40.8 g pea protein/person/day), respectively (see Section 3.2.2). The estimated exposure to PureTaste® protein from the proposed uses is 17.3 g/person/day (see Section 3.2.1), or 7.8-12.1 g pea protein/person/day assuming PureTaste® contains ~45%-70% pea protein, which is well below the current consumption of pea protein from the normal human diet.

Pea protein concentrate has been well characterized for its nutritional composition and characteristics and has been compared for its nutritional constituents and amino acid profile with other protein concentrates such as whey, casein and soy, and has been found to be substantially similar (Young and Pellett, 1994). Pea protein concentrate is digested in the human gastrointestinal tract like all dietary proteins (Nicolas, 1996). Pea Protein is not a major allergen (Food Allergen Labeling and Consumer Protection Act of 2004.).

Pea protein has not been reported to be associated with adverse effects. For example, Abou-Samra et al. (2011) reported a study in which 32 healthy male volunteers (25 ± 4 years) consumed 20 g of pea protein dissolved in 250 ml of water. No adverse events were reported. Babault et al. (2015) reported that the feeding of 25 g of pea protein dissolved in water to 161 males (18-35 years) healthy males twice daily for 6 weeks was well tolerated and no adverse events were reported.

In addition to the estimated exposure of PureTaste®, the common food use information of peas and pea protein can be considered as relevant safety support for the pea protein in PureTaste®. Peas are one of the oldest cultivated crops in the world and an important source of protein for humans and animals (Encyclopedia Britannica, 2017) and have been consumed as a food around the world since ancient times (Li, 2017). Peas are an excellent source of protein and the amino acid lysine (Vaclavik, 2013). The USDA Nutrient Database includes peas and its preparations as

foods (USDA, 2018a). The Reference Amount Customarily Consumed (RACC) for peas is 85 g/serving (21 CFR 101.12). Based on data collected by the USDA, the mean and 90th percentile estimated daily intake of cooked peas is approximately 85 and 170 g/person/day respectively (Smiciklas-Wright, 2002). The available information demonstrates common human consumption of peas and pea protein.

6.2 Safety of Rice Protein Raw Material

Rice protein concentrate (containing ≥80% protein) was previously determined to be GRAS for use in applications similar to that of PureTaste® at similar use rates (GRAS Notice 609; [U.S. Food & Drug Administration, 2016c]. Since PureTaste® comprises ≥95% total protein concentrates (approximately equal parts pea and rice protein delivering ≥75% protein in final product; ratio may be adjusted), the exposure to rice protein from the proposed uses of PureTaste® is concluded to be safe as the use of this ingredient is supported by GRAS Notice 609.

The mean and 90th percentile estimated daily intake of rice was estimated to be 150 and 312 g/person/day (or 12 and 25 g rice protein/person/day), respectively (see Section 3.2.2). The estimated exposure to PureTaste® protein from the proposed uses is 17.3 g/person/day (see Section 3.2.1), or 5.2-7.8 g rice protein/person/day assuming PureTaste® contains ~30%-45% rice protein, which is well below the current consumption of rice protein from the normal human diet.

Bran and germ from the brown rice are concentrated sources of vitamins, minerals, flavones, and other phytonutrients. Brown rice is used in the preparation of various foods including breakfast cereals, baked goods, rice cakes, tea, pasta, and noodles. The consumption of rice in developing countries is approximately 68.5 kg/person/year (188 g/person/day) and 12.8 kg/person/year (35 g/person/day) in developed countries (Kahlon, 2009).

In addition to the estimated exposure of PureTaste®, the common food use information of rice protein can be considered as relevant safety support for the rice protein in PureTaste®. Rice, brown rice, and their derivatives have a long history of human consumption, with rice cultivation documented back to prehistoric times, starting in Asia and eventually spreading across Europe around the sixth century (Rost, 1997). Among the cereals, rice and wheat share equal importance as leading food sources for humankind (Chang, 2000). Rice is produced on most continents and serves as a dietary staple for a majority of populations across the world (FAO, n.d.). Once harvested, the rice is hulled and the resulting brown rice can be further processed to generate derivatives such as rice bran oil, rice bran extract, and hydrolyzed rice protein (Sun Rice, n.d.). The USDA National Nutrient Database list includes rice and its preparations as foods (USDA, 2018b). The USDA National Nutrient Database has listed 3528 food products that contain rice suggesting common exposure to rice.

Based on its history of common use, rice and its protein are generally regarded as safe at current levels of consumption.

6.3 Safety of *L. edodes* (Shiitake) Mycelia Fermentation Organism

As discussed in Section 2.2.4.3 above, the strain of shiitake (*L. edodes*) used to produce PureTaste® protein was originally obtained from Pennsylvania State University and grown in aqueous culture as the vegetative form (shiitake mycelia). The relative amount of inactive shiitake mycelia in PureTaste® protein is estimated at <0.1%. Based on the weight-of-evidence evaluation described below, including read-across to the safety and historical consumption of the fruiting bodies of *L. edodes*, MycoTechnology concludes that the use of *L. edodes* (shiitake) mycelia in the fermentation of PureTaste® protein is safe.

6.3.1 Historical Consumption of L. edodes (Shiitake Mushroom)

The fruiting bodies of *Lentinula edodes*, also known as shiitake, are a common food particularly in Asia. As a source of diverse secondary metabolites, fungi have a long history of use in both culinary and medicinal applications (Van der Molen et al., 2017). The shiitake mushroom is the second most widely produced mushroom in the world (Bisen et al., 2010). The world mushroom industry markets more than 2 million tons of mushrooms per year and is still expanding (Nakamura, 1992).

Mushrooms have nutritional value since they are rich in protein (~2.26 % protein), with an important content of essential amino acids and fiber (Finimundy, 2014). Edible mushrooms are a high nutritional quality food and have been used as an alternative to dietary protein in countries with high malnutrition rate (Finimundy, 2014, Canadian Nutrient File for Shiitake Mushroom, Food code 6904). The chemical and nutritional characteristics of mushrooms vary in function after harvest, and processing (Finimundy, 2014).

In a review of the nutritional compounds found in *Lentinus edodes* (Finimundy, 2014) it was reported that the dietary fiber present in *L. edodes* consists of soluble and insoluble fractions. Water-soluble β-glucans and proteins are found in the soluble fraction. In the non-soluble fraction, polyuronide (acidic polysaccharide), hemicellulose, β-glucan chains with heterosaccharide, lignin, and chitin are found. *L. edodes* provides a nutritionally significant content of vitamins B1, B2, B12, C, D, and E. The aroma components include alcohols, ketones, sulfides, alkanes, and fatty acids. The main constituents which are volatile include matsutakeol (1-octen-3-ol) and ethyl, n-amyl ketone (Finimundy, 2014). The characteristic aroma of shiitake was identified as 1,2,3,5,6-Pentathiepane (Finimundy, 2014). *L. edodes* mycelium are composed of glycoproteins containing glucose, galactose, xylose, arabinose, mannose, and fructose (Coates, 2010).

6.3.2 Similarity of Shiitake Mushroom and Shiitake Mycelia Composition

The life cycle of mushrooms starts with a spore which produces a primary mycelium. When the mycelium originating from two spores mates, a secondary mycelium is produced. This mycelium continues to grow vegetatively. When vegetative mycelium has matured, its cells are capable of a phenomenal rate of reproduction which culminates in the erection of the mushroom fruitbody. The fruitbody represents the last functional change in the mushroom life cycle and it is tertiary

mycelium. The entire mushroom is composed of compressed mycelia (Stamets & Chilton, 1983). The Shiitake mushroom is largely made up of bundles of mycelia composing the pileus (cap) and stalk, and having only a small portion of tissue, located underside of the mushroom cap that differentiates into gills (lamella) to produce spores (basidiospores) for reproduction of the Shiitake organism. Thus, Shiitake mushroom itself, aside from gill tissue on undersides of caps producing spores, is, physically indistinguishable from its parent mycelia (Stamets & Chilton, 1983; Liu et al., 2016). From this information, MycoTechnology concludes that the Shiitake mycelia and Shiitake mushroom compositions are substantially similar on a physical level, and the safety demonstrated for Shiitake mushroom is directly applicable to Shiitake mycelia.

