Viebrock, Lauren

From: Annie Han <Annie.Han@iff.com>
Sent: Wednesday, May 26, 2021 7:18 PM
To: Viebrock, Lauren
Cc: Vincent Sewalt; Annie Han
Subject: [EXTERNAL] Re: GRN 000964 Questions
Attachments: Annie response to Email from Lauren Viebrok on GRN 000964 Question 26MAY2021.pdf; IFF - Entity Relationship Statement (Feb 2021).pdf; IFF merger statement to FDA on email change 19FEB2021.pdf; 2021_05_03 GRN 964 Questions for notifier.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Lauren,

I am so sorry! I just sent you an email from my previous DuPont email account but the email cannot be sent out. Please kindly find my message below again and the documents for the entity change in attachment.

Dear Lauren,

Hope everything going well with you and your family!

Thanks for your email and follow up!

I am so sorry for the late response as the emails for both Vince and myself have been changed due to the organization change. I have already sent the attached merger statement to FDA and updated the info on ESG submission system. It seems that you did not receive the update. Sorry about that!

When I periodically checked my previous email today, I saw your email below. We will work on your questions and get back to you as soon as we can. Would you please kindly extend the timeline for response?

I will contact you by using my new email address: annie.han@iff.com, and the new email address for Vince is vincent.sewalt@iff.com.

Please kindly let me know if you have any questions.

Thank you so much in advance!

Best regards
Annie

Best regards

Annie Han

Sr. Specialist, Global Regulatory Affairs
Annie.han@iff.com
T 650.846.4040
925 Page Mill Rd
Palo Alto, CA 94394
Iff.com

Re: GRN 000964 Questions

Han, Annie <Annie.Han@dupont.com>
Wed 5/26/2021 3:46 PM

To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Sewalt, Vincent <Vincent.Sewalt@dupont.com>; vincent.sewalt@iff.com <vincent.sewalt@iff.com>; Annie Han <Annie.Han@iff.com>

3 attachments (188 KB)
IFF merger statement to FDA on email change 19FEB2021.pdf; IFF - Entity Relationship Statement (Feb 2021).pdf; 2021_05_03 GRN 964 Questions for notifier.pdf;

Dear Lauren,

Hope everything going well with you and your family!

Thanks for your email and follow up!

I am so sorry for the late response as the emails for both Vince and myself have been changed due to the organization change. I have already sent the attached merger statement to FDA and updated the info on ESG submission system. It seems that you did not receive the update. Sorry about that!

When I periodically checked my previous email today, I saw your below email. We will work on your questions and get back to you as soon as we can. Would you please kindly extend the timeline for response?

I will contact you by using my new email address: annie.han@iff.com, and the new email address for Vince is vincent.sewalt@iff.com.

Thank you so much in advance!

Best regards
Annie

Best regards
Annie Han
Senior Regulatory Affairs Specialist

DuPont Industrial Biosciences

925 Page Mill Road | Palo Alto, CA 94304
Tel: 650-846-4040 | www.dupont.com
Email: annie.han@dupont.com

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Wednesday, May 19, 2021 7:41 AM
To: Han, Annie <Annie.Han@dupont.com>
Cc: Sewalt, Vincent <Vincent.Sewalt@dupont.com>
Subject: [EXTERNAL] FW: GRN 000964 Questions

Dear Ms. Han,

I am following up on an email that I sent to Mr. Sewalt as you are also listed as a contact person for GRN 000964. During our review of GRAS Notice No. 000964, we noted questions that need to be addressed. Please find the questions attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

From: Viebrock, Lauren
Sent: Monday, May 03, 2021 2:42 PM
To: Sewalt, Vincent <Vincent.Sewalt@dupont.com>
Subject: GRN 000964 Questions

Dear Mr. Sewalt,

During our review of GRAS Notice No. 000964, we noted questions that need to be addressed. Please find the questions attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,
Lauren

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

Where science
& creativity meet

IFF Corporate
521 W 57th Street
New York, New York 10019
iff.com

February 1, 2021

STATEMENT REGARDING SUBSIDIARY ENTITIES

We are pleased to inform you that on February 1, 2021, International Flavors & Fragrances Inc. ("IFF") completed its previously announced combination of IFF and the Nutrition & Biosciences business (the "N&B Business") of DuPont de Nemours, Inc. ("DuPont"), pursuant to the terms of the Agreement and Plan of Merger, dated as of December 15, 2019, as amended (the "Merger Agreement"), by and among IFF, Neptune Merger Sub I Inc. ("Merger Sub"), a Delaware corporation and a wholly-owned subsidiary of IFF, Nutrition & Biosciences, Inc. ("N&B"), a Delaware corporation and a wholly-owned subsidiary of DuPont, and DuPont, whereby, at the effective time (the "Effective Time"), Merger Sub merged with and into N&B, with N&B continuing as the surviving company and wholly-owned subsidiary of IFF (the "Merger").

