Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
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, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I 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)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

3b. 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)*: _____

PART II INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Yuri Gleba, Ph.D.	Chief Executive Officer

Company *(if applicable)*: Nomad Bioscience GmbH

Mailing Address *(number and street)*: Biozentrum Halle, Weinbergweg 22

City	State or Province	Zip Code/Postal Code	Country
Halle/Saale	Saxon - lt	-	Germany

Telephone Number	Fax Number	E-Mail Address
49 345 1314 2606	49 345 1314 2601	gleba@nomadbioscience.com

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

Name of Contact Person	Position
Kristi O. Smedley, Ph.D.	Sponsor's US Regulatory Representative

Company *(if applicable)*: Center for Regulatory Services, Inc.

Mailing Address *(number and street)*: 5200 Wolf Run Shoals Rd.

City	State or Province	Zip Code/Postal Code	Country
Woodbridge	Virginia	22192	United States of America

Telephone Number	Fax Number	E-Mail Address
70 -	70 -	smedley@cf - ices.com

PART III GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

THAUMATIN II

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

☐ Electronic Submission Gateway

☒ Electronic files on physical media with paper signature page

☐ Paper

If applicable give number and type of physical media

Submission consists of one (1) CD containing electronic files of GRAS Notice

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☒ a) GRAS Notice No. GRN 910

☐ 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)* GRN 738; GRN 775

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

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

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

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, see attached Designation of Confidential Information

☐ 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

PART IV INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

THAUMATIN II is a plant-made flavor modifying product comprised of the protein Thaumatin II. Depending on its concentration in a food or beverage matrix, it can potentiate desirable flavors and/or mask undesirable flavors. THAUMATIN II is produced by a plant-based process described in GRN 910, GRN 738 and GRN 775. The sole active ingredient, Thaumatin II protein, is (a) identical in amino acid sequence to commercially available thaumatin II (a.k.a. thaumatin 2) extracted from the fruit of the Katemfe bush; (b) a simple polypeptide with no glycosylation or other non-natural post-translational modifications; and (c) produced in high purity. Extracted and recombinant thaumatins are allowed for use in multiple foods and beverages at application rates up to 150 ppm (mg/kg or mg/L) in the USA (FEMA GRASa 3732 and FEMA GRASa 3814). The same application rates are envisioned for THAUMATIN II as those for commercially available products. THAUMATIN II is not intended for use in infant formulas. This Notice excludes uses of THAUMATIN II in USDA/FSIS-regulated food products because the information to support suitability is such products is currently under development.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

PART V IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used (*CAS, EC*)	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
	in ll CAS no. and UniProt entry no			lant, recombinant	
	in" consisting of single proteins or mixtures is CAS No. 5385 - inant TH - is CAS No. 55385				

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s)),* and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

Detailed information regarding the identity, manufacturing process, functionality, uses and safety of the notified substance is incorporated in the Notice documents provided.

3. Synonyms

Provide as available or relevant:

	i

PART VI OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete check all that apply)

- ☒ Any additional information about identity not covered in Part V of this form
- ☒ Method of Manufacture
- ☒ Specifications for food-grade material
- ☒ Information about dietary exposure
- ☒ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☒ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☒ Comprehensive discussion of the basis for the determination of GRAS status
- ☒ Bibliography

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

PART VII SIGNATURE

1. The undersigned is informing FDA that NOMAD BIOSCIENCE GMBH *(name of notifier)*

has concluded that the intended use*(s)* of THAUMATIN II *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☐ _____ *(name of notifier)* agrees to make the data and information that are the basis for the determination 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.

_____ *(address of notifier or other location)*

_____ *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☒ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ---------- *(GRAS Affirmation Petition No.)*

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Kristi Smedley Digitally signed by Kristi Smedley DN: cn=Kristi Smedley, o=Center for Regulatory Services, Inc., ou, email=smedley@cfr-services.com, c=US Date: 2020.03.20 09:39:39 -04'00'	Kristi O. Smedley, Ph.D.	03/20/2020

PART VIII 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)
	GRN for THAUMATIN II as a flavor modifier for use in foods and beverages (PDF of Notification	issio
	THAUMATIN II GRN Reference - Internal Review Only (PDFs of All Cited Reference - lication	issio

OMB Statement: Public reporting burden for this collection of information is estimated to average 150 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, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (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.

NOMAD BIOSCIENCE GmbH
Biozentrum Halle
Weinbergweg 22
D-06120 Halle/Saale
Germany
Tel. 49 345 1314 2606
Fax. 49 345 1314 2601

20 March 2020

Susan Carlson, Ph.D.
Director, Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: GRAS Notice for THAUMATIN II Flavor Modifier

Dear Dr. Carlson,

Nomad Bioscience GmbH ("Nomad"; "Notifier") is submitting this GRAS Notice for its **THAUMATIN II flavor modifier product,** which contains the protein Thaumatin II as its sole active ingredient. Nomad has concluded, under FDA's Final Rule pertaining to 21 CFR 170 (August 17, 2016), that its THAUMATIN II product containing the naturally occurring protein Thaumatin II produced recombinantly in plants, including the species *Nicotiana benthamiana,* is Generally Recognized as Safe (GRAS) for use as a food/beverages flavor modifier.

This Notice follows Notifier's GRN 738 for THAUMATIN (containing thaumatin I and/or thaumatin II) produced in edible plant species (e.g., spinach, leafy beet and lettuce) for use as a low-calorie, high-intensity sweetener, for which Nomad received from CFSAN a "No Questions" letter on 18 April 2018. GRN 738 established THAUMATIN as a GRAS sweetener in the USA. The present Notice also follows Notifier's GRN 910 for "THAUMATIN II sweetener manufactured in *Nicotiana benthamiana*" (filed by CFSAN on March 12, 2020). The resultant Thaumatin II protein produced in the non-food plant species *N. benthamiana* was shown to have the same molecular identity and sweetening properties as thaumatin II from other sources. The equivalence of identity and functionality of thaumatins produced by Notifier in multiple plant hosts led to favorable comparisons of the safety of Notifier's product to extracted and recombinant thaumatins already marketed as acceptable flavor additives. The data provided in Notifier's GRAS notices and the extensive public record on the safety of thaumatins from multiple sources, enabled us to use scientific procedures to reach a GRAS conclusion about our THAUMATIN II sweetener product regardless of the manufacturing host used.

Prior to Notifier's GRN 738, the GRAS status of thaumatins in the USA pertained to the use of these proteins only as flavor modifiers, as specifically described in FEMA GRASa No. 3732 (Oser 1984) for natural (extracted) thaumatins, and in FEMA GRASa 3814 (Smith 1996) for recombinant versions of these proteins. In the present Notice, Notifier provides additional functional information showing that its THAUMATIN II product is also a flavor modifier, impacting perception of sour, bitter and savory flavors in addition to sweetness. Results of independent sensory evaluation studies conducted by qualified organizations are included in this submission to support Notifier's flavor modification claims.

The flavor-modifying product THAUMATIN II described in this Notice contains the protein Thaumatin II as the only active ingredient, which is produced recombinantly by Notifier's proprietary manufacturing technology using the host species *Nicotiana benthamiana*. Use of this host plant and its advantages, the process employed to express and purify Thaumatin II protein from this species, the molecular characteristics of Thaumatin II, and the impurity profile of the resultant final product, were described in detail in GRN 910. The same THAUMATIN II product used in the studies of sweetness and sugar-replacement potential was used in the studies of flavor modification described herein. Consequently, for consistency, some sections of this Notice are reproduced from GRN 910. In addition, some technical properties already discussed in GRN 738 and/or GRN 910 are omitted here and instead references are made to specific sections of our prior notices where the pertinent information can be found.

In the current Notice, Nomad provides summaries of the identity, manufacturing process, product quality, safety, dietary exposure and potential risks from consumption of its THAUMATIN II flavor modifier product produced in the plant host *N. benthamiana*. Because much of this information was described in prior GRAS notices and the published literature, this Notice frequently references GRN 738, GRN 775 and GRN 910 for brevity and to rely on FDA's original review memoranda for our prior GRAS notices.

Our submission complies with the 7-part format prescribed by FDA in its Final Rule for the GRAS Notice process (August 17, 2016), and includes a CD containing PDFs of the following documents:

1. FDA Form 3667 Nomad Bioscience GRN for THAUMATIN II flavor modifier
2. GRN for THAUMATIN II flavor modifier (Parts 1-7), which includes:
   APPENDIX A: Nomad's Safety Data Sheet for THAUMATIN II flavor modifier
   APPENDIX B: THAUMATIN II *Nicotiana benthamiana* Manufacturing Process
   APPENDIX C: THAUMATIN II Characterization
3. Copies of references cited in the GRN

If the Agency has any questions or requires additional information to aid their review of Nomad's findings and GRAS conclusion, please contact us at the address listed above. For convenience, you may also contact our regulatory and product development representatives in the USA, Dr. Kristi Smedley at Center for Regulatory Services Inc., Woodbridge, VA (Tel 703-590-7337; Email smedley@cfr-services.com), and Dr. Daniel Tusé at DT/Consulting Group, Sacramento, CA (Tel 707-290-9528; Email daniel@dt-cg.com).

Sincerely,

Yuri Gleba, Ph.D.
Chief Executive Officer
Nomad Bioscience GmbH

Table of Contents

Table of Contents....3
Table of Figures....5
Table of Tables....6
1. General Introduction and Claim of Exemption from Premarket Approval Requirements....7
1.1. Submission of Notice....7
1.2. Name and Address of Notifier....7
1.3. Common or Usual Name of the Notified Substance....8
1.4. Conditions of Use....8
1.5. Statutory Basis for Notifier's GRAS Conclusion....8
1.6. Not Subject to Preclearance....8
1.7. Availability of Information for FDA Review....9
1.8. Public Disclosure....9
1.9. Certification....9
2. Identity, Method of Manufacture, Specification, Technical Effect....10
2.1. Identity, Structural and Functional Information....10
2.2. Method of Manufacture....12
2.3. Composition and Specification....13
2.4. Technical Effect and Suitability of Use....15
2.5. Overall Conclusion....15
3. Dietary Exposure....16
3.1. Application rates of THAUMATIN II in various food categories....16
3.2. Estimated dietary intake of THAUMATIN II in foods and beverages....16
3.3. Additional exposure to thaumatins (intake not related to THAUMATIN II product)....18
3.4. Dietary Exposure to Host- and Process-Derived Impurities....18
4. Information on Any Self-Limiting Levels of Use....20
5. Experience Based on Common Use in Food Before 1958....20
6. Basis for Conclusion of THAUMATIN II's GRAS Status....20
6.1. THAUMATIN II Overall Safety....21
6.1.1 Allergenic potential of THAUMATIN II....22
6.1.2 Host impurities....23
6.1.3 Process modification to lower host alkaloid content in THAUMATIN II product....25
6.1.4 Process impurities in *N. benthamiana*-based THAUMATIN II manufacture....27
6.2. Safety in Relation to Dietary Intake of THAUMATIN II....29
6.3. Occupational safety....31
6.4. GRAS conclusion for *N. benthamiana*-produced THAUMATIN II flavor modifier....31
7. Supporting Data and Information....33

References 36
APPENDIX A. THAUMATIN II Safety Data Sheet 44
APPENDIX B. THAUMATIN II Manufacturing Process 50
B.1. Introduction and Rationale 50
B.2. Organism Used and Gene Expression Cassette 50
B.3. Procedure 51
B.4. Manufacturing Facilities 55
B.5. Waste Handling and Disposal 55
B.6. Justification of Specification 55
APPENDIX C. THAUMATIN II Characterization 57
C.1. Purification of Thaumatin II Protein from Plant Biomass 57
C.2. Confirmation of Identity 58
C.3. Stability of Purified Thaumatin II Protein 59
C.4. Manufacturing Yield and Consistency 60
C.5. Analysis of Residual Alkaloid Content 61
C.6. Analysis of Elemental Impurities 61
C.7. Bioburden and Residual Vector 63
C.8. Determination of Flavor Modification Characteristics 63
C.9. Summary 73

Table of Figures

Figure 2-1. Structure of thaumatin II 10
Figure 2-2. Amino acid sequence of mature Thaumatin II protein 11
Figure B-1. *Agrobacterium* expression vector for thaumatin proteins 50
Figure B-2. Schematic of vector for thaumatin expression in plants 51
Figure B-3. Diagram of modified process for producing THAUMATIN II in *Nicotiana benthamiana* 52
Figure C-1. One-step purification of cold-extracted Thaumatin II by Capto S CEX chromatography 57
Figure C-2. Molecular stability of purified Thaumatin II protein as determined by ISD 58
Figure C-3. Determination of Thaumatin II protein mass by MALDI-TOF 58
Figure C-4. Stability of purified Thaumatin II protein 59
Figure C-5. Sensory profile in evaluation of sweet/sour taste modulation 66
Figure C-6. Sourness intensity (aftertaste) in sweet/sour taste modulation 67
Figure C-7. Sensory profile in evaluation of sour taste modulation 67
Figure C-8. Sourness intensity (aftertaste) in sour taste modulation 68
Figure C-9. Sensory profile in evaluation of caffeine bitter taste modulation 70
Figure C-10. Sensory profile of savory taste modulation in low- and high-salt conditions 72
Figure C-11. Sensory profile of savory taste modulation in low- and high-MSG conditions 72

Table of Tables

Table 2-1. Specification of THAUMATIN II Flavor Modifier Product....14
Table 2-2. THAUMATIN II flavor modification characteristics....15
Table 3-1. Estimated exposure to host alkaloids from THAUMARIN II produced in *N. benthamiana*....19
Table 6-1. Bioinformatic amino acid scan for allergenic sequences in Thaumatin II protein....23
Table 6-2. Levels of nicotine and anabasine in *N. benthamiana*....25
Table 6-3. Residual levels of *N. benthamiana* alkaloids in purified Thaumatin II protein....26
Table 6-4. Average nicotine content in common vegetables....26
Table 6-5. Consumption, nicotine content and dietary nicotine exposure from common vegetables....27
Table 6-6. Estimated exposure to elemental impurities in THAUMATIN II....28
Table 6-7. FEMA Updated thaumatin use levels (2018)....30
Table 7-1. Information supporting THAUMATIN II flavor modifier GRAS conclusion....33
Table B-1. Justification of Specification release limits for THAUMATIN II product....56
Table C-1. Stability of THAUMATIN II during storage....60
Table C-2. Consistency of production yield and purity of THAUMATIN II....60
Table C-3. Residual alkaloid impurities in *N. benthamiana*-produced THAUMATIN II....61
Table C-4. Multi-batch analysis of elemental impurities in *N. benthamiana*-produced THAUMATIN II....62
Table C-5. Multi-batch analyses of bioburden and residual *Agrobacterium* inoculum....63
Table C-6. Comparative sour flavor enhancement in sweet/sour binary taste evaluation....64
Table C-7. Mean sourness intensity scores for sweet/sour taste comparisons....65
Table C-8. Mean sourness aftertaste scores for sweet/sour taste comparisons....66
Table C-9. Caffeine bitterness modulation (range-finding)....69
Table C-10. Mean quinine bitterness intensity scores....71
Table C-11. Mean quinine bitterness aftertaste scores....71

1. General Introduction and Claim of Exemption from Premarket Approval Requirements

Nomad Bioscience GmbH ("Nomad"; Notifier) THAUMATIN II product is produced using a plant-based manufacturing process to match the amino acid sequence of naturally occurring thaumatin II protein. Thaumatins (both extracted and recombinant) have been extensively studied scientifically for many decades pre and post commercialization, and a large public database of information exists regarding manufacturing methods, functionality, applications and safety. This extensive public record enables comparison of Nomad's new product to the currently available thaumatin-containing products.

Results of a literature search and analysis of publicly available information on which Nomad based its conclusions of the safety of its THAUMATIN II product were included in GRN 738 for Thaumatins I and II produced in edible plant species hosts, in GRN 910 for Thaumatin II produced in the non-food host *Nicotiana benthamiana*, and are further updated in this Notice. Regardless of the plant species used, Nomad's plant-made thaumatins are identical in amino acid composition, sequence and structure to the corresponding native thaumatins originally extracted from the aryls of the tree *Thaumatococcus daniellii*, the major source of commercial thaumatins.

This Notice provides exposure estimates from consumption of foods that would be treated with Nomad's *N. benthamiana*-produced THAUMATIN II as a flavor modifier and includes a corresponding risk assessment. Under any use scenario, we project that THAUMATIN II can be used safely at the levels needed for functionality.

Notifier therefore concludes that under the conditions of use described herein, THAUMATIN II flavor modifier is generally recognized as safe and therefore should be exempt from premarket approval procedures under 21 CFR 170.36(a)(I). THAUMATIN II is not intended for use in infant formulas.

1.1. Submission of Notice

This Notice is submitted in compliance with Subpart E of FDA's Final Rule of the GRAS Notification process (August 17, 2016) 21 CFR 170.203-170.285.

1.2. Name and Address of Notifier

NOMAD BIOSCIENCE GmbH
Biozentrum Halle
Weinbergweg 22
D-06120 Halle/Saale, Germany
Office: 49 345 1314 2606
Fax: 49 345 1314 2601

Notifier's US Representative
Kristi O. Smedley, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192
Office: 703-590-7337; Mobile: 703-786-7674
Fax: 703-580-8637
eMail: smedley@cfr-services.com

Notifier's US Representative
Daniel Tusé, Ph.D.
DT/Consulting Group
2695 13th Street
Sacramento, CA 95818
Telephone: 707-290-9528
Fax: 916-822-4124
Email: daniel@dt-cg.com

1.3. Common or Usual Name of the Notified Substance

THAUMATIN II

1.4. Conditions of Use

What is **claimed as GRAS in this Notice** is:

> A THAUMATIN II composition consisting of recombinant, *Nicotiana benthamiana*-produced Thaumatin II protein meeting the target Specification, for application to food and beverages as a flavor modifier at 1-150 ppm, per FEMA guidance for thaumatin flavor modifiers (Cohen 2016; 2018). In this submission, Notifier excludes uses of its product on foods regulated by the USDA/FSIS as data supporting the suitability in such food products are currently being generated.

Native "thaumatin" is comprised of several related proteins, but the major sweetening properties reside in two very similar proteins, thaumatin I and thaumatin II. Thaumatins are naturally occurring proteins that can modify taste perception contextually, depending on their concentration and the properties of the food matrix into/onto which the thaumatins are applied. Nomad's product can be comprised of thaumatin I and/or thaumatin II, as described in GRN 738.

However, new manufacturing process and sensory information generated by Nomad since submission of GRN 738 suggests that thaumatin II can satisfy production and functionality requirements as a single active ingredient; therefore, this Notice focuses on a low-calorie flavor modifier comprised only of thaumatin II. Hence, Notifier's product "THAUMATIN II" is formulated to contain recombinant, *N. benthamiana* plant-produced, purified **Thaumatin II** protein (capitalized in this Notice to differentiate it from the same protein obtained from other sources). Thaumatin II can be applied to foods up to the safety limits allowed for thaumatins from native sources. The sources of information used to document and support Thaumatin II's properties, functionality, safety attributes and our GRAS conclusion are listed in Table 7-1.

Thaumatins are used to modify the flavor of foods by imparting a sensation of sweetness, enhancing savoriness, or by modulating sour, bitter or other, less desirable flavors. Different rates of application are anticipated depending on the desired effect. This Notice pertains to Thaumatin II's use as a flavor modifier, as Thaumatin II's functionality as a sweetener was described in GRN 738 and GRN 910. As a flavor modifier, we anticipate safe consumption of Thaumatin II at maximum levels that are comparable to those specified for the currently available thaumatins (i.e., maximum permitted level (MPL) for safe consumption of up to 1.1 mg/kg-day body weight basis for all age groups, EFSA E957 October 28, 2015).

Additional intake and safety information for subpopulations potentially consuming THAUMATIN II, including children, adults and the elderly, is provided in Section 3 (Dietary Exposure) of this Notice.

1.5. Statutory Basis for Notifier's GRAS Conclusion

The statutory basis of the GRAS status in through scientific procedures in accordance with 21 CFR 170.30(b): GRAS Conclusion. In accordance with the information provided in this Notice, it is Nomad Bioscience's conclusion that *Nicotiana benthamiana*-produced THAUMATIN II is generally recognized as safe when used as a flavor modifier on foods and beverages at application rates that would collectively amount to not more than 1.1 mg/kg-day (body weight basis) additive exposure in a typical human diet.

1.6. Not Subject to Preclearance

Notifier has concluded that THAUMATIN II as manufactured via its plant-based process using the host *N. benthamiana* is generally recognized as safe, and as such the substance is not subject to pre-market approval requirements of the Federal Food Drug and Cosmetic Act.

1.7. Availability of Information for FDA Review

All data and information that serve as a basis for the GRAS conclusions are included in this Notice.

1.8. Public Disclosure

The information provided in this Notice is publicly available and not subject to exception under 170.225(c)(8). All information contained in this Notice can be shared without restriction.

1.9. Certification

On behalf of Nomad Bioscience GmbH (Notifier), I certify that to the best of my knowledge, this GRAS Notice is complete, representative, and balanced with respect to the information provided, favorable or unfavorable, known to me and pertinent to the evaluation of the safety and GRAS status of our *Nicotiana benthamiana*-produced THAUMATIN II flavor modifier product.

Yuri Gleba, Ph.D.
Chief Executive Officer
NOMAD BIOSCIENCE GmbH
Biozentrum Halle
Weinbergweg 22
D-06120 Halle/Saale
Germany

2. Identity, Method of Manufacture, Specification, Technical Effect

2.1. Identity, Structural and Functional Information

Identity

In GRN 738, Notifier claimed substitutable use of Thaumatin I or Thaumatin II and/or their mixtures to achieve the desired sweetening effect. Either protein could also be used in food and beverage flavor modification. However, recent studies summarized in GRN 910 showed that Thaumatin II can be produced more efficiently than Thaumatin I and also to possess a superior flavor profile relative to Thaumatin I, including fewer off-flavor notes. Consequently, Notifier's commercial flavor modification product will contain Thaumatin II as the sole flavor modifying active ingredient.

The history of thaumatin development and commercialization was included in GRN 738 (Section 2.1, pg 9; Section 6.1, pp 15-16) and is only summarized here for brevity. The product Talin® was commercialized beginning in the 1970s by Tate & Lyle (UK) with a claimed sweetness of 1,600-2,700 times that of a 7-10% solution of sucrose (van der Wel 1972). A similar, mixture was sold in Japan under the brand San Sweet T-100®. Thaumatins are currently available from several vendors, including Naturex, Beneo Palatinit, Natex, KF Specialty Ingredients and several others. The chemistry and applications of thaumatins have been extensively described in several original and review articles (e.g. Greenly 2003; Hough 1993b; Joseph 2019; Kim 1988; Lee 1987; Shallenberger 1993; Suami 1997; Witty 1998; Zemanek 1995).

A molecular diagram of thaumatin II determined from crystallography at 0.99-Å resolution is shown in Figure 2-1 (from Masuda 2014). Thaumatins I and II have similar properties, amino acid composition, sweetness, molecular weight (both are ~22 kDa) and highly similar amino acid sequences, differing by only 5 amino acid residues. Each protein is a single polypeptide chain of 207 amino acids with 8 intramolecular disulphide linkages (Suami 1997). X-ray crystallography of thaumatin I revealed the features of the protein's backbone (de Vos 1985; Kim 1988; Ogata 1992). Circular dichroism studies (van der Wel 1984) showed few α-helices, but many β-pleated sheet strands and bends. It is thought that the constrained structure of thaumatins is responsible for inducing taste sensation. Either heat denaturation or cleavage of the disulphide bridges results in loss of sweetness, suggesting that the tertiary structure of the protein triggers taste modulation through a highly stereoselective process (Kaneko 2001; Hough 1993b).

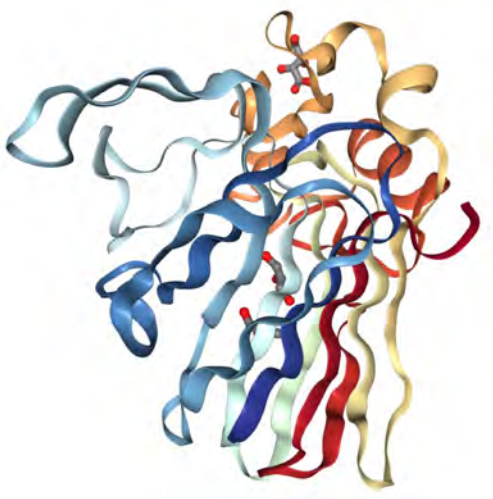


Figure 2-1. Structure of thaumatin II

Structural Information for Plant-Made Thaumatin II Protein

The amino acid sequences of thaumatins I and II, including Notifier's plant-expressed thaumatins, were included in GRN 738 (Section 2.1, pg 10). The amino acid sequence of Thaumatin II is shown in Figure 2-2 (Faus 2008). Notifier's plant-expressed, mature Thaumatin II has an identical amino acid sequence to the corresponding protein from *Thaumatococcus* (APPENDIX C, Section C.2).

The sequence shown is for the mature protein; thaumatins including Thaumatin II are natively expressed as pre-proproteins containing N-terminal signal and C-terminal peptide additions. These precursors are processed *in planta* to yield the intermediate proprotein devoid of the N-terminal peptide but still containing the C-terminal addition. Subsequent processing *in planta* yields the nature-identical sequence of amino acids in the mature, isolated thaumatin. Thaumatin II does not contain glycosylation sites on its 207-aa backbone; like the protein derived from *Thaumatococcus*, Notifier's Thaumatin II is a non-glycosylated polypeptide.

```
ATFEIVNRCSYTVWAAASKGDAALDAGGRQLNSGESWTINVEPGTKGGKIWARTDCYFDD  60
SGRGICRTGDCGGLLQCKRFGRPPTTLAEFSLNQYGKDYIDISNIKGFNVPMDFSPTTRG  120
CRGVRCAADIVGQCPAKLKAPGGGCNDACTVFQTSEYCCTTGKCGPTEYSRFFKRLCPDA  180
FSYVLDKPTTVTCPGSSNYRVTFCPTA                                    207
```

Figure 2-2. Amino acid sequence of mature Thaumatin II protein

A Safety Data Sheet (SDS) for THAUMATIN II (Thaumatin II produced in *N. benthamiana*) is found in APPENDIX A. The method applied to manufacture Notifier's Thaumatin II protein in the plant host *N. benthamiana* is described in APPENDIX B. Analytical methods used to define the physicochemical and functional properties of Notifier's recombinant Thaumatin II are summarized in APPENDIX C. Notifier's *Nicotiana*-expressed plant-made Thaumatin II conforms to its predicted composition and shares the amino acid sequence of the native *Thaumatococcus* protein.

Quantitative Composition

Bulk THAUMATIN II is prepared as a dry powder (see APPENDIX B)**.** The **Specification** for the bulk product is found in Table 2-1**.** THAUMATIN II is supplied as a solid of >95% purity, with host proteins and buffer salts comprising <5% of the total composition. Thaumatins are highly water soluble (>20% w/v). The bulk product is dissolved/diluted in a suitable food-compatible vehicle or directly mixed into foods or beverages to achieve the desired effect. THAUMATIN II can be used singly or in combination with other food additives to enhance the flavor profile of foods and beverages or to mask undesirable flavor notes. The amount of THAUMATIN II used is food- and application-specific and depends on the end result desired in each flavor formulation.

Mode of Action

The mode of action of thaumatins was reviewed in GRN 738 (Section 2.1, pg 11) and results of more recent studies are included in this submission. Thaumatins and other sweet-tasting and flavor-modifying proteins interact with taste receptors in the tongue to impart the neurophysiological sensation of taste. Sweet-tasting proteins as well as aspartame can be perceived by humans, apes, and Old World monkeys but not New World monkeys and rodents (Masuda 2013). Natural sweetness perception occurs when a sugar such as sucrose or other sweetener dissolves in saliva and binds to the heterodimeric T1R2–T1R3 receptors, which belong to the G-protein-coupled receptors (GPCRs) family (Li 2002; Margolskee 2001; Nelson 2001). These receptors have multiple binding sites (Vigues 2008) that are activated upon interaction with compounds that elicit sweet taste. Each sweetener exhibits different binding properties on the same receptors (Fernstrom 2012), leading to the varying perceptions of sweetness or flavor-modifying effects for the different proteins.

In addition to their history of use by African, Western and Eastern cultures, thaumatins have been studied systematically for their flavor modifying properties *in vitro* and *ex vivo* as well as in animal studies *in vivo*. As defined in whole animal studies using various species and in isolated gustatory cells *in vitro*, thaumatins have a demonstrable effect on various groups of taste receptors and these interactions affect perception of taste (Bartoszewski 2003; Faus 2008; Glaser 2000; Greenly 2003; Hellekant 1996; Kaneko 2001; Kinghorn 1986; Lim

2012; Nagarajan 2017; Ohta 2011; Sardesai 1991; Schiffman 1995; Suami 1997; Tinti 2000; van der Wel 1972; Witty 1990).

At the molecular level, the electrical charge distribution on the thaumatin molecules appears to mediate their interaction with the taste receptors. In addition to sweetness, thaumatins' effect in the presence of more complex flavor compounds suggests that flavor modification depends on the nature of the individual molecules present (Natex 2017). The strength of the interaction between the thaumatin molecules and the taste receptors may account for thaumatins' intensity and the duration of flavor perception.

Natural thaumatin was originally found to consist of six closely related proteins (I, II, III, a, b and c), all with a MW of ~22 kDa (207 amino acids) (van der Wel 1972; Ledeboer 1984). Thaumatin I and II are robust because of their multiple disulfide bridges, but can lose their sweetness on heating at alkaline pH; the proteins are stable at neutral to acidic pH even at elevated temperatures (Lim 2012). The 3-D structure of thaumatin I (de Vos 1985; Ogata 1992) revealed that the protein consists of three domains: (a) an 11 strand, flattened β-sandwich (aa 1–53, 85–127 and 178–207, domain I); (b) a small disulfide-rich region (54–84, domain III); and (c) a large disulfide-rich region (128–177, domain II). Subsequently, the crystal structure of plant-extracted thaumatin II was determined at 1.27-Å resolution (Masuda 2011) and the structure of a recombinant version of the same molecule was determined at 0.99-Å resolution (Masuda 2014). These studies revealed very similar structures for thaumatin I and II and enabled subsequent in-depth structure-activity studies (Masuda et. al. (2016; 2018).

Thaumatins find multiple uses in food technology because they can impart a sweet flavor, enhance desirable flavor notes, or mask or ameliorate unpleasant flavors in food components. Additional structure-activity information was provided in GRN 910 (pp 11-12). The current Notice focuses on Thaumatin II's ability to modulate specific flavors impacting the organoleptic properties of food and beverages.

2.2. Method of Manufacture

The THAUMATIN II plant-based manufacturing process was described in APPENDIX B of GRN 738 and included expression and extraction of Thaumatin I and/or II proteins in food species of plants. Plant hosts included *Spinacia oleracea* (spinach), *Beta vulgaris* (red beet) or *Lactuca sativa* (lettuce). These same hosts were found acceptable for the manufacture of antimicrobial proteins as food safety processing aids, as described in Notifier's GRN 593 and GRN 676. The safety features of these host species were detailed in GRN 593.

In GRN 910, Notifier asserted that other plant hosts can be used to produce thaumatin proteins without impacting the safety or functionality of the final product. Such species include members of the genus *Nicotiana*, specifically the species ***N. benthamiana***. Reduction in the levels of *N. benthamiana*-associated impurities such as pyridine alkaloids that are not found in edible species hosts is achieved through the **process modification** described in APPENDIX B of GRN 910. The modification enables inclusion of *N. benthamiana* to the list of candidate plant species for producing thaumatin proteins.

Nomad includes *N. benthamiana* as an additional host for its manufacturing process because the species is amenable to large-scale indoor cultivation and yields better process economics relative to its food-species counterparts. Nomad has described use of *N. benthamiana* in the manufacture of its food safety processing aids and has used scientific procedures to conclude GRAS status for the products COLICIN (GRN 775), ENDOLYSIN (GRN 802) and SALMOCIN (GRN 824).

In GRN 910 and in the present Notice, Nomad asserts its GRAS conclusion for *N. benthamiana*-produced THAUMATIN II product containing Thaumatin II protein.

Regardless of host used, the plant-derived biomass remaining after thaumatin protein extraction is treated and discarded (disposed) and is not used as a human food or animal feed product, additive or supplement.

2.3. Composition and Specification

Characteristic Properties

The characteristic properties of plant-made THAUMATIN II are summarized in the Specification. In recent studies Notifier has determined that the manufacture of Thaumatin II is more efficient than the manufacture of Thaumatin I. Additionally, Notifier has determined in sensory studies conducted in house and at independent sensory evaluation organizations, that Thaumatin II has superior flavor characteristics over Thaumatin I, including comparable flavor modification with fewer/shorter-lasting off-flavor effects. Hence, a current target **Specification** for a product consisting of Thaumatin II as the preferred active ingredient is provided in Table 2-1.

As reported in GRN 738 (Section 6.2, pp 17 -21), Notifier studied at the molecular level the allergenic potential of plant-expressed thaumatins for use in food and determined, from published information, that Notifier's thaumatins should pose no higher allergenic risk than the *Thaumatococcus*-derived commercial thaumatins currently on the market (see current Notice's Section 6.1.1 Allergenic potential of THAUMATIN II).

Formulation

THAUMATIN II is provided in bulk as a dry powder, which the end-user can dissolve and/or dilute in water or other food-acceptable vehicle according to instructions and mixed in foods and beverages at levels sufficient to achieve the desired flavor modification.

Content of Potential Human Toxicants in THAUMATIN II

Thaumatin proteins comprise authorized food ingredients and are non-toxic when used as intended. Process impurities derived from the manufacture of thaumatins in edible plants are also non-toxic as described in GRN 738. There are two potential human toxicants that may remain in low levels in the THAUMATIN II product when it is manufactured using the host plant *N. benthamiana*; namely, the alkaloids nicotine and anabasine. As first discussed by Notifier in GRN 775, the host plant *N. benthamiana* shares metabolic pathways with other members of the plant family Solanaceae, which includes tomato, pepper, potato, eggplant and others. All these plants contain low residual yet measurable levels of pyridine alkaloids.

The purpose of including a chromatographic purification step during downstream processing is to produce Thaumatin II with levels of residual alkaloids that are in the same range as the levels consumed in common vegetables in a typical diet.

In addition to residual alkaloids, some heavy metals may be present in low amounts in the final product. The residual levels of all impurities in the final product are low, and at those levels the impurities are not expected to pose a risk to any subpopulation of consumers when the product THAUMATIN II is used as specified.

Formulation excipients included in the final product are approved for food applications. There are no other known sources of potential human toxicants in THAUMATIN II.

Specification

The current Specification for THAUMATIN II is shown in Table 2-1. The process used to manufacture the product in *N. benthamiana* with this specification, including the current experimentally determined values used to justify this specification, are shown in APPENDIX B (THAUMATIN II Manufacturing Process).