Van der Molen et al. (2017) (discussed further below in § 6.3.3) in a comparison to culinary mushrooms using 1:1 methanol-chloroform (MeOH-$CHCl_3$) extracts identified similarity of 98% in the composition of shiitake mycelia culture to grocery store Shiitake mushrooms per ultrahigh-performance liquid chromatography-photodiode array-evaporative light scattering-high resolution mass spectrometry (UHPLCPDA-ELS-HRMS) analysis, confirming that the mycelium were not substantially different from the fruiting bodies used as food. The polarity of 1:1 MeOH-$CHCl_3$ is such that most organic soluble molecules are extracted efficiently. Total unevaluated peak area was 2%. In another approach, Shiitake fungal raw material (i.e. mycelia) extract was subjected to a targeted UHPLC-PDA-HRMS/MS protocol that screened for the presence of cytotoxins and mycotoxins from a database of over 300 fungal secondary metabolites (El-Elimat et al., 2013 as reported in Van der Molen et al., 2017). The Shiitake fungal raw material extract yielded matches for fungal metabolites from this database based on retention time, UV data, HRMS data, and MS/MS data. The cytotoxic metabolite ergosterol peroxide was detected in the Shiitake fungal raw material extract, but was also detected in the store-bought culinary Shiitake mushroom extract. From this information, MycoTechnology concludes that the Shiitake mycelia and Shiitake mushroom compositions are virtually identical and the safety demonstrated for Shiitake mushroom is directly applicable to Shiitake mycelia.

Song et al. (2018) reported on the differential expression of 11,675 total genes known to Shiitake and identified that 9,595 of these are <u>not</u> differentially expressed between mycelia and fruit body. There is an approximately 82% identity in expression activity between Shiitake mycelia and Shiitake fruiting body tissue. While Song et al. (2018) reports that gene expression levels differ, the authors attribute the differential expression to overexpression of genes in the mature fruiting body stage (the mushroom) related to "DNA replication, recombination, repair, chromatin structure, and the associated dynamics" and the transcripts from the fruiting body are "significantly enriched in 'replication and repair' and 'transcription' pathways for premeiotic replication, karyogamy, or meiosis." In other words, the differential expression is observed by Song et al. (2018) to be primarily related to the reproductive activity related to Shiitake fruiting in the fruiting body, which does not occur for the Shiitake mycelia. From this information, MycoTechnology concludes that the differences in gene expression between Shiitake mycelia and mature fruiting body tissues of the Shiitake mushroom are of little to no consequence to the safety of consumption of Shiitake mycelia.

6.3.3 Safety of consumption of Shiitake mycelia at estimated dietary exposures in PureTaste® protein

As discussed in § 3.3, estimated dietary exposure to Shiitake mycelia from consumption of PureTaste® protein at estimated mean and 90th percentile levels are 10 mg/person/day and 17 mg/person/day, respectively. The safety of this exposure level for Shiitake mycelia was evaluated using a weight-of-evidence approach as described by Van der Molen et al. (2017) for the safety assessment of mushrooms in dietary supplements by combining analytical data with *in silico* toxicology evaluation.

Van der Molen et al. (2017) assessed the safety of seven fungal raw materials including Shiitake (*Lentinula edodes*) consisting primarily of mycelium. Consumption of Shiitake mycelia in dietary supplements at a maximum dose of 1,500 mg and a median dose of 50 mg was evaluated by a decision tree driven weight-of-evidence approach consisting of five key principles as outlined below. MycoTechnology has also addressed these five key principles in its weight-of-evidence approach to the safety assessment of the shiitake mycelia used to produce PureTaste® protein.

1) Identification by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region (commonly referred to as ITS barcoding)
    - Van der Molen et al. (2017) verified, by ITS barcoding, that an obtained fungal raw material analyzed was Shiitake mycelia
    - MycoTechnology similarly verified, by ITS barcoding, that the microorganism used in the manufacturing process to produce PureTaste® protein is *Lentinula edodes* which is grown in aqueous culture as the vegetative form *(*mycelia)(see section 2.2.4.3)
2) Screening an extract of each fungal raw material against a database of known fungal metabolites
    - Van der Molen et al. (2017) screened the Shiitake mycelia 1:1 MeOH-CHCl3 extract against a database of 300 known cytotoxic metabolites. Table 9 of Van der Molen et al. (2017) confirms that Shiitake mycelia in commerce produced no unique detectable toxins.
    - MycoTechnology has similarly confirmed that no unique fungal toxins were detected in PureTaste® protein (see Section 6.3.4 below)
3) Similarity of the Shiitake mycelia extract to culinary mushroom extracts
    - Van der Molen et al. (2017) performed UHPLC-PDA-ELS-HRMS analysis to assign individual peaks for the Shiitake mycelia extract a percent similarity or difference to grocery store-bought Shiitake culinary mushroom. Van der Molen et al. (2017) showed that there was a 98% similarity of shiitake mycelia to grocery store-bought Shiitake mushrooms, per UHPLCPDA-ELS-HRMS analysis (2% unevaluated) and that the Shiitake mycelia is "[c]hemically very similar to food." (see Van der Molen et al., 2017; Table 9).
    - As described above (Section 6.3.2), MycoTechnology concludes that the Shiitake mycelia used to produce PureTaste® protein is substantially equivalent to Shiitake culinary mushrooms (fruiting body).

4) Review of the toxicological and chemical literature for each fungus
    - Van der Molen et al. (2017) performed a literature review of the current toxicological and chemical literature for Shiitake mushrooms. The authors concluded that shiitake has a long history of use as food (fruiting body) and numerous toxicological studies were available showing minimal toxicity.
    - MycoTechnology also performed a review of the available literature regarding the safety of shiitake mycelia (see Section 6.3.5, below). MycoTechnology concludes that the available literature supports the safety of shiitake mycelia for use in the production of PureTaste® protein.
5) Evaluation of data establishing presence in-market.
    - Van der Molen et al. (2017) reviewed in-market data using the Dietary Supplements Labels Database (DSLD) maintained by the National Institutes of Health (NIH) for a total of 223 Shiitake products with the most common ingredients being "Shiitake" (98 products) and "Shiitake Mushroom" (43 products). Most products did not report the dose of the ingredient, instead listing only the dose of a proprietary blend in which Shiitake was included (assumed to be Shiitake mycelia since commercial fungal raw materials predominantly consist of mycelia because of its rapid growth characteristics compared to the mushroom / fruiting body). Of the doses reported, the maximum dose was 1500 mg, and the median dose was 50 mg.
    - MycoTechnology concludes that the current market use of Shiitake mycelia in the form of a dietary supplement supports the safety of Shiitake mycelia at much lower exposure levels in PureTaste® protein (i.e. mean and 90th percentile levels are 10 mg/person/day and 17 mg/person/day, respectively). In contrast to Shiitake mycelia dietary exposure in the form of a dietary supplement, in PureTaste® protein the Shiitake mycelia is dispersed, i.e. it is intermixed with all other components (primarily pea and rice proteins) in PureTaste® protein. In both cases, the dietary exposure results from a heat-treated and killed Shiitake mycelia.

Based on the above analysis, Van der Molen et al. (2017) concludes that:

> "Shiitake and Maitake are commonly eaten as foods, and Shiitake, at least, has a wealth of available toxicological data supporting its safe use. The apparent prevalence in the marketplace, the lack of reported adverse events, as determined by the literature review and the very high degree of similarity between their mycelial growths (the raw materials investigated) and the culinary fruiting bodies to which they were compared give confidence that these materials are safe for consumption at doses consistent with dietary intakes of culinary mushrooms."

In summary, using a weight-of-evidence safety assessment approach Van der Molen et al. (2017) concluded that a dietary exposure to Shiitake mycelia of 50 mg to 1,500 mg (assumed daily) is safe, with the existing history of use and available toxicological data indicating no greater risk than culinary mushrooms (Van der Molen et al., 2017). Using the approach described by Van der Molen et al. (2017), including ITS barcoding results, demonstrated absence of fungal toxins, confirmed similarity of shiitake mycelia to culinary mushrooms, a review of toxicology and clinical safety literature, and comparison to in-market use of dietary supplements containing shiitake ingredients, MycoTechnology similarly concludes that the dietary exposure to Shiitake mycelia in PureTaste® protein at lower exposure levels is safe.

6.3.4 Absence of Fungal Toxins in PureTaste® Protein

The cultivation of shiitake mycelia in solid culture to produce shiitake mushrooms for use in food is well known (Van der Molen et al., 2017). Shiitake culture is grown in solid-state as mycelial tissue ("spawn") usually on grain or wood chips which, after running out of nutrient substrate, fruits mushrooms (basidiocarps) which produce spores (basidiospores) (Przybylowicz & Donoghue, 1988). The literature shows that neither shiitake mushrooms nor shiitake mycelia are known to produce mycotoxins during the growth of the mycelia or during the fruiting phase (production of mushrooms) (Han et al., 2014).