As part of the Merger, various legal entities associated with the N&B business were transferred by DuPont to IFF. The N&B legal entities listed on the following website are among the legal entities associated with the N&B business that were transferred by DuPont to IFF:

https://www.iff.com/where-we-operate/subsidiaries

IFF continues to operate under a regional entity structure. Many of the numerous legacy N&B regional entities under which IFF will continue to operate are listed on the above-referenced website.

Should you have any questions, please contact Coren Adams-DeJesus with IFF Corporate at coren.adams-dejesus@iff.com.

IFF Corporate

February 19, 2021
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Sir/Madam,

We are excited to announce that International Flavors and Fragrances and DuPont Nutrition & Biosciences have officially completed their combination into the new IFF, a purpose-driven, category-defining leader in the global consumer goods and commercial products value chain. This is a pivotal milestone, strengthening our role as an essential innovation partner and positioning us to redefine our industry in support of a healthier and more sustainable world.

IFF is a purpose-driven organization, and it is in that spirit that I am also pleased to share the new corporate positioning statements that will drive IFF forward:

- Our new IFF **brand identity and tagline** – *Where science and creativity meet* – underpins our foundational purpose and captures our obsession with combining creativity and the rigors of science.
- Our **purpose** – *Applying science and creativity for a better world*.
- Finally, our **three commitments**: *Question Everything, Champion Creators* and *Do More Good*. Collectively, these commitments encourage our team members to inspire and empower others, unleash new discoveries and challenge the status quo to ignite lasting societal impact.

Given our new IFF identity, our email addresses have changed with immediate effect. In general, our legacy DuPont usernames remain the same as before, followed by the IFF domain name. For example, annie.han@dupont.com becomes annie.han@iff.com. Please update our email distribution lists accordingly.

Finally, as the vast majority of our legal entities will not change.

Please do not hesitate to reach out to me should there be any questions or concerns.

Sincerely,

Annie Han
Sr. Specialist, Regulatory Affairs & Product Stewardship
Global Regulatory Affairs, IFF

925 Page Mill Road
Palo Alto, CA 94304
USA
T 650.846.4040
iff.com

May 3, 2021

GRN 964 Questions:

1. Please update the reference for the edition of Food Chemicals Codex specifications (FCC 12 edition, 2020).

2. You state that glucose used in manufacturing may be derived from soy and will be consumed during fermentation. Please clarify whether the final enzyme preparation contains any major allergens.

3. Please provide the methods used for analysis of lead and arsenic are high specs and the actual values from your batch analyses results.

4. On page 16, you provide a range of 1972-2018 for the literature search on the production organism. Please confirm that an updated literature search was performed beyond 2018.

Viebrock, Lauren

From: Annie Han <Annie.Han@iff.com>
Sent: Thursday, June 10, 2021 3:11 PM
To: Viebrock, Lauren
Cc: Vincent Sewalt; Annie Han
Subject: RE: [EXTERNAL] Re: GRN 000964 Questions
Attachments: Letter to FDA in response to GRN964 09JUN2021 Final signed.pdf

Dear Lauren,

Sorry for my late response! Thank you for reviewing our GRAS submission of GRN964!

Please kindly find the letter in response to your questions on GRN964 in attachment and review our feedbacks. If you have any further questions, please feel free to reach out to us.

Thank you so much in advance!

Best regards
Annie

Best regards

Annie Han
Sr. Specialist, Global Regulatory Affairs
Annie.han@iff.com
T 650.846.4040
925 Page Mill Rd
Palo Alto, CA 94394
Iff.com

From: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Sent: Tuesday, June 8, 2021 8:10 AM
To: Annie Han <Annie.Han@iff.com>
Cc: Vincent Sewalt <Vincent.Sewalt@iff.com>
Subject: RE: [EXTERNAL] Re: GRN 000964 Questions

Dear Annie,

I apologize for misspeaking in my email response below. The responses to our questions were not yet received for GRN 964. Can you please provide an update on when you anticipate submitting the amendment to GRN 964?

