Chemical Contaminants in Plant Based Protein Survey

Report by Fera Science Ltd.

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Fera

1. Chemical Contaminants in Plant Based Protein Survey

Report of Chemical Contaminants in Plant Based Protein Survey for Food Standards Scotland
Fera Science Ltd.

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Note: Whilst care has been taken to ensure that the web links contained in this report are correct at the time of issue, changes may occur.

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3. Glossary

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
ALT	Altenuene
AOH	Alternariol
AME	Alternariol monomethylether
At	Atropine
BML	Benchmark level
CV	Co-efficient of variation
DON	Deoxynivalenol
DON-3Glc	Deoxynivalenol-3-glucoside
Em	Echimidine
Er	Erucifoline
EU	European Union
EURL-MP	European Reference Laboratory for Mycotoxins and Plant Toxins
FB1	Fumonisin B_1
FB2	Fumonisin B_2
FB3	Fumonisin B_3
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
Ht	Heliotrine
HT-2	HT-2 toxin
IAC	Immunoaffinity Column
Id	Indicine
Im	Intermedine
Jb	Jacobine
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LOQ	Limit of Quantification
Lc	Lasiocarpine
Ly	Lycopsamine

Mn	Monocrotaline
NI	Northern Ireland
OTA	Ochratoxin A
PAs	Pyrrolizidine alkaloids
PBS	Phosphate Buffered Saline
PTFE	Polytetrafluoroethylene
RSD	Relative standard deviation
Rt	Retrorsine
Sc	Scopolamine
SPE	Solid Phase Extraction
Sk	Senkirkine
Sn	Senecionine
Sp	Seneciphylline
STG	Sterigmatocystin
TAs	Tropane alkaloids
T-2	T-2 toxin
TEA	Tenuazonic acid
TEN	Tentoxin
UKAS	United Kingdom Accreditation Service
a-ZOL	α-zearalenol
a-ZOL-14-Glc	α-zearalenol-14-glucoside
b-ZOL	β-zearalenol
b-ZOL-14-Glc	β-zearalenol-14-glucoside
ZON	Zearalenone
ZON-14-Glc	Zearalenone-14-glucoside

4. Executive Summary

Food Standards Scotland (FSS) commissioned Fera Science Ltd. to carry out a survey of chemical contaminants in plant-based protein products. The aim was to provide evidence on levels of mycotoxins, plant alkaloids, acrylamide and erucic acid that can be found in plant-based protein products made from wheat, soy, pea protein, or a mixture of these proteins.

Study Design

The samples analysed were products containing plant-based protein. This included protein powders, drinks and bars, tofu, and vegetarian burgers, mince and similar products. Samples were made from soy, pea or wheat protein or combinations of these (the most commonly consumed categories of plant protein products). Products were purchased online or from retail stores in Scotland, with 26 products purchased in Phase 1 in 2024 and a further 26 purchased in Phase 2 in 2025. Phase 1 samples concentrated on products with minimal ingredients and Phase 2 included more complex samples.

Phase 1 of the study tested for the *Fusarium* mycotoxins (deoxynivalenol, zearalenone, T-2 and HT-2 toxin and fumonisins B1, B2 and B3), aflatoxins (B1, B2, G1 and G2) and ochratoxin A and the plant toxins, tropane alkaloids (atropine and scopolamine). While the mycotoxins tested are regulated in other foods, there are no specific Maximum Levels (MLs, legally enforceable limits) established in The Contaminants in Food (Scotland) Regulations 2013 and Commission Regulation (EC) No 1881/2006 (1, 2) for plant-based protein products. However, MLs for other foods (e.g. cereals, groundnuts) can be used for comparison. Ergot alkaloids, which are not regulated within GB but are within the EU and Northern Ireland, were also analysed in a subset of samples that contained wheat. The samples were also analysed for acrylamide.

Phase 2 of the project added further analytes, namely the mycotoxins, citrinin, enniatins, beauvericin, fusaric acid, Alternaria toxins and the plant toxins pyrrolizidine alkaloids. Products that contained rapeseed oil were also analysed for erucic acid.