Shiitake mycelia may also be grown in aqueous culture. In aqueous culture, shiitake mycelia are not known to produce mushrooms, instead propagating as mycelia only (Tsivileva et al., 2005; Aminuddin et al., 2013; Aminuddin et al., 2007). The literature shows that during growth of shiitake mycelia in aqueous culture, no known mycotoxins were found to be produced (Van der Molen et al., 2017; EFSA, 2010). An exhaustive literature search also failed to identify any scientific report in which *L. edodes* or closely related fungal species (*Schizophyllum commune, Gymnopus luxurians)* have been associated with the production of mycotoxins or other toxic compounds. Furthermore, inspection of the *L. edodes* genome identified a total of 32 metabolite gene clusters, none of them seem to be involved in the production of known fungal toxins (Chen et al., 2016).

To corroborate the findings of the literature search discussed above, an analysis of organic compounds was performed on PureTaste® protein compared with a sham fermentation control (pea and rice protein subjected to identical processing as PureTaste® protein but lacking a shiitake mycelia inoculation step) using LCMS-APCI-QTOF (MycoTechnology; unpublished data on file). This analysis did not identify the presence of any fungal toxins.

PureTaste® protein was further evaluated via comparison to National Biotechnology Center for Information (NCBI)'s databases "NCBI Fungi" and "NCBI green plant" (MycoTechnology; unpublished data on file). The results showed that less than 1% of the sample matched the identity of a fungal protein according to the database, and no toxic fungal proteins were identified.

From this information, MycoTechnology concludes that shiitake mycelia grown under the conditions described under the manufacturing conditions as described in § 2.2 is not expected to produce mycotoxins or toxic metabolites during the production of PureTaste® protein.

6.3.5 Toxicology and Clinical Safety Literature Review for Lentinula edodes (Shiitake) Mycelia

In order to assess the safety of oral intake of *L. edodes* mycelia used to produce PureTaste® protein, a comprehensive search of the scientific literature through January 2019 was conducted using the U.S. National Library of Medicine (NLM) PubMed and TOXLINE databases. The most recent search was performed on January 18, 2019 with no date limitations / filters applied to the results. Search terms to identify relevant literature on the mycelia included "lentinula edodes" / "shiitake mushroom" AND "mycelium" / "mycelia". Search terms to identify relevant literature on the fruiting body included "lentinula edodes" / "shiitake mushroom" AND the additional keywords (PubMed search only) "safety" / "toxicity" / "carcinogenicity" / "genotoxicity" / "adverse effect" / "tolerability"/ "consumption" / "allergen" / "allergy". Relevant literature regarding the safety of dietary consumption of Shiitake mycelia is discussed below.

Yoshioka et al. (2010) assessed the safety of an aqueous suspension of L.E.M. extract powder of *Lentinula edodes* mycelia (L.E.M.) when administered to male and female Wistar rats (10 animals/sex/group) via gavage at 2,000 mg/kg bw/day for 28 days. Cultured *L. edodes* mycelia together with the solid medium were extracted with hot water (temperature not reported) and the L.E.M. extract was prepared by filtration, concentration, sterilization and lyophilization of the raw extract. Although an L.E.M. extract will not contain insoluble portions of Shiitake mycelia cells, and is therefore not identical in composition to the Shiitake mycelia present in PureTaste® protein, both the L.E.M. extract and Shiitake mycelia present in PureTaste® protein will contain the same water-soluble (presumably bioavailable) components. Thus the Yoshioka et al. (2010) L.E.M. extract repeated-dose toxicity data is useful to evaluate safety of the low levels of Shiitake mycelia present in PureTaste® protein.

Yoshioka et al. (2010) did not report any unscheduled deaths or clinical signs suggesting toxicity. Body weight and food consumption were slightly decreased compared to the control groups, particularly for males. Although there were statistically significant differences from control groups, at the study termination male body weights were only 8% (associated with slightly decreased food consumption) and female body weights were only 5% less than control groups, respectively. These minor differences are not considered to be adverse. None of the hematological parameters were statistically significantly different from respective controls after the 28-day dosing phase. Serum biochemistry revealed very few statistically significantly different parameters compared to respective controls, including increased phosphorus in both sexes; however, all values were reported as being within the laboratory's normal reference ranges. Although females had slightly increased organ weights relative to body weight (thyroid gland, kidneys, adrenals, uterus/ovaries) as did males (thyroid gland, adrenals), these differences were minor and without histopathological correlates. There were no pathological alterations in any examined tissues or organs. Yoshioka et al. (2010) concluded that the no observed adverse effect level (NOAEL) of L.E.M. extract was 2,000 mg/kg bw/day for the rat. This rat NOAEL is the equivalent to a dietary exposure of 120 g of L.E.M. extract per day for a 60 kg human. As discussed above in § 3.3, the estimated dietary exposure of Shiitake mycelia in PureTaste® protein consumption, at the 90th percentile is 17 mg per day. Therefore a more than 7000-fold difference exists between the estimated dietary consumption of Shiitake mycelia in PureTaste®

protein and L.E.M. extract tested in Yoshioka et al. (2010). MycoTechnology, Inc. concludes from this 28-day oral repeated-dose study with Wistar rats that findings from the Yoshioka et al. (2010) study supports a conclusion of safety for PureTaste® protein at the estimated levels of consumption.

In another study, Yoshioka et al. (2009) evaluated the safety of foods containing an extract of cultured *Lentinula edodes* mycelia (L.E.M.) in healthy adult volunteers. The publication is in Japanese with a limited English abstract, so details of the methods and results are difficult to discern. Yoshioka evaluated a lyophilized hot water extract of *Lentinula edodes* mycelia (LEM) material administered to subjects in granular food. Although an L.E.M. extract will not contain insoluble portions of Shiitake mycelia cells and is therefore not identical in composition to the Shiitake mycelia present in PureTaste® protein, both the L.E.M. extract and Shiitake mycelia present in PureTaste® protein will contain the same water-soluble components. Thus L.E.M. extract clinical data is useful to evaluate the safety of PureTaste® protein. Eleven healthy adult subjects (8 males and 3 females, ages 33.4 ± 9.4 years) consumed the test foods containing 5,400 mg L.E.M. extract per day for 4 weeks. Yoshioka et al. (2009) reported no adverse effects for intake of 5,400 mg L.E.M. extract per day in physical and clinical examinations, except for mild gastrointestinal symptoms such as soft stool in one subject who had a "hypersensitive" intestine. The authors concluded that "the granular food containing L.E.M. is safe in healthy adults, even if [an] excessive amount of up to 5,400 mg a day is consumed". As discussed above in § 3.3, the estimated dietary exposure of Shiitake mycelia from PureTaste® protein consumption at the 90th percentile of intake is 17 mg/day. This dietary intake would be about 300-fold less than the dietary exposure to L.E.M. extract administrated daily for 4 weeks in the Yoshioka et al. (2009) study. From this information, MycoTechnology concludes that the Yoshioka et al. (2009) study supports a conclusion of safety for Shiitake mycelia present in PureTaste® protein.

Additional human clinical studies addressing the safety of Shiitake mycelial extract are summarized in Table 10. Although these studies were performed to assess the possible therapeutic effects of Shiitake mycelial extract on quality of life and immune function, no adverse events from treatment with Shiitake mycelial extract were reported. Therefore, these studies support a conclusion of safety for Shiitake mycelia present in PureTaste® protein.

6.3.6 Safety for Fruiting Bodies (Mushrooms) at estimated levels of consumption of PureTaste® protein

The fruiting bodies of *Lentinula edodes*, also known as Shiitake mushroom, are a common food, particularly in Asia. Shiitake mushroom is the second most popular edible mushroom in the global market (Bisen et al., 2010). Relevant literature regarding the safety of dietary consumption of Shiitake mushroom (fruiting body) is discussed below. Details regarding the literature search strategy are provided above (§6.3.5).