Thank you,
Lauren

From: Viebrock, Lauren
Sent: Tuesday, June 08, 2021 11:07 AM
To: Annie Han <Annie.Han@iff.com>
Cc: Vincent Sewalt <Vincent.Sewalt@iff.com>
Subject: RE: [EXTERNAL] Re: GRN 000964 Questions

Dear Annie,

Thank you for your email and the updated company and contact information. This is to confirm receipt of your responses to our questions for GRN 964. We will be in touch as we proceed with our evaluation of the notice.

Best,
Lauren

From: Annie Han <Annie.Han@iff.com>
Sent: Wednesday, May 26, 2021 7:18 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Cc: Vincent Sewalt <Vincent.Sewalt@iff.com>; Annie Han <Annie.Han@iff.com>
Subject: [EXTERNAL] Re: GRN 000964 Questions

Dear Lauren,

I am so sorry! I just sent you an email from my previous DuPont email account but the email cannot be sent out. Please kindly find my message below again and the documents for the entity change in attachment.

Dear Lauren,

Hope everything going well with you and your family!

Thanks for your email and follow up!

I am so sorry for the late response as the emails for both Vince and myself have been changed due to the organization change. I have already sent the attached merger statement to FDA and updated the info on ESG submission system. It seems that you did not receive the update. Sorry about that!

When I periodically checked my previous email today, I saw your email below. We will work on your questions and get back to you as soon as we can. Would you please kindly extend the timeline for response?

I will contact you by using my new email address: annie.han@iff.com, and the new email address for Vince is vincent.sewalt@iff.com.

Please kindly let me know if you have any questions.

Thank you so much in advance!

Best regards
Annie

Best regards

Annie Han
Sr. Specialist, Global Regulatory Affairs
Annie.han@iff.com
T 650.846.4040
925 Page Mill Rd
Palo Alto, CA 94394
Iff.com

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Date: June 9, 2021

Where science
& creativity meet

Lauren VieBrock
Regulatory Review Scientist/Microbiology Reviewer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
Lauren.viebrock@fda.hhs.gov

Re: GRN 964

Dear Dr. VieBrock:

Thank you for your review of our submission. We are providing this letter in response to FDA's request for information that was sent via email on May 19, 2021 regarding the *Aspergillus niger* lysophospholipase enzyme derived from *Trichoderma reesei*. We have copied your information requests above each of our responses for ease of reference:

1. *Please update the reference for the edition of Food Chemicals Codex specifications (FCC 12 edition, 2020).*

   We confirm that AnLPL lysophospholipase preparation meets the purity specifications for enzyme preparations set forth in FCC, 12th edition (USP, 2020). In addition, it also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by JECFA (2006).

2. *You state that glucose used in manufacturing may be derived from soy and will be consumed during fermentation. Please clarify whether the final enzyme preparation contains any major allergens.*

   Regarding potential major food allergens, glucose (which may be derived from wheat, not soy) will be used in the fermentation process and is consumed by the microorganism as nutrients. No other major allergen substances will be used in the fermentation, recovery processes, or

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iff.com

formulation of this product. Please refer to risk assessment on glucose in Attachment 1.

3. *Please provide the methods used for analysis of lead and arsenic are high specs and the actual values from your batch analyses results.*

   Please refer to the analytical method of lead and arsenic in Attachment 2. The actual value of lead and arsenic from the certificate of analysis are in Attachment 3.

4. *On page 16, you provide a range of 1972-2018 for the literature search on the production organism. Please confirm that an updated literature search was performed beyond 2018.*

   A literature search was conducted on the organism (2018 – now) in addition to the literature search indicated in the submission (1972-2018) which uncovered no reports that implicate *T. reesei* in any way with a disease situation, intoxication, or allergenicity among healthy adult humans and animals. The updated review includes safety evaluations by the European Food Safety Authority of numerous enzymes produced with Trichoderma reesei, all without safety concerns. In addition, a review by Frisvad *et al.* (2018) is among the new results, which is an update on current knowledge of the secondary metabolite potential of the major fungal species including *T. reesei*. This review (included as attachment 4) concluded that *Trichoderma reesei* cannot produce any recognized mycotoxins and is one of the most important production organisms for safe enzyme production in the industry.

If you have any further questions regarding GRN 964, please contact me.

Sincerely,

Vincent Sewalt, PhD
Head of Regulatory Science & Advocacy
Global Regulatory Affairs
Danisco US Inc.
(a wholly owned-subsidiary of International Flavors & Fragrances Inc.)

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& creativity meet

REFERENCES:

Frisvad, J.C., Møller, L.L., Larsen, T.O., Kumar, R. and Arnau, J., 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei. *Applied Microbiology and Biotechnology*, *102*(22), pp.9481-9515.