Mycotoxins

For the samples tested for Phase 1, at least one mycotoxin residue above the Limit of Quantification (LOQ) was detected in 14 of the 26 samples (54%). One sample of soy

protein powder contained 6 different mycotoxins. Ochratoxin A (OTA) was the most frequently detected mycotoxin, it was present in each of the protein types, comprising nine samples in total (35%) at concentrations ranging from 0.11 to 9.33 µg/kg. The highest level (9.33 µg/kg) was found in a pea protein powder, the next highest concentration (4.15 µg/kg) was found in soy protein powder. Aflatoxins were detected in three samples. One soy protein powder contained 2.24 µg/kg AFB1, as well as AFB2, AFG1 and AFG2 resulting in a total aflatoxin level of 4.94 µg/kg. This sample also contained OTA (4.15 µg/kg) and zearalenone (ZON) (1.13 µg/kg). Four of 26 (15%) samples contained DON at levels from 2.85 to 7.35 µg/kg. Three samples (12%) contained ZON at 1.1 to 1.75 µg/kg, one sample contained fumonisins FB1, FB2 and FB3 and one sample contained sterigmatocystin (STG).

The 26 samples for Phase 2 were also analysed using this method. The results were broadly similar to Phase 1, although the concentrations measured were lower. OTA was the most commonly detected mycotoxin, 7 out of 26 (27%) samples contained OTA at levels ranging from 0.13 to 0.55 µg/kg. Five samples contained DON at levels from 2.32 to 14.55 µg/kg, and five samples (19%) contained ZON at 1.28 to 4.85 µg/kg. Three samples each contained AFB1 (0.15 – 0.23 µg/kg) and STG (0.22 – 0.54 µg/kg). One sample contained HT-2 toxin at 4.16 µg/kg. Fumonisins and T-2 toxin were not detected in any of the samples.

The OTA results for the pea protein and soy protein powders in Phase 1 are higher than MLs in other foods such as cereals (3 and 5 µg/kg), and wheat gluten (8 µg/kg), and similar to the MLs for dried vine fruit (10 ug/kg) and coffee (5 and 10 ug/kg). The soy protein powder sample also contained AFB1 and total aflatoxins just above the MLs (2 and 4 µg/kg respectively) for nuts and cereals.

In Phase 1 five samples that contained wheat protein were analysed for ergot alkaloids. Two samples were found to contain ergot alkaloids, eight ergot alkaloids were detected in one sample giving a sum of ergot alkaloids of 5.71 µg/kg. The other sample contained 9 compounds with a sum of 6.9 µg/kg.
All 26 Phase 2 samples were analysed for ergot alkaloids, three samples (12%) contained the sum of ergot alkaloids at levels of 2.81, 3.41 and 11.01 µg/kg. These three products contained wheat (flour and or gluten) as ingredients. There are no MLs for ergot alkaloids

in GB. There are MLs in force in the EU (4), and these apply for cereals and cereal products. Plant-based protein products are not mentioned specifically. The lowest ML is 20 µg/kg for processed cereal based food for infants and young children.

Plant alkaloids (tropane and pyrrolizidine)

All Phase 1 samples were analysed for the tropane alkaloids atropine and scopolamine. Four samples contained tropane alkaloids above the individual LOQs of 0.1 µg/kg. Two samples contained only atropine (at 0.19 and 0.2 µg/kg) while two contained both atropine and scopolamine. All four samples were soy based, the highest concentration of tropane alkaloids (1.71 µg/kg) was found in a tofu (soy) sample.

All Phase 2 samples were analysed for atropine and scopolamine, 3 out of 26 samples contained atropine at levels of 0.52 to 1.15 µg/kg. One sample contained scopolamine at 0.38 µg/kg.

There are no MLs for tropane alkaloids in plant-based protein products, the only MLs in force in GB are for baby food and processed cereal-based food for infants and young children, where MLs are set at 1.0 µg/kg each for atropine and scopolamine (2). MLs for the sum of atropine and scopolamine (tropane alkaloids) are set in the EU for cereal products at 5 to 15 µg/kg, and 25 and 50 µg/kg for dry herbal infusions (3). All of the samples in this survey contained tropane alkaloid levels well below the MLs for cereals (4).