Table 2-1. Specification of THAUMATIN II Flavor Modifier Product

THAUMATIN II Bulk Product (Thaumatin II active ingredient)		
Parameter	**Specification limit**	**Method**
Appearance	Powder, yellowish to light tan	Visual
Minimum total thaumatin content (as percent of total protein)	≥ 95%	CGE (capillary gel electrophoresis)
Solubility (DI water)	≥ 200 mg/mL	Visual
pH of a 1% solution (excipient-dependent)	2.7 – 6.0	Potentiometric
Heavy metals (sum of Class 1 metals Pb, Cd, Hg, As)	≤ 5 ng/mg thaumatin	USP38<233>
Lead (Pb)	≤ 1 ng/mg thaumatin	USP38<233>
Host alkaloid: Nicotine	≤ 20 ng/mg thaumatin	HPLC/MS
Host alkaloid: Anabasine	≤ 5 ng/mg thaumatin	HPLC/MS
Bioburden	≤ 5,000 CFU total per g	USP32<61>
Agrobacterium (vector) per 10 g sample	0 (absent)	Selective plate-based assay
Undesirable microorganisms, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella spp.* or coagulase-positive *Staphylococcus spp.*, per 25 g sample	0 (absent)	USP32<1111>
Stability (dry powder; 0-20°C) as <3% loss of thaumatin protein	≥ 6 months	CGE; thaumatin peak in 0.1% (w/v) solution, T_m relative to T_0

2.4. Technical Effect and Suitability of Use

Notifier has determined that the intended use of THAUMATIN II as a flavor modifier does not raise safety concerns. Technical effect information was developed by Notifier in its efforts to characterize the quality of its product and to assess its flavor-modifying characteristics. This information is included in this Notice (below and APPENDIX C, Section C.8) to enable additional estimates of application rates and dietary exposure (see Section 3 Dietary Exposure).

Table 2-2 summarizes THAUMATIN II's technical effects. The methods used and the results obtained in determining the product's flavor modulation characteristics are found in APPENDIX C, Section C.8 (Determination of Flavor Modification Characteristics). Addition of THAUMATIN II enables reductions in the levels of native tastants (e.g. sugar, acid, salt, MSG) typically added to various foods and beverages.

Table 2-2. THAUMATIN II flavor modification characteristics

Flavor (reference tastant)	Taste Effect
Sour (citric acid in the presence of sucrose)	Enhancement (potentiation) of sour taste
Sour (citric acid only; no sweetener)	Reduction (masking) of sour taste
Bitter (low caffeine and low Thaumatin II levels)	Reduction (masking) of bitter taste
Bitter (high caffeine and high Thaumatin II levels)	Enhancement (potentiation) of bitter taste
Bitter (quinine and low Thaumatin II level)	Reduction (masking) of bitter taste
Bitter (quinine and high Thaumatin II level)	Enhancement (potentiation) of bitter taste
Savory (broth with low salt or low MSG)	Enhancement (potentiation) of savory taste
Savory (broth with high salt or high MSG)	No/low impact on savory taste

2.5. Overall Conclusion

The results of studies supporting this Notice show that Thaumatin II can be produced consistently using recombinant expression in the plant host *Nicotiana benthamiana*. The recombinant thaumatin can be readily purified from plant biomass and exhibits the characteristic properties of extracted thaumatin II. The molecular composition of Notifier's Thaumatin II is the same as that of their native counterpart. The gene sequence used in the expression vector encodes protein precursors that are correctly processed *in planta* to yield the same mature thaumatin protein that is isolated from *Thaumatococcus*, including identical amino acid sequence. Regardless of source, including the new host *N. benthamiana*, the mature Thaumatin II protein lacks glycosylation because the polypeptide backbone does not contain glycan addition sites.

The flavor modification characteristics of Notifier's THAUMATIN II product generally match those of commercial thaumatin products, including contextual modulation of taste characteristics (Table 2-2). The safety, physicochemical and taste-modifying properties of fermentation-derived recombinant thaumatins match those of native (extracted) thaumatins (Smith 1996) and both are available commercially. Ample evidence of safety of thaumatins exists in the published literature. Thaumatin's approval for use in food and beverages in the USA, EU countries, Japan, Australia, Israel and elsewhere underscore thaumatin's excellent record of safety obtained during more than 40-years of pre- and post-market surveillance.

In sum, the main difference between Notifier's *N. benthamiana*-produced Thaumatin II protein and commercially available extracted or fermentation-derived thaumatin II is the method of manufacture.

3. Dietary Exposure

3.1. Application rates of THAUMATIN II in various food categories

Average maximum application rates in all allowed uses

Thaumatin is listed in the Codex General Standard for Food Additives and permitted for general use in food (Codex Alimentarius 2016). In Europe, it is approved as a Sweetener or as a Flavour Enhancer (EFSA E 957) for use in a number of different categories. In the USA, natural (extracted) thaumatin has GRAS classification (FEMA GRASa No. 3732; Oser 1984) as a flavor modifier, as does a recombinant version of the thaumatin B protein variant ("thaumatin B-recombinant"; FEMA GRASa 3814; Smith 1996).

Notifier's GRN 738 established plant-made thaumatins (Thaumatin I and/or Thaumatin II) as a GRAS sweetener in the USA. In the EU and Japan, the acceptable daily intake (ADI) limit for thaumatin table-top sweeteners is "not specified" (*Quantum satis*) because the proteins are considered to be non-toxic. Based on their sweetening and flavor-modifying profile, thaumatins are applied to foods in the 1-400 ppm range, or 1-400 mg thaumatin/kg or /L food or beverage. When used at these maximum allowed safe levels and on the same foods, the use rates and dietary exposure of Notifier's product described in GRN 738 were expected to be the same as those for currently available thaumatin products. In the USA, FEMA's suggested application rates range from 1-150 ppm, because they exclude sweetener uses of thaumatins (Cohen 2016; 2018).

In GRN 738, Notifier used the more inclusive EU database for estimating the maximum safe dietary intake of thaumatins from THAUMATIN because it represented a higher potential intake scenario (**1-400 ppm**) by covering all food categories and use rates encompassed by EU statutes (EFSA 2015). Based on the use rates in those statutes, a safe maximum intake from a typical diet of thaumatin-treated products was defined as 1.1 mg/kg-day; for an adult person of 70 kg body mass, maximum safe intake would be 77 mg/person-day.

Average moderate application rate as a flavor modifier

Although the 400 ppm maximum application rate and 1.1 mg/kg body mass/day upper limit of consumption of thaumatin in all uses provides a safety reference that is indicative of thaumatin's very low toxicity, it is unlikely that anyone would consume the proteins in that amount. Most uses of thaumatin are in flavor modification (Green 1999). In the current Notice, Notifier follows US guidance provided by FEMA, which suggests application rates of thaumatins for flavor modification in the range **1-150 ppm** in various foods and beverages (Cohen 2016; 2018). Per FEMA guidance, the average/moderate application rate to most foods is 7 mg thaumatin/kg or L of food or beverage (**7 ppm**). Notifier envisions equivalent application rates of its product due to the similarities between its THAUMARIN II and commercial thaumatin products.

These THAUMATIN II application rates are corroborated by results of sensory evaluation studies conducted by qualified external organizations, as described in APPENDIX C, Section C.8 (Determination of Flavor Modification Characteristics).

3.2. Estimated dietary intake of THAUMATIN II in foods and beverages

Maximum estimated intake of THAUMATIN II from all allowed uses

With respect to human dietary exposure, we expect no higher levels of consumption of Notifier's thaumatins per weight or volume of treated food as those allowed for extracted thaumatins. The European Food Safety Authority (EFSA) provides guidance on maximum permitted levels (MPL) of thaumatin on various foods and beverages according to Annex II to Regulation (EC) No 1333/2008 (OJEU 2011).

The EU's MPL for thaumatin food additive E 957 ranges from a low of 5 mg/kg in "flavored fermented milk products" (e.g. yogurt) to a high of 400 mg/kg in "food supplements supplied in a syrup-type or chewable form" (EFSA 2015). The same regulations allow the unlimited addition of thaumatin (*Quantum satis*[1]) when the protein is used as a table-top sweetener. It is envisioned that THAUMATIN II, regardless of production host, could be used at equivalent maximum safe application rates on various foods. Until GRN 738, thaumatins had not been considered a sweetener *per se* in the USA. As a flavor enhancer or modifier, FEMA cites use ranges from a low of 1 ppm for baked goods to a high of 150 ppm for chewing gum (Cohen 2016).

The application rates of thaumatins to various food products (maximum permissible level, MPL) were derived by assessing the consumption of each food type and estimating the additive intake to reach a maximum exposure of **1.1 mg thaumatin/kg body weight (bw) per day**, or **77 mg/day for a 70-kg person.** Consumption values were derived from typical European diets, taking into consideration country specific dietary preferences in Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden and the UK and various population age groups (**adult, infant, elderly**).

The original application rates allowed by EFSA (2008) were later amended (EFSA 2011) to include more food types and higher allowed thaumatin levels (OJEU 2011). Revision of statutes for expanded use was based on thaumatin's functionality and post-market safety record. Based on this safety record and on physicochemical equivalency, in GRN 738 Nomad claimed GRAS conclusion for its THAUMATIN product produced in edible plants when consumed at these same levels of intake. Additional detail is provided in Section 6.2.

Moderate estimated intake of THAUMATIN II from use as a flavor modifier

FEMA's food categories for which thaumatin flavor modification application rates are listed include a wide range of raw (e.g., "poultry") as well as finished foods comprised of multiple ingredients (e.g., "gravies", "condiments", "baked goods"). The maximum application rate in FEMA's guidance documents is 7 ppm. Current data matching the original consumption values used by FEMA to establish thaumatin addition limits in each category are difficult to find. Therefore, to estimate exposure from THAUMATIN II-treated foods, Notifier used the average per capita food consumption values published in the USDA/ERS Food Availability (Per Capita) Data System (FADS) (USDA ERS FADS 2015).

The ERS databases list US consumption levels of 474 g carbohydrates, 120 g protein, 190 g fat (total fat), and 25 g dietary fiber, for a total food intake of **809 g/person-day** based on food availability. However, food losses at all levels reduce this value by 31% (USDA ERS FADS 2015) to 40% (Buzby 2014). Discounting for losses, net per capita food consumption can be estimated as 0.46 kg/person-day (at 40% loss) to 0.56 kg/person-day (at 31% loss). We used the higher food consumption figure of **0.56 kg/person-day** to estimate exposure to THAUMATIN II when applied as a flavor modifier. Based on the 0.56 kg/person-day food intake estimate, and assuming all such foods are treated at 7 mg THAUMATIN II per kg food or beverage (7 ppm maximum), the total maximum THAUMATIN II intake would equate to **3.92 mg/person-day** (0.56 kg x 7 mg/kg).

The one food category for which FEMA lists a much higher application rate of thaumatin as a flavor modifier/enhancer is chewing gum (150 μg/g; 150 ppm). The domestic per capita daily consumption of chewing gum ranges from a median of 3.2 g per NHANES 2013-2014 to more than 8 g (reviewed in Martyn 2019). Given this range, a moderate value of **5 g/person-day** was adopted for our calculations. At that consumption level, exposure to THAUMATIN II from gum would be **0.75 mg/person-day.**

[1] With respect to food additives, the term "*Quantum satis*" indicates that no maximum level is specified. However, additives must be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided that they do not mislead the consumer. The EFSA *Quantum satis* designation was based on 10 mg/kg application rate.

Hence, in a hypothetical diet where Notifier's THAUMATIN II product replaced all current thaumatins used for flavor modification at the FEMA-specified application rates, consumers would be exposed to 3.92 (main food groups) + 0.75 (chewing gum) = 4.67 mg Thaumatin II/person-day. To simplify exposure calculations, the total was rounded upwards to **5 mg/person-day** THAUMATIN II from all food sources.

3.3. Additional exposure to thaumatins (intake not related to THAUMATIN II product)

On the basis of the estimates presented in Section 3.2 for THAUMATIN II used as a flavor modifier, 5 mg THAUMATIN II/person-day is expected to be the maximum intake contribution of Notifier's product in an extreme scenario where the product displaces all current flavor modifier uses of existing thaumatins. If introduction of Notifier's product were to result in no displacement but rather market expansion by a factor of 2, total consumption from Notifier's product and existing products would be 10 mg/person-day.

If calculated on the basis of maximum permitted level (MPL = 77 mg/person-day) of thaumatin for safe consumption, and assuming that 2/3 of thaumatin's current MPL represents food and beverage flavor modification applications, the maximum safe daily per capita intake would be ~50 mg (2/3 of 77 mg). Hence, the additive consumer intake for thaumatins in flavor modification (5 mg from THAUMATIN II plus 50 mg from existing sources) would be 55 mg. Even if the MPL were to double from 77 mg/day to 150-200 mg/day for all uses (including sweetener and flavor modification applications), thaumatin's safety factor of 1,300 based on chronic toxicity studies suggest that any additional safety risk to consumers would be minimal (Hagiwara 2005; OJEU 2011; EFSA 2015). Additional perspective on the safety risks of such intake from Notifier's THAUMATIN II product and from all sources of thaumatin is provided in Section 6.2 (Safety in Relation to Dietary Intake of THAUMATIN II).

3.4. Dietary Exposure to Host- and Process-Derived Impurities

Although the safety of thaumatin proteins has been documented (Notifier's equivalency data and referenced studies in GRN 738, GRN 910, plus this Notice), the use of non-food plant species as manufacturing hosts introduces the potential for new risks not found in the original list of production plant varieties. A description of risk factors and their mitigation is found in Section 6 of this Notice.

On a protein basis, the THAUMATIN II product consisting of Thaumatin II produced in *N. benthamiana* has a purity of >95% (typically ~98%); hence the Specification was set at $\geq$95%. The residual host-derived constituents of chromatographically purified thaumatins are mainly proteinaceous, and there are no unusual proteins in the host that would introduce risk, especially at such low levels (Leffingwell 1999).

Assuming a worst-case scenario where the purity of the product is only 95% (i.e. the Specification minimum), at the thaumatin adult MPL of 77 mg/day, **proteinaceous host-derived impurities** would amount to <4 mg/day (i.e. 5% of 77 = 3.85). With a US food consumption average of 0.56 kg/day, the **maximum exposure to host-derived proteins would be ≤7 ppm/day** (3.85 mg protein impurity/0.56 kg-day food intake), which is an insignificant level and not expected to impact either safety or nutritional content.

Extending this calculation to the **moderate exposure** from Thaumatin II used in foods as a flavor modifier (i.e., 5 mg/person-day), a 95%-pure product would expose consumers to **<0.45 ppm of host-derived proteins** (i.e. 0.25 mg protein impurity/0.56 kg/day food).

Some elemental impurities derived from plant growth media could be co-purified with thaumatin proteins (see Section 6.1.4 Process impurities in *N. benthamiana*-based THAUMATIN II manufacture). The Specification limit set for **total Class 1 heavy metal content** (sum of Pb, Cd, Hg, As) in *N. benthamiana*-purified THAUMATIN II of 5 ng/mg would expose consumers to **25 nanogram/person-day** of these metals in flavor

modification uses (5 mg Thaumatin II/day) and to **<400 ng/person-day** at the thaumatin MPL. Both levels are well below the **55 micrograms/person-day** combined oral permitted daily exposure **(PDE)** for Class 1 elements (ICH 2015).

The remaining inorganics include salts derived from the buffer (phosphate, NaCl) that are safe and allowed for food use. Hence, we assess that non-alkaloidal host- and process-derived impurities in *N. benthamiana*-produced THAUMATIN II would pose little to no risk to consumers.

The main organic impurities of concern are the alkaloids **nicotine** and **anabasine**, given their presence in *N. benthamiana* and their pharmacological activity. The other alkaloids potentially present, namely nornicotine and anatabine, have similar activities but are present at very low levels in the plant and pose no significant risk. Hence, our focus has been on assessing risk from the two main alkaloids present. The multi-batch average values for nicotine and anabasine (nanograms; ng) per milligram (mg) of Thaumatin II protein (i.e., ppm) were determined as described in APPENDIX C, Section C.5.

The results were used to support Specification limits of 20 ng/mg and 5 ng/mg for nicotine and anabasine, respectively, with a combined limit for total alkaloid established at 25 ng/mg protein. To estimate a **maximum exposure** level at the thaumatin adult MPL of 77 mg/person-day, the limit value (25 ng total alkaloid/mg protein) was multiplied by 77. Similarly, the **moderate exposure** from THAUMATIN II flavor modifier was determined by multiplying the Specification limit value by a consumption level of 5 mg/person-day. The per capita daily exposure maxima at both intake levels are shown in Table 3-1.

Table 3-1. Estimated exposure to host alkaloids from THAUMARIN II produced in *N. benthamiana*

THAUMARIN II consumption and estimated alkaloid exposure	Nicotine		Anabasine		Total Alkaloid Exposure
	Specification (ng/mg)	Daily exposure (ng/day)	Specification (ng/mg)	Daily exposure (ng/day)	Specification (25 ng/mg)
Thaumatin MPL (77 mg/d)	20	1,540	5	385	1,925 ng/day
Flavor modification (5 mg/d)	20	100	5	25	125 ng/day

Host alkaloid exposure potential was estimated from the maximum permitted level (MPL) from all uses of thaumatins (77 mg/adult person-day) as well as from consumption of THAUMATIN II flavor modifier (5 mg/person-day). The Specification limits for nicotine (20 ng/mg) and anabasine (5 ng/mg) were multiplied by 77 mg (MPL) or by 5 mg (THAUMATIN II flavor modifier) consumed per person-day, to arrive at the daily exposure estimates, in nanograms (ng) per person-day.

At the limit values the total alkaloid exposures are estimated to be <2 μg/person-day and ~125 ng/person-day at the thaumatin MPL and THAUMATIN II flavor modifier use levels, respectively. These are comparable or lower exposures than from other sources of pyridine alkaloid-containing foods, consumer products and the environment. For comparison, several studies have reported the average per capita daily food-borne exposure to nicotine from consumptions of common vegetables as ~1,000 ng (900-1,300 ng)/person-day (Andersson 2003; Davis 1991; Domino 1993; Liu 2013; Moldoveanu 2016; Nielsen 2013; Siegmund 1999).

Given THAUMATIN II's maximum alkaloid Specification limit of 25 ng/mg protein, and assuming full systemic distribution of alkaloids upon ingestion, a 70-kg person would experience <0.03 ppb level of alkaloids (Table 3-1, last column, ~2 μg total alkaloid/70 kg body mass). Moderate consumption of 5 mg/day of THAUMATIN II flavor modifier by a 70 kg person would lead to <0.002 ppb concentration of total alkaloid.

For perspective, the amount of nicotine absorbed in second-hand (passive) smoke is 100 micrograms (μg)/day for those reporting exposure, and 20 μg/day for those not reporting exposure, due to the pervasive nature of environmental nicotine (Karačonji 2005). Even in a low-use state like California, 1/2 of consumers are exposed to second-hand cigarette smoke, and 1/3 are exposed to second-hand nicotine and related alkaloids emitted from vaping devices (Vuong 2019). Legally sold nicotine-containing products contain milligram (mg) levels of nicotine. A single cigarette may contain from 2 to >10 mg of nicotine, of which 85-90% is absorbed into the blood stream through inhalation (Digard 2013). Because exposure is very rapid, up to ~9 mg nicotine may be systemically absorbed within 5 min of smoking (Digard 2013). That level corresponds to up to 130 μg/kg nicotine exposure for a 70-kg person (130 ppb), which produces pharmacologic but not toxic effects.

Nicotine-containing gum has ≥4 mg nicotine per dose (4.2 mg for Nicorette®) of which only 63% is absorbed via the oral route over a 45 min period (Digard 2013). This translates to 2.65 mg nicotine/70 kg person, or an exposure level of ~40 μg/kg (or ~38 ppb). This oral dose produces a mild, non-toxic pharmacologic effect. And these values are from only a single exposure to nicotine-containing products; multiple exposures per day are common.

Therefore, Thaumatin II alkaloid exposure is, at worst, thousands of times lower than from a single exposure to nicotine-containing consumer products. Although the nicotine analog anabasine may be similarly bioactive as nicotine, it is present at only a fraction of the level of nicotine in *N. benthamiana*-produced Thaumatin II, and at that level it too is not impactful on safety.

4. Information on Any Self-Limiting Levels of Use

Like other thaumatin products, THAUMATIN II can modify the flavor of a wide range of foods but excessive amounts can cause undesirable flavor notes and mouthfeel. Self-limiting unpalatability is expected at ≤50 ppm for most foods and beverages and at ≤150 ppm for low-volume products such as chewing gum. Based on average per capita food intake values, these upper limits would lead to exposures that are still well below thaumatins' safety based MPL of 1.1 mg/kg body mass-day (EFSA 2015).

5. Experience Based on Common Use in Food Before 1958

Notifier's GRAS Notice is based on scientific procedures. Thaumatins from *Thaumatococcus* have been used in indigenous human diets in West Africa for more than 200 years, as documented in British surgeon W.F. Daniell's report when he discovered the native use of *Thaumatococcus* pods in Nigeria in 1839 (Kinghorn 1986).

6. Basis for Conclusion of THAUMATIN II's GRAS Status

Notifier has used scientific procedures to conclude that its *N. benthamiana*-produced THAUMATIN II flavor modifier is GRAS under the conditions of intended use. Notifier has relied on detailed physicochemical analyses of its Thaumatin II protein and on extensive comparisons of its properties to a large volume of public information on thaumatins, including thaumatin's excellent safety record since these proteins were first commercially used as sweeteners and food flavor modifiers in multiple countries.

We conclude that the low residual levels of host alkaloids and elemental impurities in the *N. benthamiana*-produced THAUMATIN II product should not pose a risk to consumers, as those levels are lower than, or in the same range as, the levels consumers are exposed to in their present diets or daily environment. We further assert that the additive content of alkaloids (THAUMATIN II-derived and dietary) should not expose consumers to undue risk because of the small contribution of the product to total alkaloid intake.

Section 6.1 through Section 6.5 of this Notice summarize the data and information on *N. benthamiana*-produced THAUMATIN II that were used by Notifier to support its GRAS conclusion. In Section 7 (Supporting Data and Information), Table 7-1 presents a tabulated summary of this information from all sources.

6.1. THAUMATIN II Overall Safety

Prior to their broad approval for consumer use in the EU, Japan and other nations, thaumatin proteins were subjected to extensive safety assessments in animals and in humans, which have led to definition of application rates and daily exposure limits. Guidelines for thaumatin use and dietary exposure limits have been published in a number of regulatory documents issued by the European Food Safety Authority (EFSA 2015), based on reviews conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA 1983, 1986) and the EU Scientific Committee on Food (SCF 1985, 1989).

Thaumatin is considered safe for use in food and beverages. It is approved by EFSA in the EU (E 957) and in Japan, Australia, Israel and other countries as a food additive. In the USA thaumatin was granted GRAS status by FEMA (GRASa No. 3732, Oser 1984; and GRASa No. 3814, Smith 1996) as a flavor modifier, but not as a sweetener. FDA issued a "No Questions" letter to Nomad's GRAS Notice GRN 738 on 18 April 2018, for THAUMATIN (recombinant, plant-produced thaumatin I and/or II) as a food and beverage sweetener.

Discussions of thaumatin's safety, fully documented and supported by literature references, were presented in detail in GRN 738 and in GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*). For brevity, some details of those discussions are omitted from this Notice and instead specific references are made to prior submissions.

The safety profile of THAUMATIN II's **active ingredient** (i.e. Thaumatin II protein) can be deduced from (1) its equivalence to commercially available thaumatins, and (2) published results of detailed studies on the safety of thaumatin conducted over the last 40 years.

The safety of the **gene expression system** and of residual **impurities from edible species of host plants** that Notifier uses to produce THAUMATIN II were discussed in detail in GRN 593 (GRN 593 Section A.2.2; pp 25-28). The **impurities from *N. benthamiana*** were discussed in detail in GRN 775 (GRN 775 Section 6.1.1 – 6.1.6; pp 28-39) and in our THAUMATIN II sweetener Notice, GRN 910. Regardless of host plant, the manufacturing processes defined in GRN 738 (THAUMATIN from edible species), GRN 593 (COLICIN from edible species), GRN 775 (COLICIN from *N. benthamiana*) and GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*) are analogous and differ mainly in the higher downstream purification applied for THAUMATIN II when using *N. benthamiana* as the host.

With respect to the thaumatin proteins themselves, studies by Higginbotham et al. (1983) and Hagiwara et al. (2005) have been pivotal to establishing safety and have helped craft regulations for the application rate and safe usage levels of these substances. Higginbotham et al. (1983) found that thaumatin, when used as a sweetener/flavor enhancer, was unlikely to be hazardous at the anticipated level of consumption. They studied the safety of thaumatin over a 7-year period when various purities and formulations of thaumatin were under development and the initial product was being commercially manufactured. A summary of their findings includes:

Thaumatin digestibility. Thaumatin was readily digested prior to absorption in rats. Groups of 20 animals were split into 2 x 10 animals each, and fed diets containing no (0%) or 5.73 wt% thaumatin, for 10 consecutive days with sampling at two time periods. The results of metabolic analyses showed that thaumatin was 89 to >90% digested when fed as a mixed additive to the diet. Digestibility was high even though thaumatins I and II have 8 intramolecular disulfide bridges.

Subacute toxicity in rats and dogs. Thaumatin-containing diets were fed to groups of 20 male and 20 female rats, and to groups of 4 male and 4 female dogs, at concentrations of 0%, 0.3%, 1.0% and 3.0% (w/w), daily for 13 weeks. Behavioral, food consumption, weight gain, clinical chemistry, cytology, ophthalmoscopy, urinalysis, and histopathology observations were conducted on all animals at every dose group. No adverse effects were found for any dose of thaumatin administered.

Teratogenicity. Thaumatin was not teratogenic when administered orally by gavage to groups of 20 pregnant rats at 0, 200, 600 and 2,000 mg/kg body weight/day from day 6 to 15 of gestation. No thaumatin- or dose-related adverse effects on the fetus were found.

Mutagenicity. Thaumatin was evaluated for mutagenic potential using two standard assays, the dominant-lethal test, and the Ames' *in vitro* mutagenicity assay. In the first study, groups of 15 male mice each of proven fertility were intubated daily for 5 consecutive days with thaumatin or negative or positive controls. Thaumatin administration had no effect on the incidence of dominant lethal mutations when administered at doses of 200 and 2,000 mg/kg-day.

In the second study, the lack of mutagenic potential was confirmed in bacterial mutagenicity assays with *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98 and TA100) and *Escherichia coli* WP2, at levels of addition of 0.05–50 mg thaumatin/plate. All results were negative for mutagenicity.

No observed adverse effect level. The multi-species preclinical studies by Higginbotham et al. helped establish the **NOAEL** (no observed adverse effect level) for thaumatins at **1.4 g/kg** (dog) to **2.4-2.8 g/kg** (M and F rat). By conventional criteria, thaumatins are non-toxic. Allergenic risk potential was low.

Persistence of thaumatin proteins and genes. To complement Higginbotham (1983), Szwacka (2012) studied digestibility of thaumatins and the uptake of the thaumatin genes in animals fed transgenic fruit. The proteins were fully digested, and there was no gene transfer to the intestinal microflora.

6.1.1 Allergenic potential of THAUMATIN II

Amino acid sequences in Thaumatin II protein with similarity to known allergens

Because Notifier's Thaumatin II is identical in amino acid sequence and no less pure than commercial compositions containing thaumatin II, the allergenic risk for the recombinant proteins is deemed to be not higher than that of existing commercial products. Internal and external data were used to reach this conclusion. Baniulis et al. (2008) had conducted a computational analysis of thaumatin II allergenicity and assessed potentially antigenic elements in thaumatin-like family proteins.

Of the various candidate antigenic domains in thaumatin II, only four amino acid residues (Thr12, Leu74, Gln133 and Thr161) were found to possess high surface exposure and antigenic propensity, in theory. Using a similar technique, Notifier conducted an updated search *in silico* for potentially antigenic domains in Thaumatin II.

The results of Notifier's sequence searches were included and discussed in detail in GRN 738 (Section 6.2, Allergenic potential of THAUMATIN, pp 17-21). The majority of identity hits were for thaumatin-like proteins ("TLP"), which are widely distributed across many genera. It is thought that TLP serve a protective function in the plants that synthesize them, including protection from fungal attack and/or stress; TLP may also be involved in fruit ripening (Barre 2000; Liu 2010).

Table 6-1 summarizes the results of Notifier's amino acid sequence analyses performed *in silico*.

Table 6-1. Bioinformatic amino acid scan for allergenic sequences in Thaumatin II protein

Thaumatin	>35% allergen similarity at indicated search granularity			TLP Species Prevalence	Allergenicity Potential
	Full seq	80-mer	8-mer		
II	35 identified	33 similar seq	17 matches	Banana (17/17)	Low

The published literature show that TLP are consumed from multiple sources in our daily diet. Like any foreign protein, they have the potential for allergenicity. Yet, the lack of literature linking TLP to severe food allergies or anaphylactic reactions suggests that TLP, including thaumatins, present a low allergenic risk relative to other allergens in common foods, such as peanut, shellfish or gluten.

Allergenicity of *Thaumatococcus* thaumatins determined in preclinical and clinical studies

Results of prior allergenicity studies in humans and animals were included and discussed in detail in GRN 738 (Section 6.2, pp 19-21). Briefly, Higginbotham et al. (1983) performed detailed acute and sub-chronic toxicological evaluations of *Thaumatococcus*-extracted thaumatins, including determining the allergenic and anaphylactic potential of these proteins in rodents, NHP (non-human primates), and human volunteers.

Their multi-species studies were comprehensive, and included evaluating the potential toxicity of purified thaumatin proteins and plant-derived impurities from *Thaumatococcus,* as well as formulation excipients. For example, they compared the safety of purified thaumatins I and II (95-98% protein) to that of less pure (78-85%) formulations.

These studies showed no evidence of thaumatin-related toxicity. Extensive preclinical and two human clinical studies verified the low allergenicity of thaumatins. Some commercial thaumatins from *Thaumatococcus* available even today may contain potentially allergenic and/or toxic impurities, including proteases, thaumatin variants and thaumatin-like proteins (some of which are known allergens), aluminum salts and other impurities and contaminants (Asherie 2008; Nikolic 2014).

In contrast, Notifier's THAUMATIN II is devoid of impurities and contaminants associated with *Thaumatococcus* extraction, and any residual host-derived proteins or other host components are found in low concentration (see APPENDIX C THAUMATIN II Characterization, for detail).

6.1.2 Host impurities

Edible plant species hosts

The acceptability and safety of food species of plants, such as **red or leafy beet, spinach** or **lettuce,** as manufacturing hosts for recombinant proteins was described in GRN 593 (Section A.2.2; pp 25-28), and the safe use of the same species in thaumatin production was described in GRN 738 (Section 6.1, pp 15-16). Edible food plants used in manufacturing recombinant thaumatins are in fact “food" and are therefore generally recognized as safe for ingestion at unrestricted levels.

Nicotiana benthamiana as a preferred manufacturing host

Although the original list of production hosts described in GRN 738 included only food (edible) species, Notifier has determined that additional plant species could be included in the manufacturing host list, and that these additional hosts also yield thaumatin proteins that are safe when produced using Notifier’s manufacturing process, even though such species have not been traditionally consumed in human diets.

Nicotiana benthamiana is an ideal plant host for producing a wide range of biologics, including food additives. A thorough review of *N. benthamiana* was published by Goodin et al. (2008). The composition of this species, its genetic homogeneity, permissiveness to plant viruses, and its adoption as the preferred host for heterologous protein expression using plant virus-derived transient expression systems based on tobacco mosaic virus (TMV), potato virus X (PVX), and other vector backbones have been comprehensively reviewed (e.g., Gleba 2014). The homogeneity of the host, the ease of viral transduction for heterologous protein expression, and the high levels of accumulated protein product achievable after 7-10 days post infection, have made *N. benthamiana* a widely adopted "platform" species for plant-made pharmaceutical (PMP) R&D.

PMP is a rapidly developing field and its progress has been the subject of a number of reviews, many of which cover *N. benthamiana* as the preferred host (Cañizares 2005a; Cañizares 2005b; Chen 2011; Daniell 2009; Gleba 2004; Gleba 2005, 2007; Karg 2009; Klimyuk 2014; Komarova 2010; Lico 2008; McCormick 2008a; Mett 2008; Mortimer 2012; Pelosi 2012; Pogue 2010; Rybicki 2009; Sainsbury 2009; Saunders 2013; Smith 2009; Tusé 2011; Whaley 2011; Yusibov 2008) and others.

It is poignant that most of the PMP products that have or will enter clinical studies are manufactured in *N. benthamiana*; notably those manufactured by MAPP, KBP, Fraunhofer IMB, iBio CMO, University of Louisville/Intrucept Biomedicine, Icon Genetics and Nomad Bioscience. Universally, the products produced in *Nicotiana* have shown a high level of safety in clinical studies, even products whose release specifications for purity were set at only ≥90% (Tusé 2011). This means that *Nicotiana* host-derived impurities allowed in clinical products at up to 10% levels have failed to produce adverse events in clinical studies, even when the products were injectable vaccines co-administered with an adjuvant (McCormick 2008b; Tusé 2015).

Host impurities from *Nicotiana* species and their impact on safety

As extensively reviewed by (Leffingwell 1999), constituents of *Nicotiana* species include (a) carbohydrates, including starch, sugars, sugar esters, cellulose and pectin; (b) nitrogenous constituents, including protein, soluble amino acids, nitrate, and certain alkaloids; (c) plastid pigments, including chlorophyll and carotenoids; (d) isoprenoids and diterpenoids (both carotenoid-derived and non-carotenoid-derived), cembranoids and labdanoids; (e) phenolics, including polyphenols, lignin and various other phenolics; (f) sterols, and (g) various inorganics, including calcium, potassium, magnesium, sodium, chloride, and various other minerals that are absorbed from the soil (Leffingwell 1999).

These major structural, proteinaceous and biochemical components of *Nicotiana* species, including *N. benthamiana*, are shared with other plant species, including edible species, and are not considered inherently toxic. In fact, Wildman (1983) assessed *Nicotiana* species as a source of nutritional protein and other valuable biochemicals.

Of the minor constituents of *Nicotiana* species, it is the alkaloids that could have an impact on safety. Hence, potential toxicants need to be removed or diluted to ensure that the final product is safe. *Nicotiana* species synthesize a number of bioactive substances, some of which are toxic in sufficiently high doses. The major bioactive alkaloids in the genus include **nicotine**, **nornicotine**, **anabasine** and **anatabine**. The biosynthesis of these pyridine alkaloids, their accumulation, extraction, absorption and metabolism from various routes of exposure, and pharmacologic effects in humans and animals were discussed in detail in GRN 775 (Sections 6.1.1 – 6.1.6; pp 28-39) and GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*; Sections 6.1.2 – 6.1.4; pp 24 - 35), and are omitted here for brevity.

Table 6-2 summarizes levels of nicotine and anabasine found in *N. benthamiana* by Notifier's own analyses compared to levels reported in the literature. Nicotine is the most abundant bioactive alkaloid in

N. benthamiana, followed distantly by anabasine. Nicotine constitutes 80-90% of the total alkaloid content of *N. benthamiana* and anabasine 8-12% of the total; the typical ratio of nicotine to anabasine is ~10:1 (Sisson 1990).

Nornicotine and **anatabine** may be present in trace amounts (<1% of total alkaloids each), and at the projected use levels of thaumatins these two alkaloids are not a risk. The genetic homogeneity of *N. benthamiana* cultivars suggests that alkaloid levels and ratios will remain consistent (Goodin 2008).

Table 6-2. Levels of nicotine and anabasine in *N. benthamiana*

A	**Notifier's Internal Data**		**Data From Literature**	
Sample	**Nicotine** content ng/g fresh weight	**Nicotine** content ng/g dry weight	**Nicotine** content ng/g fresh weight	**Nicotine** content ng/g dry weight
N. benthamiana Buffer 1	**61,830 ± 29,590**	**690,000 ± 340,000**	~500,000 (Todd 2010)	~14,000,000 (Sisson 1984) ~3,000,000 (Saitoh 1985)
N. benthamiana Buffer 2	**103,330 ± 15,610**	**1,140,000 ± 190,000**		
B	**Notifier's Internal Data**		**Data From Literature**	
Sample	**Anabasine** content ng/g fresh weight	**Anabasine** content ng/g dry weight	**Anabasine** content ng/g fresh weight	**Anabasine** content ng/g dry weight
N. benthamiana Buffer 1	**7,070 ± 1,290**	**80,000 ± 20,000**		~1,300,000 (Sisson 1984) (9.3% of nicotine)
N. benthamiana Buffer 2	**12,930 ± 400**	**140,000 ± 10,000**		

Panels A and B show the levels of nicotine and anabasine, respectively, found by Notifier in analyses of *N. benthamiana* using two different buffer extraction systems, compared to results reported in the literature.