Frizo et al. (2014) tested the effects of reconstituted Shiitake mushroom powder consumption in a rat (strain not reported) developmental toxicity model at daily gavage doses of 400 mg/kg and 800 mg/kg (0.53 g β-glucan per 100 g mushroom) from the 1st to the 20th day of gestation and saline was administered to the control group. Only a scientific meeting abstract is available for Frizo et al. (2014), so details of the methods and quantitative results cannot be discerned. A

further publication of this study was not identified in the literature. The fetuses were removed before birth on the 21st day of gestation by caesarean section. Maternal kidney and liver toxicity were assessed and oxidative stress was determined by measurement of glutathione (GSH). The corpora lutea, implantations, resorptions, live and dead fetuses were counted. The placentae and fetuses were weighed. External and visceral morphology examinations of fetuses were performed following fixation with Boudin solution. Skeletal evaluations were performed following diaphonization and staining with alizarin red-S. Although there was an absence of maternal toxicity, the glutathione (GSH) plasma ratio was reduced at 400 and 800 mg/kg/day, suggesting antioxidant properties of Shiitake mushroom at the relatively high doses used in this study. No changes were reported in urea plasma ratio, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). There was an increase in post implantation loss, reduced fetal body weights and external measurements (no further details reported). However, no visceral or skeletal abnormalities of the fetuses were found. As discussed in § 3.3, the estimated dietary exposure of Shiitake mycelia in PureTaste® protein is 17 mg/day at the 90th percentile of intake, corresponding to 0.28 mg/kg/day level of consumption at a mean body weight of 60 kg. The Shiitake mushroom dietary exposure in Frizo et al. (2014) is more than 1400-fold higher than that from PureTaste® protein. In the absence of additional details from this study, interpretation of the reported findings is limited. However, MycoTechnology concludes that the level of dietary exposure to Shiitake mycelia in PureTaste® protein is significantly less than the dietary exposure levels of Shiitake mushroom powder reported to show developmental toxicity in fetal rats following *in utero* exposure for 20 days.

Female mice (*Mus musculus* NIH/S; n = 6/group) were administered to 0, 3, 6 or 9 g dry Lentinus edodes/kg bw/day (fresh mushroom equivalents 19.4, 41.9, and 61.4 g/kg bw/day) in the diet for 5 days (Nieminen et al., 2009). These were extremely high doses, i.e. with human equivalent doses of fresh Shitake mushrooms for a 60 kg person being 1164, 2514, and 3684 g/ day. Food and water consumption, plasma clinical chemistry and liver and muscle histopathology were evaluated. Although there were statistically significant, somewhat dose-related decreases of HDL/total cholesterol (mid- and high-dose groups), and statistically significant, somewhat dose-related increases of total protein (low-, mid- and high-dose groups), creatinine kinase (high-dose group) and bilirubin (low-, mid- and high-dose groups) following 5 days of extremely high dry Shiitake mushroom consumption in the diet, small group sizes limits definitive interpretation of statistical significance and dose-dependency. No adverse findings were reported for histopathology. The estimated dietary exposure of 17 mg per day to Shiitake mycelia (§ 3.3) from consumption of PureTaste® protein is approximately 10,000 times less than the low-dose level of dry Shiitake mushroom (i.e. 3 g/kg bw/day) showing minimal toxicity in female mice (males were not tested) in the Nieminen et al. (2009) study.

In a study reported by Grotto et al. (2016), four groups of male Wistar rats received dry and powdered *L. edodes* (shiitake mushroom) reconstituted in water at gavage doses of 100, 400 or 800 mg/kg for 30 days. Reductions in hemoglobin concentration and leukocytes were reported at 400 and 800 mg/kg compared to controls. The authors concluded that the safe daily intake of *L. edodes* determined in this study was 100 mg/kg based on the observed effects on hematological parameters at higher doses. The human equivalent dose of *L. edodes* for a 60 kg person would be 6000 mg/day; therefore, the estimated dietary exposure of 17 mg per day to Shiitake mycelia (§ 3.3) from consumption of PureTaste® protein is more than 350 times less than the safe daily intake level of *L. edodes* identified in this study.

During a 10-week clinical study of the cholesterol-lowering effect of 4 g/day shiitake mushroom powder ingestion (Unpublished Personal Communication, D. Jacobson, J. O. Hill, University of Colorado, 1994 summarized by Levy et al., 1998), 17 of 49 subjects (no demographic details provided) in the treatment arm withdrew from the trial because of either rash (seven subjects) or abdominal discomfort (10 subjects). Two subjects had marked peripheral blood eosinophilia at the time they stopped ingesting mushrooms. However, their eosinophilia resolved after discontinuation of mushrooms. The cause for the findings was unknown; however, food allergy was considered unlikely because the reaction developed after prolonged exposure (Levy et al., 1998). In the absence of additional information, it is difficult to interpret the findings from this limited study report.

Levy et al. (1998) reported on the effect of ingestion of Shiitake mushroom powder on the induction of eosinophilia, changes in eosinophil-active cytokines and eosinophil proteins in blood and stool, or gastrointestinal symptoms. This published study was conducted in 10 normal subjects (9 males and 1 female; average age 40.6 years; range, 31 to 63 years). Exclusion criteria included a history of allergy to mushrooms, disease associated with significant eosinophilia, and gastrointestinal disease. Additional exclusion criteria included baseline blood eosinophil counts greater than 500/mm^3, abnormal serum IgE levels, use of prescription medication (except oral contraceptives), and pregnancy. Four (4) g Shiitake mushroom powder (open label) were ingested daily by each subject for 10 weeks (trial 1), and the same protocol was repeated in these subjects after 3 to 6 months (trial 2). The investigators defined responders as subjects having peak eosinophil counts four or more times their average baseline counts. Each trial had four responders, and trial 2 had one new and three repeat responders. Responders had increased blood eosinophils, serum major basic protein, stool eosinophil-derived neurotoxin, and factors that enhanced eosinophil viability. Anti-Shiitake IgE was not detected, but anti-Shiitake IgG was increased in two responders. Gastrointestinal symptoms coincided with eosinophilia in two subjects. Gastrointestinal symptoms (e.g. abdominal cramping, more frequent and looser stools) and eosinophilia resolved after discontinuing shiitake ingestion. The authors stated that eosinophilic response to Shiitake does not appear to be a typical allergic reaction because of the inability to detect anti-Shiitake IgE and by the delayed and gradual time-course of the response. However, the authors concluded that "the response is likely immune-mediated because it is associated with cytokines that enhance eosinophil viability and elevations in anti-Shiitake IgG in two of the five responders".

Although the results in these unpublished and published clinical trials reported by Levy et al. (1998) show transient effects for consumption of Shiitake mushroom powder at 4 g per day for 10 weeks, this intake level is at least 200-fold higher than the 90th percentile daily estimated intake of 17 mg of Shiitake mycelium from PureTaste® protein dietary consumption (§ 3.3).

Therefore, MycoTechnology concludes that similar adverse effects from PureTaste® protein consumption are highly unlikely.

Nguyen et al. (2017) discussed the clinical features of Shiitake dermatitis in a systematic review of the literature. Nguyen's review identified 50 total reported patient cases (38 males, 12 females; mean age: 44.58 years) of this "rare" cutaneous reaction, and identified that "the majority" of cases resulted after consumption of raw mushrooms (93% of cases were associated with raw, lightly or undercooked mushrooms; Nguyen et al. (2017), Table 2). The authors reported that Shiitake dermatitis "is self-limiting, resolving in approximately 12.5 d without treatment." The authors postulated that a heat-labile b-glucan in the cell walls of Shiitake mushrooms (lentinan) may be responsible for the clinical dermatitis. Experiments by another investigator have shown a presumed association between lentinan exposure and dermatitis by demonstrating a cutaneous response to the consumption of shiitake mushrooms cooked at 100 °C but not to those cooked at 150 °C (Corazza et al., 2015 as cited in Nguyen et al., 2017). Since lentinan would be expected to degrade at 150 °C, heat processing of Shiitake mycelia would seem likely to minimize shiitake dermatitis from PureTaste® protein. As noted in § 2.2, the PureTaste® protein manufacturing process involves thermal deactivation (80°C for 1 minute) and high temperature concentration and spray drying steps (air inlet 250°C; powder outlet 75°C), which is sufficient to denature and deactivate enzymes (§6.3.7) and lentinan (§ 6.3.6). MycoTechnology, Inc. concludes that the rare cutaneous Shiitake dermatitis effect is very unlikely to occur with PureTaste® protein consumption due to heat treatment steps during the manufacturing process, and the low dietary exposure to Shiitake mycelia in PureTaste® protein (17 mg per day at the 90th percentile of intake).

Additional human clinical studies reporting Shiitake dermatitis are summarized in Table 11. As discussed above, MycoTechnology, Inc. concludes that the rare cutaneous Shiitake dermatitis effect is unlikely to occur with PureTaste® protein consumption due to heat treatment steps during the manufacturing process, and to the low dietary exposure to Shiitake mycelia in PureTaste® protein (17 mg per day at the 90th percentile of intake).