FAO/WHO, 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. *FAO JECFA Monographs*, *3*, pp.63-67.

U.S. Pharmacopeia, 2020. Food Chemicals Codex (FCC), 12th Edition. United States Pharmacopeial Convention (USP), Rockville, MD.

ATTACHMENT LIST:

1. Risk Assessment: Residual protein levels in glucose and sorbitol products derived from wheat
2. Analytical Method for Lead and Arsenic
3. Updated Certificate of Analysis (3 Lots)
4. Frisvad *et al.* (2018) Review paper in Applied Microbiology and Biotechnology (open access copy)

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Attachment 1: Risk Assessment: Residual protein levels in glucose and sorbitol products derived from wheat

iff.com

May 17, 2021

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To Whom It May Concern,

Labelling provisions for food enzymes and food enzyme preparations are established by Regulation (EC) No 1332/2008(3). Article 11 of said Regulation states that food enzymes and food enzyme preparations which are *not* intended for the final consumer shall be labelled, where relevant, with information about the presence of substances that are listed as substances with allergenic or intolerance effects. It is the responsibility of the food enzyme manufacturer to comply with the labelling provisions for food enzymes and food enzyme preparations. The Association of Manufacturers and Formulators of Enzyme Products (AMFEP), therefore, recommends that the relevance of labeling allergenic substances when added during fermentation should be addressed in a *risk assessment*.

Risk Assessment: Residual protein levels in glucose and sorbitol products derived from wheat

IFF uses glucose as a nutrient raw material in various fermentation processes to produce enzymes and glucose or sorbitol can be added to enzyme formulations. IFF purchases both glucose and sorbitol that may be derived from wheat from commercial sources. To determine if the disclosure of wheat on the Product Specification/Product Description is necessary for enzyme products using glucose or sorbitol derived from wheat, a risk assessment was conducted. The risk assessment focused on measuring the amount of total protein remaining in various glucose and sorbitol products. Samples were measured for total protein levels using Thermo Scientific Coomasie Plus assay (Bradford assay)—colorimetric method since this assay has the highest tolerance to glucose. All samples of sorbitol and glucose contained less than 3 ppm (<LOD) total protein except for one glucose sample from wheat that was 10 ppm. Further, gluten levels were below quantification level of <5 ppm, based on ELISA analysis.

For this assessment, a worst-case was assumed in which all sorbitol and glucose products contain 10 ppm wheat protein. Additionally, it is assumed that all the protein ends up in the enzyme product following recovery (worst-case). Such residual levels of protein or potential fragments, however, will not likely pose a risk to the consumer for the following reasons:

1) In our fermentation process, the glucose syrup would be diluted approximately 50% in the fermentation mix. Therefore, the fermentation mix would contain 5 ppm wheat protein. 5 ppm total wheat protein in the enzyme product results in 5 ppb protein in the final food processed with 0.1% enzyme product, a *de minimis* amount of protein.

   10 ppm total wheat protein in sorbitol or glucose results in 2.5-3 ppm protein in our enzyme formulations, which means 2-3 ppb protein in the final food processed with 0.1% enzyme product, a *de minimis* amount of protein. Based on these various sources, the highest

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contribution of wheat protein would result in 5 ppb wheat protein in the final food (worst case);

2) as noted above by the Food Allergy Research and Resource Program at the University of Nebraska, 'if any residual but undetected fragments of the food allergen remain, the relevance of any such residual material to food allergenicity is unproven';

3) the Voluntary Incidental Trace Allergen Labelling (VITAL) program (http://allergenbureau.net/vital/) in Australia specifies a reference dose of 1 mg cereal protein, (the eliciting dose for an allergic reaction in 1% of the population), below which only extremely sensitive allergic persons will experience an adverse reaction (Allen, K.J. et al., Allergen reference doses for precautionary labelling (VITAL 2.0): Clinical implications, J. Allergy Clin. Immunol., 133:156-164). Protein levels are in the ppb range (i.e., µg) in the final food processed with our enzyme product (worst-case).

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Based on the above risk assessments, IFF concludes that the amount of wheat proteins or protein fragments in the final food product to be *de minimis* and not likely to pose a risk to the final consumer.