6.1.3 Process modification to lower host alkaloid content in THAUMATIN II product

The downstream process used to reduce the level of nicotine and anabasine in the purified Thaumatin II protein expressed in *N. benthamiana* is an adaptation of the method described in GRN 738 for thaumatins produced in edible plants and in Notifier's GRN 910 (THAUMATIN II Sweetener produced in *N. benthamiana*). The general approach was first described by Stephan et al. (2017) and is further detailed in APPENDIX B of this GRN, with adaptations for the properties of thaumatin proteins.

The process successfully reduces the level of host alkaloids in purified proteins relative to initial levels in the plant. Table 6-3 summarizes results from 3 batches showing average residual level of host alkaloids that are currently achievable. The process is subject to additional optimization that is expected to further reduce alkaloid residues.

Determination of alkaloid levels is discussed in APPENDIX C, Section C.5. The results were used to develop the upper alkaloid limits in the Specification, namely, 20 ng/mg protein for nicotine and 5 ng/mg protein for anabasine, for a combined limit of 25 ng total alkaloid/mg THAUMATIN II.

Table 6-3. Residual levels of *N. benthamiana* alkaloids in purified Thaumatin II protein

Protein	Residual alkaloid level in purified protein samples				
	Nicotine (ng/mg protein)		Anabasine (ng/mg protein)		No. of batches analyzed
	Single Batches	Mean ± SD	Single Batches	Mean ± SD	
Thaumatin II	14.09 [a]	14.41 ± 0.67	3.37 [a]	2.52 ± 1.55	3
	13.78 [a]		<3.84 [a]		
	15.37 [b]		0.34 [b]		

[a] Batches produced at bench scale at Notifier's facilities. [b] Batch produced at pilot-scale by Notifier's CRO.

For perspective, humans chronically ingest low levels of nicotine from their diet. Table 6-4 from Andersson (2003) summarizes the nicotine content of common vegetables as reported in multiple published studies.

Table 6-4. Average nicotine content in common vegetables

Sample	Data from literature		
	Nicotine content ng/g dry weight (Moldoveanu 2016)	Nicotine content ng/g dry weight	References
Cherry tomato, fruit	181.9 (tomato)	79-98 (a) 78-148 (b) 28-115 (c)	(a) Castro and Monji 1986 (d) Domino et al. 1993 (b) Davis et al. 1991 (e) Davis et al. 1986 (c) Liu et al. 2013 (f) Siegmund et al. 1999 (g) Andersson et al. 2003
Eggplant, fruit	174.3	1,000 (a) 52-150 (e)	
Potato (whole), tuber	42.6	71+/-59 (d) 76 (b) 45-123 (c)	
Cauliflower		3.8 (d)	
Sweet pepper		5.4 (g)	

Hence, from these published results the natural dietary intake of nicotine can be calculated. Table 6-5 (Andersson 2003) summarizes the estimated food-borne nicotine exposure in Scandinavia; although daily consumption values for each vegetable sampled may differ from US consumption estimates, nicotine ingestion ranges from **900-1,300 ng/person-day** depending on dietary habits.

Table 6-5. Consumption, nicotine content and dietary nicotine exposure from common vegetables

Food Source	Average daily consumption (g/day)	Average nicotine content (μg/kg)	Average daily dietary nicotine exposure (ng/day)
TOMATO - Sweden - Denmark	21.6 62	4.4	95.0 273
POTATO - Sweden - Denmark	168.6 156	5.8	977.9 905
EGGPLANT - Sweden - Denmark	0.71¤ 2.7	34.4	24.4 93
SWEET PEPPER - Sweden - Denmark	0.27* 5.5**	5.4	1.5 30

¤ Swedish import of eggplant in 2000 (2 297 000 kg) divided with number of citizens in 2000 (8872294) and 365 days; * only green pepper; ** green, yellow or red pepper

In Section 3.4 of this Notice, Table 3-1, we presented the estimated maximum intake of alkaloids at the daily adult thaumatin MPL of 77 mg/person. At the Specification limit for total alkaloid (25 ng/mg protein), the estimated per capita daily exposure to all host alkaloids in that "worst case" scenario was <2 μg/person-day, which is in the same range of the level consumed daily in common vegetables.

Importantly, when THAUMATIN II is used for flavor modification with a per capita consumption estimated at 5 mg per day, the total alkaloid intake would be ~125 nanograms/day, or <15% of the level in vegetables.

6.1.4 Process impurities in *N. benthamiana*-based THAUMATIN II manufacture

Other than the low risk from natural alkaloids present in *N. benthamiana* discussed above, the only remaining potential risks would come from the **vector or induction process** used to express the thaumatin genes and/or any residual reagents added in the manufacturing process.

Agrobacterial vector and ethanol induction

Gene expression and thaumatin synthesis can be induced via infection of non-GM plants with agrobacteria or via chemical induction (e.g. ethanol) in transgenic hosts, as first defined in GRN 593 and subsequently described in APPENDIX B of GRN 738 (THAUMATIN from edible species), and in GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*; APPENDIX B, pp 61-67).

When agroinfection is used, the bacterial vectors carrying viral amplicons (TMV or PVX) with the thaumatin genes are recombinantly produced and become part of the manufacturing process. When ethanol induction is used, the thaumatin genes are a stable but silent trait in the host and are expressed when a dilute solution of ethanol is applied to the plant (Werner 2011). The ethanol is removed during thaumatin purification.

Either agrobacterial induction process yields non-viable vector after the plant biomass is treated and the thaumatin protein is extracted (APPENDIX C, Section C.7 Bioburden and Residual Vector). Even if residual vector were found as a process impurity in the final product (it is not), it is worth mentioning that neither plant virus used in our process would pose a health risk.

Humans are routinely exposed to significant amounts of TMV and PVX through consumption of uncooked vegetables (e.g. spinach, beets, collard greens, tomato, cucumber, etc.). A large percentage of smokers have serum antibodies against TMV (Liu 2013) with no ill effects.

Neither virus as used in these expression systems (Gleba 2014) has been implicated as a health risk. Therefore, even if viable vector became an impurity in the final product, the levels would be lower than what humans naturally consume from ingesting produce.

Abiotic process impurities

Purified Thaumatin II protein obtained from two bench-scale developmental batches and from one pilot-scale manufacturing batch have been analyzed for the **elemental impurities** of most concern in food (i.e. Class 1 heavy metals due to their high toxicities; see APPENDIX C, Section C.6 Analysis of Elemental Impurities). The results are summarized in Table 6-6.

These values were used to set Specification limits of 1 ng for Pb and 5 ng total Class 1 metals per mg Thaumatin II protein. Hence, hypothetical exposure to these most toxic metals (Pb, Cd, Hg, As) from consumption of THAUMATIN II as a flavor modifier (5 mg/day) and at the maximum permitted level (MPL; 77 mg/day) are projected to be **~25 nanograms/person-day** and **<400 nanograms/person-day**, respectively.

Considering that the cumulative PDE for this combination of Class 1 elements is **55 micrograms/person-day** (ICH 2015), we conclude that the intake of heavy metal impurities from consumption of THAUMATIN II at either flavor modifier or MPL quantities will be inconsequential and will constitute a very low safety risk.

Table 6-6. Estimated exposure to elemental impurities in THAUMATIN II

Heavy Metal Specification, THAUMATIN II			Estimated Exposure		PDE
Element	**Class**	**Spec. Limit**	**Flavor Mod.**	**MPL**	**(Oral/Daily)**
		(ng/mg protein)	**(ng/day)**	**(ng/day)**	**(μg/day)**
Pb	1	1	5	77	5
Sum of Pb, Cd, Hg, As	1	5	25	385	55

Specification limits for Pb and for all Class 1 heavy metals (Pb, Cd, Hg, As) were set at 1 ng/mg protein and 5 ng/mg protein, respectively, based on results of multi-batch analyses described in APPENDIX C, Section C.6. The Specification limits were multiplied by 77 or by 5, to estimate total potential exposure from consumption of Thaumatin II at the thaumatin MPL or at the level used as a flavor modifier; values are expressed as nanograms/person-day (ng/day). The estimated elemental intake values at each level of consumption are compared to the permitted daily exposure (PDE) for oral intake of Class 1 elements and are expressed in micrograms/person-day (μg/day; last column in the table; ICH 2015).

The conclusion that can be drawn with these new data is that consumption of 5 mg THAUMATIN II/day as a flavor modifier would expose consumers to 5 x 1 = 5 ng/day of Pb and to 77 x 1 = 77 ng Pb/day from all uses at the MPL. Likewise, exposure to all Class 1 heavy metals would lead to consumption of 5 x 5 = 25 ng heavy metals/day from flavor modifier uses and to 77 x 5 = 385 ng heavy metals/day from all uses at the MPL.

Compared to the 5 micrograms/day (Pb) and 55 micrograms/day (sum of Class 1 elements) permitted daily exposures (PDE) to these same heavy metals, respectively, exposure from THAUMATIN II consumption would lead to less than 0.1% and ~1.5% Pb exposure from flavor modifier uses and MPL from all uses, respectively, relative to the PDE. Similarly, consumption of THAUMATIN II would expose consumers to <0.045% and ~0.7% of the total Class 1 metals PDE from consumption of the product as a flavor modifier and from all uses at the MPL, respectively. For perspective, using the NHEXAS database, Ryan (2001) estimated the actual oral **daily intake** of heavy metals in Maryland residents as 28 **mg** for arsenic, 10 **mg** for cadmium, and 8 **mg** for lead.

ASTSDR (1999) states that the mercury intake through food is approximately 3.5 **mg**/day. Compared to these reported levels of heavy metal intake and even the guidance limits (PDEs), the level of exposure to these elements from THAUMATIN II should be of little consequence with respect to health risks. The levels of Class 2 and Class 3 elements in a pilot-scale batch of THAUMATIN II were analyzed and found to total less than 1 ng/mg protein combined. These very low levels are inconsequential to safety and hence Class 2 and Class 3 metals were omitted from the Specification.

With respect to abiotic safety risks from **process reagents**, all processing aids, aqueous buffers/salts and solvents are selected from lists of approved food additives and food processing aids. Any residual levels of processing reagents would introduce no more risk than those derived from the consumption of other food products that also utilize them.

6.2. Safety in Relation to Dietary Intake of THAUMATIN II

We expect the same maximum levels of consumption of Notifier's plant-made THAUMATIN II, whether produced in edible hosts or in *N. benthamiana*, per weight or volume of treated food as those allowed for all uses of plant-extracted (i.e. *Thaumatococcus*) thaumatins. We also expect a much-reduced, moderate level of Thaumatin II protein consumption when THAUMATIN II is used as a flavor modifier. We do not compare use rates of THAUMATIN II to those allowed by FEMA for recombinant thaumatin B (FEMA 3814) because that material is derived from microbial fermentation, has a different amino acid composition, and its impurity profile is expected to differ from those of the plant-derived thaumatin proteins.

FEMA's GRAS List (Cohen 2016; 2018) provides guidance on US application rates for thaumatins when used in flavor modification. Table 6-7 lists the original and expanded-use levels for extracted and recombinantly produced thaumatins for US food products. Although Notifier evaluated functionality of THAUMATIN II at concentrations ranging from 0.6 ppm to 28 ppm (APPENDIC C, Section C.8), the higher levels introduced undesirable sweetness and Notifier expects that, for flavor modification, THAUMATIN II can be used on the various foods listed in Table 6-7 at equivalent application rates as those of current thaumatin products. Notwithstanding these use rates, Notifier excludes from this Notice applications of THAUMATIN II to any USDA/FSIS-regulated product, as the data supporting suitability in such products are under development.

Extensive preclinical safety studies, followed by focused clinical trials, led to definition of MPL for thaumatin in various foods. The most comprehensive guidance appears in the EU because thaumatins are allowed for both sweetening uses and flavor modification in a wide range of foods. The statutes were established by the European Food Safety Authority (EFSA 2015) based on reviews by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1983, 1986) and the EU Scientific Committee on Food (SCF 1985, 1989).

Detail discussions on Notifier's adoption of EFSA guidance concerning MPL and exposure calculations were presented in GRN 738 and GRN 910. In its Notices, Notifier concluded that an assessment of the potential dietary intake of thaumatins can best be derived from EU maximum usage data, which show that, from all dietary sources, continuous thaumatin ingestion levels of up to 1.1 mg/kg (bw)-day in all age populations, or 77 mg/person-day for a 70-kg adult, are safe.

The commercial introduction of Notifier's THAUMATIN II product for either sweetener uses or flavor modification is expected to displace, initially, thaumatin that is currently derived from extraction of the same proteins from *Thaumatococcus*. Should industrial thaumatin usage expand due to greater availability of THAUMATIN II, consumption of thaumatin would be expected to increase. Even if daily per capita intake limit were to double to 150-200 mg from the current 77 mg MPL, thaumatin's safety factor of 1,300 based on chronic toxicity studies suggest that any additional safety risk to consumers would be minimal (EFSA 2015; Hagiwara 2005; OJEU 2011).

Table 6-7. FEMA Updated thaumatin use levels (2018)

	Thaumatin	Thaumatin B-Recombinant		Thaumatin	Thaumatin B-Recombinant
FEMA NO.	3732	3814	FEMA NO.	3732	3814
GRAS PUBLICATION	13	17	GRAS PUBLICATION	13	17
CATEGORY			CATEGORY		
Baked goods	7[a]/7[a]	1/1	Jams and jellies	7[a]/7[a]	2/5
Beverages, non-alcoholic	7[a]/7[a]	5/7[a]	Meat products	7[a]/7[a]	2/2
Beverages, alcoholic	7[a]/7[a]	5/7[a]	Milk products	7[a]/7[a]	3/6
Breakfast cereals	7[a]/7[a]	1/2	Nut products	7[a]/7[a]	5/7[a]
Cheeses	7[a]/7[a]	7[a]/7[a]	Other grains	7[a]/7[a]	
Chewing gum	150[a]/150[a]	150/150[a]	Poultry	7[a]/7[a]	2/5
Condiments and relishes	7[a]/7[a]	1/2	Processed fruits	7[a]/7[a]	2/5
Confections and frostings	7[a]/7[a]	2/5	Processed vegetables	7[a]/7[a]	2/5
Egg products	7[a]/7[a]	2/5	Reconstituted vegetables	7[a]/7[a]	2/5
Fats and oils	7[a]/7[a]		Seasonings and flavors	7[a]/7[a]	0.5/1
Fish products	7[a]/7[a]	5/7[a]	Snack foods	7[a]/7[a]	1/2
Frozen dairy	7[a]/7[a]	1/2	Soft candy	7[a]/7[a]	2/5
Fruit ices	7[a]/7[a]	2/5	Soups	7[a]/7[a]	2/5
Gelatins and puddings	7[a]/7[a]	1/2	Sugar substitutes		0[a]/0[a]
Granulated sugar			Sweet sauces	7[a]/7[a]	2/5
Gravies	7[a]/7[a]	2/5			
Hard candy	7[a]/7[a]	2/5			
Imitation dairy	7[a]/7[a]	7[a]/7[a]			
Instant coffee and tea	7[a]/7[a]	2/5			

Abstracted from FEMA GRAS™ 28 List (July 2018). Columns show allowed use levels for extracted and recombinant thaumatins in various food products. FEMA Updated average usual use levels (ppm)/average maximum use levels (ppm) for flavoring substances previously recognized as FEMA GRAS. Superscript "a" represents a new use level (Cohen 2016; 2018).

6.3. Occupational safety

With respect to occupational safety, Notifier's Thaumatin II protein is considered non-toxic. No higher-level precautions for personnel protection are suggested for handling, preparing or disposing of THAUMATIN II powder or liquid solutions. Only general precautions for handling powdered bulk substances and protein-containing waste solutions are suggested. A draft SDS for THAUMATIN II is appended to this Notice (APPENDIX A), where additional recommendations for storage, handling and disposal of this product are listed.

6.4. GRAS conclusion for *N. benthamiana*-produced THAUMATIN II flavor modifier

The results of clinical studies by Higginbotham et al. (1983) assessing the effects of occupational pulmonary exposure to thaumatin-containing substances, as well as the effects of oral ingestion of thaumatins, corroborate the results they obtained with multiple animal species that show that thaumatins are digestible, show no systemic toxicity when administered orally, and have low allergenic potential if delivered systemically but none if delivered orally.

The sub-acute (28-day with 14-day thaumatin plus 14-day lactose cross-over) ingestion study with human volunteers administered a total of 1.4 grams of thaumatin over a two-week period, with no ill effects. Similarly, the multi-year occupational exposure to low levels of thaumatin-containing dust in the workplace had no obvious toxic effects and only low measurable sensitivity, which was lower in prevalence than allergy to dust mites in the surveyed population.

Subsequently, Hagiwara et al. (2005) reported results of a 13-week (sub-chronic) feeding study of thaumatin in Sprague–Dawley rats. Thaumatin was administered at dietary levels of 0% (control), 0.3%, 1.0% and 3.0% to groups of 10 male and 10 female Crj:CD (SD) IGS rats. There were no treatment-related clinical signs or adverse effects on the survival rate, body weight, food consumption, water consumption and urinalysis, ophthalmology, hematology, or blood biochemistry data. No treatment-related alterations in gross pathology or organ weights were found in any group. On histopathological examination, sporadic spontaneous lesions known to occur in this strain of rats were the only findings, with no specific relation to the test substance.

Safety data available at the time helped support extensive reviews conducted by the EU Scientific Committee on Food (SCF 1985, 1989) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1983, 1986). Subsequently, Hagiwara et al. (2005) calculated the no-observed-adverse-effect-level (NOAEL) in rats to be a dietary intake of 2,502 mg/kg body weight-day for males and 2,889 mg/kg body weight-day for females. A study in dogs established a lower average NOAEL of 1,400 mg/kg body weight/day. Collectively, these studies have helped update the application rates of thaumatin. The levels of usage of thaumatin on various food groups, the consumption of each food type, and the anticipated total exposure to thaumatin as a food additive have since been aggregated and updated (EFSA 2015).

Based on the lowest NOAEL of 1,400 mg/kg bw-day for dogs calculated from results by Higginbotham et al. (1983) and Hagiwara et al. (2005) and others, EFSA applied a **safety factor of ~1,300 for E 957** (1,400 mg/kg bw-day in dogs and the then-current human maximum daily intake level of 1.03 mg/kg body wt-day) to arrive at an MPL for thaumatin by food product group, including solids, semi-solids and liquid beverages, for all age groups. The cumulative daily limit for chronic human consumption was set at 1.1 mg/kg-day, or 77 mg/day for a 70-kg adult person. At this intake level, thaumatin protein would represent <0.13% of the total adult daily protein intake (i.e. nutritionally insignificant). Even at 2x the current intake level, the safety factor would be nearly 700:1.

The safety of Thaumatin II protein comprising Notifier's THAUMATIN II product can be deduced by analogy to the thaumatin products evaluated in the studies cited herein. Higginbotham et al. (1983) evaluated thaumatin compositions of various degrees of purity, including commercial thaumatin (Thalin®) that contained 3% residual host impurities, including non-thaumatin proteins, carbohydrates, pectin, and glucuronoxylan from the arils of the source fruit. Ramsohoye (1989) found non-thaumatin protease and esterase impurities in commercial thaumatin products. In addition, because the original Tate & Lyle thaumatin extraction process involved the use of aluminum salts to assist with purification, the final product contained small amounts of the heavy metal. Importantly, in spite of these findings, the toxicity studies of Higginbotham et al. included evaluation of isolated host and process impurities, which proved to be innocuous with respect to safety. Since Notifier's submission of GRN 738 (THAUMATIN from edible plant species; 16 October 2017), and GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*; 3 February 2020), no new literature was identified citing thaumatin safety concerns.

In the current Notice, Notifier has concluded using scientific procedures that its Thaumatin II protein produced in the host *Nicotiana benthamiana* is generally recognized as safe when used at up to 100% of the MPL allowed by current statutes for commercially available thaumatins. When used as a flavor modifier, Thaumatin II would be ingested at not more than 6.5% of the daily MPL (i.e., 5 mg/77 mg person-day). To ensure a high safety margin, host-derived alkaloids are monitored and controlled to minimize risk. In our target Specification for Thaumatin II, we have set 20 ng/mg and 5 ng/mg as the maximum nicotine and anabasine levels, respectively, and total alkaloids of ≤25 ng/mg protein in a final product. At the Specification limit, ingestion of the adult thaumatin MPL of 77 mg/day would lead to total alkaloids consumed of ~2 μg/person-day, or the equivalent of 1-2 extra servings of vegetables at the current average rate of US food consumption (0.56 kg food/day). At the same Specification limit, consumption of 5 mg Thaumatin II flavor modifier/person-day would result in exposure to ~125 ng total alkaloid.

We present data from multiple public sources that these levels of pyridine alkaloids are below the threshold of pharmacologic or toxic activity even for intravenous administration. We reiterate that these low exposures from ingestion of residual alkaloids from Thaumatin II are exaggerated by the assumption of 100% adoption of Notifier's product in all food categories claimed and at the maximum permitted level (MPL) of the product.

Notifier describes a process modification introduced into its manufacturing method when using *N. benthamiana* as the expression host for thaumatins (APPENDIX B). The modification involves cool ambient temperature neutral extraction of biomass, heat treatment, and cation-exchange chromatography followed by diafiltration. These downstream steps effectively reduce the levels of host alkaloids and other impurities in the purified Thaumatin II protein.

We also present the content of Class 1 elemental impurities in purified Thaumatin II protein derived from analyses of multiple batches. Our process is undergoing optimization, yet current results enabled us to set a Specification limit of 5 ng total Class 1 metals (sum of Pb, Cd, Hg, As) per mg THAUMATIN II. At that limit, total heavy metal exposure resulting from the consumption of THAUMATIN II at the adult MPL (77 mg/day) would be <400 ng/person-day, whereas 25 ng/person-day elemental intake would be realized from THAUMATIN II's use only as a flavor modifier (5 mg/day).

These levels pose no undue risk to consumers relative to the cumulative Class 1 metal PDE of 55 micrograms/day. Preliminary analyses of the less toxic Class 2 and Class 3 metals in a pilot-scale manufacturing batch of THAUMATIN II have yielded very low impurity levels (sum of Ag, Bi, Cu, Mo, Sb and Sn = <1 ng/mg protein; Table C-4), and hence these additional elements are not included in the Specification.

There are no other identifiable toxic entities in *N. benthamiana* that we could find from extensive literature surveys or from our own analyses. The protein, lipid, carbohydrate and mineral components of the plant have been studied and are found in the public record, and reveal no unusual properties of the plant that are not also present in solanaceous plant species consumed as food.

Similarly, no allergenic or hypersensitivity inducing components have been reported for this species. In an average diet, exposure to residual *N. benthamiana* proteins would be ≤ 7 ppm/day (MPL worst case); hence, host proteins are not a risk factor in the product.

Conclusion

The totality of evidence, from Notifier's internal studies and from the extensive public record cited herein, in GRN 738, and in GRN 910 for THAUMATIN II Sweetener from *N. benthamiana*, suggests that consumer risk associated with ingestion of thaumatins themselves, or with ingestion of trace amounts of impurities derived from manufacturing Thaumatin II protein in *N. benthamiana*, is low. Consumers already ingest equivalent or even higher levels of the same impurities in their daily diet, and any contribution of additional alkaloids or elemental impurities from the use of THAUMATIN II as a flavor modifier is not expected to significantly raise dietary risk.

7. Supporting Data and Information

Multiple sources of information were used to support the conclusion that Notifier's THAUMATIN II product produced in the host *N. benthamiana* is GRAS. Table 7-1 lists the various data and other information discussed in this Notice and used in reaching this conclusion. Also listed in the table is whether the specific information cited was generated by Notifier and/or obtained from databases or references in the public domain.

The active ingredient, Thaumatin II protein, in Notifier's THAUMATIN II product is of the same molecular composition as the active ingredient in thaumatin II-containing products derived from extracts of *Thaumatococcus*, and has the same functional properties. Therefore, we draw analogies to those referenced products with respect to flavor modification functionality, dietary use levels, and safety.

Table 7-1. Information supporting THAUMATIN II flavor modifier GRAS conclusion

Topic	Location in this GRN	Source of data or information	Availability
Thaumatin identity	Section 2.1	Greenly 2003; Hough 1993a; Lee 1987; Suami 1997; van der Wel 1972; Zemanek 1995;	Public
Thaumatin structure and function	Section 2.1	de Vos 1985; Faus 2008; Hough 1993a; Kim 1988; Masuda 2011; Masuda 2013; Masuda 2014; Ogata 1992; Suami 1997; van der Wel 1984	Public
Mode of action and mechanisms of taste perception	Section 2.1	Bartoszewski 2003; Faus 2008; Fernstrom 2012; Glaser 2000; Greenly 2003; Hellekant 1996; Kaneko 2001; Kinghorn 1986; Ledeboer 1984; Li 2002; Lim 2012; Margolskee 2001; Masuda 2013; Masuda 2016; Masuda 2018; Nagarajan 2017; Nelson 2001; Ohta 2011; Sardesai 1991; Schiffman 1995; Suami 1997; Tinti 2000; van der Wel 1972; Witty 1990	Public

Topic	Location in this GRN	Source of data or information	Availability
Method of manufacture and QC	Section 2.2 APPENDIX B	Nomad Bioscience GmbH	This GRN
Host, vector and process impurities	Section 2.2 APPENDIX B APPENDIX C	Nomad Bioscience GmbH	GRN 738, Dec 9, 2019 GRN and This GRN
Composition and Specification	Section 2.3 Table 2-1	Nomad Bioscience GmbH	Dec 9, 2019 GRN and This GRN
Technical effect and suitability of use	Section 2.4 Table 2-2 APPENDIX C	Nomad Bioscience GmbH	This GRN
Allowed maximum and moderate application rates; Estimated dietary intake; Additional exposure not related to Notifier's product	Sections 3.1, 3.2 and 3.3	Codex Alimentarius 2016; Cohen 2016; Cohen 2018; EFSA 2008, 2011, 2015; OJEU 2011; Nomad Bioscience GmbH	Public and This GRN
Dietary exposure to host- and process-derived impurities	Section 3.4 Table 3-1	Nomad Bioscience GmbH	This GRN
Thaumatin overall safety; digestibility; potential for gene transfer to intestinal microflora	Section 6.1	EFSA 2011, 2015; Hagiwara 2005; Higginbotham 1983; JECFA 1983, 1986; SCF 1985, 1989; Szwacka 2012	Public
Estimation of thaumatin's NOAEL	Section 6.1	Hagiwara 2005; Higginbotham 1983	Public
Determination of thaumatin's allergenic potential	Section 6.1.1	a. Computational estimates / *in silico* modeling: Baniulis 2008; Nomad Bioscience GmbH b. Empirical studies, preclinical and clinical, Higginbotham 1983	a. Public and GRN 738 b. Public
Commonly consumed and environmentally contacted thaumatin-like proteins (TLP)	Section 6.1.1	Barre 2000; Christensen 2002; Liu 2010; van Loon 2006	Public and GRN 738
Allergenicity and occupational safety	Sections 6.1.1 and 6.4	Higginbotham 1983	Public

Topic	Location in this GRN	Source of data or information	Availability
Safety in relation to allowed dietary intake of thaumatin	Sections 3.1, 3.2, 6.1 and 6.2	Codex Alimentarius 2016; Cohen 2016; Cohen 2018; EFSA 2015; OJEU 2011; Oser 1984; Smith 1996; Nomad Bioscience GmbH.	Public and This GRN
Potential additional exposure if consumption of thaumatins increases	Section 6.2	EFSA 2015; Hagiwara 2005; OJEU 2011	Public
THAUMATIN II Safety Data Sheet (SDS)	APPENDIX A	a. Nomad Bioscience GmbH b. SCBT 2015; SFC 2006; Sigma-Aldrich 2017	a. This GRN b. Public
Thaumatin II analysis and characterization	APPENDIX C	Nomad Bioscience GmbH	This GRN
Determination of Thaumatin II's flavor modification profiles	APPENDIX C	Nomad Bioscience GmbH	This GRN

References

Adesina SK and JD Higginbotham. 1977. Studies on a novel polysaccharide gel from the fruit of Thaumatococcus daniellii (Benth). *Carbohydrate Research* 59(2):517-524.

Adesina SK and JB Harborne. 1978. Occurrence and identification of falvonoids in Thaumatococcus daniellii Benth. *Journal of Medicinal Plant Research* 34:323-327.

Andersson C, P Wennström and J Gry. 2003. Nicotine alkaloids in Solanaceous food plants. Nordic Working Group on Food Toxicology and Risk Evaluation. Nordic Council of Ministers. 92-893-0905-9. p 1-37.

Asherie N, C Ginsberg, A Greenbaum, S Blass and S Knafo. 2008. Effects of Protein Purity and Precipitant Stereochemistry on the Crystallization of Thaumatin†. *Crystal Growth & Design* 8(12):4200-4207.

ASTSDR. 1999. Public Health Statement: Mercury CAS#: 7439-97-6. CDC. https://www.atsdr.cdc.gov/PHS/PHS.asp?id=112&tid=24. Accessed Nov 21, 2018.

Baniulis D, V Stanys, G Vaitiekaitis, A Sasnauskas, D Gelvonauskienė and J Liobikas. 2008. Computational analysis of thaumatin-II allergenicity and prediction of antigenic elements of thaumatin-like family proteins. *Biologija* 54(3):202-207.

Barre A, WJ Peumans, L Menu-Bouaouiche, et al. 2000. Purification and structural analysis of an abundant thaumatin-like protein from ripe banana fruit. *Planta* 211(6):791-799.

Bartoszewski G, A Niedziela, M Szwacka and K Niemirowicz-Szczytt. 2003. Modification of tomato taste in transgenic plants carrying a thaumatin gene from Thaumatococcus daniellii Benth. *Plant Breeding* 122(4):347-351.

Buzby JC, HF Wells and J Hyman. 2014. The Estimated Amount, Value, and Calories of Postharvest Food Losses at the Retail and Consumer Levels in the United States, EIB-121. *US Department of Agriculture, Economic Research Service*.

Cañizares MC, GP Lomonossoff and L Nicholson. 2005a. Development of cowpea mosaic virus-based vectors for the production of vaccines in plants. *Expert Rev Vaccines* 4(5):687-697.

Cañizares MC, L Nicholson and GP Lomonossoff. 2005b. Use of viral vectors for vaccine production in plants. *Immunol Cell Biol* 83(3):263-270.

Chen Q, J He, W Phoolcharoen and HS Mason. 2011. Geminiviral vectors based on bean yellow dwarf virus for production of vaccine antigens and monoclonal antibodies in plants. *Hum Vaccin* 7(3):331-338.

Christensen AB, BH Cho, M Naesby, et al. 2002. The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins. *Mol Plant Pathol* 3(3):135-144.

Codex Alimentarius. 2016. General Standard for Food Additives, CODEX STAN 192-1995 (Adopted in 1995. Revision 1997, 1999, 2001, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016). FAO/WHO.

Cohen SM, S Fukushima, NJ Gooderham, et al. 2016. FEMA GRAS Flavoring Substances 28 (Interim).

Cohen SM, G Eisenbrand, S Fukushima, et al. 2018. FEMA GRAS 28 Flavoring Substances.

Cusack M, AG Stephen, R Powls and RJ Beynon. 1991. Purification and characterization of thaumatopain, a cysteine protease from the arils of the plant Thaumatococcus daniellii. *Biochem J* 274 (Pt 1):231-236.

Daniell H, ND Singh, H Mason and SJ Streatfield. 2009. Plant-made vaccine antigens and biopharmaceuticals. *Trends Plant Sci* 14(12):669-679.

Davis RA, MF Stiles, JD deBethizy and JH Reynolds. 1991. Dietary nicotine: a source of urinary cotinine. *Food Chem Toxicol* 29(12):821-827.

de Vos AM, M Hatada, H van der Wel, H Krabbendam, AF Peerdeman and SH Kim. 1985. Three-dimensional structure of thaumatin I, an intensely sweet protein. *Proc Natl Acad Sci U S A* 82(5):1406-1409.

Digard H, C Proctor, A Kulasekaran, U Malmqvist and A Richter. 2013. Determination of nicotine absorption from multiple tobacco products and nicotine gum. *Nicotine Tob Res* 15(1):255-261.

Domino EF, E Hornbach and T Demana. 1993. The nicotine content of common vegetables. *N Engl J Med* 329(6):437.

EFSA. 2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union*.

EFSA. 2011. Scientific Opinion on the Safety and Efficacy of thaumatin for all animal species. *EFSA Journal* 9(9):1-10.

EFSA. 2015. Scientific Opinion on the safety of the extension of use of thaumatin (E 957). *EFSA Journal* 13(11):4290.

Faus I and H Sisniega. 2008. Sweet-tasting proteins. *Biopolymers* 8:203-210.

Fernstrom JD, SD Munger, A Sclafani, IE de Araujo, A Roberts and S Molinary. 2012. Mechanisms for sweetness. *J Nutr* 142(6):1134S-1141S.

Glaser D, M Wanner, JM Tinti and C Nofre. 2000. Gustatory responses of pigs to various natural and artificial compounds known to be sweet in man. *Food Chemistry* 68(4):375-385.

Gleba Y, S Marillonnet and V Klimyuk. 2004. Engineering viral expression vectors for plants: the 'full virus' and the 'deconstructed virus' strategies. *Curr Opin Plant Biol* 7(2):182-188.

Gleba Y, V Klimyuk and S Marillonnet. 2005. Magnifection--a new platform for expressing recombinant vaccines in plants. *Vaccine* 23(17-18):2042-2048.

Gleba Y, V Klimyuk and S Marillonnet. 2007. Viral vectors for the expression of proteins in plants. *Curr Opin Biotechnol* 18(2):134-141.

Gleba YY, D Tuse and A Giritch. 2014. Plant viral vectors for delivery by *Agrobacterium*. *Curr Top Microbiol Immunol* 375:155-192.

Goodin MM, D Zaitlin, RA Naidu and SA Lommel. 2008. *Nicotiana benthamiana*: its history and future as a model for plant-pathogen interactions. *Mol Plant Microbe Interact* 21(8):1015-1026.

Green C. 1999. Thaumatin: a natural flavour ingredient. *World Rev Nutr Diet* 85:129-132.

Greenly LW. 2003. A doctor's guide to sweeteners. *Journal of Chiropractic Medicine* 2(2):80-86.

Hagiwara A, H Yoshino, M Sano, et al. 2005. Thirteen-week feeding study of thaumatin (a natural proteinaceous sweetener), sterilized by electron beam irradiation, in Sprague-Dawley rats. *Food Chem Toxicol* 43(8):1297-1302.

Hahn S, A Giritch, D Bartels, L Bortesi and Y Gleba. 2015. A novel and fully scalable *Agrobacterium* spray-based process for manufacturing cellulases and other cost-sensitive proteins in plants. *Plant Biotechnol J* 13(5):708-716.

Hellekant G and V Danilova. 1996. Species differences toward sweeteners. *Food Chemistry* 56(3):323-328.

Higginbotham JD, DJ Snodin, KK Eaton and JW Daniel. 1983. Safety evaluation of thaumatin (Talin protein). *Food Chem Toxicol* 21(6):815-823.