Miyaji et al. (2004) assessed the *in vitro* genotoxic and antigenotoxic effects of aqueous extracts of Shiitake mushroom using the Comet assay with Hep-2 cells at test concentrations of 0.5, 1.0, and 1.5 mg/ml and three temperatures (4°, 22° and 60°C). The authors reported a "low level" of genotoxic activity at all aqueous extract test concentrations prepared at 22 ± 2 and 60 °C and two concentrations (1.0 and 1.5 mg/mL) of extract prepared at 4 °C. As cytotoxicity data was not reported for this test and the validation status of the performing laboratory is unknown, the results of this study are considered to be of limited reliability. The International Workshop on Genotoxicity Testing (IWGT) has repeatedly concluded that cytotoxicity could be a confounder of Comet assay results, adding that comet assay results are more reliable if obtained in laboratories with demonstrated proficiency (IWGT, 2015). It is also noted that a standardized and validated regulatory testing guideline for the *in vitro* Comet assay is not available and the OECD guideline for the *in vivo* mammalian alkaline Comet assay did not exist until recently (i.e. OECD Testing Guideline 489; Adopted 29 July 2016). Therefore, Miyaji et al. (2004) predated a standardized and validated Comet assay protocol. Furthermore, the Comet assay is not among the standard battery of *in vitro* tests for genetic toxicity assessment of food ingredients or pharmaceuticals recommended by regulatory authorities (e.g. ICH S2(R1), 2011; EFSA, 2012;

FDA, 2007). In summary, MycoTechnology concludes that this study is of limited relevance to the current safety assessment of Shiitake mycelia in PureTaste® protein.

The same investigators studied the possible "antigenotoxicity" effects of shiitake mushroom extracts via modulation of micronuclei induction after treatment with alkylating agents *in vitro* or *in vivo* (de Lima et al., 2001; Miyaji et al., 2006). These studies are of limited relevance to the current safety assessment.

In an Ames test performed prior to standardized testing guidelines or Good Laboratory Practice (GLP) regulations, a crude ethanol extract of *L. edodes* was reported to have mutagenic activity to tester strains TA100 and TA1535, which are sensitive to base-pair substitutions (von Wright et al., 1982). As neither statistical analysis nor cytotoxicity data were reported, the results of this study are of limited reliability. Additionally, the crude ethanol extract used as the test article is not representative of the *L. edodes* mycelia used in the production of PureTaste® protein. Based on this information,

In consideration of the information above, MycoTechnology concludes that a genotoxic hazard is not likely to occur upon extremely low dietary exposure to Shiitake mycelia in PureTaste® protein.

In its Scientific Opinion on the safety of "Lentinus edodes extract" (Lentinex®) as a Novel Food ingredient, the EFSA Panel on Dietetic Products, Nutrition and Allergies concluded that "owing to the fermentative production of the novel food ingredient [Lentinex®] from the mycelium and the final application of a heat-induced sterilisation step, adverse effects reported after the consumption of the fruiting body of the Shiitake mushroom are not considered relevant" (EFSA, 2010). MycoTechnology. similarly concludes that any adverse effects reported after the consumption of the fruiting body of the Shiitake mushroom are not likely to occur upon exposure to low amounts (<0.1%) of heat-inactivated Shiitake mycelia in PureTaste® protein.

6.3.6 Exposure to lentinan ((1-3)-β-D-glucan) in PureTaste® protein

The composition of Shiitake mushroom (and mycelia) cell walls includes the polysaccharide lentinan ((1-3)-β-D-glucan) (Bisen et al., 2010). This polysaccharide occurs only in fungi, and has been asserted to have beneficial immunomodulatory effects in humans (EFSA, 2010; Bisen et al., 2010; Lull et al., 2005).

The safety of lentinan exposure from a 60°C hot water aqueous extract of Shiitake mycelia, called Lentinex®, as a food was addressed in the European approval as a novel food (EFSA, 2010). The EFSA (2010) Scientific Opinion confirmed that lentinan, as a component of Lentinex®, was safe for consumers at a level of 41.7 µg/kg/day. This intake level is the equivalent of approximately 2.5 mg/day of lentinan for a 60 kg person. As reported in the EFSA (2010) Opinion and European Commission Decision of 2 February 2011Annex 1 (EC, 2011), the mycelial extract from *L. edodes* contains 2% dry matter which is comprised of β-glucan lentinan (0.8 – 1.2 mg/ml), free glucose, protein, and N-containing constituents. Conservatively assuming that the shiitake mycelia in PureTaste® protein contains no more than 2% lentinan, a 17 mg daily dietary exposure of shiitake mycelia in PureTaste® protein (§ 3.3) is equivalent to a

dietary exposure to lentinan from PureTaste® protein of 0.34 mg lentinan/day. Therefore, the dietary exposure to lentinan from PureTaste® protein using a worst-case scenario is approximately 7-fold lower than the amount determined to be safe in the EFSA Scientific Opinion (EFSA, 2010). Consequently, MycoTechnology concludes that the calculated margin of exposure for lentinan from dietary exposure of Shiitake mycelia in PureTaste® protein compared to the dose confirmed as safe by EFSA is considered to be safe.

The toxicity of lentinan has been evaluated in short-term, subchronic, and chronic studies via i.v. administration to rats, monkeys, and dogs (Ishi et al., 1980; Shimazu et al., 1980; Sortwell et al., 1981; Chesterman et al., 1981). Although some treatment-related effects were reported by the authors, these i.v. studies via were not considered relevant to the safety assessment of the Lentinex® novel food ingredient by EFSA (2010) and are not considered relevant to the current safety assessment of lentinan from dietary exposure of Shiitake mycelia in PureTaste® protein.

Cozens et al. (1981a,b,c,d) reported on the effects of lentinan on fertility, reproductive performance, *in utero* fetal development and postnatal development of rats and rabbits that received once daily i.v. administration during mating, gestation and/or lactation at doses of 0.01, 0.1, 0.3, 1.0, or 5.0 mg/kg bw/day. There was no evidence that treatment of the F0 parents adversely affected *in utero* embryonic or fetal development, post-weaning development or reproductive performance of the F1 offspring; however, in male rats administered 1.0 mg/kg/day, the authors reported "clear evidence of gonadal damage and impairment of reproductive capacity", with the effect less marked at 0.1 mg/kg bw/day and much reduced at 0.01 mg/kg bw/day (Cozens et al., 1981a). A treatment-related increase in mean spleen weights was also reported the three studies performed in rats (Cozens et al., 1981a,c,d). These four i,v, studies were not considered relevant to the safety assessment of the Lentinex® novel food ingredient by EFSA (2010) and are not considered relevant to the current safety assessment of lentinan from dietary exposure of Shiitake mycelia in PureTaste® protein.

No reports of adverse effects of lentinan via dietary exposure were identified in the literature. MycoTechnology concludes that the low level of dietary exposure to lentinan from dietary exposure to Shiitake mycelia in PureTaste® protein supports a conclusion of safety for PureTaste® protein.

6.3.7 Safety of Fungal Enzymes

The use of fungal enzymes to modify and improve food products is well established. *Aspergillus*, a genus of filamentous fungus closely related to the filamentous fungus genus *Lentinula*, has been identified as a source of enzymes used in industrial food processing applications, several of which are recognized as GRAS for use in food (Soares et al., 2012; FDA 2018a; FDA, 2018b). *Lentinula edodes* is also known to secrete a number of these fungal enzymes with GRAS status, such as pectinase, cellulase, amylase, laminarinase (beta-*glucanase*), and xylanase (Mata et al., 2016; Soares et al., 2012). In particular, it is known that Shiitake mycelia constitutively express laccases, and expression of laccases in Shiitake mycelia may be upregulated or stimulated by the presence of lignin-derived phenols and or polymeric lignin materials (Matjuškova et al., 2017). MycoTechnology concludes that there are no safety concerns associated with these enzymes commonly used for food processing that may be present

during the manufacturing process for PureTaste® protein.