Phase 2 samples were also analysed for pyrrolizidine alkaloids (PAs), none were detected in any of the 26 samples.

Other Mycotoxins

Two additional LC-MS/MS methods (MM2 and multi-mycotoxin) were used to analyse an extended range of Fusarium mycotoxins for the Phase 2 samples. Four out of 26 samples (15 %) contained ZON, three just below the LOQ (two at 2.3 µg/kg and one at 1.7 µg/kg), the other contained 4.6 µg/kg. These results were very similar to the ZON results found for the same samples using the 11+ IAC method. DON was found in one sample at 12.6 µg/kg, compared to 11.22 µg/kg found by the 11+ IAC method.

Beauvericin was found in 8 samples (31 %) at levels of 0.8 – 1.8 µg/kg. Enniatin A was found in 6 out of 26 samples (0.7 – 1.2 µg/kg) and enniatin A1 was found in 7 samples (0.6 -1.4 µg/kg). Enniatin B was found in 14 out of 26 samples at levels from 0.6 – 11.8 µg/kg and enniatin B1 was found in 9 out 26 samples at levels from 0.7 – 29.1 µg/kg. There are

no MLs for enniatins and beauvericin. Sixteen samples contained levels of fusaric acid from 4.1 – 118.4 µg/kg. The highest level quantified was 118.4 µg/kg, but there was also a sample that flagged as >1000 µg/kg. This is indicative as the initial extract result was outside the calibration range and the recovery was low which increases the uncertainty about the result. There are no MLs for fusaric acid. All Phase 2 samples were also analysed for citrinin, no residues above the LOQ of 2.5 µg/kg were detected.

Alternaria toxins

The Phase 2 samples were analysed for Alternaria toxins. Tenuazonic acid was most frequently detected and was found in 14 out of 26 (54 %) of samples at levels from 3.8 – 62.7 µg/kg. Alternariol was the next most frequently found, five samples contained levels from 1.1 – 13.5 µg/kg, four samples contained alternariol monomethylether at levels from 2.1 – 6.1 µg/kg. Altenuene and tentoxin were not detected in any samples. There are no regulations in force for Alternaria toxins, so there are no MLs to compare these results to. However, these findings are similar to results reported in other published studies (6-10).

Acrylamide

Phase 1 samples were analysed for acrylamide, 2 of out 26 samples contained acrylamide above the LOQ of 30 µg/kg. One sample, a plant based burger, contained 36.8 µg/kg and the other product, a vegetarian sausage, made from soy and wheat, contained 41.7 µg/kg. There are no MLs for acrylamide but there are benchmark levels (BMLs) in place in GB. BMLs are guidance values, not legal limits, that are used as reference points for monitoring and risk management. The BMLs for acrylamide range from 40 to 4000 µg/kg for baby food and chicory based coffee substitutes respectively (5).

Erucic acid

A subset of 10 Phase 2 samples were analysed for erucic acid. The selected samples contained rapeseed as an ingredient. The levels of erucic acid measured ranged from 0 to 6.0 g/kg erucic acid in the oil / fat fraction of the sample. These were all below the ML of 20 g/kg erucic acid for oils used as an ingredient in food or for direct consumption.

Summary

All of the samples analysed were compliant with GB MLs where they exist. Two samples in Phase 1 contained aflatoxins and OTA at concentrations at or above MLs for foods that might be considered comparable such as wheat gluten, nuts and cereals.

5. Introduction

5.1 Background to the study

Food Standards Scotland (FSS) requested a survey of chemical contaminants in plant-based protein products (PBPP). There are various types of PBPP, these include meat alternative products such as tofu, meat free or meat substitute mince, non-meat pieces, and protein powders. PBPP can be made from a variety of different protein sources; soy, wheat or wheat gluten, pea, other legumes and vegetable proteins are amongst the most common found in supermarket plant-based meat replacement products. Plant-based protein powders are also made from a range of different proteins such as pea and soy protein. There is a risk of mycotoxin and plant alkaloid contamination in the raw materials and therefore FSS want to assess risk in the processed products to inform future policy.