Hough L. 1993a. High-intensity, low-calorie sweeteners. In: *Low-calorie Foods and Food Ingredients* Khan R (ed). Blackie Academic & Professional, London.

Hough L and R Khan. 1993b. The relationship between sweetness and the molecular structure of sucrose and its derivatives. In: *Sweet-Taste Chemoreception* M. Mathlouthi JAKGGB (ed). Elsevier Applied Science, London.

ICH. 2015. Guidance for Industry Q3D Elemental Impurities.

JECFA. 1983. Evaluation of certain food additives and contaninants. WHO. vol 696.

JECFA. 1986. Evaluation of certain food additives and contaminants. WHO. vol 733.

Joseph JA, S Akkermans, P Nimmegeers and JFM Van Impe. 2019. Bioproduction of the Recombinant Sweet Protein Thaumatin: Current State of the Art and Perspectives. *Front Microbiol* 10(695).

Kaneko R and N Kitabatake. 2001. Sweetness of sweet protein thaumatin is more thermoresistant under acid conditions than under neutral or alkaline conditions. *Biosci Biotechnol Biochem* 65(2):409-413.

Karačonji IB. 2005. Facts about nicotine toxicity. *Arh Hig Rada Toksikol* 56(4):363-371.

Karg SR and PT Kallio. 2009. The production of biopharmaceuticals in plant systems. *Biotechnol Adv* 27(6):879-894.

Kim S-H, A de Vos and C Ogata. 1988. Crystal structures of two intensely sweet proteins. *Trends Biochem Sci* 13(1):13-15.

Kinghorn AD, DD Soejarto and GE Inglett. 1986. Sweetening agents of plant origin*. *Critical Reviews in Plant Sciences* 4(2):79-120.

Klimyuk V, G Pogue, S Herz, J Butler and H Haydon. 2014. Production of Recombinant Antigens and Antibodies in *Nicotiana benthamiana* Using 'Magnifection' Technology: GMP-Compliant Facilities for Small- and Large-Scale Manufacturing. *Curr Top Microbiol Immunol* 375:127-154.

Komarova TV, S Baschieri, M Donini, C Marusic, E Benvenuto and YL Dorokhov. 2010. Transient expression systems for plant-derived biopharmaceuticals. *Expert Rev Vaccines* 9(8):859-876.

Ledeboer AM, CT Verrips and BM Dekker. 1984. Cloning of the natural gene for the sweet-tasting plant protein thaumatin. *Gene* 30(1-3):23-32.

Lee C-K. 1987. The Chemistry and Biochemistry of the Sweetness of Sugars. *Advances in Carbohydrate Chemistry and Biochemistry* 45:199-351.

Leffingwell JC. 1999. Basic Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types. In: *Tobacco: Production, Chemistry, And Technology* Davis DL, Nielson MT (eds). Blackwell Science.

Li X, L Staszewski, H Xu, K Durick, M Zoller and E Adler. 2002. Human receptors for sweet and umami taste. *Proc Natl Acad Sci U S A* 99(7):4692-4696.

Lico C, Q Chen and L Santi. 2008. Viral vectors for production of recombinant proteins in plants. *J Cell Physiol* 216(2):366-377.

Lim TK. 2012. *Thaumatococcus daniellii*. In: *Edible Medicinal And Non Medicinal Plants: Volume 3, Fruits*. Springer Netherlands, Dordrecht, p 259-264.

Liu JJ, R Sturrock and AK Ekramoddoullah. 2010. The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. *Plant Cell Rep* 29(5):419-436.

Liu R, RA Vaishnav, AM Roberts and RP Friedland. 2013. Humans have antibodies against a plant virus: evidence from tobacco mosaic virus. *PLoS One* 8(4):e60621.

Margolskee RF. 2001. Molecular mechanisms of bitter and sweet taste transduction. *J Biol Chem* 277(1):1-4.

Martyn DM and A Lau. 2019. Chewing gum consumption in the United States among children, adolescents and adults. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 36(3):350-358.

Masuda T, K Ohta, F Tani, B Mikami and N Kitabatake. 2011. Crystal structure of the sweet-tasting protein thaumatin II at 1.27Å. *Biochem Biophys Res Commun* 410(3):457-460.

Masuda T, W Taguchi, A Sano, K Ohta, N Kitabatake and F Tani. 2013. Five amino acid residues in cysteine-rich domain of human T1R3 were involved in the response for sweet-tasting protein, thaumatin. *Biochimie* 95(7):1502-1505.

Masuda T, B Mikami and F Tani. 2014. Atomic structure of recombinant thaumatin II reveals flexible conformations in two residues critical for sweetness and three consecutive glycine residues. *Biochimie* 106:33-38.

Masuda T, K Ohta, N Ojiro, et al. 2016. A Hypersweet Protein: Removal of The Specific Negative Charge at Asp21 Enhances Thaumatin Sweetness. *Sci Rep* 6:20255.

Masuda T, S Kigo, M Mitsumoto, et al. 2018. Positive Charges on the Surface of Thaumatin Are Crucial for the Multi-Point Interaction with the Sweet Receptor. *Front Mol Biosci* 5:10.

McCormick AA and KE Palmer. 2008a. Genetically engineered Tobacco mosaic virus as nanoparticle vaccines. *Expert Rev Vaccines* 7(1):33-41.

McCormick AA, S Reddy, SJ Reinl, et al. 2008b. Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical study. *Proc Natl Acad Sci U S A* 105(29):10131-10136.

Mett V, CE Farrance, BJ Green and V Yusibov. 2008. Plants as biofactories. *Biologicals* 36(6):354-358.

Moldoveanu SC, WA Scott and DM Lawson. 2016. Nicotine Analysis in Several Non-Tobacco Plant Materials. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research* 27(2).

Mortimer E, JM Maclean, S Mbewana, et al. 2012. Setting up a platform for plant-based influenza virus vaccine production in South Africa. *BMC Biotechnol* 12:14.

Most BH, RJ Summerfield and M Boxall. 1978. Tropical Plants with Sweetening Properties Physiological and Agronomic Problems of Protected Cropping: 2. *Thaumatococcus daniellii*. *Economic Botany* 32(3):321-335.

Nagarajan R. 2017. Amazing, super-sweet natural proteins: A look at three alternate natural sweeteners that could be safe for our health. O'Reilly Media. https://www.oreilly.com/ideas/amazing-super-sweet-natural-proteins. Accessed 7/1/2017 2017.

Natex. 2017. The Science Of Thaumatin. http://www.thaumatin.com/science-of-thaumatin. Accessed 8/16/2017 2017.

Nelson G, MA Hoon, J Chandrashekar, Y Zhang, NJ Ryba and CS Zuker. 2001. Mammalian sweet taste receptors. *Cell* 106(3):381-390.

Nielsen SS, GM Franklin, WT Longstreth, PD Swanson and H Checkoway. 2013. Nicotine from edible *Solanaceae* and risk of Parkinson disease. *Ann Neurol* 74(3):472-477.

Nikolic J, I Mrkic, M Grozdanovic, et al. 2014. Protocol for simultaneous isolation of three important banana allergens. *J Chromatogr B Analyt Technol Biomed Life Sci* 962:30-36.

Ogata CM, PF Gordon, AM de Vos and SH Kim. 1992. Crystal structure of a sweet tasting protein thaumatin I, at 1.65 Å resolution. *J Mol Biol* 228(3):893-908.

Ohta K, T Masuda, F Tani and N Kitabatake. 2011. The cysteine-rich domain of human T1R3 is necessary for the interaction between human T1R2-T1R3 sweet receptors and a sweet-tasting protein, thaumatin. *Biochem Biophys Res Commun* 406(3):435-438.

OJEU. 2011. Annex II Regulations: Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union*.

Oser BL, RA Ford and BK Bernard. 1984. Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. 13. GRAS Substances. *Food Technology* 38(10):66-89.

Pelosi A, R Shepherd and AM Walmsley. 2012. Delivery of plant-made vaccines and therapeutics. *Biotechnol Adv* 30(2):440-448.

Pogue GP, F Vojdani, KE Palmer, et al. 2010. Production of pharmaceutical-grade recombinant aprotinin and a monoclonal antibody product using plant-based transient expression systems. *Plant Biotechnol J* 8(5):638-654.

Ramsohoye PV and IA Kozlov. 1989. An investigation of enzyme activities found in thaumatin preparations. *International Journal of Food Science & Technology* 24(6):619-627.

Ryan PB, KA Scanlon and DL MacIntosh. 2001. Analysis of dietary intake of selected metals in the NHEXAS-Maryland investigation. *Environ Health Perspect* 109(2):121-128.

Rybicki EP. 2009. Plant-produced vaccines: promise and reality. *Drug Discov Today* 14(1-2):16-24.

Sainsbury F, L Liu and GP Lomonossoff. 2009. Cowpea mosaic virus-based systems for the expression of antigens and antibodies in plants. *Methods Mol Biol* 483:25-39.

Sardesai VM and TH Waldshan. 1991. Natural and synthetic intense sweeteners. *The Journal of Nutritional Biochemistry* 2(5):236-244.

Saunders K and GP Lomonossoff. 2013. Exploiting plant virus-derived components to achieve *in planta* expression and for templates for synthetic biology applications. *New Phytol* 200(1):16-26.

SCBT. 2015. Thaumatin Safety Data Sheet. *Santa Cruz Biotechnology I*. SC-296528; CAS 53850-34-3.

SCF. 1985. Reports of the Scientific Committee for Food concerning sweeteners (opinion expressed by the SCF on 14 September 1984). In: *Food Science and Techniques Reports of the Scientific Committee for Food (16th series)*. Commission of the European Communities, Directorate-General for Health and Consumer Protection, Scientific Committee for Food, Brussels, Belgium.

SCF. 1989. Reports of the Scientific Committee for Food on Sweeteners (opinion expressed 11 December 1987 and 10 November 1988, and adopted 10 November 1988). In: *Food Science and Techniques Reports of the Scientific Committee for Food (21st series)*. Commission of the European Communities, Directorate-General for Health and Consumer Protection, Scientific Committee for Food, Brussels, Belgium.

Schiffman SS, BJ Booth, ML Losee, SD Pecore and ZS Warwick. 1995. Bitterness of sweeteners as a function of concentration. *Brain Research Bulletin* 36(5):505-513.

SFC. 2006. Thaumatin Safety Data Sheet. *Chemical SF*. CAS 53850-34-3.

Shallenberger RS. 1993. Amino Acids, Peptides, and Proteins. In: *Taste Chemistry*. Springer US, Boston, MA, p 250-252.

Siegmund B, E Leitner and W Pfannhauser. 1999. Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. *J Agric Food Chem* 47(8):3113-3120.

Sigma-Aldrich. 2017. Thaumatin Safety Data Sheet. *Aldrich S*. T7638.

Sisson VA and RF Severson. 1990. Alkaloid Composition of the *Nicotiana* Species Beiträge zur Tabakforschung International/Contributions to Tobacco Research. vol 14, p 327.

Smith ML, WP Fitzmaurice, TH Turpen and KE Palmer. 2009. Display of peptides on the surface of tobacco mosaic virus particles. *Curr Top Microbiol Immunol* 332:13-31.

Smith RL, P Newberne, TB Adams, RA Ford, JB Hallagan and FEMA. 1996. GRAS Flavoring Substances 17. *Food Technology* 50(10):72-78, 80-81.

Stephan A, S Hahn-Lobmann, F Rosche, M Buchholz, A Giritch and Y Gleba. 2017. Simple Purification of *Nicotiana benthamiana*-Produced Recombinant Colicins: High-Yield Recovery of Purified Proteins with Minimum Alkaloid Content Supports the Suitability of the Host for Manufacturing Food Additives. *Int J Mol Sci* 19(1).

Stephen AG, R Powls and RJ Beynon. 1991. The relationship between thaumatin, a sweet protein and thaumatopain, a cysteine protease, from the arils of *Thaumatococcus daniellii*. *Biochem Soc Trans* 19:297S-297S.

Suami T, L Hough, T Machinami, N Watanabe and R Nakamura. 1997. Molecular mechanisms of sweet taste 7: The sweet protein, Thaumatin I. *Food Chemistry* 60(3):277-285.

Szwacka M, W Burza, R Zawirska-Wojtasiak, et al. 2012. Genetically Modified Crops Expressing 35S-Thaumatin II Transgene: Sensory Properties and Food Safety Aspects. *Comprehensive Reviews in Food Science and Food Safety* 11(2):174-186.

Tinti JM, D Glaser, M Wanner and C Nofre. 2000. Comparison of Gustatory Responses to Amino Acids in Pigs and in Humans. *LWT - Food Science and Technology* 33(8):578-583.

Tusé D. 2011. Safety of plant-made pharmaceuticals: Product development and regulatory considerations based on case studies of two autologous human cancer vaccines. *Human Vaccines* 7(3):322-330.

Tusé D, T Tu and KA McDonald. 2014. Manufacturing economics of plant-made biologics: case studies in therapeutic and industrial enzymes. *Biomed Res Int* 2014:256135.

Tusé D, N Ku, M Bendandi, et al. 2015. Clinical Safety and Immunogenicity of Tumor-Targeted, Plant-Made Id-KLH Conjugate Vaccines for Follicular Lymphoma. *Biomed Res Int* 2015:648143.

USDA ERS. 2015. Nutrients (food energy, nutrients, and dietary components). https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/food-availability-per-capita-data-system/#Food%20Availability. Accessed March 06, 2020.

van der Wel H and K Loeve. 1972. Isolation and characterization of thaumatin I and II, the sweet-tasting proteins from *Thaumatococcus daniellii* Benth. *Eur J Biochem* 31(2):221-225.

van der Wel H, RB Iyengar, J van Brouwershaven, PD van Wassenaar, WJ Bel and FJ van der Ouderaa. 1984. Assignment of the disulphide bonds in the sweet-tasting protein thaumatin I. *Eur J Biochem* 144(1):41-45.

van Loon LC, M Rep and CM Pieterse. 2006. Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol* 44:135-162.

Vigues S, CD Dotson and SD Munger. 2008. The receptor basis of sweet taste in mammals. *Results Probl Cell Differ* 47:187-202.

Vuong T, X Zhang and A Roeseler. 2019. California Tobacco Facts and Figures 2019. Sacramento, CA: California Department of Public Health. May 2019.

Werner S, O Breus, Y Symonenko, S Marillonnet and Y Gleba. 2011. High-level recombinant protein expression in transgenic plants by using a double-inducible viral vector. *Proc Natl Acad Sci U S A* 108(34):14061-14066.

Whaley KJ, A Hiatt and L Zeitlin. 2011. Emerging antibody products and Nicotiana manufacturing. *Hum Vaccin* 7(3):349-356.

Wildman S. 1983. An Alternate Use for Tobacco Agriculture: Proteins for Food Plus a Safer Smoking Material. In: *Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals - Workshop Proceedings*. U.S. Congress, Office of Techology Assessment, OTA-BP-F-23, Washington, D.C., p 63-77.

Witty M. 1990. Thaumatin II — a palatability protein. *Trends in Biotechnology* 8:113-116.

Witty M. 1998. New technologies for taste modifying proteins. *Trends in Food Science & Technology* 9(7):275-280.

Yusibov V and S Rabindran. 2008. Recent progress in the development of plant derived vaccines. *Expert Rev Vaccines* 7(8):1173-1183.

Zemanek EC and BP Wasserman. 1995. Issues and advances in the use of transgenic organisms for the production of thaumatin, the intensely sweet protein from *Thaumatococcus danielli*. *Crit Rev Food Sci Nutr* 35(5):455-466.

APPENDIX A. THAUMATIN II Safety Data Sheet

NOMAD

DRAFT– THAUMATIN II SAFETY DATA SHEET
Version 3.0
Revision Date 03/07/2020
Print Date 03/17/2020

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name	:	THAUMATIN II from plants (e.g. lettuce, red beet, spinach, *Nicotiana benthamiana*)
	:	CAS Number 53850-34-3 (extracted); CAS Number 553850-34-3 (recombinant)
	:	Consists of Thaumatin II of identical amino acid sequence to thaumatin II from *Thaumatococcus daniellii*

1.2 Functional uses

Identified uses	:	Flavor modifier/enhancer for use in food and beverages

1.3 Manufacturer

Company	:	Nomad Bioscience GmbH Weinbergweg 22 Halle 02160, Germany
Telephone	:	+49 345 1314 2606
Fax	:	+49 345 1314 2601

1.4 Emergency telephone number

Emergency Phone Number	:	+49 345 1314 2424 in the EU (US emergency phone number to be provided)

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture
Not a hazardous substance or mixture

2.2 GHS Label elements, including precautionary statements
Not a hazardous substance or mixture

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS
None

2.4 HMIS Classification

Health hazard:	0
Flammability:	0
Physical hazards:	0

2.5 NFPA Rating

Health hazard:	0
Fire:	0
Reactivity hazard:	0

2.6 Potential Health Effects of Bulk Powder/Concentrate

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation
Skin	May be harmful if absorbed through skin. May cause skin irritation
Eyes	May cause eye irritation
Ingestion	May be harmful if swallowed

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

CAS Registry Number : 53850-34-3
No ingredients are hazardous according to OSHA criteria
No components need to be disclosed according to the applicable regulations

Page 1

4. FIRST AID MEASURES

4.1 Description of first aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration

In case of skin contact
Wash off with soap and plenty of water

In case of eye contact
Flush eyes with water as a precaution

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water

5. FIREFIGHTING MEASURES

5.1 Extinguishing media - Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide

5.2 Hazardous combustion products
Hazardous decomposition products formed under fire conditions – Carbon oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary

5.4 Additional information
No additional information is available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
As with any concentrated protein, avoid dust formation and inhalation of particulates or aerosols. For personal protection see Section 8

6.2 Environmental precautions
Product active ingredients are biodegradable. No special environmental precautions are necessary

6.3 Methods and materials for containment and cleaning up
Sweep up and shovel solid. Water-wash surfaces. Use closed containers for disposal of any unused product

6.4 Reference to other sections
For disposal see Section 13

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Provide appropriate exhaust ventilation during preparation and use. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry well-ventilated place. Recommended storage temperature 2-8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values

8.2 Exposure controls

Appropriate engineering controls
General industrial hygiene practice

Personal protective equipment

Eye/face protection

Use government tested and approved eye protection devices (e.g. NIOSH - US or EN 166 - EU)

Skin protection

Handle with gloves that are inspected prior to use. Use proper glove removal technique to avoid skin contact. Dispose of used gloves in accordance with applicable laws and good laboratory practices. Wash and dry hands

Body Protection

Wear lab coat or similar cover during preparation, application and disposal of product in keeping with specific practices in the work environment

Respiratory protection

Use type N95 (US) or type P1 (EN 143) dust masks, or respirators, depending on the product formulation and preparation and use environment. Use devices approved under appropriate government standards such as NIOSH (US) or CEN (EU)

Control of environmental exposure

Product components are biodegradable and will be diluted during use. No special procedures for controlling environmental exposure are recommended

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance form:	Solid; yellowish-white to light tan
b)	Odor	No specific odor
c)	Odor threshold	No odor threshold identified
d)	pH	pH 2.7 – 6, depending on the formulation
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	No data available
l)	Vapor density	No data available
m)	Relative density	No data available
n)	Water solubility	>25 g/L
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information

No additional information available

10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under recommended storage conditions

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid
Strong oxidizing agents

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
Other decomposition products – Carbon oxides. In the event of fire: See Section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
No data available

Inhalation
No data available

Dermal
No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as a probable, possible or confirmed human carcinogen by IARC

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: Not available. Product is not a hazardous substance or mixture

11.2 Potential health effects

Inhalation: May be harmful if inhaled. May cause respiratory tract irritation
Ingestion: May be harmful if swallowed
Skin: May be harmful if absorbed through skin. May cause skin irritation
Eyes: May cause eye irritation

12. ECOLOGICAL INFORMATION

12.1 Toxicity
No data available

12.2 Persistence and degradability
Active ingredient is a protein that is biodegradable and can also be destroyed by heat and extremes in pH

12.3 Bioaccumulative potential
None anticipated

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
No data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company

Contaminated packaging
Dispose of any unused product

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA
No known OSHA hazards

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right to Know Components
No components are subject to the Massachusetts Right to Know Act

Pennsylvania Right to Know Components
Thaumatin II from plants. See Section 1.1

New Jersey Right to Know Components
Thaumatin II from plants. See Section 1.1

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm

16. OTHER INFORMATION

Additional information

Copyright © 2020 Nomad Bioscience GmbH. All Rights Reserved Worldwide.

The information contained in this Safety Data Sheet is believed to be correct as of the time of its release. It should be used as a guide for safe handling, storage, preparation, and disposal of the product. Assessment of product safety under conditions of normal use is based on information available at the time. Because information in some categories is lacking, this SDS is not all-inclusive and is subject to periodic updates. Nomad Bioscience GmbH and its Affiliates shall not be held liable for any damage resulting from handling, use, disposal or from contact with the above product.

APPENDIX B. THAUMATIN II Manufacturing Process

B.1. Introduction and Rationale

Notifier manufactures thaumatins recombinantly using a plant-based process very similar to the process described in GRN 593, modified for thaumatin proteins. This approach offers a more environmentally sustainable source of thaumatins in comparison to harvesting *Thaumatococcus* in the wild (Most 1978), and a more scalable and cost-effective manufacturing option relative to fermentation.

Notifier's THAUMATIN manufacturing process using the food host plants *Beta vulgaris* (**beet**), *Spinacia oleracea* (**spinach**), or *Lectuca sativa* (**lettuce**) was described in detail in GRN 738. In Notifier's GRAS Notice for THAUMATIN II Sweetener (GRN 910) as well as in this Notice, ***Nicotiana benthamiana*** is added to the list of production host plants. With any species, plant leaves can be transfected to express thaumatins by transient expression of a plant viral vector, such as tobacco mosaic virus (TMV) or potato virus X (PVX), containing the gene for a thaumatin protein. The safety of host and vector impurities was described in GRN 593 (edible species) and in GRN 775 (*N. benthamiana*). The components of the expression system and host plants are prepared independently and subsequently combined.

Alternatively, thaumatins can be produced in the same host plants carrying transgenically the thaumatin gene under the control of an ethanol-inducible promoter, with induction by dilute ethanol. After induction with either method, thaumatin protein accumulates in leaf tissues for several days. Plants are subsequently harvested and thaumatin is extracted and concentrated from the plant biomass.

THAUMATIN II contains no live biological materials that were introduced in the upstream steps of the process (e.g. when using *Agrobacterium* and viral replicons). The process is generic in that it is applicable to the expression and isolation of a wide range of proteins. A description of the process with respect to production of Thaumatin II is summarized.

B.2. Organism Used and Gene Expression Cassette

In the agroinduction method, the production organism *Agrobacterium tumefaciens* harboring binary plasmid vector comprising a TMV replicon with inserted thaumatin gene is depicted in Figure B-1. Thaumatins are natively expressed as pre-pro-proteins and Notifier has developed vectors that express pre-pro-proteins with native signal peptide (SP), as well as pro-proteins with native SP replaced by rice alpha amylase SP, or mature thaumatin protein without native SP and C-terminal peptide, co-expressed with rice SP. Comparison of protein quality and yield led to optimization of constructs and selection of pICH95105 and pICH95397; the latter is used preferentially for thaumatin manufacture.

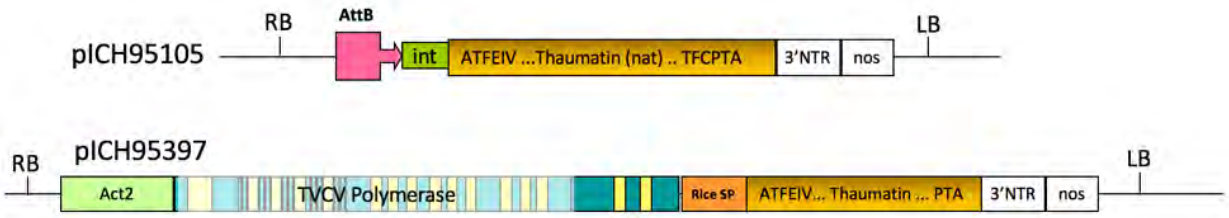


Figure B-1. *Agrobacterium* expression vector for thaumatin proteins

Mature thaumatin proteins of the correct (native) amino acid composition and sequence are efficiently expressed in agrobacterial vectors

After polypeptide processing *in planta*, the mature thaumatin protein accumulates in leaf tissue over several days post inoculation. Neither the native *Thaumatococcus* thaumatins nor the recombinant equivalents are glycosylated as there are no glycan addition sites along the backbone.

Preparation of vectors and inoculum are depicted in Figure B-2. Vectors are constructed by conventional molecular biology methods and maintained as Master and Working Plasmid Banks in *E. coli* (Figure B-2-**A**). The T-DNA vector encoding TMV-Thm replicon is transfected into *A. tumefaciens* to prepare the inoculum (Figure B-2-**B**). Each bacterium in the inoculum contains the T-DNA-TMV-Thm plasmid (Figure B-2-**C**).

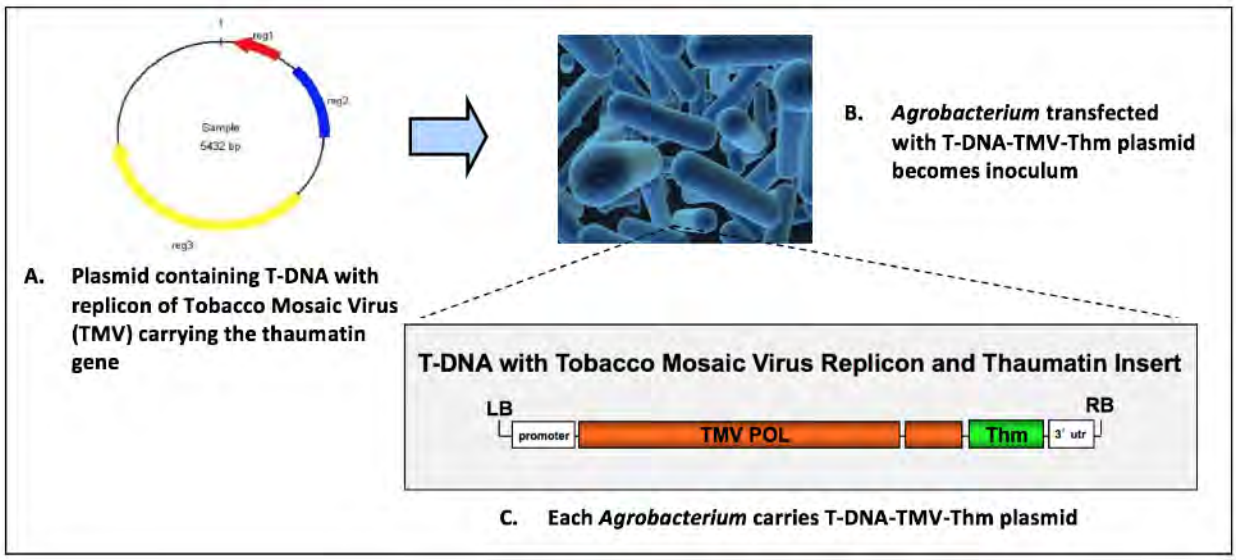


Figure B-2. Schematic of vector for thaumatin expression in plants

B.3. Procedure

A flow diagram summarizing the key steps in producing thaumatin proteins in edible species of plants was included in APPENDIX B of GRN 738 (Figure B-3, p 43). A **process modification** to ensure reduction in host and process impurities when using *N. benthamiana* as the plant host is shown in Figure B-3 of this Notice. This process adaptation yields the same THAUMATIN II product described in Notifier's GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*). Summary descriptions of key process steps follow; step numbers correspond to the process steps indicated in Figure B-3. The induction of gene expression can be accomplished by one of two alternative methods (described below), which share common downstream purification unit operations.

Step 1a. Inoculum production for *Agrobacterium* induction method

A proprietary industrial strain of *Agrobacterium tumefaciens* harboring a binary plasmid vector containing a TMV replicon with inserted Thaumatin II gene is grown in defined medium under aseptic conditions following strict quality SOPs; this bacterial suspension constitutes the inoculum. Notifier's *Agrobacterium* strain is grown in medium containing de-mineralized water, yeast extract, peptones, minerals, kanamycin and rifampicin.

The removal of residual antibiotics and fermentation chemicals is achieved by high dilution of the bacterial suspension before inoculation of plants and the ultra- and dia-filtration procedures during plant biomass extraction and processing.

All raw materials and processing aids are food grade. A multi-vial Master Vector Bank of the vector is prepared and stored at -80°C, from which aliquots are removed as Working Vector Batch of the inoculum for each manufacturing batch.

Each Working Batch of *Agrobacterium* is handled in a way to reduce the risk of contamination by foreign microorganisms. This includes use of sterile materials for bacterial cultivation, quality control checks to ensure axenic culture, and confirmation of strain identity before plant inoculation. Samples not meeting criteria are rejected and disposed, and new aliquots are drawn from the Master Bank. If a problem is identified at the Master Bank level, a new Master Bank is generated and subjected to quality control procedures before further use.

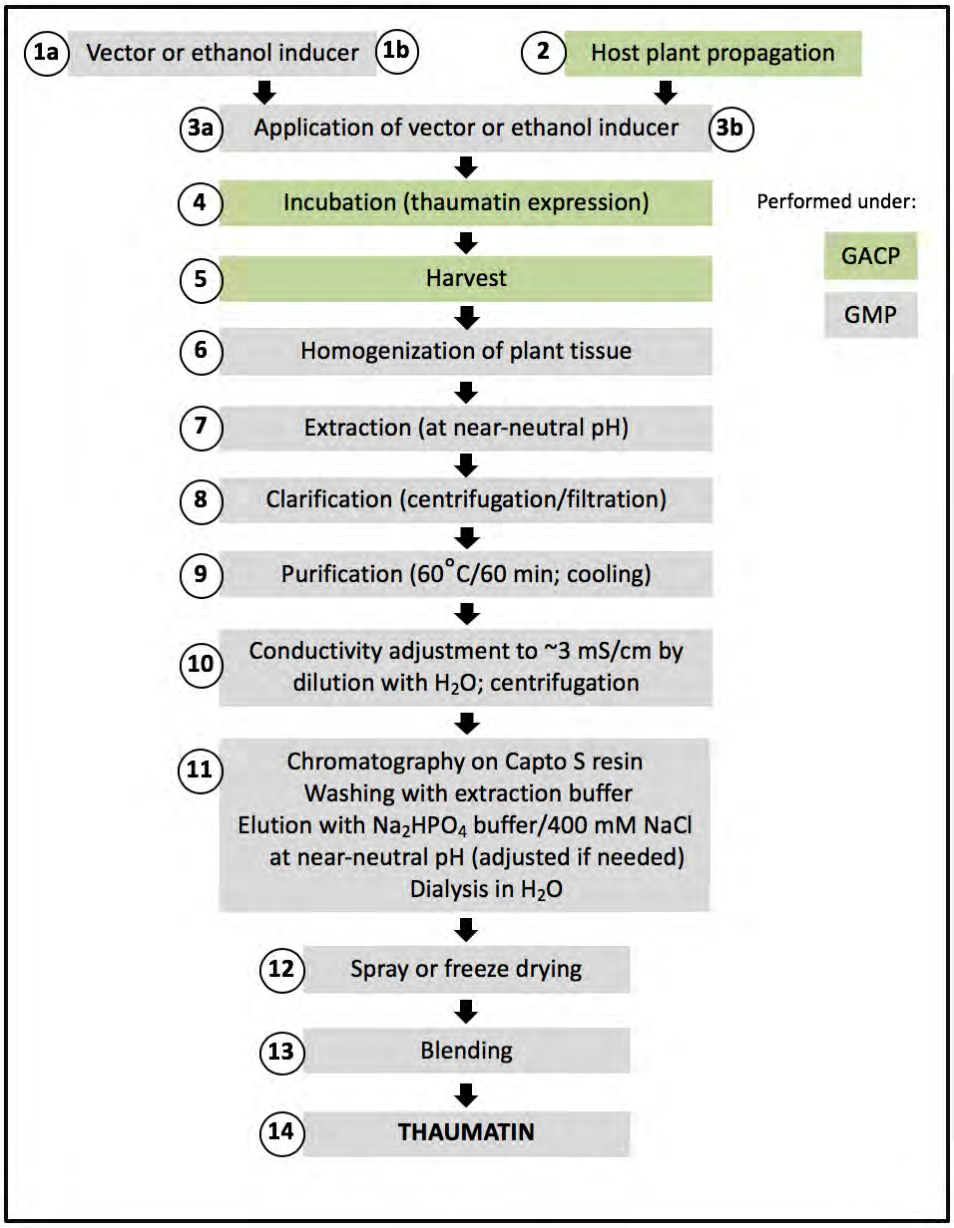


Figure B-3. Diagram of modified process for producing THAUMATIN II in *Nicotiana benthamiana*

Step 1b. Ethanol induction of transgenic plants

This variation of the method employs transgenic plants carrying an ethanol-inducible promoter. The procedure was developed by Notifier and described by Werner (2011). The process is based on inducible release of viral RNA replicons from stably integrated DNA pro-replicons. A simple treatment with dilute ethanol releases the replicon leading to RNA amplification and high-level production of thaumatin protein.

Step 2. Host plant preparation

For agroinduction, normal seeds of *Nicotiana benthamiana* host plant are obtained from qualified seed producers. For ethanol induction, transgenic seeds of these host plant developed by Notifier are used, which contain the gene insert for the desired thaumatin driven by an ethanol-inducible promoter. With either method of induction, plants are propagated in trays using either aeroponics or a food-crop compatible soil-based substrate, fertilizer and water. For seeding, plant propagation, target expression and plant harvest, the principles of Good Agriculture and Collection Practices (GACP) are applied. All used materials underlie a quality management system ensuring a predefined quality.

Step 3a. Inoculation of host plants with agrobacterial vector

The *A. tumefaciens* inoculum carrying the selected thaumatin replicon is applied to greenhouse-grown and quality tested host plants through the stomata (pores) in the leaves. The plant hence takes the place of a conventional "fermenter" in the production of the product. The *Agrobacterium* inoculum and the host plants are cultured under predefined and controlled conditions. At a specified timepoint after seeding, the plants are treated with a defined concentration of *Agrobacterium* in dilution buffer. Inoculation of plants is accomplished by either vacuum-mediated infiltration after immersing the plant leaves in a suspension of the inoculum, or via a procedure wherein the inoculum is sprayed onto plant leaves mixed with a surfactant (Gleba 2014; Hahn 2015; Tusé 2014). Via either method, the agrobacteria are efficiently internalized into the plant and gain systemic distribution.

The agrobacteria infect the plant cells and insert the T-DNA plasmid into the nucleus, which initiates synthesis of thaumatin-encoding RNA transcripts. Amplification of the transcript and translation of the thaumatin RNA message into thaumatin occurs in the cytoplasm of each cell. Neither the vector nor thaumatin genes are integrated into seed or passed on to subsequent generations (i.e. no stable integration); thus, the expression of proteins via viral vectors is transient and the production plants are not genetically modified.

Step 3b. Ethanol induction

In this variation of the method, treatment of the transgenic plants carrying a thaumatin gene with dilute ethanol (2.5% v/v) releases the replicon leading to RNA amplification and high-level thaumatin expression. To achieve tight control of replicon activation and spread in the non-induced state, the viral vector has been deconstructed, and its two components, the replicon and the cell-to-cell movement protein, have each been placed separately under the control of an inducible promoter (Werner 2011). Throughout the induction period, thaumatin protein accumulates in the tissues of the host plant. The inducer (ethyl alcohol) is evaporated or metabolized during plant growth and is not found in the final product.