Consistent with the literature, testing has confirmed the secretion of endogenous laccase during the manufacturing process for PureTaste® protein. Activity of the laccase enzyme is attributed to the improved organoleptic properties (via oxidation of volatile taste and aroma compounds) of the input pea and rice protein raw materials (see Section 2.2). However, the manufacturing conditions used for concentration and spray-drying steps of the manufacturing process as described in § 2.2 are consistent with conditions that are known to denature and deactivate enzymes (i.e. the fermentation process is terminated by heat treatment followed by an evaporator/concentration step; a thermal deactivation step is carried out at 80 °C for 1 minute, followed by spray drying for 1 to 3 minutes (air inlet 250 °C; powder outlet 75 °C). Testing for residual laccase enzyme activity in PureTaste® protein after termination of fermentation was performed according to published methodology[1] and confirmed that no residual laccase enzyme activity was present (MycoTechnology; unpublished data on file). From this information, MycoTechnology concludes that any enzymes secreted by the shiitake mycelia during the manufacturing process for PureTaste® are inactivated in PureTaste® protein.

[1] https://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-laccase.html

Table 10 - Shiitake Mushroom Mycelia, Safety Evidence from Human Studies

Study Title (reference)	Study Design	Outcome Measures/Endpoint	Results
Consuming *Lentinula edodes* (Shiitake) Mushrooms Daily Improves Human Immunity (Dai, 2015)	A randomized dietary intervention study; to determine whether consumption of whole, dried *Lentinula edodes* (shiitake) mushrooms could improve human immune function.	Fifty-two healthy males and females (21-41 years), participated in a 4 weeks parallel group study, consuming either 5 or 10 g of shiitake mushrooms daily.	Conclusion: Dosage was well tolerated. Safety and adverse events were not reported in the study.
Safety of orally administered *Lentinula edodes* mycelia extract for patients undergoing cancer chemotherapy: a pilot study. (Yamaguchi, 2011)	Observational study to investigate safety of *Lentinula edodes* on quality of life (QOL) and the immune response in patients undergoing cancer chemotherapy.	Seven patients were studied in total. The patients were undergoing post-operative adjuvant chemotherapy for breast cancer (n = 3) or gastrointestinal cancer (n = 2), or were receiving chemotherapy to prevent recurrence of gastrointestinal cancer (n = 2). The first course of treatment was chemotherapy alone and the second was chemotherapy plus concomitant administration of *Lentinula edodes* extract. Outcome measures: Adverse events and changes in the QOL score were evaluated during the study period.	Conclusion: Treatment with *Lentinula edodes* extract with chemotherapy is safe and no adverse events were attributable to *Lentinula edodes* extract.
Oral Administration of *Lentinula edodes* Mycelia Extract for Breast Cancer Patients Undergoing Postoperative Hormone Therapy. (Suzuki, 2013)	This was a 12-week, single-arm, open-label study. All subjects first entered a 4-week observation period, followed by an 8-week period of oral *Lentinula edodes* extract ingestion at 1800 mg daily. Preparation: *Lentinula edodes* mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran.	This study investigated the influence of *Lentinula edodes* on the quality of life (QOL) and immune response in breast cancer patients undergoing postoperative adjuvant hormone therapy. Twenty patients* were studied in total. They received only hormone therapy in the first 4 weeks followed by hormone therapy and *Lentinula edodes* (1800 mg/day) during the next 8 weeks. *As subjects are breast cancer patients, this suggests strongly	Conclusion: No subjects reported any serious adverse events. Safety of oral administration of *Lentinula eododes* Mycelia Extract was supported by this study.

Study Title (reference)	Study Design	Outcome Measures/Endpoint	Results
	Medium containing the mycelia was incubated in hot water, and then the soluble fraction was dried and used as *Lentinula edodes* extract.	that all subjects are female.	
Efficacy of Orally Administered *Lentinula edodes* Mycelia Extract for Advanced Gastrointestinal Cancer Patients Undergoing Cancer Chemotherapy: a Pilot Study. (Okuno, 2011).	This study was conducted as an 8-week single-group open label study. During the study period, each subject took two courses of chemotherapy. *Lentinula edodes* extract was orally ingested during the second course at a dose of 1800 mg/day for 4 weeks. Preparation: Lentinula edodes mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran. Medium containing the mycelia was incubated in hot water, and then the soluble fraction was dried and used as LEM	This study investigated the influence of *Lentinula edodes* mycelia extract (LEM), an oral immunomodulator, on immune function and adverse events from chemotherapy. Subjects comprised 1 gastric (male) and 7 colorectal (5 females, 2 males) cancer patients. Ages ranged from 52 to 71. The first course of treatment was chemotherapy alone and the second was chemotherapy plus concomitant administration of LEM. Adverse events and interferon (IFN)-γ production by CD4+ T, CD8+ T and CD56+ NK/NKT cells were evaluated at the end of each course.	Conclusion: Concomitant use of *Lentinula edodes* Mycelia Extract with chemotherapy can decrease the incidence of adverse effects from cancer chemotherapy among patients with advanced cancer. Safety of *Lentinula edodes* is supported by this study.
Dietary supplementation with rice bran fermented with *Lentinus edodes* increases interferon-γ activity without causing adverse effects: a randomized, double-blind, placebo-controlled, parallel-group study. (Choi, 2014)	A randomized, double-blind, placebo-controlled, and parallel-group investigated the hypothesis that dietary supplementation with rice bran fermented with *Lentinus edodes* (rice bran exo-biopolymer, RBEP), a substance known to contain arabinoxylan, enhances natural killer (NK) cell activity and modulates cytokine production in healthy adults.	Dosage: 80 healthy (non-pregnant/lactating adults, aged 25-70 years old comprised of 31 females and 49 males) participants were randomly assigned to take six capsules per day of either 3g RBEP or 3g placebo for 8 weeks.	Conclusion: This well designed RCT demonstrates the safety of rice bran fermented with *Lentinus edodes*. No adverse events were reported.

Study Title (reference)	Study Design	Outcome Measures/Endpoint	Results
Evaluation of host quality of life and immune function in breast cancer patients treated with combination of adjuvant chemotherapy and oral administration of *Lentinula edodes* mycelia extract (Nagashima et al., 2013)	Ten breast cancer patients with nodal metastases receiving surgery were enrolled in this study. This was an open-label trial with a single group. Subjects were treated with two courses of FEC75 chemotherapy for 3 weeks as one course. The first course comprised FEC75 chemotherapy alone, whereas the second course used LEM in combination with FEC.	Dosage: *Lentinula edodes* mycelia extract (LEM; 1800 mg/day by mouth) was administered for 21 days during the second course.	The authors concluded that concomitant use of *Lentinula edodes* mycelia extract with FEC75 therapy can maintain host QOL and immune function, and offer important implications for an application of LEM as a useful oral adjuvant to anthracycline-based chemotherapies. No adverse events associated with LEM treatment were reported.
Lentinula edodes mycelia extract plus adjuvant chemotherapy for breast cancer patients: Results of a randomized study on host quality of life and immune function improvement. (Nagashima et al., 2017)	A randomized double-blind study was conducted to evaluate the effectiveness of Lentinula edodes mycelia extract (LEM), which is an oral biological response modifier (BRM) medicine for cancer patients as such an adjuvant. A total of 47 breast cancer patients who were scheduled to receive postoperative adjuvant anthracycline-based chemotherapy were enrolled in the study.	Dosage: *Lentinula edodes* mycelia extract (LEM; 1800 mg/day by mouth) was ingested daily over two 3-week courses, for a total of 6 weeks	The authors concluded that LEM appears to be a useful oral adjuvant for patients receiving anthracycline-based chemotherapy. No adverse events associated with LEM treatment were reported.