Prepared and reviewed by:

Gregory S. Ladics, Ph.D., DABT, Fellow ATS
Technical Fellow
Head of Global Product Safety and Chemical Management
302-695-6782
Gregory.s.ladics@iff.com

iff.com

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Attachment 2: Analytical Method for Lead and Arsenic

iff.com

SILLIKER

GLOBAL CHEMISTRY METHOD SYNOPSIS

Determination of heavy metals and minerals in food and feed matrices by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave digestion

AS-CC-012

Effective: 1 February 2012

This uncontrolled method synopsis has been created to give Silliker customers a clear understanding of how the method is performed at Silliker, and where the method has been derived. The complete Silliker method incorporates all of our knowledge and experience performing these procedures, and thus is proprietary.

TABLE OF CONTENTS

1. PRINCIPLE

The method al ows determination of trace meta s or minera s in food matrices. Al the ana ytes subject to this method are general y natural y present in food matrices, but their concentrations may be higher than the natural evel due to the effects of anthropo ogical contamination of environmental origin or as a resu t of treatments during the food production chain. Many of the ana ytes subject to this method must respect maximum concentration imits in many food matrices.

Required methods and SOPs

Internal references

P ease see Attachment 7 for ocal internal references and SOPs.

External references

- UNI EN 13804
- UNI EN 13805
- Reg. CE 1881/2006
- Reg. CE 629/2008
- Reg. UE 420/2011

Test methods

Not app icab e

Other methods referencing this method

None

LIMS test code

The internal code is: **U I E 15763:2010**

Methods reviews and changes

N/A

Scope and field of application

Analytes and Matrices

Method UNI EN 15763:2010 reports the determination of Cd, Pb, As and Hg (Cadmium, Lead, Arsenic, Mercury) in food matrices; this method is extended to inc ude the additional ana ytes:

Ag, A , Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Sb, Se, V, Zn

(Si ver, A uminum, Barium, Ca cium, Coba t, Chromium, Copper, Iron, Potassium, Lithium, Magnesium, Manganese, Mo ybdenum, Sodium, Nicke , Phosphorous, Antimony, Se enium, Vanadium, Zinc).

The food matrices to which this method app ies are as fol ows:

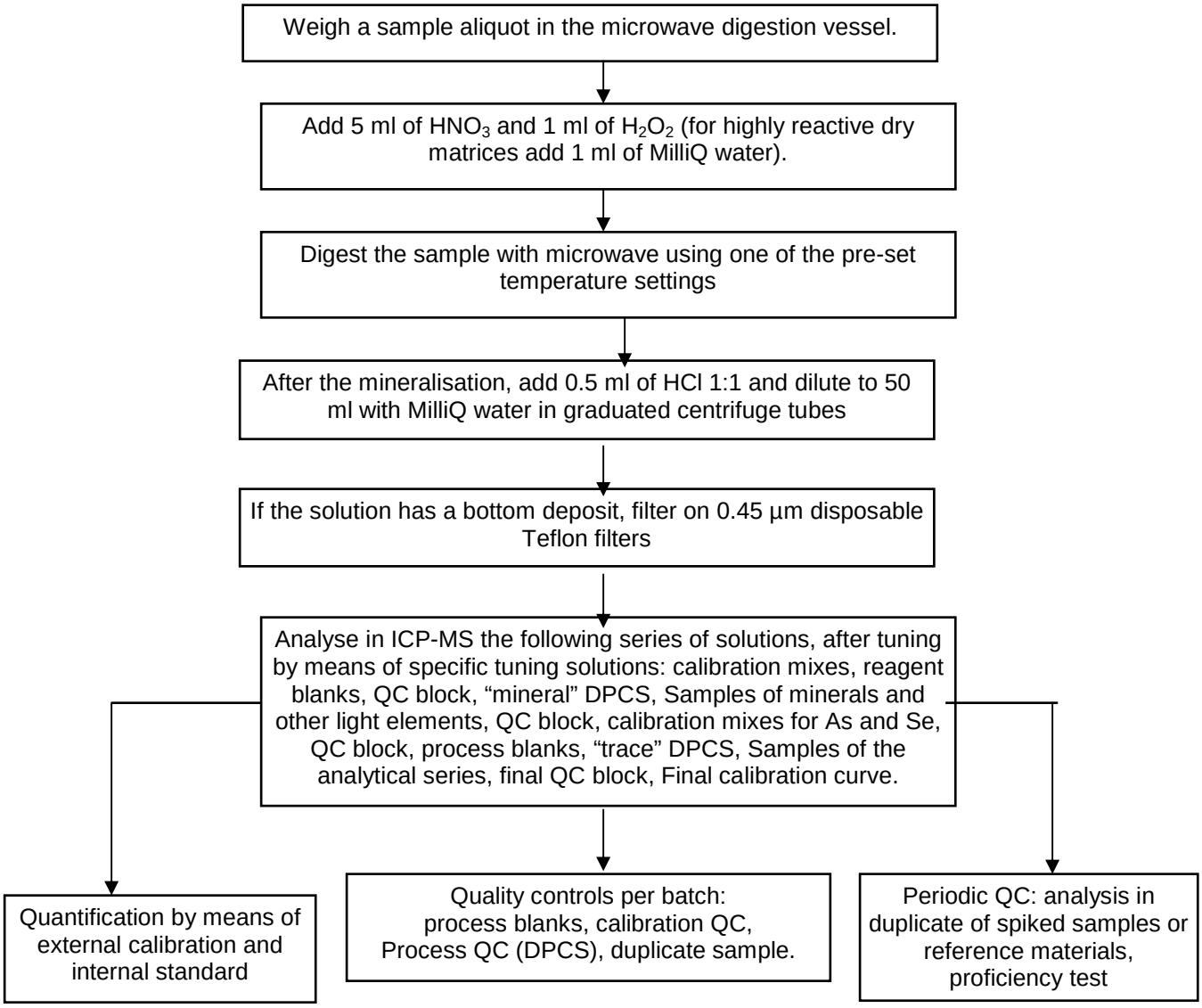
- Cocoa and choco ate
- Cerea s products
- Preserves and semi-preserves
- Nuts and simi ar
- Mi k
- Dairy products
- Feed
- Animal oi s and fats
- Vegetab e oi s and fats
- Pasta products
- Meat products
- Marine products
- Fruits and vegetab es
- Sausage products
- Wine and spirits