Step 4. Incubation

After agro-inoculation or ethanol induction, the plants are incubated for 5-10 days under controlled temperature, humidity and illumination to allow for accumulation of thaumatin. During this incubation, there is rapid systemic replication of the vector and expression and accumulation of the induced product.

Step 5. Harvest

Plants producing thaumatin protein are harvested typically <10 days post inoculation/induction. Samples of plant biomass are taken for analyses of thaumatin protein content, general health and other process QC procedures prior to large-scale extraction. Plants in trays are transported to the cutting operation. The plants' aerial biomass (i.e. leaves and part of the stems) are mechanically cut and harvested into bins, which are transported to the extraction room.

Step 6. Homogenization of plant tissue

Cut plant biomass is disintegrated by homogenization in a grinder using an extraction buffer; the course plant material and fibers are removed, and the protein-containing soluble stream is further purified through a series of physicochemical treatments, precipitations and filtration steps.

Step 7. Extraction

The homogenate from Step 6 is subjected to near-neutral pH extraction in sodium phosphate buffer at cool ambient temperature. Precipitating proteins and other cellular debris are removed by centrifugation or filtration to enrich the thaumatin-containing supernatant/filtrate.

Step 8. Clarification

The extraction step is followed by filtration and/or centrifugation to remove bulk host impurities.

Step 9. Purification

The product-containing solution is then heated to 60°C and held at that temperature for 60 min, followed by cooling. This procedure causes precipitation of residual proteins and impurities and further enriches the process stream for thaumatin protein.

Step 10. Conductivity adjustment

To maximize thaumatin recovery, the conductivity of the solution is adjusted to approximately 3 mS/cm with water, followed by centrifugation.

Step 11. Chromatography

Chromatography is included when using *N. benthamiana* to reduce alkaloid and elemental impurities. This step utilizes Capto S cation exchange resin (GE Healthcare) and sodium phosphate/NaCl extraction. For efficient recovery, the buffer pH can be adjusted as needed. Extraction is followed by dialysis/diafiltration against water.

Steps 12 – 14. Drying, blending, fill and finish

The final Thaumatin II precursor solution is stabilized and standardized by the addition of water and food-compatible pH regulators, as needed. The solution is either spray dried or lyophilized to produce a dry, off-white, yellowish to light tan powder. Prior to release, the bulk product is tested to ensure compliance with the final product specification.

In-Process controls and quality assurance

Notifier applies rigorous in-process controls to manage the quality of process intermediates and final products throughout the manufacturing process. Materials not meeting pre-determined specifications are rejected. Product release is done after each batch passes identity and purity tests. A Quality Management system is in place to ensure conformance with industry standards and federal and local regulatory guidelines.

B.4. Manufacturing Facilities

Notifier can manufacture THAUMATIN II product at various locations in Europe and the United States. For commercial manufacture, semi-automated plant cultivation, inoculation, incubation and harvesting systems can be applied. Depending on the scale needed, Notifier can manufacture at its own facilities or use a contract manufacturing organization to produce and formulate thaumatin proteins meeting Notifier's specification. Features of an existing US facility's upstream and downstream processing capabilities include:

Upstream

- 80,000 sq ft of controlled growth space with 672 tables holding 30,240 plant trays in 3 levels. Each tray holds 104 plants; controlled conditions for the growth and harvest of transfected plants
- An automated plant transport system allowing movement, irrigation, lighting and environmental control (temperature and humidity) of trays for plant growth

Downstream

- 32,000 sq ft manufacturing area
- Linear scalability: 1 metric ton (mt)/shift pilot scale – 68 mt/shift commercial scale
- 75 L of Green Juice (post-grind/pre-clarification extract) per minute; continuous processing up to UF
- 35,000 L of tank storage capacity; heating and cooling control of in-process material
- Manufacturing clean rooms with controlled environments
- Computer-controlled processing and data collection
- Clarification options (UF/DF/Microfiltration/Nanofiltration/Reverse Osmosis)

Regardless of manufacturing venue, all substances, materials and reagents used in manufacturing THAUMATIN II by Notifier's process conform to food grade or higher standards. All processing equipment is high-grade stainless steel meeting food-industry criteria. All cleaning and sterilization procedures are validated in accordance with FDA guidelines for food-grade materials.

B.5. Waste Handling and Disposal

Waste streams containing plant-derived residuals are treated per local regulations and discarded. No by-products of the process are used in food or feed products, supplements, additives or processing aids.

B.6. Justification of Specification

A Specification for the plant-expressed THAUMATIN II product (final bulk) produced through the manufacturing process described herein and using *N. benthamiana* as the host plant is shown in Table 2-1. The current values for parameters used to establish and justify the target Specification for *N. benthamiana*-produced THAUMATIN II flavor modifier containing Thaumatin II active ingredient are shown in Table B-1.

The Specification limits for alkaloid impurities in the final product shown in Table B-1 were derived from analyses of two bench-scale and one pilot-scale THAUMATIN II batches (see APPENDIX C, Section C.5).

The Specification limits for lead (Pb) and the sum of Class 1 (Pb, Cd, Hg, As) elemental impurities were also established from results of analyses of two bench-scale developmental batches and one pilot-scale manufacturing batch (APPENDIX C, Section C.6). Limits for the less toxic Class 2 and Class 3 metals were not included in the Specification because analyses of the pilot-scale manufacturing batch yielded very low levels of these impurities (i.e. sum of Ag, Bi, Cu, Mo, Sb and Sn = < 1 ng total metal/mg THAUMATIN II).

Table B-1. Justification of Specification release limits for THAUMATIN II product

Summary of current THAUMATIN II Flavor Modifier's Conformance with Target Specification			
Parameter	**Method**	**Specification limit**	**Results of analyses [1]**
Appearance	Visual	Powder, yellowish-lt. tan	Conforms
Minimum total thaumatin content	CGE	≥ 95% of total protein	Ave: 97.7 ± 1.19% Range: 96.1% – 99.4%
Solubility (DI water)	Visual	≥ 200 mg/mL	Conforms
pH of a 1% solution in water	Potentiometric	2.7 – 6.0	Conforms
Heavy metals (sum of Class 1 metals Pb, Cd, Hg, As) [2]	USP38<233>	≤ 5 ng/mg thaumatin	Ave: 0.232 ± 0.048 ng/mg
Lead [2]	USP38<233	≤ 1 ng/mg thaumatin	Ave: 0.126 ± 0.22 ng/mg
Nicotine (per total thaumatin) [2]	HPLC/MS	≤ 20 ng/mg thaumatin	Ave: 14.41 ± 0.67 ng/mg
Anabasine (per total thaumatin) [2]	HPLC/MS	≤ 5 ng/mg thaumatin	Ave: 2.52 **±** 1.55 ng/mg
Bioburden	USP32<61>	≤ 5,000 CFU total per g	0 (absent)
Agrobacterium (CFU/10 g sample)	Selective plate based assay	0 (absent)	0 (absent)
Undesirable microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Salmonella spp.* or coagulase-positive *Staphylococcus spp.*, per 25 g final product	USP32<1111>	0 (absent)	0 (absent)
Stability of dry product (0-20°C) [3] as <3% loss of thaumatin protein	CGE; THM peak at T_m vs. T_0	≥ 6 months	> 11 months (at time of GRN development)

[1] Results of analyses of dry, purified Thaumatin II protein were obtained from three non-consecutive batches. Methods of analysis and results to support the Specification are discussed in APPENDIX C.

[2] Elemental and alkaloid impurity Specification limits were derived from results of analyses of 3 non-consecutive developmental batches, including two bench-scale batches and one pilot-scale manufacturing batch.

[3] Stability results were interim at the time of submission and were based on samples in an on-going stability program. Stability is calculated from Thaumatin II protein peak determined by CGE for 1% (w/v) Thaumatin II solution in water at T_0 and at various durations of storage expressed in months (T_m) using samples from a minimum of 3 non-consecutive developmental batches. Product is considered stable if there is ≤3% loss of Thaumatin II protein at the time of sampling.

APPENDIX C. THAUMATIN II Characterization

The methods used by Notifier to characterize its plant-produced thaumatin proteins and the results obtained were described in detail in GRN 738 for THAUMATIN Sweetener from edible plant species and in GRN 910 for THAUMATIN II Sweetener from *N. benthamiana*, and are only summarized in this Notice for brevity.

Briefly, the Thaumatin II protein expressed in the process described in APPENDIX B was analyzed for quality independently and for equivalency to *Thaumatococcus*-derived thaumatin, both extracted and recombinant, using publicly available information on thaumatin sequence, structure and function. Representative characterization methods and results are included.

C.1. Purification of Thaumatin II Protein from Plant Biomass

The plant homogenates obtained as described in APPENDIX B are purified through a series of physicochemical treatments, precipitations and/or filtration steps. In GRN 738, several methods were evaluated for extracting thaumatins. In this Notice, the *N. benthamiana* biomass was subjected to extraction at near-neutral pH.

Extraction at near-neutral pH. This method was described in GRN 738 (APPENDIX C, pp 49-50). Briefly, after clarification of the process stream by centrifugation and/or filtration, the semipurified thaumatin-containing solution is subjected to Capto S chromatography at cool ambient temperature, to yield a pure and homogeneous product.

The chromatographic separation method takes advantage of the relatively high isoelectric points of thaumatins (pI 11.5-12.5). A strong cation exchange (CEX) resin such as Capto S (GE Healthcare) is used and results in a highly pure and homogeneous protein that can be readily recovered.

Figure C-1 shows typical results of chromatographic purification of Thaumatin II and the resultant level of purity (>95-98%) of isolated protein.

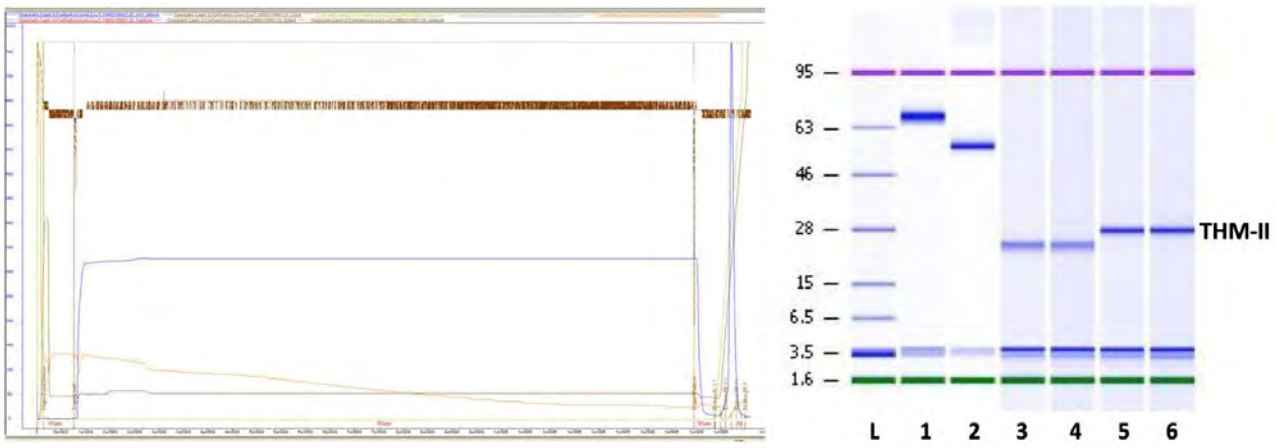
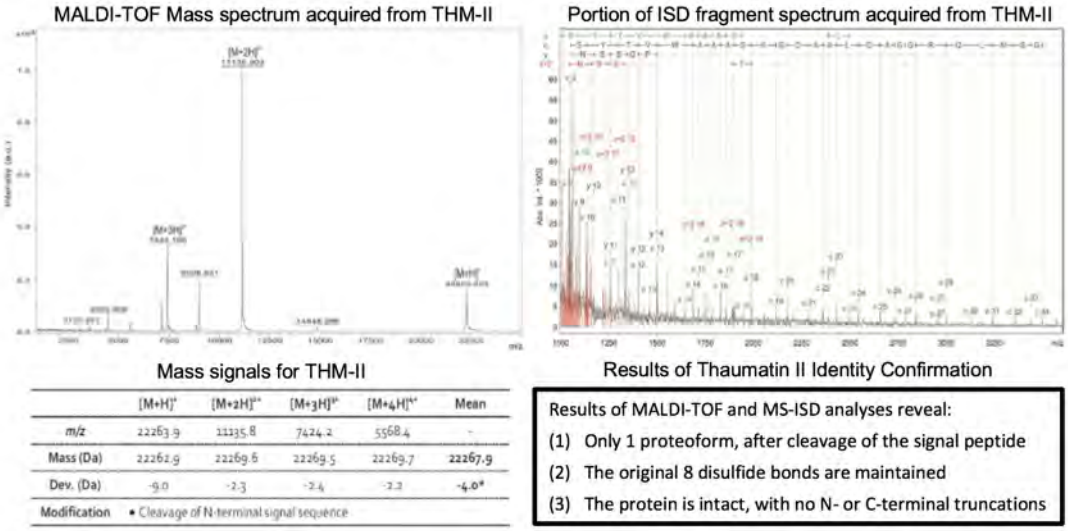


Figure C-1. One-step purification of cold-extracted Thaumatin II by Capto S CEX chromatography

Chromatographic purification of Thaumatin II with the strong cation exchange (CEX) resin Capto S (GE Healthcare) results is highly pure and homogeneous protein. The purity of Thaumatin II (mature protein) is shown as a single band by capillary gel electrophoresis (CGE) on the right side of the panel. Lanes 1 and 2, BSA standard, reduced and non-reduced, respectively. Lanes 3 and 4 and 5 and 6 show replicates of Thaumatin II (THM-II), reduced and non-reduced, respectively.

C.2. Confirmation of Identity

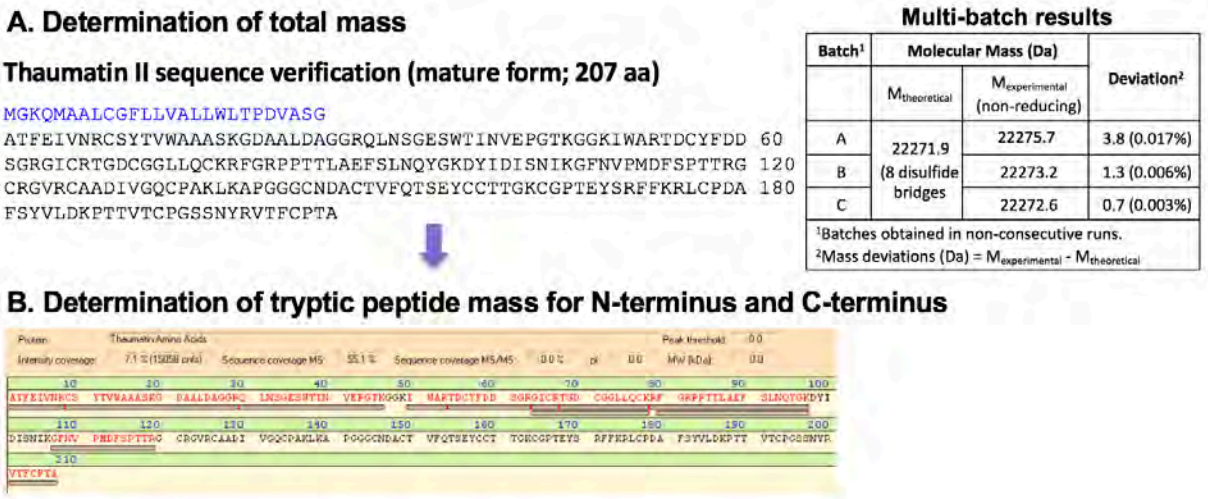
MS-ISD analysis. In addition to CGE, in-source decay (ISD) mass spectrometry (MS-ISD) was used as a more precise method of identity confirmation of plant-produced Thaumatin II protein. Representative results are shown in Figure C-2, which confirm the stability of the 8 disulfide bridges and the lack of truncation.



	$[M+H]^+$	$[M+2H]^{2+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$	Mean
m/z	22263.9	11135.8	7424.2	5568.4	-
Mass (Da)	22262.9	22269.6	22269.5	22269.7	22267.9
Dev. (Da)	-9.0	-2.3	-2.4	-2.3	-4.0*
Modification	• Cleavage of N-terminal signal sequence				

Figure C-2. Molecular stability of purified Thaumatin II protein as determined by ISD

MALDI-TOF and tryptic MALDI MS analysis. Isolated Thaumatin II was also subjected to MALDI-TOF and tryptic MALDI MS analyses to determine the molecular mass of the expressed protein, and to confirm that accessory sequences and signal peptide were cleaved to yield the expected mature protein. Figure C-3 shows representative results, as well as mass determinations from 3 non-consecutive Thaumatin II batches.



Batch[1]	Molecular Mass (Da)		Deviation[2]
	$M_{theoretical}$	$M_{experimental}$ (non-reducing)	
A	22271.9 (8 disulfide bridges)	22275.7	3.8 (0.017%)
B		22273.2	1.3 (0.006%)
C		22272.6	0.7 (0.003%)

[1]Batches obtained in non-consecutive runs.
[2]Mass deviations (Da) = $M_{experimental}$ - $M_{theoretical}$

Figure C-3. Determination of Thaumatin II protein mass by MALDI-TOF

Top panel (**A**) shows results of MALDI-TOF analysis for total mass of the Thaumatin II protein, including multi-batch results. Expression of the pre- and proprotein leads to post-translational processing *in planta* to yield the expected mature Thaumatin II. The signal peptide added to aid expression and target accumulation is cleaved off to yield a product with the expected mass. Bottom panel (**B**) shows typical results of N- and C-terminal tryptic mass fingerprinting, confirming total mass and the cleavage of accessory sequences to yield the expected mature protein.

There are slight discrepancies among the molecular masses estimated using CGE and mass spectrometry, with MS yielding the more precise values. Mass values also depend on the conformation of the protein; for example, formation of disulfide bonds leads to loss of protons and hence lower masses. Regardless, the average molecular masses determined by various methods match the values in reference databases, and the amino acid sequence of the mature, plant-produced Thaumatin II exactly matches the reference sequence (Figure 2-2).

C.3. Stability of Purified Thaumatin II Protein

The stability of purified, *N. benthamiana*-produced Thaumatin II protein powders was assessed during storage at 4°C and at room temperature (~22°C). Stability was determined by CGE (capillary gel electrophoresis) using an Agilent 2100 Bioanalyzer and Agilent Protein 80 reagent kit (Agilent Technologies). For analysis, one milligram (1 mg) of stored purified thaumatin protein powder produced in non-sequential batches sampled at various timepoints was dissolved in 1 ml water. Typical electropherograms of samples from a developmental batch of Thaumatin II stored at room temperature are shown in Figure C-4.

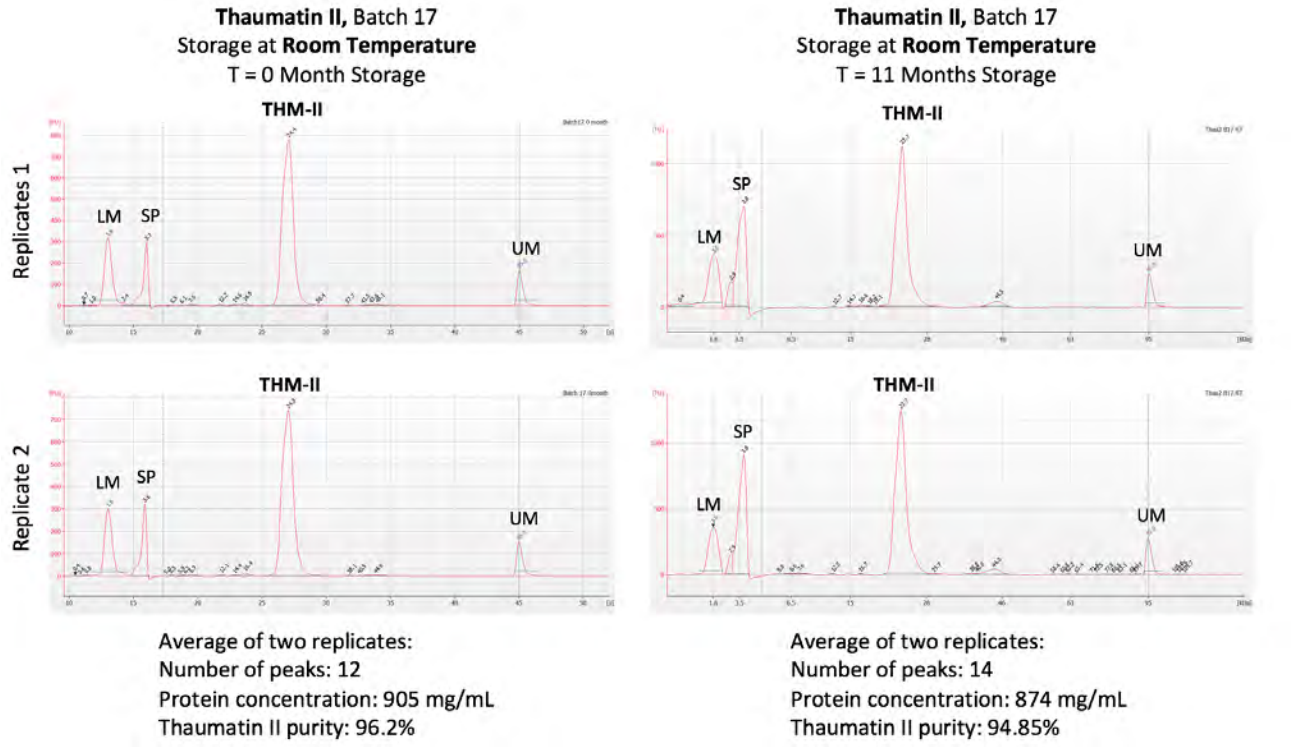


Figure C-4. Stability of purified Thaumatin II protein

The CGE electropherograms show the Thaumatin II (THM-II) peak and several other protein peaks, identified as follows: LM – lower marker; SP – system peak; THM-II – Thaumatin II; UM – upper marker. The identity and percent purity of Thaumatin II was followed over time in storage at 4°C and at room temperature (~22°C); room temperature graphs are shown.

Using such data, Thaumatin II protein purity over time of storage was used to help establish the stability component for the Specification. The percent purity of THAUMATIN II was determined from analyses (average of duplicate replicate experiments) of non-sequentially produced batches sampled at various times of storage at two temperatures. Stability was defined as acceptable for product release if the loss of purity over time is <3%. A compilation of results for THAUMATIN II is shown in Table C-1.

THAUMATIN II was stable during storage under the conditions indicated. The percent purity values shown in Table C-1 are averages of two replicate analyses. The active ingredient in THAUMATIN II flavor modifier, Thaumatin II, was found stable for 12 months when stored at 4°C, with less than 3% loss of purity over that storage time. Some batches of THAUMATIN II were also stable at room temperature (RT) for up to 11 months. Generally, cold storage (4°C-10°C) is expected to provide greater stability, hence enabling longer duration of product storage. Storage conditions will continue to be defined and optimized during product development.

Table C-1. Stability of THAUMATIN II during storage

	THAUMATIN II Protein Purity During Storage							
	Storage at 4°C				**Storage at RT (~22°C)**			
Product	**Time of storage (months)**				**Time of storage (months)**			
	0	9	11	12	0	9	11	12
THAUMATIN II								
Batch 5	98.0			96.15				
Batch 17	96.2			95.35	96.2		94.85	
Batch 31	99.4	96.15			99.4	94.55		
Thaumatin II protein purity (thaumatin protein as percent of total protein) was maintained when dry protein powders were stored over prolonged periods. None of the samples of THAUMATIN II showed degradation fragments or aggregation upon storage at either 4°C or at room temperature (~22°C).								

C.4. Manufacturing Yield and Consistency

The manufacturing yield, consistency, and product purity were determined from multiple non-sequential batches of THAUMATIN II produced during product and process development at bench scale. Consistency is expected to increase as the process is upgraded and optimized at pilot-scale and eventually at industrial scale. Table C-2 summarizes multi-batch yield and purity of *N. nicotiana*-produced THAUMATIN II.

Table C-2. Consistency of production yield and purity of THAUMATIN II

Product	**Yield of purified protein (mg/kg FW plant biomass)**		**Number non-consecutive batches analyzed**	**Thaumatin purity (% of total protein)**		**Number non-consecutive batches analyzed**
	Single batches	**Ave. ± SD**		**Single batches**	**Ave. ± SD**	
THAUMATIN II (bench-scale)	710	550 ± 160	6	98.0	97.8 ± 1.55	4
	240			96.1		
	420			97.3		
	470			99.4		
	540					
	610					
THAUMATIN II (pilot-scale)	117	-	1	98.2	-	1

Table C-2 lists results of analyses of individual production batches at developmental (bench) scale. The yield of purified protein per unit of biomass processed (fresh weight; FW) was determined for each batch, as well as the purify of the isolated protein obtained. Results from 6 independent production batches of THAUMATIN II show the recoverable yield of protein and the resultant protein purity. In addition to data from bench-scale developmental studies, data from one pilot-scale run for THAUMATIN II are included in Table C-2. Process scale-up focused on purity first, and results suggest that the purity release criterion of >95% can be achieved at larger scale.

C.5. Analysis of Residual Alkaloid Content

The process modification described in APPENDIX B was applied to *N. benthamiana*-derived THAUMATIN II process streams to reduce the level of host impurities. These results were used by Notifier to assess safety.

Alkaloid content was determined by HPLC/MS analysis and was conducted on behalf of Notifier by the Fraunhofer Institute of Cell Therapy and Immunology (Halle, Germany). The method has a LLOQ of ~20 ng/mL (20 ppb) and linearity of 20-1,500 ng/mL (20-1,500 ppb).

Table C-3 is a summary of results of THAUMATIN II analysis, showing the nicotine and anabasine alkaloid content of purified protein powders. Batches were produced non-sequentially. Results include alkaloid levels in two bench-scale batches produced at Notifier's facilities and in one pilot-scale batch produced by Notifier's CRO (KBP Inc., Owensboro, KY, USA).

As shown by the results obtained, there is high reproducibility and consistency among batches for the most prevalent alkaloid, nicotine, regardless of the manufacturing scale used. These results help verify the control of quality as well as the scalability of the manufacturing process. The pilot-scale process reduced the level of the minor impurity, anabasine, to approximately 1/10th the level found in bench-scale batches.

Table C-3. Residual alkaloid impurities in *N. benthamiana*-produced THAUMATIN II

Product	Residual alkaloid level in purified protein samples				
	Nicotine (ng/mg protein)		Anabasine (ng/mg protein)		No. of batches analyzed
	Single Batches	Mean ± SD	Single Batches	Mean ± SD	
THAUMATIN II	14.09 [a]	14.41 ± 0.67	3.37 [a]	2.52 ± 1.55	3
	13.78 [a]		<3.84 [a]		
	15.37 [b]		0.34 [b]		

[a] Batches produced at bench scale at Notifier's facilities. [b] Batch produced at pilot-scale by Notifier's CRO.

The average values for nicotine and anabasine content in purified Thaumatin II were used to establish the alkaloid limits in the **Specification**, namely, **20 ng/mg protein for nicotine** and **5 ng/mg protein for anabasine**.

C.6. Analysis of Elemental Impurities

Elemental analyses of dried, *N. benthamiana*-purified Thaumatin II protein produced in three non-consecutive batches were conducted to assess residual heavy metal content. Analyses for elemental impurities were conducted on behalf of Notifier by Wolfener Analytik GmbH (Bitterfeld-Wolfen, Germany; Prüfbericht Nr. 00146/20, 9 January 2020). Developmental batches were analyzed with priority for the most toxic heavy metals first (Class 1), followed by additional elemental analyses for Class 2 and 3 metals in Thaumatin II protein produced in a pilot-scale manufacturing batch. The Class 1 metals Cd, Pb and As were

determined per DIN 38406-29 (Determination of Selected Elements by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)), which is similar to USP38<2332, "Elemental Contaminants in Dietary Supplements." The Class 1 metal Hg was determined according to Wolfener Analytik in-house SOP also using ICP-MS, as above.

The results of multi-batch analyses are shown in Table C-4. Uniformly low levels of residual Class 1 elements were detected in the protein. In the table, Batch A and Batch B were produced at smaller bench-scale at Notifier's facilities, whereas Batch C was produced at larger pilot manufacturing scale by Notifier's CRO using the same process. The consistently low levels of Class 1 elemental impurities across production batch sizes support the scalability and control of quality in the manufacturing process. As process development, scale-up and optimization continue, even lower levels of elemental impurities might be achieved. Importantly, the current levels offer no reason for safety concerns.

The current results were used to set **Specification** limits for **Pb of 1 ng/mg** protein and for the **sum of Class 1 metals (i.e. Pb, Cd, Hg, As) of 5 ng/mg** protein. Analyses of Class 2 and 3 elements in the pilot-scale batch yielded very low levels of these less-toxic metals (~0.85 ng/mg for all 6 metals combined); therefore, these elements were omitted from inclusion in the Specification.

The projected intake of these elemental impurities from consumption of the THAUMATIN II product are insignificant compared to elemental exposures from food and environmental sources, the levels of which being in milligrams/person-day, as discussed in Section 6.1.4.

Table C-4. Multi-batch analysis of elemental impurities in *N. benthamiana*-produced THAUMATIN II

Heavy Metal Content, THAUMATIN II					
Element	**Class**	**Batch A**	**Batch B**	**Batch C**	**3-Batch Mean ± SD** (ng/mg)
		(ng/mg)	(ng/mg)	(ng/mg)	
Pb	1	<0.125	<0.154	<0.100	0.126 ± 0.22
Cd	1	<0.023	<0.031	0.014	0.022 ± 0.001
Hg	1	<0.057	<0.077	<0.050	0.061 ± 0.011
As	1	<0.023	<0.031	<0.010	0.021 ± 0.008
Sum (Class 1 metals)		**<0.228**	**<0.293**	**0.174**	**0.232 ± 0.048**
Ag	2B			<0.010	
Bi	-			<0.050	
Cu	3			0.56	
Mo	3			0.075	
Sb	3			<0.100	
Sn	3			<0.050	
Sum (all metals)				**1.02**	

Three independent batches of purified, *N.* benthamiana-produced THAUMATIN II were analyzed for the most toxic Class 1 elemental impurities. Approximately 300-350 mg of dry protein powder were analyzed in 2 replicates per batch. Results of individual batches are shown, as well as the calculated means and standard deviations (SD) for each element. Elemental levels are recorded as ng metal per mg of Thaumatin II protein. The values were used to set upper limits of elemental impurities in the Specification. Batches A and B were produced at bench scale, whereas Batch C was produced at pilot scale. Batch C protein was also analyzed for Class 2 and 3 elements, with results showing very low levels of these less toxic metal species.

C.7. Bioburden and Residual Vector

The bioburden level and level of residual *Agrobacterium* vector was determined for *N. nicotiana*-produced THAUMATIN II from non-consecutive batches. Results shown in Table C-5 demonstrate that the isolates had no bioburden or residual *Agrobacterium* vector (inoculum), therefore meeting the Specification.

Table C-5. Multi-batch analyses of bioburden and residual *Agrobacterium* inoculum

Batch[1]	Bioburden (CFU/g THAUMATIN II; average of 3 replicates per batch)[2]		
	Total aerobic microbial content	Total combined yeast and mold (TYMC)	Total residual *Agrobacterium* (inoculum) content
Batch A	0	0	0
Batch B	0	0	0
Batch C	0	0	0

[1]Batches analyzed were obtained from non-consecutive production runs

[2]THAUMATIN II solution at 1 mg/ml stock, 100 µl aliquots plated in 3 replicates on different media. Total aerobic microbial count (TAMC) was determined in Soybean-Casein Digest Agar (Roth #X937.1) plates incubated at 33°C for 3 days. Total combined yeast and molds count (TYMC) was determined in Sabouraud-Glucose Agar (Roth #932.1) plates incubated at 20-25°C for 6 days. Residual *Agrobacterium* inoculum was determined in LB-Bacto Agar + 50 µg/ml rifampicin plates incubated at 28°C for 2 days. Agrobacteria containing binary plasmid were enumerated in plates of LB-Bacto Agar + 50 µg/ml rifampicin and 50 µg/ml kanamycin incubated at 28°C for 2 days.

C.8. Determination of Flavor Modification Characteristics

Previously, the sweetness profile, sensory threshold, intensity, and duration of effect (lingering taste) of THAUMATIN II were determined in independent studies using panels of tasters, as described in Notifier's GRN 910 (THAUMATIN II Sweetener from *N. benthamiana*). In this Notice, Notifier summarizes results of flavor modification studies with the same THAUMATIN II product that was evaluated for sweetener applications. Studies were conducted on behalf of Notifier by qualified, independent sensory evaluation organizations, including **University of Georgia** (Athens, GA)**, BevNology** (Fayetteville, GA), **and Covance/Eurofins USA** (Princeton, NJ).

All taste evaluations had a similar goal and followed a comparable protocol. The objective of these evaluations was to identify THAUMATIN II aqueous formulations with similar sensory profiles relative to reference control solutions. Initial range-findings studies were conducted to assess the functionality of Notifier's THAUMATIN II because no sensory evaluations had been conducted before with this protein, other than evaluations for sweetness and sugar-replacement potential, as described in GRN 910.

The taste modification characteristics evaluated included THAUMATIN II's effect on **sweet/sour**, **sour** and **bitter** tastes, and its effects on a **savory** base (chicken broth) under low and high salt and low and high monosodium glutamate (MSG; umami flavor) levels. These evaluations were blinded to minimize bias, and statistical analyses were performed to arrive at the concentrations of THAUMATIN II providing the desired functionality.

The results of these studies are proprietary but the methods used and key outcomes are excerpted from the original reports. The specifics of each study and the results obtained are summarized in this Notice.

1. Sweet/Sour and Sour Flavor Modulation

Study 1 (BevNology 062118)

Method

A hybrid descriptive method was used that combined a "difference from control" technique and an "attribute intensity" rating. Evaluations were done by 6 trained panelists. This comparative study focused on understanding the relative flavor modification contributions of both Notifier's THAUMATIN II and a commercial thaumatin obtained from Natex UK Ltd. (Letchworth Garden City, UK). First, a comparable concentration set for both commercial thaumatin and THAUMATIN II standard sucrose sugar solutions was developed, containing either 10% or reduced (8%) sugar and varying levels of citric acid (CA). To the 8% reduced-sugar standard plus 0.06%-0.1% CA, thaumatin or THAUMATIN II were added to either 3 ppm, 7 ppm, 14 ppm or 28 ppm final concentrations. Using these blends, the degree of acid flavor masking or enhancement by thaumatin and THAUMATIN II were assessed by conducting sensory evaluations. In parallel, each protein's potential for reducing sugar and acid content while maintaining flavor was also determined.

Results

Modulation of sweet/sour taste intensity – THAUMATIN II and commercial thaumatin. Summary results from this evaluation are shown in Table C-6. The solution blends in samples M and P (shown in shaded columns) were detected as having equivalent flavor intensities. At 28 ppm, THAUMATIN II maintained the same perception of sourness as the control standard with 20% less citric acid (Sample M), whereas at 20 ppm the commercial (NX-TH) sample maintained sourness equivalent to the control with 30-45% less citric acid.