Table 11 – Human Clinical Evidence of Shiitake Mushroom Dermatitis

Study Title (reference)	Study Design	Outcome Measures/Endpoint	Results
Shiitake *(Lentinus edodes)* dermatitis (Nakamura, 1992).	Retrospective study (from 1974 to 1991) to examine 51 patients with shiitake dermatitis due to the intake of half-baked raw shiitake.	Shiitake dermatitis in 41 men and 10 women (15-76 years) was analyzed retrospectively. Dosage: varies and not reported.	All patients (n=51) had truncal involvement of Shiitake dermatitis. Extremities, neck, face and head were involved in decreasing order of frequency. No patients had digestive or nervous system symptoms, nor were the mucous membranes affected. Conclusion: Shiitake dermatitis can be avoided by eating sufficiently boiled raw shiitake.
Flagellate dermatitis after consumption of Shiitake Mushrooms. (Czarnecka et al, 2014).	Case report, investigated Flagellate dermatitis occurrence in patients who had eaten Shiitake mushrooms.	A 55-year-old German patient (male) was diagnosed with Flagellate dermatitis after eating Shiitake mushroom at a restaurant. Dosage: not reported.	Examination revealed severely-itching parallel, striped whiplash-like infiltrated erythema with severe itching on the trunk and upper extremities. In addition, there were papulovesicles on urticarial erythemas on the shoulders. Conclusion: Flagellate dermatitis could be avoided by eating adequately cooked shiitake mushrooms.
Flagellate mushroom (Shiitake) dermatitis and photosensitivity. (Hanada, 1998).	Case report, investigated Flagellate skin lesions in a patient after eating the mushroom *Lentinus edodes.*	A 44-year-old man was diagnosed with Flagellate dermatitis after eating Shiitake mushroom.	This patient was diagnosed with flagellate skin lesions on his trunk after eating *L. edodes*. The patient also developed photosensitive lesions on skin exposed to sunlight . Analysis of the case histories of 94 Japanese patients with shiitake dermatitis revealed that 44 (47%) cases developed dermatitis on the skin exposed to sunlight. Conclusion: Despite the high consumption of Shiitake mushrooms, the incidence of

Study Title (reference)	Study Design	Outcome Measures/Endpoint	Results
			severe allergic reactions appears to be very low.
Systemic allergic contact dermatitis due to consumption of raw shiitake mushroom. (Kopp, 2009).	Case report, investigated the effect of raw Shiitake mushroom (*Lentinus edodes*) on contact dermatitis.	A 52-year-old man who developed a generalized pruritic papulovesicular eruption 2 weeks after daily consumption of uncooked shiitake mushrooms. Prick-to-prick and scratch tests with uncooked mushrooms resulted in an eczematous reaction at 24 h that peaked at 72 h and persisted for 1 week.	This patient had systemic allergic contact dermatitis due to consumption of raw shiitake mushroom. Conclusion: Shiitake dermatitis could be avoided by eating adequately cooked or processed shiitake mushrooms.
Eosinophilia and gastrointestinal symptoms after ingestion of shiitake mushrooms. (Levy, 1998)	An open label, observational study; investigated whether ingestion of shiitake mushroom powder (freeze dried powder) induces eosinophilia or symptoms.	Dosage: Each capsule contained 250 mg of Shiitake mushroom powder (freeze dried). 10 Subjects (9 men and 1 woman*, with an average age of 40.6 years, range of 31 – 63 years) ingested 4 grams (16 capsules = 4 medium sized mushrooms) of freeze dried shiitake powder daily for up to 10 weeks (trial 1) or 3 to 6 months (trial 2). *The woman was of child-bearing age and she was requested to use contraception to prevent pregnancy during the study.	Conclusion: At 4g per day of raw shiitake mushrooms some abdominal cramping and eosinophilia were reported.

6.4 Safety of Additional PureTaste® Raw Materials

6.4.1 Maltodextrin

Maltodextrin ($(C_6H_{10}O_5)n$, CAS Reg. No. 9050-36-6) is a non-sweet nutritive saccharide polymer that consists of D-glucose units linked primarily by [alpha]-1-4 bonds and a dextrose equivalent (D.E.) of less than 20. It is prepared as a white powder or concentrated solution by partial hydrolysis of corn starch, potato starch, or rice starch with safe and suitable acids and enzymes, meeting specifications in Food Chemicals Codex, 3d ed., 3d supp. (1992), p. 125. Maltodextrin as used in PureTaste® is listed in 21 CFR 184.1444 as affirmed as GRAS. This ingredient is used in PureTaste® consistent with current Good Manufacturing Practice (cGMP, 21 CFR 184.1444)

6.4.2 Carrot Powder

The carrot powder used in PureTaste® is composed of 100% organic carrots. There is common knowledge of a long history of human consumption of carrot, both fresh and cooked. Traces of carrot have been discovered at archeological sites (pre-historic lake dwellings in Switzerland), and carrot was included in the listing of vegetables in the Babylonian royal gardens (8th century B.C.) (Davidson, 1999). Carrots were also among the vegetables eaten by early Mediterranean civilizations in Sumer and Egypt (around 3000 B.C.) (McGee, 1984). These early references likely refer to carrot's use as an aromatic herb rather than root vegetable (Davidson, 1999).

The first description of the modern carrot was in the early 12th century in Andalusia. Carrots reached Western Europe and Britain in the 14th century. The violet/purple carrot was grown in Italy by the early 1300s (Schneider, 2001). The early descriptions of carrots were of two types, purple/red and pale yellow/white. The orange carrot first appeared in the 17th century and soon dominated carrot production. Cultivated carrots were brought to the New World before 1565, likely by the Spanish and were then adopted by Native Americans (Davidson, 1999). Based on the long history of use of carrots as a food product and the minimal amount present in PureTaste® this ingredient is not expected to pose a safety risk from consumption of PureTaste®.

6.4.3 Antifoam

The antifoam (TRANS-4062) used in the production of PureTaste® is made from vegetable oil, which is a safe and suitable food processing aid. TRANS-4062 is considered a secondary direct food additive according to the Code of Federal Regulations, Title 21, and is compliant with 21 CFR 173.340(a)(2). It is degraded during the production process and residues are not expected in the final food PureTaste® product.

6.5 Allergenicity Assessment for PureTaste® Protein

MycoTechnology uses an allergen control program to ensure that the facility has evaluated processes and the premises to mitigate with proper use, storage and labeling any risk of allergen related food safety incidents. MycoTechnology also maintains a complete HACCP program including personnel training as it relates to allergens in its facility.

Although at least 170 foods have been reported to cause allergic reactions, there are only eight major food allergens – milk, egg, peanut, tree nuts, wheat, soy, fish and crustacean shellfish that are responsible for most of the serious food allergy reactions in the US (FARE, n.d.). It is estimated that up to 15 million Americans have food allergies, including 5.9 million children under the age of 18 years (FARE, n.d.). Each year in the U.S., it is estimated that anaphylaxis to food results in 30,000 emergency room visits, 2,000 hospitalizations, and 150 deaths (FDA CFSAN, 2018).

MycoTechnology acknowledges that PureTaste® does not contain any milk, egg, fish, crustacean shellfish, tree nuts, peanuts, soybeans, or wheat, the major food allergens under the U.S. Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). For a more extensive review of potential allergens which may be present in PureTaste® please refer to Table 12.

PureTaste® is primarily composed of pea and rice protein. The following discussion is limited to the potential allergenicity of pea and rice protein.

Cereal grains have been reported to be a cause of food allergies. Rice specifically has been reported as an inhalant or contact allergen, rather than a food allergen (GRAS 609). In Japan, the prevalence of IgE- mediated rice allergy is approximately 10% in atopic subjects; however, it is much lower in Europe and the US, <1% (Besler, 2001). As indicated in GRAS notice 609, allergy to rice is rare and the frequency may differ among varying populations. GRAS notice 609 speaks to the use of rice as one of the earliest solid foods given to babies due to its intrinsic hypoallergenic nature.

Peas are part of a family of plants called legumes, which also include alfalfa, clover, beans, lentils, mesquite, carob, soybeans, peanuts, tamarind, and wisteria (Grains & Legumes Nutrition Council, 2017). Allergenic response to legumes may range from mild skin reactions to life-threatening anaphylactic reactions (Verma, 2013). Legumes have been reported to be a cause of food allergies, especially peanut allergy (Sicherer, 1999). Peanuts and soybeans are the major legume allergies in the United States, United Kingdom, and Japan, while lentils, chickpeas and pea allergies are more common in the Mediterranean area and India (Sanchez-Monge, 2004). Peanut and/or Tree Nut allergy affects approximately 1.1% of the general population, or about 3 million Americans (Sicherer, 1999). Legume cross-reactivity varies by region - while extensive cross-reactivity among lentil, chickpea and pea were reported in the Mediterranean area, only minimal cross-reactivity among legumes (mainly reported between peanut and soy) have been reported in North America (Abrams, 2015). As indicated in GRAS notice 608 for pea protein, the available information for allergenicity of pea proteins indicates that persons with peanut allergies may be sensitive to peas; however, allergic reactions to peas appear to be rare and may fluctuate among different populations.

The low anticipated allergenicity concern with the pea and rice proteins in PureTaste® can be mitigated by the listing of the common name of this product on a label, which is pea and rice protein fermented with shiitake mycelia. As such, appropriate labelling by use of the common name of PureTaste® does not hinder the safety and GRAS status that is the subject of this notification.