No food matrixes are known to be out of this scope.

Measuring field

Quantification Limits (LOQ) and Upper Limit of Quantitation (ULQ) of the meta s subject to this method are detai ed in Attachment 2.

2. FLOW CHART



3 QUICK REFERENCE

1) Depending on the matrix, weigh the correct amount of sample in a suitab e (quartz or g ass) vessel for microwave digestion.
2) Add 5 ml of HNO3 and 1 ml of H2O2.
3) In case of high y reactive, dry matrixes, add 1 ml of Mil iQ water.
4) Digest the samp e with a microwave digester equipped with automatic samp er for sequential digestions (or ana ogous microwave digester), using one of the pre-set temperature programs).
5) After digestion, add 0.5 ml of HCl 1:1 and di ute to 50 ml with Mil iQ water in centrifuge tubes (diameter 35 mm).
6) If after digestion the so ution is not clear (presence of so id residue inso ub e in the applied ana ytical conditions), fi ter the iquid with 0.45 µm disposab e Tef on fi ters.
7) Wine or spirits samples are di uted not ess than 30 times with Mil iQ water.
8) Ana yse by ICP-MS, previous y set up by ana ysis of specific tuning so utions, together with the external ca ibration curve and qua ity contro s.
9) Qua ity contro s per batch: Method b ank, calibration QCs, DPCS, dup icate samp e.
10) Periodic QC: dup icate determination of spiked samp es or reference materia s, proficiency tests.

CALCULATIONS AND EXPRESSION OF RESULTS

The va ue of the C_V concentration in the final extract (in µg/l or mg/ , shown in Tab e 10) is obtained by interpo ating the samp e signal (ratio between cps of the ana yte and of its associated ISTD) on the respective calibration curve.
If the Upper Quantification Limit set for an analyte (Attachment 2) is exceeded, the samp e is di uted according y with the so ution and ana yzed again. the di ution factor X_F is detai ed in the "Di ution" co umn of the MassHunter program and wil be considered in the ca cu ation of the final concentration.
The *Concentration* C_S of the ana yte is obtained by means of the fol owing formu a (depending on the matrix and the measuring unit):

All matrices except vinegar, balsamic vinegar, spirits, wine and grape must:

$$C_S = \left(\frac{C_V \cdot V_F}{P_S \cdot 1000} \cdot X_F \right)$$ for al ana ytes (u.d.m. mg/kg) except:

$$C_S = \left(\frac{C_V \cdot V_F}{P_S} \cdot X_F \right)$$ for Fe (u.d.m. mg/kg)

$$C_S = \left(\frac{C_V \cdot V_F}{P_S \cdot 10} \cdot X_F \right)$$ for Ca, K, Mg, P (u.d.m. mg/100g)

$$C_S = \left(\frac{C_V \cdot V_F}{P_S \cdot 10000} \cdot X_F \right)$$ for Na (u.d.m. g/100g).