Table C-6. Comparative sour flavor enhancement in sweet/sour binary taste evaluation

	Concentration of Solution Components								
THAUMATIN II		28 ppm	20 ppm	28 ppm	20 ppm	28 ppm	20 ppm	28 ppm	20 ppm
Sample code	J	K	L	M	N	O	P	Q	R
Source	10 brix	TH-2	NX TH	TH-2	NX TH	TH-2	NX-TH	TH-2	NX-TH
Sucrose	100%	20% less	20% less	20% less	20% less	20% less	20% less	20% less	20% less
Citric acid target	0.1% CA	0.1% CA	0.1% CA	0.08%	0.08%	0.07%	0.07%	0.06%	0.06%
Composition									
Water	450	458.6	459	458.6	459	458.6	459	458.6	459
Sucrose	50	40	40	40	40	40	40	40	40
Thaumatin (1%)	0	1.4	1	1.4	1	1.4	1	1.4	1
Citric acid	0.5	0.5	0.5	0.4	0.4	0.35	0.35	0.3	0.3

Model set at 10 Brix. All acid reductions were based on 20% less sugar sweetness made up with thaumatins.
Key: "TH-2", Notifier's THAUMATIN II; "NX TH", commercially sourced thaumatin; "CA", citric acid.

In this pilot comparison, the tasting panel reported that at the concentrations used THAUMATIN II was subjectively more potent as a flavor enhancer than as a sweetener. Both THAUMATIN II and commercial thaumatin enhanced perception of sourness from citric acid. It is likely that the purified commercial thaumatin was slightly more potent as a flavor enhancer than the THAUMATIN II from a developmental batch, but it is clear that both proteins enhanced sour flavor perception at the range of concentrations studied.

Study 2 (BevNology 061219)

Method

A hybrid descriptive method was also used in this study and combined a "difference from control" technique and an "attribute intensity" rating. Evaluations were done by 6 trained panelists. The intensity ratings for the sweet and sour controls first determined in pilot studies were used in the full evaluation, with 10% (w/v) sucrose without and with 0.1% (w/v) citric acid used as the reference sweetener and acidulant, respectively. The perceived intensities for sweet/sour and sour flavors without and with varying levels of Notifier's THAUMATIN II were determined and rated relative to controls. A 15-point scale with 0.5 increments was used. Because thaumatins are known to modulate some flavors more slowly than native inducers (tastants) of the same flavor, assessments were made for "first sip" as well as after holding the sample in the mouth for up to 2 minutes. The timing for sensory evaluation was controlled by a panel leader. Each evaluation was replicated 5 times to determine reproducibility and statistical significance.

Results

Modulation of sweet/sour taste intensity. The sourness intensity of a sweet/sour binary flavor reference control solution (10% sucrose + 0.1% citric acid) was 8.0. The perceived sourness intensity of test solutions increased in direct proportion to THAUMATIN II concentration. Summary results are shown in Table C-7. Notifier's THAUMATIN II was confirmed to enhance perception of sour taste ("sourness") in a sweet/sour flavor evaluation.

In these evaluations, sucrose content was reduced by 25% relative to control (7.5% vs. 10%) to compensate for THAUMATIN II's inherent sweetness. The sample with the lowest THAUMATIN II level (7.5% sucrose + 0.08% citric acid ("CA") + 2 ppm THAUMATIN II) had the lowest sourness intensity, while the sample with the highest THAUMATIN II level (7.5% Sugar + 0.08% CA + 5 ppm THAUMATIN II) had the highest sourness score, indicating sour flavor potentiation (enhancement) by THAUMATIN II in the presence of sucrose.

Samples with 7.5% sucrose + 0.08% CA + 3.5 ppm THAUMATIN II had equivalent sourness relative to the control, indicating that 3.5 ppm THAUMATIN II in combination with sucrose was able to reduce the level of CA by 20% (from 0.1% to 0.08%) while retaining an equivalent perception of sour taste (shaded rows in table).

Table C-7. Mean sourness intensity scores for sweet/sour taste comparisons

Solutions	Sourness (P ≤ 0.05)
7.5% sucrose + 0.08% CA + 5 ppm THAUMATIN II	8.8 a ± 0.07
7.5% sucrose + 0.08% CA + 3.5 ppm THAUMATIN II	8.0 b ± 0.09
Control (10% sucrose + 0.1% CA)	8.0 b
7.5% Sugar + 0.08% CA + 2 ppm THAUMATIN II	7.0 c ± 0.08

Modulation of sour aftertaste. The sour aftertaste intensity of the control solution (10% sucrose + 0.1% citric acid) was 4.0. The perceived sour aftertaste intensity increased in direct proportion to THAUMATIN II concentration. Therefore, THAUMATIN II was found to also enhance sour aftertaste. Summary results are shown in Table C-8. The sample with the lowest THAUMATIN II level (7.5% sucrose + 0.08% CA + 2 ppm THUMATIN II) had a similar sour aftertaste intensity as the control (no significant difference $p \leq 0.05$; shaded rows), while samples with 3.5 ppm and 5 ppm THAUMATIN II had higher sour aftertaste intensities than the control. The sour aftertaste was scored 20 seconds after expectoration of the sample.

Table C-8. Mean sourness aftertaste scores for sweet/sour taste comparisons

Solutions	Sour Aftertaste (P ≤ 0.05)
7.5% sucrose + 0.08% CA + 5 ppm THAUMATIN II	6.3 a ± 0.026
7.5% sucrose + 0.08% CA + 3.5 ppm THAUMATIN II	5.3 b ± 0.23
Control (10% sucrose + 0.1% CA)	4.0 c
7.5% sucrose + 0.08% CA + 2 ppm THAUMATIN II	3.7 c ± 0.16

Study 3 (Covance 8399119)

Method

Quantitative descriptive testing was conducted by Eurofins/Covance using 10 trained panelists and 2 replications of each evaluation. Panelists participated in three 2.5-hour orientation session to develop the ballot, discuss the samples, and review the references. Panelists rated attribute intensities on 15-point line scales. Sweet and sour flavors were evaluated independently in-mouth after 1st and 2nd sips. Additionally, total flavor and characteristic or off flavors were also recorded, including "artificial sweetener," "sweet aromatic," and "bitter." Mouthfeel factors were also evaluated, including "powdery," "astringent," and "soft tissue irritation." To evaluate lingering or extended flavor modulation, the panel recorded perceived flavors at initial exposure and again at 30 and 60 seconds post initial exposure. The test solutions included Full Sucrose (10% sucrose) + Citric Acid Control (0.1% Citric Acid); - 25% Sucrose, - 20% Citric Acid Control; - 25% Sucrose, - 20% Citric Acid + 28 ppm THAUMATIN II.

Results

Modulation of sweet/sour taste intensity. Figure C-5 summarizes in-mouth results of this binary evaluation. The graph shows taste values along a 15-point intensity scale for each flavor attribute. Highlighted (circles and arrows) are results of 1st sip followed by 2nd sip of each test solution. Notably, very similar sourness was recorded with solutions containing THAUMATIN II and reduced concentrations of both sucrose (-25%) and citric acid (-20%), confirming that THAUMATIN II enhances (potentiates) acid flavor in combination with sugar.

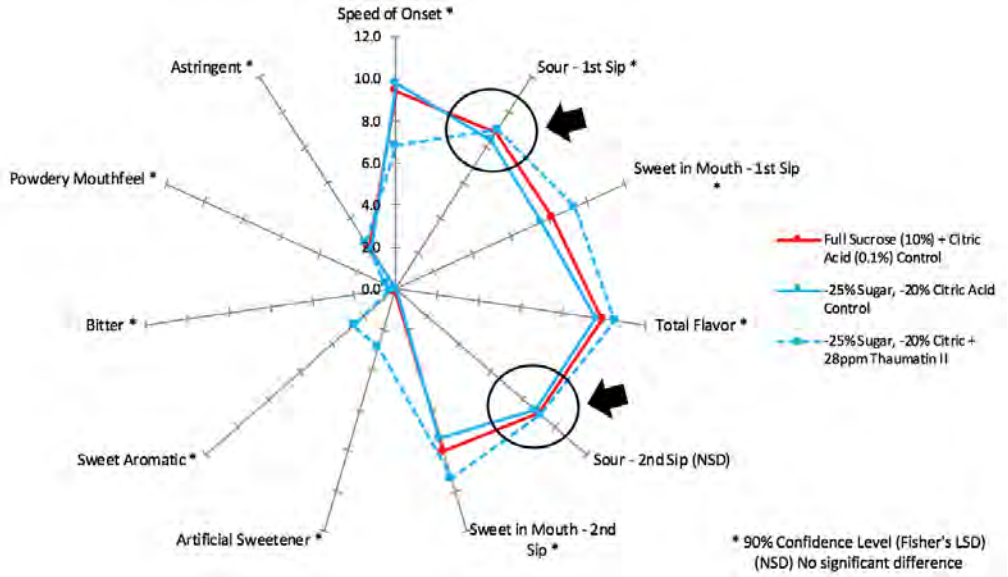


Figure C-5. Sensory profile in evaluation of sweet/sour taste modulation

Results in-mouth depicted in Figure C-5 show that a very small difference was noted among the full sugar + acid reference solution, -25% sugar/-20% acid solution, and the -25% sugar/-20% acid + THAUMATIN II solution. While the slight difference was noticed at 1st sip (90% CL, Fisher's Least Significant Difference; "LSD"), the difference disappeared (no significant difference; "NSD") at 2nd sip. The same study assessed sourness intensity and duration (sour "aftertaste") of the same solutions; results are shown in Figure C-6. Although the sour aftertaste is more pronounced in the THAUMATIN II-containing solution relative to both control solutions, the difference (90% CL; Fisher's LSD) is not dramatic.

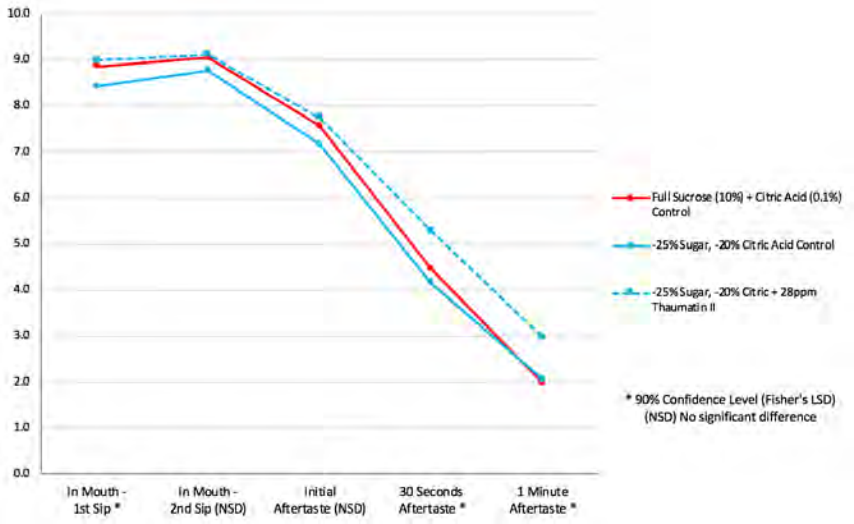


Figure C-6. Sourness intensity (aftertaste) in sweet/sour taste modulation

Modulation of sour taste intensity. A similar evaluation was conducted using only citric acid, omitting the sweetener. In this series, reference control solutions consisted of 0.1% citric acid and -20% (0.08%) citric acid, whereas the test solution consisted of reduced (-20%) citric acid supplemented with 28 ppm THAUMATIN II. The results of this cell are summarized in Figure C-7 and Figure C-8.

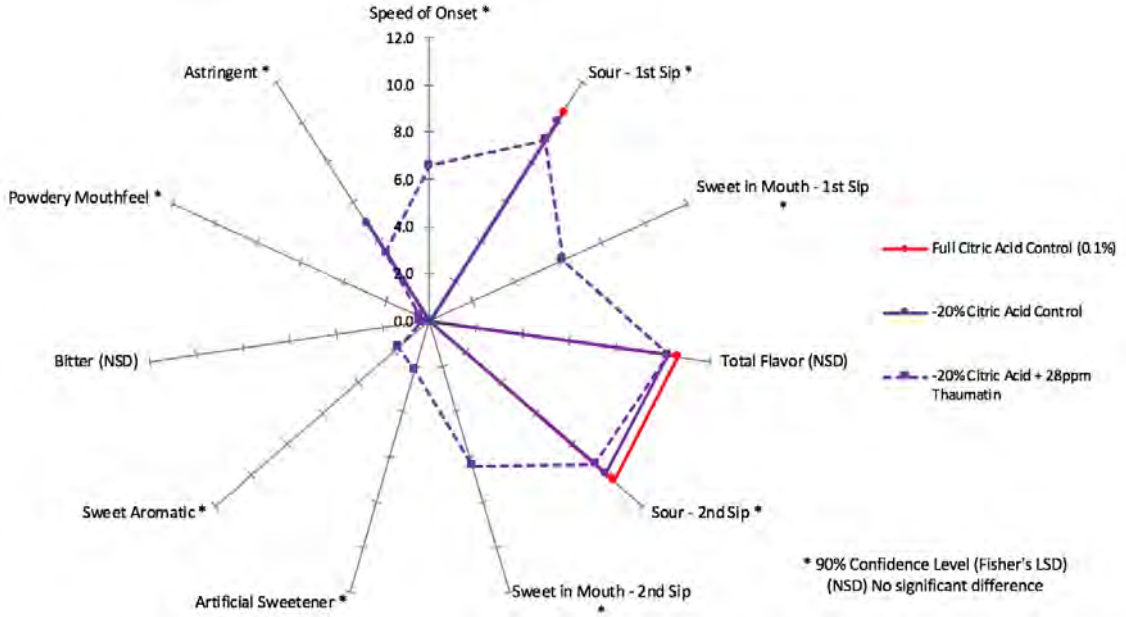


Figure C-7. Sensory profile in evaluation of sour taste modulation

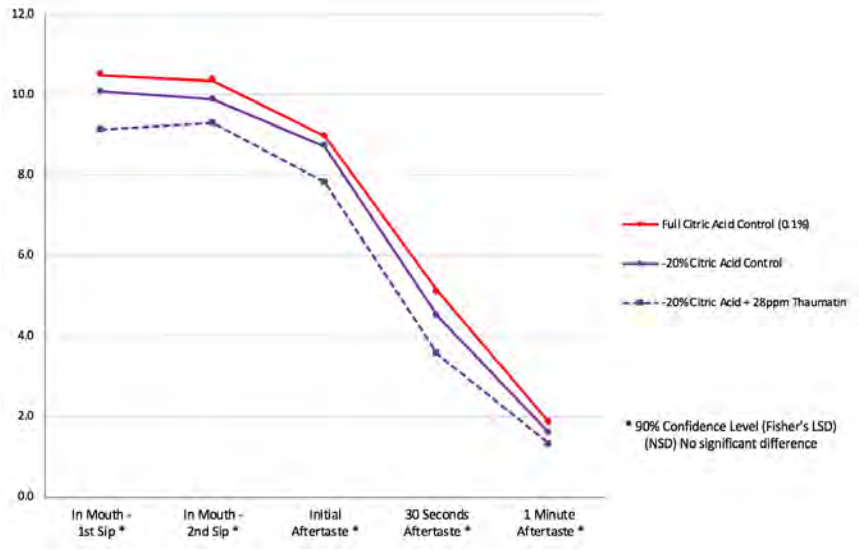


Figure C-8. Sourness intensity (aftertaste) in sour taste modulation

Results of evaluations of sweet/sour and sour solutions at reference standard concentrations and reduced tastant levels showed that THAUMATIN II-supplemented (28 ppm) solutions were significantly lower than both controls in "sourness" at all time points. In this series, THAUMATIN II did not demonstrate sour enhancement in a citric acid only solution, but it did demonstrate reduction of sour taste in the absence of sugar (90% CL; Fisher's LSD). That is, THAUMATIN II was shown to have a sour taste-masking effect in the presence of citric acid and in the absence of sucrose.

2. Bitter Flavor Modulation

Study 4 (BevNology SOW3A)

Method

A hybrid descriptive method was also used in this study and combined a "difference from control" technique and an "attribute intensity" rating. Evaluations were done by 6 trained panelists. In this range-finding study, the concentrations of caffeine (bitter tastant) required to induce the taste sensation of bitterness were defined, aiming for moderate rather than extreme bitterness. Previous experience by the expert panel indicated that between 300 and 500 ppm caffeine should be sufficient to assess bitter flavor modulation by THAUMATIN II. Anhydrous caffeine was added to purified water to final concentrations of 1250, 1375, 1500, 1675 and 1750 ppm (w/w) in solution. The approach called for establishing bitterness intensity first, followed by sequential increases of caffeine by 10%, 20%, 30% and 40% (w/w) while augmenting the solutions with THAUMATIN II to 7 ppm, 14 ppm or 28 ppm final concentrations. Depending on initial findings, THAUMATIN II levels were to be modified as necessary to achieve goals. The objective of the study was to identify the point at which trained tasters could detect a recognizable difference in bitterness.

Results

Bitterness modulation with caffeine. Notwithstanding the original design of the study, THAUMATIN II at 7, 3.5 and even 1.25 ppm was detected to be slightly sweet. Hence, even lower concentrations of THAUMATIN II (e.g. down to 0.6 ppm) were selected because at those low concentration the solutions had no detectable sweet taste. The test matrix used in this bitterness modulation study is shown in Table C-9.

Table C-9. Caffeine bitterness modulation (range-finding)

Ingredient	Caffeine (anhydrous)				
Final conc. (ppm)	1250	1375	1500	1675	1750
	Control	10% more	20% more	30% more	40% more
Water (gr)	499.345	499.2825	499.22	499.1325	499.095
Caffeine (mg)	625	687.5	750	837.5	875
THAUMATIN II (mg) 1%	30	30	30	30	30

In the final evaluation, THAUMATIN II (3 mL of a 1% solution = 30 mg) was added to a final concentration of 0.6 ppm (0.6 mg/L). All solutions that contained sub-threshold (i.e. no detectable sweetness) concentrations of THAUMATIN II (i.e. 0.6 ppm) and the specified levels of caffeine successfully masked (reduced the perception of) bitterness. However, there was wide variability among the panel members' subjective perception of bitterness when caffeine was used as the reference tastant. Samples containing 10%, 20% and up to 30% more caffeine were scored by some tasters as being equal to or less bitter than the lowest caffeine concentration of 1250 ppm. Caffeine is consumed daily at different levels, and it is possible that the variation found in this study was related to the degree of sensitivity/desensitization to caffeine by panel members.

Study 5 (Covance 8399119)

Method

This study also evaluated modulation of caffeine bitterness by THAUMATIN II. Quantitative descriptive testing was conducted by Eurofins using 10 trained panelists, with 2 replications per test series. Panelists participated in three 2.5-hour orientation session to develop the ballot and discuss the samples. Panelists rated attribute intensities on 15-point linear scales. Caffeine control water solution consisted of 400 ppm caffeine, with variants including +15% caffeine control and +15% caffeine + 15 ppm THAUMATIN II. The relevance of caffeine concentrations for sensory evaluation was determined beforehand by benchtop evaluations conducted by sensory staff. Tasting attributes rated included overall bitter flavor sensation (in mouth) rated at 1st sip and 2nd sip, sweetness (in mouth) at 1st and 2nd sips, plus total flavor perceived, "artificial sweetener," "sweet aromatic" and "sour qualities." Feel factors included "powdery mouthfeel" and "astringency." Aftertaste (duration of effect) was assessed for "bitter," "sweet," "artificial," "sweet aromatic" and "soft tissue irritation" at first tasting, and again at 30 seconds and one minute after first tasting.

Results

Bitterness modulation with caffeine. The results of this cell are summarized in Figure C-9. The sample containing THAUMATIN II (15 ppm) was significantly higher in sweetness qualities at all time points compared to the two control samples that only contained caffeine. The THAUMATIN II-containing sample was rated significantly higher in bitterness sensation than the full caffeine control (400 ppm caffeine) and the increased bitter control (+15% caffeine) at all time points except initial aftertaste.

Under the conditions used, THAUMATIN II did not demonstrate bitter masking in a caffeine-only solution. These findings contrast results of the previous study with caffeine (Study 4), which used higher concentrations of caffeine (1250 – 1750 ppm) relative to the levels used in Study 5 (400 – 460 ppm) as well as lower concentrations of THAUMATIN II (0.6 – 2 ppm instead of 15 ppm) to avoid introducing sweetness in the test solutions.

Caffeine's bitterness is a desirable taste component in some beverages (tea, coffee) and hence masking of excessive bitterness is a more practical target. Under higher caffeine bitterness levels and lower THAUMATIN II concentrations, THAUMATIN II did reduce (mask) excessive bitterness, as shown by the results of Study 4.

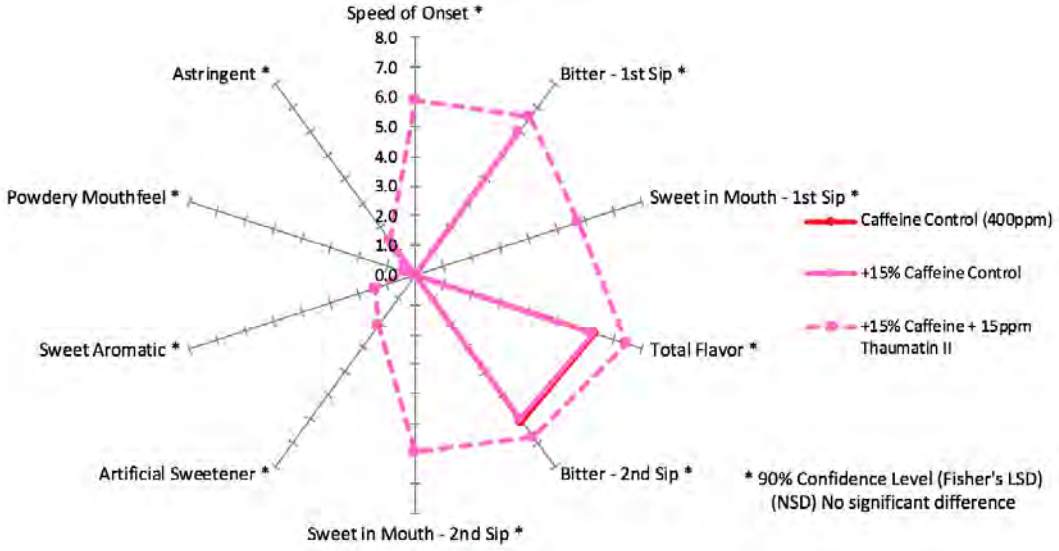


Figure C-9. Sensory profile in evaluation of caffeine bitter taste modulation

Because of the variability in sensing and scoring caffeine bitterness in Study 4 and Study 5, possibly due to panelists' familiarity and desensitization to caffeine-containing beverages, a subsequent study (Study 6) used a less common bitter tastant, quinine, as the reference flavor to assess THAUMATIN II's modulation effects.

Study 6 (University of Georgia 60219)

Method

A hybrid descriptive method was also used in this study. Evaluations were done by 5 trained panelists. The bitter flavor control solution containing 5 ppm quinine was used in this study because it was perceived to have a similar bitterness intensity to 500 ppm caffeine solution. The bitterness intensity ratings were determined using a 15-point linear scale with 0.5 increments. These evaluations determined bitter taste modulation as well as duration of effect (i.e. bitter aftertaste). All attributes were rated after holding the sample in the mouth for 5 seconds, followed by re-scoring every 20 seconds for 2 minutes. The timing was controlled by a panel leader. The test was replicated five times.

Results

Bitterness modulation with quinine. The results of this evaluation are summarized in Table C-10. The bitterness intensity of the control solution (5 ppm quinine) was 2.0. Taste modulation was assessed by adding THAUMATIN II to 0.6, 1.0 and 2.0 ppm to the quinine-containing solutions. Over this THAUMATIN II concentration range, the perceived bitterness intensity increased in proportion to the level of THAUMATIN II added. Therefore, THAUMATIN II appeared to enhance bitterness at the higher levels. Notably, however, the sample with the lowest THAUMATIN II level (5 ppm quinine + 0.6 ppm THAUMATIN II) had the lowest bitterness intensity while the one with the highest THAUMATIN II level (5 ppm quinine + 2 ppm THAUMATIN II) had the highest bitterness intensity score. The sample with 5 ppm quinine + 1 ppm THAUMATIN II had similar bitterness intensity as the control (no significant difference $p \leq 0.05$).

Table C-10. Mean quinine bitterness intensity scores

Solutions	Bitterness (P ≤ 0.05)
5 ppm quinine + 2 ppm THAUMATIN II	2.86a ± 0.11
5 ppm quinine + 1 ppm THAUMATIN II	2.10b ± 0.10
Control (5 ppm quinine)	2.00b
5 ppm quinine + 0.6 ppm THAUMATIN II	1.50c ± 0.00

These results indicate that bitterness modulation is dependent on the tastant used (caffeine vs. quinine), the concentration of the reference tastant, and the concentration of THAUMATIN II added. Bitter flavor modulation therefore appears to be contextual, with higher levels of THAUMATIN II enhancing bitterness (flavor potentiator) and lower levels suppressing (masking) bitterness. Note that at 0.6 ppm, THAUMATIN II significantly ($p<0.05$) suppressed the sensation of bitterness relative to the no-THAUMATIN II control solution.

Modulation of bitter aftertaste (duration of modulation effect). The bitter aftertaste intensity of the control solution (5 ppm quinine) was 2.0. Perceived bitter aftertaste intensity increased proportionally to the THAUMATIN II level added to each solution. Therefore, THAUMATIN II also appeared to enhance bitter aftertaste sensation under the described test conditions. The sample with the lowest THAUMATIN II level (5 ppm quinine + 0.6 ppm THAUMATIN II) had similar bitter aftertaste intensity as the control, while samples with 1 ppm and 2 ppm THAUMATIN II had higher bitter aftertaste intensities than the control. These results are summarized in Table C-11.

Table C-11. Mean quinine bitterness aftertaste scores

Solutions	Bitter Aftertaste (P ≤ 0.05)
5 ppm quinine + 2 ppm THAUMATIN II	4.08a ± 0.13
5 ppm quinine + 1 ppm THAUMATIN II	3.28b ± 0.20
Control (5 ppm quinine)	2.00c
5 ppm quinine + 0.6 ppm THAUMATIN II	2.03c ± 0.05

Notably, the lowest concentration of THAUMATIN II evaluated, 0.6 ppm, had no significant ($p\leq0.05$) impact on quinine's bitter aftertaste even though at that concentration THAUMATIN II effectively decreased (masked) the intensity of bitter taste perceived by the panelists (Table C-10).

3. Salty and Savory Flavor Modulation

Study 7 (Covance 61818)

Method

The goal of this study was to assess the impact of THAUMATIN II and a commercially available thaumatin (Natex UK Ltd.) to enhance or mask certain flavor components in savory applications. Tastant bases included unsalted chicken broth with added bitterness tastants (e.g., caffeine, quinine or hop extract) or saltiness tastants (salt or monosodium glutamate; MSG). Preliminary work identified low- and high-levels for each tastant (between 20 ppm and 5,000 ppm), as well as two levels of THAUMATIN II with noticeable but not unacceptable effects, namely, 7 ppm and 15 ppm. Assessments were conducted by 3 food scientists trained

in benchmarking. Scoring was done on a linear scale with units ranging from -4 (masking) to +4 enhancement. Only representative flavor modulation results with salt and monosodium glutamate (MSG) are summarized here, as results showed that neither thaumatin impacted bitter flavor modulation in savory broths.

Results

Modulation of taste in salty and savory systems. Figure C-10 summarizes the in-mouth sensory profiles in flavor assessments under **low-salt** and **high-salt** conditions without (Control) or with addition of commercial (Natex) thaumatin or Notifier's THAUMATIN II.

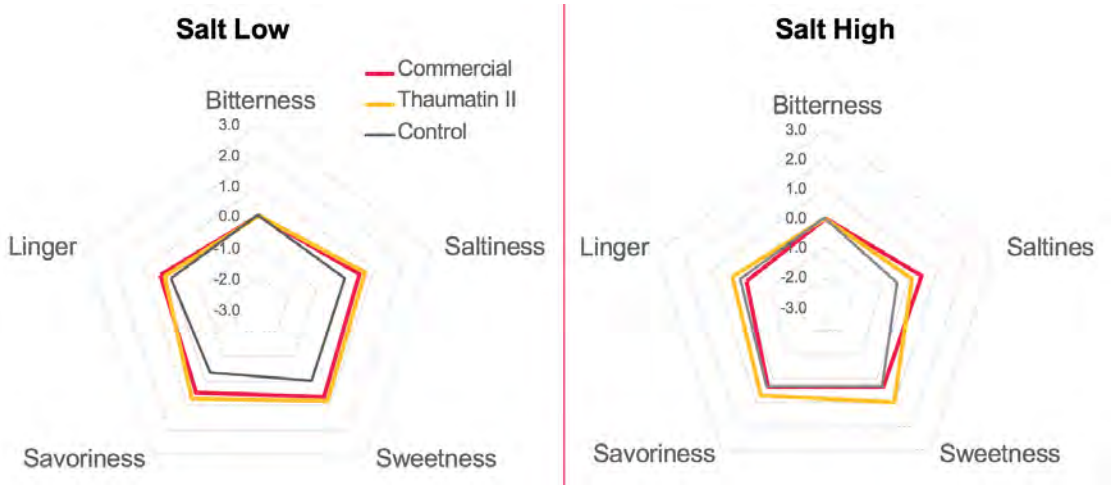


Figure C-10. Sensory profile of savory taste modulation in low- and high-salt conditions

Under low-salt (NaCl) conditions, the sweetness, saltiness and savoriness attributes of both thaumatin samples were rated to be slightly higher than the control. Under high-salt conditions, the sweetness, saltiness, and savoriness of both thaumatin samples were rated to be relatively similar to the control. There was no impact on bitterness in either taste cell. With respect to aftertaste (linger), the slightly sweet taste of both thaumatins was described as pleasant and savory, but not very strong.

Figure C-11 summarizes results of taste modulation in the presence of **low** and **high** levels of **MSG**.

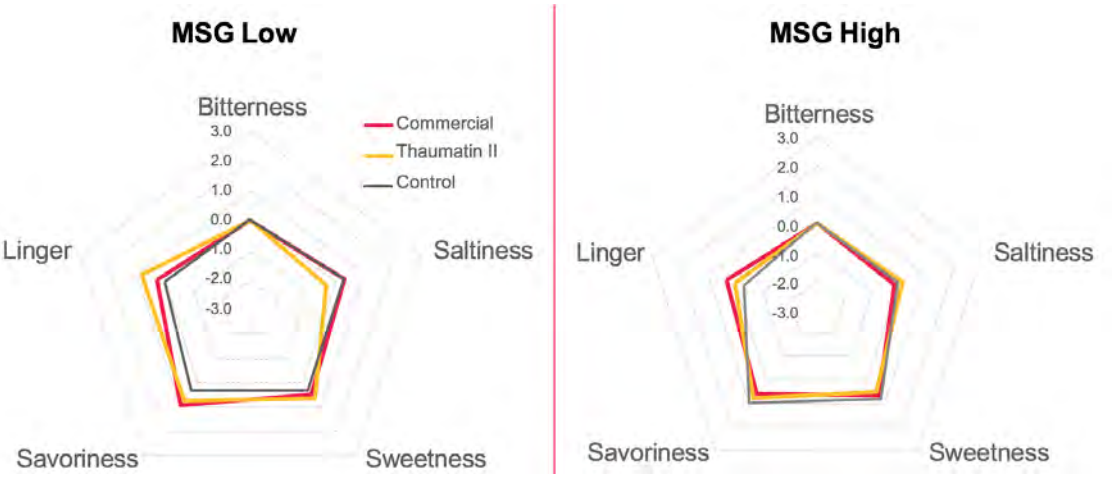


Figure C-11. Sensory profile of savory taste modulation in low- and high-MSG conditions

Under low MSG conditions, the sweetness and saltiness attributes of both thaumatin samples were rated as relatively similar to the control. Both the commercial thaumatin and THAUMATIN II increased savoriness (umami flavor) slightly, though the difference from the control sample was relatively small.

With higher MSG concentration, the sweetness, saltiness and savoriness attributes of both thaumatin samples were rated as relatively similar to the control. Addition of either thaumatin to low or high MSG had no impact on bitterness attributes. There was no clear difference of linger (aftertaste) between either thaumatin sample and the control.

The conclusion from these evaluations is that addition of THAUMATIN II to a salty/savory base with low salt or low MSG content will enhance savory attributes, while the effect is less noticeable when the base contains higher levels of either salt or MSG. These findings suggest that THAUMATIN II could help reduce the level of either salt (NaCl), MSG, or both, in foods where salt or MSG are commonly used.

An additional finding is that THAUMATIN II modulates flavor very similarly to the commercial thaumatin product employed for comparison in these studies. The flavor modulation (masking and/or potentiation) of these thaumatin products is not identical because the composition, including impurities and excipients, and actual concentration of protein might differ slightly between the two samples.

C.9. Summary

APPENDIX C summarizes the various methods used to characterize Notifier's THAUMATIN II product produced recombinantly in the plant host *Nicotiana benthamiana*. The Thaumatin II protein that comprises Notifier's THAUMATIN II product is expressed with signal peptide to enhance its accumulation in plant tissue and facilitate isolation. The precursor protein is correctly processed *in planta* post-translationally to produce the final mature Thaumatin II protein.

All three methods described in GRN 738 (APPENDIX C, pp 47-51) for purifying thaumatins from edible plant biomass (heat/acid-induced precipitation, cool ambient temperature plus chromatography, or tartrate-induced crystallization) can yield highly pure product (95-98%). Cool neutral extraction followed by cation-exchange chromatography applied to *N. benthamiana*-produced process stream yielded similarly pure protein (~95-98%). The batch-to-batch yield in developmental studies has been generally reproducible and the process generates proteins of consistent quality. Improvements in overall yield and quality are expected as the process is scaled and optimized, as suggested by results of a pilot-scale manufacturing run with THAUMATIN II.

The amino acid composition, amino acid sequence, and molecular mass of the resultant protein are identical to the corresponding thaumatins II found in *Thaumatococcus daniellii*, which forms the basis of currently marketed thaumatin products (GRN 738; GRN 910; this Notice).

The methods used to characterize thaumatins, summarized in GRN 738 and in this section of the Notice, are industry standard and are validated and conducted by licensed external analytical laboratories. Other release tests (e.g. pH with various excipients) are applied to each batch of THAUMATIN II (not shown here) to ensure that the product meets its specification. THAUMATIN II product is stable for >6 months in storage as dry powder and is also stable (>3 months) when mixed and stored with sucrose. The stability program is in progress and longer stable-storage times should be supported by subsequent results.

Notifier's purification process is undergoing optimization during scale up. The residual host-derived alkaloid impurities in THAUMATIN II are consistently low and preliminary results suggest that they can be further reduced during purification at larger (i.e. pilot) manufacturing scale, which employs more rigorous process

controls. Even at the current level of total alkaloids, these impurities do not constitute a health risk due to their low concentration and the low level of ingestion of thaumatin proteins in the diet. Likewise, the levels of elemental impurities in THAUMATIN II are not considered a health risk due to their low concentrations and the low per capita consumption of thaumatin.

It is important to note that thaumatin products approved as food additives in multiple countries are not 100% pure thaumatin, and contain a number of host- and process-derived impurities as well, including extraneous protein and heavy metals (Adesina 1977; Adesina 1978; Cusack 1991; Lim 2012; Stephen 1991). Regardless, the safety of the commercial products has been conclusively shown and, by comparison, the safety of Notifier's final product has also been affirmed (see Section 6.1 (overall safety), Section 6.1.1 (allergenic potential), and Section 6.2 (safety in relation to dietary intake).