Table 12 – Absence of Allergens in PureTaste®

Component	Present in the product	Present in other products produced on the same line	Present in the same plant
1. Barley, Rye, Oats	NO	NO	NO
2. Celery (not including seeds)	NO	NO	NO
3. Corn	NO	NO	NO
4. Egg or egg product	NO	NO	NO
5. Fish	NO	NO	NO
6. Mille & Mille by-product	NO	NO	NO
7. Monosodium Glutamate (MSG)	NO	NO	NO
8. Peanuts or peanut products	NO	NO	NO
9. Seeds (Poppy, Sunflower, Cottonseed)	NO	NO	NO
10. Sesame Seeds	NO	NO	NO
11. Shell Fish & Crustaceans	NO	NO	NO
12. Soybean Oil (excluding refined soy oil)	NO	NO	NO
13. Soybean (not including oil)	NO	NO	NO
14. Sulphites (enter maximum ppm)	NO	NO	NO
15. Tree Nuts	NO	NO	NO
16. Wheat or wheat products	NO	NO	NO
17. Gluten <l O ppm	NO	NO	NO
18. Yellow 5 (Tartrazine)	NO	NO	NO
19. Animal Fat	NO	NO	NO
20. Grains containing gluten	NO	NO	NO
21. Mustard	NO	NO	NO
22. Lupin	NO	NO	NO
23. Lactose	NO	NO	NO

6.6 Safety Narrative Summary

PureTaste® is a product manufactured with fermentation technology composed of proven safe food ingredients with a long history of common use and regulatory acceptance in the worldwide food supply. MycoTechnology Inc. has concluded the Generally Recognized as Safe (GRAS) status of PureTaste® based on the following:

- The PureTaste® protein is manufactured within a BRC inspected facility under current Good Manufacturing Practices (cGMPs) and meets appropriate food grade specifications.
- The identity of PureTaste® protein has been clearly defined and confirmed through scientific data and information.
- Both pea and rice proteins, the main constituents of PureTaste® protein, have been consumed for centuries through the consumption of peas and rice and through the consumption of the protein products as affirmed GRAS (GRNs 581, 608, 609 and 788).
- All ingredients included in PureTaste® protein, including Shiitake mycelia, are concluded to be safe for use in food at inclusion levels and food categories proposed for PureTaste®.
- No PureTaste® protein raw materials are listed as major allergens (Food Allergen Labeling and Consumer Protection Act of 2004).
- The fermentation organism used to produce PureTaste® protein, *Lentinula edodes* (shiitake mushroom), is commonly consumed as food and there are no identified hazards associated with the use of shiitake mycelia described herein
- Following fermentation, the absence of live shiitake mycelia or fungal enzymes in the final PureTaste® protein is achieved through multiple heat treatment steps and thermal deactivation
- Consumption data and information pertaining to the individual proposed food uses of pea protein and rice protein were used to estimate the all-person and all-user intakes of PureTaste® for the U.S. population. In summary, the mean and 90th percentile intake of PureTaste® protein for "all users" is estimated to be 10.3 g/person/day and 17.3 g /person/day, respectively. The maximum intake associated with the use of PureTaste® as a supplemental protein in sports nutrition applications is estimated as 30 g/person/day.
- PureTaste® will substitute for other protein sources in the diet, and thus will not increase the overall increase of consumption of protein in the diet.
- The conservative estimate of PureTaste® protein intake is below current FDA and IOM recommendations for protein in the diet further supporting a conclusion of safety.
- The weight of evidence from reliable published toxicological and human clinical studies using the same or closely-related (e.g. Shitake mycelial extracts, reconstituted powdered Shiitake mushroom) test materials as those components included in PureTaste® support a conclusion that no adverse health effects are expected at dietary intake levels which are proposed for PureTaste® protein.

6.7 Conclusion of the GRAS Panel

At the request of MycoTechnology, Inc., a panel of experts, (the "GRAS Panel"), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, independently and collectively critically evaluated the information summarized in this GRAS dossier on the safety of pea and rice protein fermented by Shiitake mycelia. The GRAS Panel also considered other published data and information deemed appropriate. The GRAS Panel consisted of Professor Michael W. Pariza, Ph.D., chair, Madhusudan Soni, Ph.D., and Professor Joseph F. Borzelleca, Ph.D.

Following their critical evaluation, the GRAS Panel unanimously concluded that the proposed use as a nutritional food ingredient of the pea and rice protein fermented by Shiitake mycelia, produced consistent with current Good Manufacturing Practices (cGMP) and meeting the food ingredient specifications described herein, is safe and suitable and GRAS based on scientific procedures.

The GRAS Panel unanimously opined that other qualified scientists reviewing the same body of information would concur with these conclusions.

The signed opinion of the GRAS Panel is provided as Appendix 1.

Part 7: 170.255 List of Supporting Data and Information

All the references used in this GRAS including animal and human studies are generally available and listed below.

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GRAS Panel Statement on the Generally Recognized As Safe (GRAS) Conclusion for Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein)

January 31, 2019

Background

MycoTechnology, Inc. intends to market pea and rice protein fermented by Shiitake mycelia (PureTaste®) as a protein source, formulation aid and texturizer in conventional foods. A panel of independent experts (the "GRAS Panel"), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was convened to conduct an independent, critical and comprehensive evaluation of the available technical and safety information on PureTaste® protein, and to determine if the proposed use of PureTaste® protein as a nutritional food ingredient is safe and suitable and can be considered Generally Recognized As Safe (GRAS) based on scientific procedures in accordance with 21 CFR §170.30(a) and (b). The GRAS Panel consisted of Professor Michael W. Pariza, Ph.D., chair, Madhusudan Soni, Ph.D., and Professor Joseph F. Borzelleca, Ph.D.

A technical dossier, "*Generally Recognized as Safe (GRAS) Determination for the Use of Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein) in Conventional Foods*" (issued 29 January 2019), was prepared on behalf of MycoTechnology, Inc. and made available to the GRAS Panel. This document represents an update to and replacement of a previous dossier regarding the GRAS conclusion for "fermented shiitake, pea and brown rice protein" reviewed by the Panel in March 2018. The current dossier contains data and information on the characterization, method of manufacture, product specifications, composition and stability, analytical testing, proposed levels of use, consumer exposure estimates, and safety assessment of PureTaste® raw materials (including pea protein, rice protein, and Shiitake mushroom mycelia). The GRAS Panel, independently and collectively, critically evaluated this document and other published information deemed appropriate, and convened by telephone on 31 January 2019.

Following their critical evaluation, the GRAS Panel unanimously concluded that the proposed use of pea and rice protein fermented by Shiitake mycelia (PureTaste®) as a nutritional food ingredient, manufactured consistent with current Good Manufacturing Practices (cGMPs) and meeting appropriate food-grade specifications, is safe and suitable and GRAS based on scientific procedures. The GRAS Panel unanimously opined that other qualified scientists reviewing the same body of information would concur with these conclusions.

Conclusion

We, the independent qualified members of the GRAS Panel, have individually and collectively critically evaluated the data and information summarized in the technical dossier, "*Generally Recognized as Safe (GRAS) Determination for the Use of Pea and Rice Protein Fermented by Shiitake Mycelia (PureTaste® Protein) in Conventional Foods*" (29 January 2019), and other published data and information that we deemed pertinent to the safety of the proposed uses of pea and rice protein fermented by Shiitake mycelia (PureTaste®) in food. We unanimously conclude that the proposed use of PureTaste® protein as a nutritional food ingredient, produced consistent with current good manufacturing practices (cGMPs) and meeting appropriate food grade specifications, is safe and suitable and Generally Recognized As Safe (GRAS) based on scientific procedures.

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

(b) (6)

Professor Michael W. Pariza, Ph.D., Chair
University of Wisconsin

31 January 2019
Date

(b) (6)

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth School of Medicine

31 January 2019
Date

(b) (6)

Madhusudan Soni, Ph.D.
President, Soni & Associates, Inc.

31 January 2019
Date

Bonnette, Richard

Subject: FW: Your submission to the FDA GRAS notification program - Uses in meat and poultry (USDA)

From: Nicole Berzins <nberzins@mycotechcorp.com>
Sent: Wednesday, February 27, 2019 10:37 AM
To: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Subject: RE: Your submission to the FDA GRAS notification program - Uses in meat and poultry (USDA)

Richard,

Thank you for the clarification.

We would like to remove all USDA category information to support the progression of the dossier.

We will prepare the information for later submission review for USDA food categories.

I have reached out to dr. abley to determine details required.

Nicole

Nicole Berzins

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Email: nberzins@mycotechcorp.com **Website:** www.mycotechcorp.com