For vinegar, spirits, wine and grape must:

$$C_S = \frac{C_V \cdot X_F}{1000}$$ for al ana ytes (u.d.m. mg/) except:

$$C_S = C_V \cdot X_F$$ for Ca, K, Mg, Fe, Na, P (u.d.m. mg/)

For balsamic vinegar:

$$C_S = \left(\frac{C_V \cdot V_F}{P_S \cdot 1000} \cdot X_F \right) \cdot \rho$$ for al ana ytes (u.d.m. mg/) except:

$$C_S = \left(\frac{C_V \cdot V_F}{P_S} \cdot X_F \right) \cdot \rho$$ for Ca, K, Mg, Fe, Na, P (u.d.m. mg/)

C_S is the concentration in the samp e
C_V is the concentration read on the ca ibration curve
V_F is the final vo ume, and is equal to 50 ml
P_S: is the samp e weight (in grams)
F if needed, it is the di ution factor for solid foods and the di ution factor used for vinegar, spirits, wine and grape must
ρ is the density of the samp e expressed in g/ml. The density is determined by weighing on an ana ytical ba ance a known quantity of samp e (for examp e, in a calibrated f ask) and dividing the weight (in g) by the weighed vo ume (in m).

The Concentration CS in the samp e is ca cu ated direct y by the MassHunter program, after entry in the "Di ution" co umn of the correct samp e weight, final vo ume and any di ution va ues.
The final concentration C_X of the ana yte in the samp e is then obtained from C_S by subtraction of the Blank (as recommended in UNI EN 17294-2):

$$C_X = C_S - C_B$$

In this formu a C_B is the concentration in the process b ank, ca cu ated at the correct mean weighing evel (see Tab e 1) and expressed in the same measuring units as C_S and C_X. The ca cu ation of the concentration C_X of the ana yte in the samp e (subtraction of the C_B b ank from C_S) may be done manual y or by means of a Microsoft Excel ca cu ation sheet that has the MassHunter resu ts tab e as input.
The C_X resu t is expressed in the correct measuring unit (see tab e 10 be ow) and with 3 significant figures. The recovery factor is NOT app ied to the ca cu ation of the final concentration of the samp es.

Table 10: Units of measure

Analyte	Calibration curve MU	Sample MU
Silver	µg/l	mg/kg
Aluminium	µg/l	mg/kg
Arsenic	µg/l	mg/kg
Barium	µg/l	mg/kg
Calcium	µg/l	mg/100g
Cadmium	µg/l	mg/kg
Cobalt	µg/l	mg/kg
Chromium	µg/l	mg/kg
Copper	µg/l	mg/kg
Iron	mg/l	mg/kg
Mercury	µg/l	mg/kg
Potassium	mg/l	mg/100g
Lithium	µg/l	mg/kg
Magnesium	mg/l	mg/100g
Manganese	µg/l	mg/kg
Molybdenum	µg/l	mg/kg
Sodium	mg/l	g/100g

Analyte	Calibration curve MU	Sample MU
Nickel	µg/l	mg/kg
Phosphorous	mg/l	mg/100g
Lead	µg/l	mg/kg
Antimony	µg/l	mg/kg
Selenium	µg/l	mg/kg
Vanadium	µg/l	mg/kg
Zinc	µg/l	mg/kg
Vinegar, Alcohols (al analytes)	As above	mg/l
Wine, Grape Must (al analytes except Pb)	As above	mg/l
Wine, Grape Must (Pb)	As above	mg/kg
Balsamic vinegar (al analytes)	As above	mg/l

7. REFERE CE

ATTACHME TS

Attachment 2: LOQ-ULQ Tab e
Attachment 3: Drawing up of Ca ibration Curve
Attachment 4: Minera ization Tab e
Attachment 5: Extended Uncertainties
Attachment 6: "Repeatabi ity Limits

Source	Original title and authors
UNI EN ISO 17294-1:2007	Water qua ity App ication of the mass spectrometry to the inductive y coupled plasma (ICP-MS) Part 1: General guide ines
EPA 6020A	Standard EPA 6020A - Revision 1 (February 2007)
Book	Practical Guide to ICP-MS A Tutorial for Beginners, 2nd ed (2008) – Robert Thomas
Book	Statistics and chemometrics for analytical chemistry, 5th ed (2005) - James N Mil er and Jane C Mil er

Journal Artic e Food Chemistry 126 (2011) 1498–1504	Results from two inter aboratory comparisons on the measurement of trace element contents in food supplements – State of the art of control aboratories in Europe. Ines Baer, Håkan Emteborg, Beatriz de a Cal e
Agilent Technical note	Simp e, Rapid Ana ysis of Trace Metals in Foods Using the Agilent 7700x ICP-MS (2009) – Steve Wilbur