The flavor-modifying profiles of Notifier's THAUMATIN II product using various test matrices have been defined under controlled conditions in seven (7) sensory evaluation studies conducted to date. These studies have determined that THAUMATIN II (a) behaves similarly to commercially sold thaumatin; (b) potentiates (enhances) sour taste in reduced citric acid cells in the presence of sucrose; (c) has a sour flavor-masking effect in the presence of acid and in the absence of sucrose; (d) enhances bitter flavor notes under certain concentration ranges of caffeine or quinine, but reduces (masks) bitterness of either caffeine or quinine depending on the concentrations of the tastant and THAUMATIN II; and (e) enhances the savoriness of broths with low salt or low MSG content but the enhancement is less noticeable in broths with higher salt and higher MSG. These findings are detailed here and summarized in Section 2.4, Table 2-2.

Importantly, consumption of THAUMATIN II at the projected rates of application to achieve any of the flavor modifications described is expected to be safe with respect to the intake of Thaumatin II protein itself and of any residual host or process-derived impurities remaining in the final THAUMATIN II product.

END OF NOTICE.

Eighteen pages have been removed in accordance with the Privacy Act of 1974

The references have been removed in accordance with copyright laws. The removed reference citations are found in the "References" section.

From: Kristi Smedley
To: Harry, Molly; daniel@dt-cg.com
Subject: RE: GRN 000920 - Thaumatin II as a Flavor Modifier
Date: Tuesday, July 7, 2020 1:14:39 PM
Attachments: image001.png
GRN 920 Amendment Notifier Response 7 July 2020.pdf

Dr. Harry:

Thank you for giving us the opportunity to amend the GRAS Notice 920 (THAUMATIN II as a Flavor Modifier). We have attached the direct response to your questions as well as the supporting published articles.

Should you need additional information or clarification, please contact us and we can immediately respond.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell 703-786-7674
Fax 703-580-8637

From: Harry, Molly [mailto:Molly.Harry@fda.hhs.gov]
Sent: Friday, June 26, 2020 11:57 AM
To: Kristi Smedley; daniel@dt-cg.com
Subject: RE: GRN 000920 - Thaumatin II as a Flavor Modifier

Dears Drs. Smedley and Tuse'

Our review of GRN 000920 is ongoing and the reviewers have some questions that we would like you to respond to. In some cases, the questions are the same as we asked for GRN 910, but since we consider each GRN as a stand alone document, we are asking that you respond to them for GRN 920 also.

1. Please confirm whether all the analytical methods used for the batch analyses have been validated and are fit for purpose.

2. Your estimated alkaloid exposure (nicotine and anabasine combined) is nearly 2 µg/p/d (Table 3-1), which is about twice as high as the intake from common vegetables in the diet ~1 µg/p/d (0.9-1.3). However, on page 20, you conclude that *"… host alkaloids …should not pose a risk to consumers, as those levels are lower than, or in the same range as, the levels consumers are exposed to in their present diets or daily environment.*" Your proposed alkaloid exposure combined with the background alkaloid exposure will raise the exposure 3-times the background level. Please explain why this will not pose a safety concern for the 90th percentile consumers.

3. On page 24 of the notice, regarding the safety information on host impurities, you state that *"The major bioactive alkaloids … were discussed in detail in GRN 775 (Sections 6.1.1 – 6.1.6; pp 28-39) and GRN 910 (THAUMATIN II Sweetener from N. benthamiana; Sections 6.1.2 – 6.1.4; pp 24 - 35) and are omitted here for brevity."* While detailed discussions on all the safety studies can be incorporated by referencing previous GRNs, a brief summary of the key information is still needed in order to provide the basis for safety in this GRN. Some examples of key information (at the minimum) include the doses in the pivotal study (studies) and the

study design, the NOAEL, any treatment-related effects that could be interpreted as potentially adverse, and an explanation of why such effects are of no safety concern etc.

Please note that the comparison of nicotine exposure from the use of thaumatin II products and that from second hand smoke or nicotine-containing consumer products does not provide appropriate and valid support for the GRAS claim, because there are reported health problems and toxic effects associated with these exposures (References 1 and 2 below). Please explain why the alkaloid exposure from thaumatin II products does not present a safety concern. You may carry out the discussion within the context of the toxicity/clinical data that you summarize (see above) and refer to the risk assessment method used in GRN 775, i.e. margin of safety approach, based on your estimated exposure.

Reference 1. CDC: Health Effects of secondhand smoke: https://www.cdc.gov/tobacco/data_statistics/fact_sheets/secondhand_smoke/health_effects/index.htm

Reference 2. Smolinske SC et al (1988). Cigarette and nicotine chewing gum toxicity in children. Human Toxicology 7(1):27-31.

Please contact me if you have any questions regarding this notice.

Sincerely,
Molly A. Harry
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1075
Molly.Harry@fda.hhs.gov

Notifier's Responses to Agency Questions

GRN 920 – THAUMATIN II Flavor Modifier

Nomad Bioscience GmbH, 7 July 2020

On June 26, 2020 CFSAN provided Notifier with questions regarding GRN 920, THAUMATIN II manufactured in *N. benthamiana* for use as a flavor modifier. The Agency stated that its review of the GRN is ongoing and that the reviewers had several questions to which they requested our response. Our answers and comments are provided below. For convenience, the questions are reproduced in numbered order in ***bold italic*** *font*, followed by Notifier's responses to each.

1. ***Please confirm whether all the analytical methods used for the batch analyses have been validated and are fit for purpose.***

   Nomad confirms that all analytical methods used for batch analyses are performed using validated compendial methods. The analyses for heavy metal content are performed for Notifier by Wolfener Analytik GmbH (Bitterfeld, Germany), and the analyses for alkaloid content and protein identity by mass spectrometry are performed by Fraunhofer Institute of Cell Therapy and Immunology (Halle, Germany); both CROs are certified.

2. ***Your estimated alkaloid exposure (nicotine and anabasine combined) is nearly 2 µg/p/d (Table 3-1), which is about twice as high as the intake from common vegetables in the diet ~1 µg/p/d (0.9-1.3). However, on page 20, you conclude that "… host alkaloids …should not pose a risk to consumers, as those levels are lower than, or in the same range as, the levels consumers are exposed to in their present diets or daily environment." Your proposed alkaloid exposure combined with the background alkaloid exposure will raise the exposure 3-times the background level. Please explain why this will not pose a safety concern for the 90th percentile consumers.***

   The Agency had suggested that we apply a margin-of-safety approach to estimating risk of exposure to low levels of alkaloid impurities in the Thaumatin II product. The maximum adult exposure to alkaloids (at the thaumatin MPL of 1.1 mg/kg body mass-day) of 2 µg/person-day equates to <0.03 µg/kg (2 µg/70 kg adult). Adding the dietary background level would equate to an adult intake of 3 µg/p-d, or 0.042 µg/kg (3 µg/70 kg). As we have provided in GRN 775 (Section 6.1.4, pp 32-37; Table 6-1, pg 35) and from Baumung et al. (2016), the threshold (first measurable) physiologic but non-toxic response to alkaloids in humans (slight, transient and rapidly reversible increase of the heart rate) occurs at 8-13 µg/kg. Hence the margin of safety of our product ranges from ~190- to ~310-fold (8/0.042 to 13/0.042). That is, an adult would have to ingest between ~200-300 times the MPL of thaumatin plus a full ration of pyridine-containing vegetables in a single day to consume enough alkaloid to induce the first threshold physiologic but non-toxic effect. Of course this would far exceed the upper limit allowed for consumption of thaumatin protein itself.

   These estimates are based on body mass and the thaumatin MPL and apply across all consumer percentiles, including high-level vegetable consumers (e.g., vegetarians; vegans). For children, proportional scale-down of food intake and lower body mass still yields similarly low levels of exposure and a proportionately high safety margin. For example, a child weighing 1/3 as much as an adult (~23 kg or ~50 lbs) eating the same Thaumatin II-containing food products plus the same vegetables as adults but consuming only 1/3 as much, would benefit from the same 200-300-fold safety margin.

   We remind the Agency that the thaumatin MPL established by EFSA is 15-times higher than the maximum level of Thaumatin II flavor modifier specified for Notifier's product. A more relevant exposure scenario than the MPL for a risk assessment is one using our calculated daily consumption of Thaumatin II at the specified use level of 5 mg/p-d. Even when including other dietary sources of alkaloids, product-associated (0.125 µg; i.e. 5 mg Thaumatin II/p-d x 25 ng/mg total alkaloid impurity per specification limit) plus dietary (ave. 1 µg/p-d) alkaloids would total 1.125 µg/p-d. These very low levels project exposures in adults and children (based on relative food consumption and body mass) of 0.016 µg/kg. Such a level yields a calculated ~500-800-fold margin of safety.

Therefore, doubling or even tripling the alkaloid consumption from the combined ingestion of Thaumatin II and daily dietary sources should not impact consumer safety, given that the concentrations of total dietary alkaloids will be from ~200-fold (thaumatin MPL + dietary) to ~800-fold (Thaumatin II flavor modifier + dietary) lower than the alkaloid levels that have been documented to elicit the mildest physiologic but non-toxic effects in humans (Baumung et. al., 2016 and response to Question 3).

3. On page 24 of the notice, regarding the safety information on host impurities, you state that "The major bioactive alkaloids … were discussed in detail in GRN 775 (Sections 6.1.1 – 6.1.6; pp 28-39) and GRN 910 (THAUMATIN II Sweetener from N. benthamiana; Sections 6.1.2 – 6.1.4; pp 24 - 35) and are omitted here for brevity." While detailed discussions on all the safety studies can be incorporated by referencing previous GRNs, a brief summary of the key information is still needed in order to provide the basis for safety in this GRN. Some examples of key information (at the minimum) include the doses in the pivotal study (studies) and the study design, the NOAEL, any treatment-related effects that could be interpreted as potentially adverse, and an explanation of why such effects are of no safety concern.

Notifier has relied on reported human physiologic effects of nicotine as the type alkaloid from *Nicotiana* species in its assessment of consumer risk due to ingestion of trace amounts of such alkaloids present as impurities in its Thaumatin II product. Results of controlled clinical studies of nicotine exposure via various routes of administration do exist, as cited by Notifier in prior GRNs, including landmark work by Lindgren et al. (1999) as later reviewed and updated by Baumung et al. (2016).

Lindgren et al. (1999) conducted a well-controlled study in humans administered nicotine via the intravenous (i.v.) route to assess pharmacologic effects. The trial was designed as a single-blind randomised, placebo-controlled crossover dose-response study. Doses administered ranged from 0 (saline control) to 28 μg/kg nicotine delivered over 10 minutes with timed periodic blood draw, constant monitoring (cardiovascular and electroencephalographic) and follow-up. The minimum dose of nicotine reported to induce a measurable pharmacologic effect in human subjects (slight, transient and rapidly reversible increase in heart rate) was used to establish an i.v. LOAEL of 3.5 μg/kg. As subsequently discussed by Baumung et al. (2016), a correction factor of 0.44 was applied to account for ADME differences between the i.v. and oral routes of exposure in humans (Russell 1983), and an uncertainty factor of 10 was used to account for species variability from animals studies to human results, to establish an oral LOAEL of 8 μg/kg.

Using Lindgren's and other data, Baumung et al. (2016) also calculated the Benchmark Dose Level (BMDL) for first measurable effects to be 13 μg/kg. Therefore, from these studies and published analyses, the LOAEL for nicotine in humans can be considered to range from 8 to 13 μg/kg via the oral route. Presumably, the no observed adverse effect level (NOAEL) would be <8 μg/kg. Because the NOAEL is not known with certainty, we used the known LOAEL range to assess risk instead.

As in our response to Question 2, the margin of safety (or margin of exposure MOE) is calculated as the NOAEL (LOAEL)/estimated exposure (μg/kg-day). Using 0.042 μg/kg as the additive exposure from Thaumatin II impurities at the thaumatin MPL and dietary sources, then the MOS or MOE ranges from ~190- to ~310-fold (8/0.042 to 13/0.042). The MOS or MOE is even greater when calculated at the specified use level of Thaumatin II as a flavor modifier (5 mg/p-d); a calculation that yields a ~500-800-fold safety margin.

Whether trace amounts of alkaloid impurities in Thaumatin II in combination with vegetable-derived alkaloids double, triple, quadruple or quintuple the level of alkaloids normally consumed in the daily diet is inconsequential to consumer safety, because even at the MPL for thaumatin the margin of safety for alkaloids consumed at any of those multiples remains at least 2 logs.

References cited (attached to this response)

Baumung C, J Rehm, H Franke and DW Lachenmeier. 2016. Comparative risk assessment of tobacco smoke constituents using the margin of exposure approach: the neglected contribution of nicotine. *Sci Rep* 6:35577.

Lindgren M, L Molander, C Verbaan, E Lunell, I Rosén. 1999. Electroencephalographic effects of intravenous nicotine: A dose-response study. *Psychopharmacology* 145:342–350.

Russell MA, MJ Jarvis, C Feyerabend and O Ferno. 1983. Nasal nicotine solution: a potential aid to giving up smoking? *Br Med J (Clin Res Ed)* 286(6366):683-684.

From: Kristi Smedley
To: Harry, Molly
Cc: "DANIEL TUSE"
Subject: RE: GRN 000920 - Thaumatin II as a Flavor Modifier
Date: Monday, October 5, 2020 4:04:37 PM
Attachments: image001.png

Dear Ms. Harry,

In response to the questions in your October 2, 2020 email, GRN 910 and GRN 920 do not claim use of Thaumatin II on the same food categories, although there is overlap.

GRN 920 encompasses application of Thaumatin II as a flavor enhancer/modifier to the same foods and at the same application rates included in FEMA GRAS 28 (July 2018). FEMA's GRAS listing includes uses of thaumatin as a flavor enhancer/modifier at up to 7 ppm in non-alcoholic beverages (soft drinks). Notifier's GRN 910 (Thaumatin II sweetener) does not specifically include use of Thaumatin II as a sweetener in soft drinks. We do not wish to modify the food groups or application rates claimed in GRN 920, but we will seek to modify the food groups and use levels in GRN 910 to include soft drink applications. The GRN 920 exposure assessment adequately covers the uses described in the notice.

The notifier plans to submit a Supplement to GRN 910 to include use of Thaumatin II in soft drinks, as the supplement mechanism for expanding the uses and/or application rate of the sweetener was deemed appropriate based on our communications of September 21 and 22, 2020.

Does this explanation answer your questions? If not, please let us know specifically how best we may clarify any remaining issues.

If it would be helpful, we will be glad to discuss additional questions by phone.

Sincerely,

Kristi

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell 703-786-7674
Fax 703-580-8637

From: Harry, Molly [mailto:Molly.Harry@fda.hhs.gov]
Sent: Friday, October 02, 2020 8:59 AM
To: Kristi Smedley; 'DANIEL TUSE'
Subject: RE: GRN 000920 - Thaumatin II as a Flavor Modifier

Dear Drs. Smedley and Tuse'

Please clarify if thaumatin II is intended to be used as a flavor modifier in the same food categories as listed in GRN 000910 at levels ranging from 1-150ppm. If Thaumatin II is intended for use as a flavor modifier in different food categories please specify the food categories and the use levels in those categories. Please also provide a revised dietary exposure estimate that takes into account all uses of Thaumatin II as a flavor modifier.

Please provide the response as soon as possible.

Sincerely,
Molly A. Harry
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1075
Molly.Harry@fda.hhs.gov

From:	DANIEL TUSE
To:	Harry, Molly
Cc:	Kristi Smedley; DANIEL TUSE
Subject:	Re: GRN 000920 - Thaumatin II as a Flavor Modifier
Date:	Monday, November 16, 2020 7:27:13 PM
Attachments:	image001.png GRN 920 Amendment Nomad Bioscience.pdf
Importance:	High

Dear Ms Harry,

Pursuant our recent communications with respect to GRN 920, we have concluded that the best way to address the Agency's questions about Thaumatin II intake as a flavor modifier/enhancer is to submit an Amendment to the GRN. The attached Amendment should address FDA's two main questions regarding our prior notice, namely: (1) "If Thaumatin II is intended for use as a flavor modifier in different food categories (than those in GRN 910) please specify the food categories and the use levels in those categories"; and (2) "please also provide a revised dietary exposure estimate that takes into account all uses of Thaumatin II as a flavor modifier."

To more accurately assess food categories and intake values of the foods most likely to be treated with Thaumatin II in this application, Notifier recruited the assistance of Exponent Inc., the company who developed the FARE® data analysis tool. Exponent reviewed the NHANES 2013-2016 two-day surveys for intakes of nine (9) newly defined food and beverage categories, and applied specific food codes to calculate the *per user* intake in a typical diet for the US population 2 years of age and up, reporting both the mean and the 90^{th} percentile intake values as g food consumed/kg-bw/day.

Using the dietary intake information provided by Exponent for the newly selected food categories, Notifier then calculated the intake of Thaumatin II flavor modifier/enhancer if the protein were applied to those foods at a maximum of 7 ppm. In multiple sensory evaluation studies reported in GRN 920, Notifier concluded that flavor modification/flavor masking effects can be achieved at 7 ppm or less in most foods. In addition, Notifier calculated the exposure to various host- and process-derived impurities when Thaumatin II protein is applied to the specified foods and beverages at 7 ppm.

In the attached document we make reference to various sections of GRN 920 for comparison to our updated findings as described in the Amendment. We also make reference to published information previously cited in GRN 920 and/or provide executable links for those sources. However, we are not providing PDFs of those references in the Amendment as these same files were provided in GRN 920 and are already available to the Agency.

The report prepared for Notifier by Exponent Inc. does not contain any safety related information, only the intake values for the newly specified food categories. All food intake

information identified by Exponent has been integrated into the Amendment and used by Notifier to derive the safety assessments. If necessary for its evaluation, and at the Agency's request, we can provide a copy of the Exponent report commissioned by Notifier for FDA's internal review.

As provided in the Amendment, Notifier's THAUMATIN II product would be applied to a subset of the foods and beverages originally identified in GRN 920. Based on scientific procedures, we have concluded that Thaumatin II protein applied at a rate of 7 ppm to the newly defined food categories would not result in risk to consumers either from intake of Thaumatin II protein or of trace amounts of host- and process-derived impurities.

Please let us know if you have any questions regarding the attached Amendment, or if we can provide any additional information to assist the Agency in completing its review of GRN 920, as amended.

Sincerely,

Daniel Tusé, Ph.D.
CEO and Principal Consultant
DT/Consulting Group
2695 13th Street
Sacramento, CA 95818, USA
eMail daniel@dt-cg.com
Tel 707 290 9528

***

From: "Harry, Molly" <Molly.Harry@fda.hhs.gov>
Date: Friday, October 2, 2020 at 5:58 AM
To: Kristi Smedley <smedley@cfr-services.com>, 'DANIEL TUSE' <daniel@dt-cg.com>
Subject: RE: GRN 000920 - Thaumatin II as a Flavor Modifier

Dear Drs. Smedley and Tuse'

Please clarify if thaumatin II is intended to be used as a flavor modifier in the same food categories as listed in GRN 000910 at levels ranging from 1-150ppm. If Thaumatin II is intended for use as a flavor modifier in different food categories please specify the food categories and the use levels in those categories. Please also provide a revised dietary exposure estimate that takes into account all uses of Thaumatin II as a flavor modifier.

Please provide the response as soon as possible.

Sincerely,

Molly A. Harry
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1075
Molly.Harry@fda.hhs.gov

NOMAD

NOMAD BIOSCIENCE GmbH
Biozentrum Halle
Weinbergweg 22
D-06120 Halle/Saale
Germany
Tel. 49 345 1314 2606
Fax. 49 345 1314 2601

16 November 2020

Susan Carlson, Ph.D.
Director, Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: Amendment to GRN 920: THAUMATIN II Flavor Modifier/Enhancer

Dear Dr. Carlson,

Nomad Bioscience GmbH ("Nomad"; "Notifier"; "Company") is submitting this Amendment to GRN 920, **THAUMATIN II flavor modifier/enhancer**. This Amendment is in response to recent communications between Notifier's representatives and FDA/CFSAN (October 2, 8 and 15, 2020), in which the Agency asked for clarification with respect to the categories of food to which THAUMATIN II would be applied for flavor modification, and the concomitant dietary exposure of the US population to Thaumatin II protein and to host- or process-derived impurities when the product is used as intended.

Background and goal of the Amendment

In its GRAS notice now identified as GRN 920 (submitted 3 March 2020), Nomad had concluded, under FDA's Final Rule pertaining to 21 CFR 170 (August 17, 2016), that its THAUMATIN II product containing the naturally occurring protein Thaumatin II produced recombinantly in plants, including the species *Nicotiana benthamiana,* is Generally Recognized as Safe (GRAS) for use as a food/beverage flavor modifier. GRN 920 was Notifier's latest notice for thaumatins, which began with GRN 738 (Thaumatin I and/or Thaumatin II proteins produced in edible plant species for use as a low-calorie, high-intensity sweetener), for which Nomad received from CFSAN a "No Questions" letter on 18 April 2018. GRN 738 established THAUMATIN as a GRAS sweetener in the USA. Notifier then submitted GRN 910 (Thaumatin II sweetener produced in *Nicotiana benthamiana*), for which Nomad received from CFSAN a "No Questions" letter on 9 September 2020, followed by GRN 920. The data provided in these GRAS notices and the extensive public record on the safety of thaumatins from multiple sources, enabled us to use scientific procedures to reach GRAS conclusions about THAUMATIN II when used as a sweetener or a flavor modifier regardless of the manufacturing host used.

For uses in flavor modification, Notifier referenced extensive prior assessments of thaumatins conducted by the Flavor and Extracts Manufacturers Association (FEMA) expert panel, as specifically described in FEMA GRASa No. 3732 (Oser 1984) for natural (extracted) thaumatins, and in FEMA GRASa 3814 (Smith 1996) for recombinant versions of these proteins. Notifier referred to FEMA's published food categories and the safe maximum rate of application of thaumatins to each to draw comparisons to its THAUMATIN II product. Notifier adopted the FEMA application rates for each food class, to similarly arrive at a GRAS conclusion.

In the communications cited above, FDA requested Notifier to produce its own specific listing of foods and beverages in which THAUMATIN II would be used as a flavor modifier and to provide an intake analysis for the US population, ages 2 years and up. This Amendment complies with the Agency's request. Specifically, Notifier has amended its listing to include the following 9 food and beverage categories:

1. Beverages (water-based, non-alcoholic)
2. Beverages (carbonated, non-alcoholic)
3. Juice fruit drinks and ades
4. Flavored milks and milk drinks
5. Fruit juices/nectars/fruit-based smoothies
6. Fermented dairy products
7. Coffee and teas
8. Imitation dairy beverages
9. Vegetable juices

In GRN 920, Notifier adopted the same rates of application for Thaumatin II as those published in FEMA's GRASa 3732 (Cohen 2016, 2018) for natural, plant-extracted thaumatin when used for flavor modification/enhancement; namely, 7 mg/kg food or 7 mg/L beverage (i.e. 7 ppm), with the exception of chewing gum for which the maximum application rate is 150 ppm. This Amendment contains a revised listing of foods/beverages, shown above and developed independently by Notifier, to which Thaumatin II protein would be added as a flavor modifier/enhancer, but maintains the previous application rate of 7 ppm for the newly specified categories. In GRN 920 Notifier presented results of multiple independent flavor impact evaluations of THAUMATIN II which, taken together, suggested that flavor modification including enhancement of desirable flavors or masking of undesirable flavor notes can be achieved at levels not exceeding 7 ppm for most foods.

Methodology

This Amendment provides the food category and intake information requested by the Agency, plus specific exposure estimates for impurities in the product, together with an updated consumer risk assessment. To arrive at the updated information, Notifier engaged Exponent Inc. (Menlo Park, CA), the developer of the FARE® software tool, to mine food intake by category using the 2013-2014 and 2015-2016 National Health and Nutrition Examination Survey (NHANES 2013-2016) food consumption data.

The intake analyses were conducted in three steps. First, an intake assessment for the newly selected food or beverage categories was conducted based on the NHANES food consumption surveys. The list of food codes included in NHANES was reviewed and those codes that corresponded to each specified category were selected. The daily intake of each of the food categories for the U.S population (2+ years of age) is reported on a ***per user*** basis. Intake values are expressed as quantity of food consumed/kg body mass/day. Intakes are provided for each category as well as for the two-day average total daily intake based on surveyed dietary patterns. Data tables summarizing this estimated daily intake (EDI) information are provided herein and include values for the **mean** and for the **90th percentile**. Subsequently, the Thaumatin II intake was estimated by assuming that each food group consumed contained 7 ppm Thaumatin II protein. Lastly, for each category and for each EDI the level of impurities derived from the source plant and manufacturing process were estimated. To address safety concerns, a risk assessment is provided taking into consideration Thaumatin II intake vis-à-vis the maximum permitted level (MPL) for thaumatins as well as the risk of exposure to potentially toxic impurities. A summary of our findings is provided as well as our conclusion that Thaumatin II is GRAS as a flavor modifier/enhancer when applied at up to 7 ppm at the EDIs cited for the newly specified food categories.

If the Agency has any questions or requires additional information to aid their review of Nomad's findings and GRAS conclusion, please feel free to contact our regulatory and product development representatives in the USA, Dr. Kristi Smedley at Center for Regulatory Services Inc., Woodbridge, VA (Tel 703-590-7337; Email smedley@cfr-services.com), and Dr. Daniel Tusé at DT/Consulting Group, Sacramento, CA (Tel 707-290-9528; Email daniel@dt-cg.com).

Certification

On behalf of Nomad Bioscience GmbH (Notifier), I certify that to the best of my knowledge, this GRAS Notice Amendment is complete, representative, and balanced with respect to the information provided, favorable or unfavorable, known to me and pertinent to the evaluation of the safety and GRAS status of our THAUMATIN II flavor modifier/enhancer product.

Sincerely,

Yuri Gleba, Ph.D.
Chief Executive Officer
Nomad Bioscience GmbH

Attachment: Intake Analysis and Risk Assessment

Intake Analysis and Risk Assessment

1. Food Categories for Use of THAUMATIN II as a Flavor Modifier

A listing of nine (9) newly defined food categories in which THAUMATIN II is intended to be used for flavor modification or flavor enhancement is shown in **Table 1**. The categories are generally derived from 21 CFR 170.3, but specific DRIFDCD codes were used to evaluate intake (Section 2).

Table 1. Summarized list of food categories included in assessment

Cat #	Food Category	Summary of Included Codes
1	Beverages (water-based, non-alcoholic)	Flavored and enhanced waters, sport drinks, energy drinks
2	Beverages (carbonated, non-alcoholic)	Soft drinks (diet and regular), carbonated water
3	Juice fruit drinks and ades	Fruit punch, lemonade, vegetable and fruit drink blends, carbonated and non-carbonated fruit drinks
4	Flavored milks and milk drinks, RTD	Chocolate milk, hot chocolate made with dairy, strawberry milk, milk shakes, eggnog
5	Fruit juices/nectars/fruit-based smoothies	100% fruit juice[1], nectars, fruit and/or vegetable smoothie drinks, with and without dairy products
6	Fermented dairy products	Buttermilk, yogurt, kefir
7	Coffee and teas, RTD	Bottled coffee and teas
8	Imitation dairy beverages	Milk substitutes including soy milk, almond milk, rice milk, coconut milk, and other imitation milks
9	Vegetable juices	Tomato juice[1], carrot juice, celery juice, mixed vegetable juice

RTD: Ready-to-drink

[1]Excludes fruit and vegetable juices with a standard of identity (SOI) including 100% grapefruit, orange, pineapple, prune, and tomato juices per FDA SOIs (21 CFR §146.132, 21 CFR §146.135, 21 CFR §146.137, 21 CFR §146.140, 21 CFR §146.150, 21 CFR §146.146, 21 CFR §146.148, 21 CFR §146.185, 21 CFR §146.187, 21 CFR §156.145).

In its GRN 920 submission, Notifier adopted FEMA's GRAS List (Cohen 2016; 2018) of foods which could benefit from application of THAUMATIN II as a flavor modifier/enhancer, as those publications provided guidance on US application rates for thaumatins when used in flavor modification. Table 6-7 in GRN 920 (page 30) listed the original and expanded-use levels for extracted and recombinantly produced thaumatins for US food products. Although Notifier evaluated functionality of THAUMATIN II at concentrations ranging from 0.6 ppm to 28 ppm (GRN 920 APPENDIC C, Section C.8; pp 63-73), the higher levels introduced undesirable sweetness and Notifier concluded that, for flavor modification, THAUMATIN II could be used on the various foods listed in GRN 920 Table 6-7 at equivalent application rates as those of current thaumatin products; typically 7 ppm with the exception of chewing gum at 150 ppm.

Notwithstanding these use rates, Notifier excluded from GRN 920, and excludes from this Amendment, all applications of THAUMATIN II to any USDA/FSIS-regulated product, as the data supporting suitability in such products are under development.

Hence, the foods included in Table 1 of this Amendment constitute the revised food categories claimed by Notifier for addition of THAUMATIN II flavor modifier/enhancer.

2. Food Intake Estimates for Food Categories to be Treated with THAUMATIN II Flavor Modifier/Enhancer

The specific intakes of the foods listed in Section 1, Table 1, were estimated from the NHANES 2013-1016 surveys. The methods used are described below.

Consumption data

The estimated intakes of the nine food and beverage categories were based on consumption records collected in the WWEIA (what we eat in America) component of NHANES conducted in 2013-2014 and 2015-2016 cycles (NHANES 2013-2016), which were the latest datasets available that contained the desired intake information. This continuous survey is a complex, multistage, probability sample designed to be representative of the civilian US population (National Center for Health Statistics (2016, 2018).

The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection. As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview.

The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 14,601 individuals in the 2013-2016 survey period provided two complete days of dietary recalls.

Food and Nutrient Database for Dietary Studies (FNDDS)

For each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) databases translates foods as reported consumed into one or more ingredients (and gram amounts) or recipes. The FNDDS also provides information on the amount of energy and approximately 60 nutrients or food constituents per 100 g of each food based on the National Nutrient Database for Standard Reference (SR). Our analysis applied 2015-2016 FNDDS food recipes (USDA 2018) to process dietary recall data reported in NHANES 2013-2016 and 2013-2014 FNDDS recipes (USDA 2016a, 2016b) for foods that were only reported consumed in NHANES 2013-2014.

NHANES Food Selection

The list of all food codes reported consumed in NHANES 2013-2016 that fell into any of the nine food and beverage categories was reviewed. Food codes corresponding to the food and beverage categories in Table 1 were primarily identified based on the food description.

For categories where the food or beverage corresponding to the select categories of interest could be used as an ingredient (e.g., buttermilk, yogurts, milk substitutes), our analysis utilized the USDA's FNDDS recipes to identify the component of the food mixture that corresponded to the select food interest. Identification of the weight of ingredients in food mixtures allowed for the estimation of select foods that can be consumed as is or as a component in a food (e.g., yogurt in biryani and soy milk in a latte). NHANES foods corresponding to all other food categories were identified based on the food description of the food reported as consumed.

The selection of food codes excluded fruit and vegetable juices with a standard of identity (SOI) including 100% grapefruit, orange, pineapple, prune, and tomato juices per FDA SOIs (21 CFR §146.132, 21 CFR §146.135, 21 CFR §146.137, 21 CFR §146.140, 21 CFR §146.150, 21 CFR §146.146, 21 CFR §146.148, 21 CFR §146.185, 21 CFR §146.187, 21 CFR §156.145).

A summary of the NHANES foods identified for each category that were included in the analysis is presented in Table 1 and the comprehensive list of NHANES food codes and their descriptions are provided in the **Appendix**.

Analysis

Using the NHANES consumption data, our analysis estimated the 2-day average daily intake on a "per capita" and "per user" basis. Per capita estimates refer to the consumption based on the entire population of interest, whereas per user estimates refer to those who reported consuming any of the foods within a given food product category on either of the survey days.

We identified each participant who reported consuming select foods or beverages of interest on either of the survey days, and we used that individual's responses for both survey days. Zero consumption days are included in calculating that individual's average daily intake. For example, if someone reported consuming 150 grams (g) of diet cola on day 1 and 200 g of diet cola on day 2, his/her 2-day average diet cola consumption would be 175 g ([150 + 200]/2). The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS.

The 2-day average intakes by each individual were estimated using Exponent's Foods Analysis and Residues Evaluation Program (FARE® version 13.06) software, and the statistically weighted values from the survey were used in the analyses. The statistical weights compensate for variable probabilities of participant selection, adjusted for non-response, and provide intake estimates that are representative of the U.S. population.

Two-day average estimates were derived for each of the nine food and beverage categories as well as from all food uses combined. Estimates were derived on a body mass (body weight; bw) basis based on each participant's measured body weight and derived for the U.S. population 2-years of age and older.

Results

Two-day average intake estimates of the nine food and beverage categories were calculated based on consumption data collected in NHANES 2013-2016. Both the *per capita* and *per user* mean and 90th percentile results for the U.S. population 2+ years, in g/kg-bw/day, by food category and all food uses are provided in **Table 2**.

In summary, on a *per user* basis, the total combined mean intake of the nine food and beverage categories by the U.S. population 2+ years was estimated to be **8.2 g/kg-bw/day**. At the *per user* 90th percentile (i.e., heavy users), the total combined intake by the U.S. population 2+ years of the nine food and beverage categories was estimated to be **17.2 g/kg-bw/day**.

Table 2 presents food categories as summaries. A comprehensive listing of the specific NHANES food codes and corresponding descriptions used in our analyses are included in the **Appendix**.

Table 2. Two-day average estimated daily intake (EDI) of select food and beverage categories among the US population 2+ years of age (NHANES 2013-16)

Cat No	Food Category	N	% User	Food Intake Per Capita		Food Intake Per User	
				Mean	90th Percentile	Mean	90th Percentile
				g/kg-bw/day			
1	Beverages, water-based, non-alcoholic	1,452	11	0.7	1.5	6.3	12.4
2	Beverages, carbonated, non-alcoholic	6,915	53	3.2	9.1	6.0	12.5
3	Coffee and Tea, RTD	1,080	8	0.4	0.0	4.9	10.2
4	Fermented dairy products	2,068	17	0.3	1.2	1.9	3.7
5	Flavored milks and milk drinks	1,399	8	0.3	0.0	4.3	8.9
6	Fruit drinks and ades	3,370	20	0.8	2.8	4.3	8.8
7	Fruit juices, nectars, and smoothies	3,224	19	0.9	2.8	4.6	9.2
8	Imitation dairy drinks	682	6	0.2	0.0	2.8	5.6
9	Vegetable juice	200	2	<0.05	0.0	2.5	4.9
10	**Total**	**11,568**	**84**	**6.9**	**16.2**	**8.2**	**17.2**

The **Total Mean intake per user** (Column 7 in the table) is **8.2 g/kg-bw/day**, whereas the **Total 90th Percentile intake per user** (Column 8 in the table) is approximately 2.1-times the mean, or **17.2 g/kg-bw/day**.

3. Estimated Exposure to Thaumatin II Protein When Used as a Flavor Modifier/Enhancer

To arrive at Thaumatin II protein exposure estimates from use of the product as a flavor modifier/enhancer, the estimated food intakes per the DRIFDCD food codes evaluated and summarized by category in Section 2, Table 2, were assumed to be treated with Thaumatin II protein at a maximum application rate of 7 ppm (i.e. 7 mg/kg of solid, semisolid or liquid food regardless of consistency). The results are shown in **Table 3**.

Building on the intake estimates from Table 2, the last four columns on the right in Table 3 show the intake of Thaumatin II at the *per user* level when the product is applied to the foods shown at 7 ppm. The amount consumed in micrograms Thaumatin II/kg-bw/day (μg/kg-bw/day) for each food category and for the total are shown for the mean and 90th percentile levels.

Also shown are the amounts of Thaumatin II consumed relative to the **maximum permitted level (MPL)** for thaumatin. The European Food Safety Authority (EFSA) has provided guidance on thaumatin's MPL on various foods and beverages according to Annex II to Regulation (EC) No 1333/2008 (OJEU 2011).