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Attachment 3: Updated Certificate of Analysis (3 Lots)

iff.com

CERTIFICATE OF ANALYSIS

PRODUCT: **T-LPL UFC**
LOT NUMBER: **1663419842**

ASSAY	UNIT	SPECIFICATION	FOUND
ENZYME ACTIVITY			
Lysophospholipase	U/g	Report value	427,455
MICROBIOLOGICAL ANALYSIS			
Total Viable Count	CFU/ml	0 – 50000	<100
Coliforms	CFU/ml	0 - 30	<10
E. coli	/25ml	Negative by test	Negative
Salmonella	/25ml	Negative by test	Negative
Production Strain	/ml	Negative by test	Negative
Antibacterial activity	/ml	Negative by test	Negative
PHYSICAL PROPERTIES			
Specific gravity		Report	1.14
OTHER ASSAYS			
Lead	mg/kg	0 – 5	<0.01
Arsenic	mg/kg	0 - 3	<0.01
Cadmium	mg/kg	0 – 0.5	<0.001
Mercury	mg/kg	0 – 0.5	<0.01
Mycotoxins		Negative by test	Negative

This product complies with the FAO/WHO and Food Chemicals Codex recommended specifications for food grade enzymes and contains permitted levels of stabilizers and preservatives.

9-Jun-2021
Date

Kelly A. Altman
QA/QC Department

This certificate of analysis was electronically generated and therefore has not been signed.

CERTIFICATE OF ANALYSIS

PRODUCT: **T-LPL UFC**
LOT NUMBER: **GS20182463**

ASSAY	UNIT	SPECIFICATION	FOUND
ENZYME ACTIVITY			
Lysophospholipase	U/g	Report value	1,720,990
MICROBIOLOGICAL ANALYSIS			
Total Viable Count	CFU/ml	0 – 50000	<1
Coliforms	CFU/ml	0 - 30	<1
E. coli	/25ml	Negative by test	Negative
Salmonella	/25ml	Negative by test	Negative
Production Strain	/ml	Negative by test	Negative
Antibacterial activity	/ml	Negative by test	Negative
PHYSICAL PROPERTIES			
Specific gravity		Report	1.09
OTHER ASSAYS			
Lead	mg/kg	0 – 5	<0.01
Arsenic	mg/kg	0 - 3	<0.01
Cadmium	mg/kg	0 – 0.5	<0.001
Mercury	mg/kg	0 – 0.5	<0.005
Mycotoxins		Negative by test	Negative

This product complies with the FAO/WHO and Food Chemicals Codex recommended specifications for food grade enzymes and contains permitted levels of stabilizers and preservatives.

9-Jun-2021
Date

Kelly A. Altman
QA/QC Department

This certificate of analysis was electronically generated and therefore has not been signed.

CERTIFICATE OF ANALYSIS

PRODUCT: **T-LPL UFC**
LOT NUMBER: **GS20182464**

ASSAY	UNIT	SPECIFICATION	FOUND
ENZYME ACTIVITY			
Lysophospholipase	U/g	Report value	1,702,467
MICROBIOLOGICAL ANALYSIS			
Total Viable Count	CFU/ml	0 – 50000	<1
Coliforms	CFU/ml	0 - 30	<1
E. coli	/25ml	Negative by test	Negative
Salmonella	/25ml	Negative by test	Negative
Production Strain	/ml	Negative by test	Negative
Antibacterial activity	/ml	Negative by test	Negative
PHYSICAL PROPERTIES			
Specific gravity		Report	1.09
OTHER ASSAYS			
Lead	mg/kg	0 – 5	<0.01
Arsenic	mg/kg	0 - 3	<0.01
Cadmium	mg/kg	0 – 0.5	<0.001
Mercury	mg/kg	0 – 0.5	<0.005
Mycotoxins		Negative by test	Negative

This product complies with the FAO/WHO and Food Chemicals Codex recommended specifications for food grade enzymes and contains permitted levels of stabilizers and preservatives.

9-Jun-2021
Date

Kelly A. Altman
QA/QC Department

This certificate of analysis was electronically generated and therefore has not been signed.

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Attachment 4: Frisvad *et al* (2018) Review paper in Applied Microbiology and Biotechnology (open access copy).

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Thirty-five pages have been removed in accordance with copyright laws. The removed reference citation is:

Frisvad, “Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei”, Applied Microbiology and Biotechnology (2018) 102:9481–9515 https://doi.org/10.1007/s00253-018-9354-1