The thaumatin MPL was established from results of extensive preclinical and clinical safety studies, to arrive at 1.1 mg thaumatin/kg-bw/day as a safe intake level based on thaumatin's established margin of exposure (MOE) and margin of safety (MOS) (EFSA 2015). Mean and 90th percentile values *per user* are shown for individual food groups as well as for the typical dietary total.

As shown in Table 3, when applied at 7 ppm, no individual food category exceeds 90 micrograms of Thaumatin II protein intake on a body mass basis per day. Analysis of total food intake (bottom row in table) yields total *per user* Mean and total *per user* 90th Percentile Thaumatin II intake values of **57.3 μg/kg-bw/day** (7 ppm x 8.2 g/kg-bw/day) and **120.6 μg/kg-bw/day** (7 ppm x 17.2 g/kg-bw/day), respectively.

Table 3. Estimated daily intake (EDI) of Thaumatin II when applied at 7 ppm to selected food categories (NHANES 2013-16)

Cat No	Food Category	Food Intake		Thaumatin II Intake			
		Per User		Per User			
		Mean	90th Percentile	Mean		90th Percentile	
		g/kg-bw/day		At 7 ppm Amount (μg/kg/d)	Percent of MPL*	At 7 ppm Amount (μg/kg/d)	Percent of MPL*
1	Beverages, water-based, non-alcoholic	6.3	12.4	44.4	4.0	86.9	7.9
2	Beverages, carbonated, non-alcoholic	6.0	12.5	42.3	3.8	87.7	8.0
3	Coffee and Tea, RTD	4.9	10.2	34.2	3.1	71.1	6.5
4	Fermented dairy products	1.9	3.7	13.1	1.2	25.7	2.3
5	Flavored milks and milk drinks	4.3	8.9	30.3	2.8	62.4	5.7
6	Fruit drinks and ades	4.3	8.8	29.9	2.7	61.8	5.6
7	Fruit juices, nectars, and smoothies	4.6	9.2	32.0	2.9	64.6	5.9
8	Imitation dairy drinks	2.8	5.6	19.7	1.8	38.9	3.5
9	Vegetable juice	2.5	4.9	17.2	1.6	34.4	3.1
10	**Total**	**8.2**	**17.2**	**57.3**	**5.2**	**120.6**	**11.0**

*MPL = Maximum Permitted Level of thaumatin consumption per day, established by EFSA as 1.1 mg/kg-bw/day (EFSA 2015). Some products of multiplication may not match exactly due to rounding.

When evaluating Thaumatin II specific intake (μg/kg-bw/day) as a percentage of the maximum permitted daily intake level for thaumatins (MPL) of 1.1 mg/kg-bw/day (= 1,100 μg/kg-bw/day), the total *per user* Mean and total *per user* 90th Percentile intake values represent **5.2%** (57.3/1,100 μg/kg-bw/day x 100) and **11%** (120.6/1,100 μg/kg-bw/day x 100) of the MPL, respectively.

Hence, even if all the foods in the above-listed categories were treated with a maximum of 7 ppm Thaumatin II protein, consumption *per user* would amount to between 5.2 and 11 percent of the 1.1 mg/kg-bw/day MPL for thaumatin based on the protein's established safety profile.

4. Estimated Exposure to Host and Process Impurities

In GRN 920, Notifier's THAUMATIN II product Specification defined the upper limits of host- and process-derived impurities based on the Company's manufacturing experience to date. These impurity levels and their potential toxicities if consumed in large quantities were used to assess potential risk from consuming foods and beverages treated with THAUMATIN II for flavor modification/enhancement. The estimates presented in GRN 920 for the original candidate food groups derived from FEMA's assessments (Cohen 2016, 2018) were refined on the basis of the new list of foods included in this Amendment. Exposure is summarized by type of impurity.

Residual host protein

The THAUMATIN II product Specification presented in GRN 920 (GRN 920 Section 2.3, pg 14) states Thaumatin II protein purity as 95% of total protein; the ≤5% residual protein comprising plant host-derived proteins. Consumption of Thaumatin II at the thaumatin MPL of 1.1 mg/kg-bw/day would result in a maximum intake of 55 μg/kg-bw/day of plant host-derived protein, or approximately 3.85 mg protein/day for a 70-kg adult.

Consumption of the foods specified at the intake rates evaluated in this Amendment if all foods were treated with 7 ppm Thaumatin II (0.35 ppm host protein; 0.05 x 7 ppm) would result in intakes of 2.9 (0.05 x 57.3 μg/kg-bw/day) to 6 (0.05 x 120.6 μg/kg-bw/day) micrograms (μg) host proteins/kg-bw/day at the mean *per user* and the 90th percentile *per user*, respectively, or 0.2 (mean *per user*) to 0.42 (90th percentile *per user*) mg/day for a 70-kg adult. Host proteins from *N. benthamiana* are as digestible as other plant proteins and at those trace levels would not impact safety, nutrition or caloric intake.

Alkaloidal host impurities

In GRN 920, Notifier identified the main organic impurities of concern as the alkaloids nicotine and anabasine, given their presence in the *N. benthamiana* production host plant and their pharmacologic activity. The multi-batch average values for nicotine and anabasine (nanograms; ng) per milligram (mg) of Thaumatin II protein (i.e. ppm) were determined as described in GRN 920 APPENDIX C, Section C.5 (pg 61). A maximum level per Specification of 25 ng total alkaloid (20 ng nicotine + 5 ng anabasine) per mg Thaumatin II protein was adopted for calculating exposure at the food intake levels evaluated herein.

The micrograms (μg) of Thaumatin II consumed per kg body mass per day in each food class and in total for the mean and 90th percentile intake levels were presented in Table 3. The alkaloid exposure potentially occurring from those levels of Thaumatin II consumption are reported in **Table 4** for the *per user* mean and 90th percentile intake levels.

Table 4. Estimated exposure to alkaloidal host impurities in Thaumatin II when applied as a flavor enhancer/modifier

		Food Intake		Alkaloid Impurity Exposure from Thaumatin II			
		Per User		Per User			
		Mean	90th Percentile	Thaumatin Mean	Alkaloid Max at 25 pg alkaloid/μg Thaumatin	Thaumatin 90th Percentile	Alkaloid Max at 25 pg alkaloid/μg Thaumatin
Cat No	Food Category	g/kg-bw/day		Max at 7 ppm Thaumatin Amount (μg/kg/d)	Amount (ng/kg/d)	Max at 7 ppm Thaumatin Amount (μg/kg/d)	Amount (ng/kg/d)
1	Beverages, water-based, non-alcoholic	6.3	12.4	44.4	1.1	86.9	2.2
2	Beverages, carbonated, non-alcoholic	6.0	12.5	42.3	1.1	87.7	2.2
3	Coffee and Tea, RTD	4.9	10.2	34.2	0.9	71.1	1.8
4	Fermented dairy products	1.9	3.7	13.1	0.3	25.7	0.6
5	Flavored milks and milk drinks	4.3	8.9	30.3	0.8	62.4	1.6
6	Fruit drinks and ades	4.3	8.8	29.9	0.7	61.8	1.5
7	Fruit juices, nectars, and smoothies	4.6	9.2	32.0	0.8	64.6	1.6
8	Imitation dairy drinks	2.8	5.6	19.7	0.5	38.9	1.0
9	Vegetable juice	2.5	4.9	17.2	0.4	34.4	0.9
10	**Total**	**8.2**	**17.2**	**57.3**	**1.4**	**120.6**	**3.0**

At a Thaumatin II application rate of 7 ppm containing the maximum level of alkaloidal impurities per Specification (25 pg alkaloid/μg Thaumatin II protein), the potential total alkaloid exposures would be 1.4 nanograms (mean) and 3.0 nanograms (90th percentile) on a specific weight basis (ng alkaloid/kg-bw/day).

These values translate to approximately 0.1 μg (mean) and 0.21 μg (90th percentile) alkaloid per day for a 70-kg adult. These intakes are approximately 10% to 20% of the alkaloid levels consumed daily from vegetables.

Elemental impurities

As detailed in the original GRN 920 submission (GRN 920 Section 6.1.4, pg 28; APPENDIX C, Section C.6, pp 61-62), Notifier determined the levels of Class 1 – 3 heavy metals in multiple batches of its product. These values were used to set Specification limits of 1 ng for Pb and 5 ng total Class 1 metals per mg Thaumatin II protein. Class 2 and 3 metal levels were too low to present a safety risk; hence Notifier focused on analysis of Class 1 elements due to their higher toxicities.

Hypothetical exposure from consumption of THAUMATIN II even at the maximum permitted level (MPL; 1.1 mg/kg-day) was projected to be <400 nanograms/person-day. Considering that the cumulative permitted daily exposure (PDE) for a combination of Class 1 elements is 55 micrograms/person-day (ICH 2015), we concluded then that the intake of heavy metal impurities from consumption of THAUMATIN II at the MPL would be inconsequential and will constitute a very low safety risk.

We reached the same conclusion in the present analysis, since at 5 ng total Class 1 heavy metal/mg Thaumatin II protein applied at 7 ppm to the specified food groups would result in exposures of 0.29 to 0.6 ng/kg-bw/day (approximately 20 ng/day and 42 ng/day for a 70-kg adult) at the total mean (57.3 μg/kg-bw/day) and total 90th percentile (120.6 μg/kg-bw/day) food intake levels. The PDE for heavy metals is 55 micrograms/person-day (ICH 2015), or approximately 786 nanograms/kg-bw/day for a 70-kg adult. Therefore, the incremental intake of these Class 1 elements from 7 ppm Thaumatin II is not expected to impact safety, since 20 ng/day and 42 ng/day represent 0.04% (20/55,000 ng x 100) and 0.08% (42/55,000 ng x 100) of the PDE.

5. Assessment of Risk

The updated food categories and intake values derived from NHANES 2013-2016 were used to develop a risk assessment. Risk from consumption of the THAUMATIN II product when used to modify or enhance food flavors was estimated on the basis of the levels of exposure to Thaumatin II protein and to host- and process-derived impurities. Risks from exposure to each component are summarized.

Thaumatin II protein

We expect THAUMATIN II to initially replace existing sources of thaumatins used for flavor modification/enhancement in the specifically listed food categories evaluated herein. Therefore, we do not see a significant additive effect from consumption of THAUMATIN II plus existing sources of thaumatin in this product use category. Introduction of THAUMATIN II in all foods listed at 7 ppm would result in only 5-11% (mean to 90th percentile) of the safety defined daily MPL. That is, inclusion of Thaumatin II protein at 7 ppm in all the listed food categories would result, at worst, in no more than 11% of the thaumatin MPL. Therefore, we do not anticipate a risk to consumers from consumption of the Thaumatin II protein at the stated levels.

In our GRN 920 submission we had estimated Thaumatin II protein intake at 7 ppm application rate for most foods and 150 ppm for chewing gum using the more encompassing FEMA food categories list, and estimated that the maximum exposure to the protein would be about 5 mg per day for a 70-kg adult, or 6.5% of the MPL (i.e. 5 mg Thaumatin II/77 mg/person-day MPL [70 kg adult x 1.1 mg/kg-day]) based on usual consumption patterns (i.e. equivalent to "Mean" in this Amendment). For a 70-kg adult, the specific consumption of Thaumatin II protein would be 71.4 μg/kg-bw/day ([5 mg/day]/70 kg). Therefore, our initial estimates using the FEMA list suggested intakes of approximately 6.5% (5 mg/77 mg x 100) of the MPL.

Specifically, our new calculations for this Amendment were based on more accurate food intake values for revised food categories of sub-populations 2 years of age and older, reported on a specific body mass basis.

We assumed a uniform 7 ppm Thaumatin II application rate across all foods for flavor modification/flavor enhancement (Cohen 2016, 2018). Our new estimates yielded a *per user* Thaumatin II protein intake of 57.3 μg/kg-bw/day for the Mean intake, or 5.2% of the thaumatin MPL, and 120.6 μg/kg-bw/day, or 11% of the thaumatin MPL, for the 90th Percentile of food intake. We reiterate that Nomad's Thaumatin II would replace, not add to, existing permitted uses of thaumatin in the covered food products, which is a subset of current thaumatin uses.

Host and process impurities

The biotic and abiotic impurities in the THAUMATIN II flavor modifier/enhancer product include low levels of plant host-cell protein, and trace levels of pyridine alkaloids and elemental impurities. The host-derived protein impurities at the low levels defined would not impact safety, caloric or nutritional content. The elemental impurities amount to no more than 0.1% of the PDE for heavy metals in the diet. Other inorganic impurities include salts derived from the buffer (phosphate, NaCl) that are safe and allowed for food use. None of these impurities pose a safety risk at the levels defined herein.

As reported in Notifier's 7 July 2020 response to FDA regarding the estimated alkaloid exposure described in GRN 920, the levels of exposure for the originally defined food list were equivalent to 1-2 servings of vegetables, which are all uniformly too low to induce either pharmacologic or toxic effects. The alkaloidal impurities at the maximum level in the Specification (25 pg/μg Thaumatin II) impacting the newly defined food groups at the consumption levels analyzed in this Amendment, and assuming a Thaumatin II application rate of 7 ppm, yield potential *per user* exposures of 1.4 nanograms (mean) and 3.0 nanograms (90th percentile) total alkaloid on a specific weight basis (ng alkaloid/kg-bw/day). These values translate to approximately 0.1 μg (mean) and 0.21 μg (90th percentile) alkaloid per day for a 70-kg adult. For comparison, several studies have reported the average per capita daily food-borne exposure to nicotine from consumptions of common vegetables as ~1 μg (900-1,300 ng)/person-day (Andersson 2003; Davis 1991; Domino 1993; Moldoveanu 2016; Nielsen 2013; Siegmund 1999). Therefore, exposure to residual host alkaloids at the 7 ppm Thaumatin II protein application rate, even in combination with dietary intake of pyridine alkaloids, will result in only 10-20% fractional increases in alkaloid consumption relative to dietary levels, which should not pose a consumer safety risk.

6. GRAS Conclusion

Notifier evaluated in more detail a revised list of foods to which its THAUMATIN II product could be added as a flavor modifier/enhancer (**Table 1**). The intake levels for each food comprising each category were derived from the NHANES 2013-2016 surveys (**Table 2**). Results were expressed as gram food consumed per kg body mass (weight) per day (g/kg-bw/day) across all categories surveyed. In addition, mean and 90th percentile total intake values were presented for typical dietary combinations of the foods evaluated as reported by the consumer surveys. Although per capita intake values were presented, further analyses of exposure to THAUMATIN II product components were conducted using intake values at the "per user" level.

The amount of Thaumatin II protein consumed (**Table 3**) was estimated based on mean and 90th percentile food intakes and determined to be only between 5 and 11 percent of the established thaumatin maximum permitted level (MPL). Other biotic impurities including non-thaumatin protein and pyridine alkaloids from the host plant (**Table 4**) were determined to be too low to present a safety concern. Equally, the levels of inorganic elemental impurities were determined to be too low to present a safety concern.

Taken together, Notifier concludes that THAUMATIN II is generally recognized as safe (GRAS) based on scientific procedures when applied as a flavor modifier/enhancer to the foods identified in this Amendment at a level not to exceed 7 mg Thaumatin II protein per kg treated food (7 ppm).

References

Andersson C, P Wennström and J Gry. 2003. Nicotine alkaloids in Solanaceous food plants. Nordic Working Group on Food Toxicology and Risk Evaluation. Nordic Council of Ministers.

Cohen SM, S Fukushima, NJ Gooderham, et al. 2016. FEMA GRAS Flavoring Substances 28 (Interim).

Cohen SM, G Eisenbrand, S Fukushima, et al. 2018. FEMA GRAS 28 Flavoring Substances.

Davis RA, MF Stiles, JD deBethizy and JH Reynolds. 1991. Dietary nicotine: a source of urinary cotinine. *Food Chem Toxicol* 29(12):821-827.

Domino EF, E Hornbach and T Demana. 1993. The nicotine content of common vegetables. *N Engl J Med* 329(6):437.

EFSA. 2015. Scientific Opinion on the safety of the extension of use of thaumatin (E 957). *EFSA Journal* 13(11):4290.

ICH. 2015. Guidance for Industry Q3D Elemental Impurities.

Moldoveanu SC, WA Scott and DM Lawson. 2016. Nicotine Analysis in Several Non-Tobacco Plant Materials. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research* 27(2).

National Center for Health Statistics (NCHS). 2016. National Health and Nutrition Examination Survey Data 2013-2014. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available from:
https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2013.
Accessed November 10, 2020.

National Center for Health Statistics (NCHS). 2018. National Health and Nutrition Examination Survey Data 2015-2016. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available from:
https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015.
Accessed November 10, 2020.

Nielsen SS, GM Franklin, WT Longstreth, PD Swanson and H Checkoway. 2013. Nicotine from edible *Solanaceae* and risk of Parkinson disease. *Ann Neurol* 74(3):472-477.

OJEU. 2011. Annex II Regulations: Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union*.

Oser BL, RA Ford and BK Bernard. 1984. Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. 13. GRAS Substances. *Food Technology* 38(10):66-89.

Siegmund B, E Leitner and W Pfannhauser. 1999. Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. *J Agric Food Chem* 47(8):3113-3120.

Smith RL, P Newberne, TB Adams, RA Ford, JB Hallagan and FEMA. 1996. GRAS Flavoring Substances 17. *Food Technology* 50(10):72-78, 80-81.

USDA. 2016a. USDA Food and Nutrient Database for Dietary Studies (FNDDS), 2013-2014. Beltsville, MD: U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group. Available from: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-download-databases/.
Accessed November 10, 2020.

USDA. 2016b. USDA National Nutrient Database for Standard Reference, Release 28 (Slightly revised). Beltsville, MD: Agricultural Research Service, Food Surveys Research Group. Available from: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/methods-and-application-of-food-composition-laboratory/mafcl-site-pages/sr11-sr28/.
Accessed November 10, 2020.

USDA. 2018. USDA Food and Nutrient Database for Dietary Studies (FNDDS), 2015-2016. Beltsville, MD: Agricultural Research Service, Food Surveys Research Group. Available from: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-download-databases/.
Accessed November 10, 2020.

Appendix

NHANES Food Codes Used in Analysis

Food and beverage category	Food Code	Name
Beverages, carbonated, non-alcoholic	13120800*	Ice cream soda, flavors other than chocolate
	13120810*	Ice cream soda, chocolate
	92400000	Soft drink, NFS
	92400100	Soft drink, NFS, diet
	92410110	Carbonated water, sweetened
	92410210	Carbonated water, unsweetened
	92410250	Carbonated water, sweetened, with low-calorie or no-calorie sweetener
	92410310	Soft drink, cola
	92410315	Soft drink, cola, reduced sugar
	92410320	Soft drink, cola, diet
	92410340	Soft drink, cola, decaffeinated
	92410350	Soft drink, cola, decaffeinated, diet
	92410360	Soft drink, pepper type
	92410370	Soft drink, pepper type, diet
	92410390	Soft drink, pepper type, decaffeinated
	92410400	Soft drink, pepper type, decaffeinated, diet
	92410410	Soft drink, cream soda
	92410420	Soft drink, cream soda, diet
	92410510	Soft drink, fruit flavored, caffeine free
	92410520	Soft drink, fruit flavored, diet, caffeine free
	92410550	Soft drink, fruit flavored, caffeine containing
	92410560	Soft drink, fruit flavored, caffeine containing, diet
	92410610	Soft drink, ginger ale
	92410620	Soft drink, ginger ale, diet
	92410710	Soft drink, root beer
	92410720	Soft drink, root beer, diet
	92410810	Soft drink, chocolate flavored
	92410820	Soft drink, chocolate flavored, diet
	92411510	Soft drink, cola, fruit or vanilla flavored
	92411610	Soft drink, cola, fruit or vanilla flavored, diet
	92510720*	Fruit punch, made with fruit juice and soda
	92510730*	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
	93301000*	Cocktail, NFS
	93301060*	Gin and Tonic
	93301120*	Mint julep
	93301125*	Mojito
	93301130*	Old fashioned

Food and beverage category	Food Code	Name
	93301142*	Seven and Seven
	93301170*	Whiskey and soda
	93301182*	Whiskey and cola
	93301183*	Whiskey and diet cola
	93301184*	Whiskey and ginger ale
	93301190*	Rum and cola
	93301191*	Rum and diet cola
	93301205*	Brandy and cola
	93301211*	Vodka and soda
	93301214*	Vodka and cola
	93301215*	Vodka and diet cola
	93301218*	Vodka and tonic
	93301270*	Fruit punch, alcoholic
	93301360*	Long Island iced tea
	93404550*	Sangria, red
	93404560*	Sangria, white
Beverages, water-based, non-alcoholic	93301083*	Jagerbomb
	93301216*	Vodka and energy drink
	94100200	Water, bottled, sweetened, with low calorie sweetener
	94100300	Water, bottled, flavored (Capri Sun Roarin' Waters)
	94210100	Water, bottled, flavored (Propel Water)
	94210200	Water, bottled, flavored (Glaceau Vitamin Water)
	94210300	Water, bottled, flavored (SoBe Life Water)
	94220100	Propel Zero Water
	94220215	Water, bottled, flavored, sugar free (Glaceau Vitamin Water)
	94220310	Water, bottled, flavored, sugar free (SoBe)
	95310200	Energy drink (Full Throttle)
	95310400	Energy drink (Monster)
	95310500	Energy drink (Mountain Dew AMP)
	95310560	Energy drink (NOS)
	95310600	Energy drink (Red Bull)
	95310700	Energy drink (Rockstar)
	95310750	Energy drink (SoBe Energize Energy Juice Drink)
	95311000	Energy Drink
	95312400	Energy drink, low calorie (Monster)
	95312500	Energy drink, sugar free (Mountain Dew AMP)
	95312560	Energy drink (Ocean Spray Cran-Energy Juice Drink)
	95312600	Energy drink, sugar-free (Red Bull)
	95312700	Energy drink, sugar free (Rockstar)
	95312900	Energy drink (XS)
	95313200	Energy drink, sugar free

Food and beverage category	Food Code	Name
	95320200	Sports drink (Gatorade G)
	95320500	Sports drink (Powerade)
	95321000	Sports drink, NFS
	95322200	Sports drink, low calorie (Gatorade G2)
	95322500	Sports drink, low calorie (Powerade Zero)
	95323000	Sports drink, low calorie
	95330100	Fluid replacement, electrolyte solution
	95341000	FUZE Slenderize fortified low calorie fruit juice beverage
Coffee and Tea (RTD)	92100000	Coffee, NS as to type
	92171000	Coffee, bottled/canned
	92171010	Coffee, bottled/canned, light
	92307500	Iced Tea / Lemonade juice drink
	92307510	Iced Tea / Lemonade juice drink, light
	92307520	Iced Tea / Lemonade juice drink, diet
	92309000	Tea, iced, bottled, black
	92309010	Tea, iced, bottled, black, decaffeinated
	92309020	Tea, iced, bottled, black, diet
	92309030	Tea, iced, bottled, black, decaffeinated, diet
	92309040	Tea, iced, bottled, black, unsweetened
	92309050	Tea, iced, bottled, black, decaffeinated, unsweetened
	92309500	Tea, iced, bottled, green
	92309510	Tea, iced, bottled, green, diet
	92309520	Tea, iced, bottled, green, unsweetened
Fermented dairy products	11115000	Buttermilk, fat free (skim)
	11115100	Buttermilk, low fat (1%)
	11115200	Buttermilk, reduced fat (2%)
	11115300	Buttermilk, whole
	11115400	Kefir, NS as to fat content
	11400000	Yogurt, NFS
	11400010	Yogurt, Greek, NS as to type of milk or flavor
	11410000	Yogurt, NS as to type of milk or flavor
	11411010	Yogurt, NS as to type of milk, plain
	11411100	Yogurt, whole milk, plain
	11411200	Yogurt, low fat milk, plain
	11411300	Yogurt, nonfat milk, plain
	11411390	Yogurt, Greek, NS as to type of milk, plain
	11411400	Yogurt, Greek, whole milk, plain
	11411410	Yogurt, Greek, low fat milk, plain
	11411420	Yogurt, Greek, nonfat milk, plain
	11420000	Yogurt, vanilla, NS as to type of milk

Food and beverage category	Food Code	Name
	11421000	Yogurt, vanilla, whole milk
	11422000	Yogurt, vanilla, low fat milk
	11422100	Yogurt, vanilla, low fat milk, light
	11423000	Yogurt, vanilla, nonfat milk
	11424000	Yogurt, vanilla, nonfat milk, light
	11424500	Yogurt, Greek, vanilla, whole milk
	11424510	Yogurt, Greek, vanilla, low fat
	11424520	Yogurt, Greek, vanilla, nonfat
	11426000	Yogurt, chocolate, whole milk
	11427000	Yogurt, chocolate, nonfat milk
	11428000	Yogurt, Greek, chocolate, nonfat
	11430000	Yogurt, NS as to type of milk, fruit
	11431000	Yogurt, whole milk, fruit
	11432000	Yogurt, low fat milk, fruit
	11432500	Yogurt, fruit, low fat milk, light
	11433000	Yogurt, nonfat milk, fruit
	11433500	Yogurt, fruit, nonfat milk, light
	11433990	Yogurt, Greek, NS as to type of milk, fruit
	11434000	Yogurt, Greek, whole milk, fruit
	11434010	Yogurt, Greek, low fat milk, fruit
	11434020	Yogurt, Greek, nonfat milk, fruit
	11434090	Yogurt, NS as to type of milk, flavors other than fruit
	11434100	Yogurt, whole milk, flavors other than fruit
	11434200	Yogurt, low fat milk, flavors other than fruit
	11434300	Yogurt, nonfat milk, flavors other than fruit
	11435000	Yogurt, Greek, NS as to type of milk, flavors other than fruit
	11435010	Yogurt, Greek, whole milk, flavors other than fruit
	11435020	Yogurt, Greek, low fat milk, flavors other than fruit
	11435030	Yogurt, Greek, nonfat milk, flavors other than fruit
	11435100	Yogurt, Greek, with oats
	11436000	Yogurt, liquid
	11440010*	Chipotle dip, yogurt based
	11440020*	Dill dip, yogurt based
	11440040*	Ranch dip, yogurt based
	11440050*	Spinach dip, yogurt based
	11440060*	Tzatziki dip
	11440070*	Vegetable dip, yogurt based
	27116100*	Beef curry
	27120160*	Pork curry
	27130100*	Lamb or mutton curry
	27146150*	Chicken curry

Food and beverage category	Food Code	Name
	27150100*	Shrimp curry
	27150320*	Fish curry
	27213010*	Biryani with meat
	27243100*	Biryani with chicken
	27516010*	Other sandwiches (single code) *
	32101530*	Egg curry
	42401100	Yogurt, coconut milk
	52304010*	Muffin, wheat bran
	52304040*	Muffin, bran with fruit, lowfat
	52408000*	Bread, Irish soda
	53119000*	Cake, pineapple, upside down
	53341500*	Pie, buttermilk
	53366000*	Pie, yogurt, frozen
	53441210*	Basbousa
	58124500*	Pastry, filled with potatoes and peas, fried
	75440600*	Vegetable curry
	77316600*	Eggplant and meat casserole
	83115000*	Yogurt dressing
	91306040*	Dessert dip
Flavored milks and milk drinks	11220000	Milk, condensed, sweetened
	11511000	Chocolate milk, NFS
	11511100	Chocolate milk, ready to drink, whole
	11511200	Chocolate milk, ready to drink, reduced fat
	11511300	Chocolate milk, ready to drink, fat free
	11511400	Chocolate milk, ready to drink, low fat
	11511550	Chocolate milk, ready to drink, reduced sugar, NS as to milk
	11511600	Chocolate milk, ready to drink, low fat (Nesquik)
	11511610	Chocolate milk, ready to drink, fat free (Nesquik)
	11511700	Chocolate milk, ready to drink, low fat, no sugar added (Nesquik)
	11512010	Hot chocolate / Cocoa, ready to drink
	11512020	Hot chocolate / Cocoa, ready to drink, made with nonfat milk
	11512100	Hot chocolate / Cocoa, ready to drink, with whipped cream
	11512110	Hot chocolate / Cocoa, ready to drink, made with nonfat milk and whipped cream
	11519040	Strawberry milk, NFS
	11519050	Strawberry milk, whole
	11519105	Strawberry milk, reduced fat
	11519200	Strawberry milk, low fat
	11519205	Strawberry milk, fat free
	11531000	Eggnog, regular

Food and beverage category	Food Code	Name
	11531500	Eggnog, lowfat / light
	11541400	Milk shake with malt
	11542100	Milk shake, fast food, chocolate
	11542200	Milk shake, fast food, flavors other than chocolate
	11543000	Milk shake, bottled, chocolate
	11543010	Milk shake, bottled, flavors other than chocolate
	11560000	Chocolate milk drink
Fruit drinks and ades	42403010	Coconut water, unsweetened
	42404010	Coconut water, sweetened
	92432000	Fruit juice drink, citrus, carbonated
	92433000	Fruit juice drink, noncitrus, carbonated
	92510610	Fruit juice drink
	92510650	Tamarind drink
	92510720*	Fruit punch, made with fruit juice and soda
	92510730*	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
	92510955	Lemonade, fruit juice drink
	92510960	Lemonade, fruit flavored drink
	92511015	Fruit flavored drink
	92512090	Pina Colada, nonalcoholic
	92513000	Fruit flavored smoothie drink, frozen, no dairy
	92513010	Fruit flavored smoothie drink, frozen, light, no dairy
	92530410	Fruit flavored drink, with high vitamin C
	92530510	Cranberry juice drink, with high vitamin C
	92530610	Fruit juice drink, with high vitamin C
	92530950	Vegetable and fruit juice drink, with high vitamin C
	92531030	Fruit juice drink (Sunny D)
	92550030	Fruit juice drink, with high vitamin C, light
	92550035	Fruit juice drink, light
	92550040	Fruit juice drink, diet
	92550110	Cranberry juice drink, with high vitamin C, light
	92550200	Grape juice drink, light
	92550350	Orange juice beverage, 40-50% juice, light
	92550360	Apple juice beverage, 40-50% juice, light
	92550370	Lemonade, fruit juice drink, light
	92550380	Pomegranate juice beverage, 40-50% juice, light
	92550400	Vegetable and fruit juice drink, with high vitamin C, diet
	92550405	Vegetable and fruit juice drink, with high vitamin C, light
	92550610	Fruit flavored drink, with high vitamin C, diet
	92550620	Fruit flavored drink, diet
	92552020	Fruit juice drink, reduced sugar (Sunny D)
	92552030	Fruit juice drink (Capri Sun)

Food and beverage category	Food Code	Name
	92582100	Fruit juice drink, with high vitamin C, plus added calcium
	92582110	Fruit juice drink, added calcium (Sunny D)
	92612010	Sugar cane beverage
	92801000	Wine, nonalcoholic
	92803000	Nonalcoholic malt beverage
	92804000	Shirley Temple
	93301032*	Cape Cod
	93301141*	Seabreeze
	93301213*	Vodka and lemonade
	93301270*	Fruit punch, alcoholic
	93301360*	Long Island iced tea
	93301500*	Frozen daiquiri
	93301510*	Frozen margarita
Fruit juices, nectars, and smoothies	11551050	Licuado or Batido
	11553100	Fruit smoothie, NFS
	11553110	Fruit smoothie, with whole fruit and dairy
	11553120	Fruit smoothie, with whole fruit and dairy, added protein
	11553130	Fruit smoothie juice drink, with dairy
	61213220	Tangerine juice, 100%
	61213800	Fruit juice blend, citrus, 100% juice
	61213900	Fruit juice blend, citrus, 100% juice, with calcium added
	64100100	Fruit juice, NFS
	64100110	Fruit juice blend, 100% juice
	64100200	Cranberry juice blend, 100% juice
	64100220	Cranberry juice blend, 100% juice, with calcium added
	64101010	Apple cider
	64104010	Apple juice, 100%
	64104030	Apple juice, 100%, with calcium added
	64104600	Blackberry juice, 100%
	64105400	Cranberry juice, 100%, not a blend
	64116020	Grape juice, 100%
	64116060	Grape juice, 100%, with calcium added
	64120010	Papaya juice, 100%
	64121000	Passion fruit juice, 100%
	64126000	Pomegranate juice, 100%
	64132500	Strawberry juice, 100%
	64133100	Watermelon juice, 100%
	64134015	Fruit smoothie, with whole fruit, no dairy
	64134020	Fruit smoothie, with whole fruit, no dairy, added protein
	64134030	Fruit smoothie juice drink, no dairy

Food and beverage category	Food Code	Name
	64134100	Fruit smoothie, light
	64134200	Fruit smoothie, bottled
	64200100	Fruit nectar, NFS
	64201010	Apricot nectar
	64201500	Banana nectar
	64202010	Cantaloupe nectar
	64203020	Guava nectar
	64204010	Mango nectar
	64205010	Peach nectar
	64210010	Papaya nectar
	64215010	Pear nectar
	64221010	Soursop, nectar
	75200700	Aloe vera juice drink
	78101000	Vegetable and fruit juice, 100% juice, with high vitamin C
	78101100	Fruit and vegetable smoothie
	78101110	Fruit and vegetable smoothie, added protein
	78101120	Fruit and vegetable smoothie, bottled
	95342000	Fruit juice, acai blend
Imitation dairy drinks	11320000	Soy milk
	11320100	Soy milk, light
	11320200	Soy milk, nonfat
	11321000	Soy milk, chocolate
	11321100	Soy milk, light, chocolate
	11321200	Soy milk, nonfat, chocolate
	11340000	Imitation milk, non-soy, sweetened
	11350000	Almond milk, sweetened
	11350010	Almond milk, sweetened, chocolate
	11350020	Almond milk, unsweetened
	11350030	Almond milk, unsweetened, chocolate
	11360000	Rice milk
	11370000	Coconut milk
	11512030*	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
	11512120*	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream
	11513310*	Chocolate milk, made from dry mix with non-dairy milk
	11513395*	Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik)
	11513750*	Chocolate milk, made from syrup with non-dairy milk
	11513805*	Chocolate milk, made from light syrup with non-dairy milk
	11514150*	Hot chocolate / Cocoa, made with dry mix and non-dairy milk
	11514360*	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk

Food and beverage category	Food Code	Name
	11519215	Strawberry milk, non-dairy
	56203076*	Oatmeal, regular or quick, made with non-dairy milk, fat not added in cooking
	56203077*	Oatmeal, regular or quick, made with non-dairy milk, fat added in cooking
	56203106*	Oatmeal, instant, plain, made with non-dairy milk, fat not added in cooking
	56207027*	Cream of wheat, regular or quick, made with non-dairy milk, fat added in cooking
	92101903*	Coffee, Latte, with non-dairy milk
	92101906*	Coffee, Latte, with non-dairy milk, flavored
	92101923*	Frozen coffee drink, with non-dairy milk
	92101960*	Coffee, Cafe Mocha, with non-dairy milk
	92101975*	Coffee, Cafe Mocha, decaffeinated, with non-dairy milk
	92102020*	Frozen mocha coffee drink, with non-dairy milk
	92102050*	Frozen mocha coffee drink, with non-dairy milk and whipped cream
	92102502*	Coffee, Iced Latte, with non-dairy milk
	92102602*	Coffee, Iced Cafe Mocha, with non-dairy milk
	92161002*	Coffee, Cappuccino, with non-dairy milk
Vegetable juices	73105010	Carrot juice, 100%
	74302000	Tomato juice cocktail
	74303000	Tomato and vegetable juice, 100%
	74303100	Tomato and vegetable juice, 100%, low sodium
	75132000	Mixed vegetable juice
	75132100	Celery juice

*Only the select food or beverage component of the food was included towards the EDI.

End of Amendment.