Contamination with mycotoxins depends upon many factors. The presence of mycotoxins in foods can occur in the field or during storage. Agricultural practices and climate effects such as drought, heavy rain and temperature can affect the potential risk for contamination. In addition, other factors such as post-harvest handling and storage conditions of crops and raw materials are potential risk factors in mycotoxin development. Although there are mycotoxin MLs within legislation for cereal products, unprocessed cereals and other commodities such as nuts there are no specific MLs for other raw materials such as soybeans or peas. There are also no MLs for plant-based alkaloids for soybeans or peas within current legislation, either in GB or the EU.

Food law in the UK comprises European Union (EU) legislation and domestic legislation for each UK nation. All references to EU legislation should be read as either EU law in Northern Ireland (NI) or assimilated law in England, Wales and Scotland, as applicable. In NI, under the Windsor Framework, EU food law continues to apply, but for England, Wales and Scotland retained EU law was on 1 January 2024 under the Retained EU Law (Revocation and Reform) Act 2023 reclassified as assimilated law into national law.

The project was carried out in two phases, sampling and analysis were carried out at two different time points to ensure sampling was representative.

5.2 Phase 1 study design

Fera provided advice to FSS to help with the design of Phase 1 of the study and FSS purchased all samples and sent them to the laboratory. FSS requested the laboratory help with design of the testing regime, but specified the following analytes as mandatory and that all samples should be analysed for these unless there was a scientific basis to exclude them.

Table 1. Priority List of Contaminants

Mandatory Contaminants
Aflatoxins (AFB1, AFB2, AFG1, AFG2)
Ochratoxin A (OTA)
Fumonisins (FB1, FB2, FB3)
Trichothecenes (deoxynivalenol (DON), T-2 and HT-2 toxin)
Zearalenone (ZON)
Tropane alkaloids (Atropine (At) and scopolamine (Sc))
Ergot alkaloids (sum of 12 compounds as listed in Commission Regulation (EU) 2023/915 (4)

There are currently no specific maximum levels in place for mycotoxins and plant alkaloids in PBPP, although any products on sale on the retail market must meet the general provisions of food safety law, i.e. that the food is not harmful or unfit for consumption and must be of the expected nature, substance, and quality (11). The mycotoxins listed in Table 1 are included in Commission Regulation (EC) No 1881/2006 (as amended) setting maximum levels for certain contaminants in foodstuffs for a range of foods (1, 2). In addition, Commission Regulation (EU) 2016/239 introduced MLs for atropine and scopolamine (tropane alkaloids) in cereal based foods for infants and young children (12). MLs are in force for ergot alkaloids in some cereal based foods in the European Union

(EU) (4) and also Northern Ireland, however these limits do not apply in Great Britain (GB). A comparison of MLs in force in GB and the EU / NI is given in Table 2.

Table 2. Comparison of maximum levels in force for GB and EU/NI.

Contaminant	**Regulation (EC) No 1881/2006 (applies GB) (2)**	**Regulation (EU) 2023/915 (applies EU and NI) (4)**
Aflatoxins	MLs for AFB1 and total aflatoxins range from 2 µg/kg AFB1 to 15 µg/kg total AF in range of foods. ML for AFB1 in infant foods 0.1 µg/kg.	Same as Regulation (EC) No 1881/2006
Ochratoxin A	MLs from 0.1 µg/kg (infant foods) to 80 µg/kg in a range of foods.	MLs align for foods in 1881/2006. Additional MLs for foods such as pistachios, oilseeds, and bakery wares are in force in EU.
Deoxynivalenol	MLs in cereal based foods range from 200 µg/kg (infant foods) to 1750 µg/kg (unprocessed maize, durum wheat and oats). ML for unprocessed cereals other than durum wheat, maize and oats is 1250 µg/kg.	Lower MLs for same food product categories. ML for infant food is 150 µg/kg, unprocessed cereals other than durum wheat, maize and oats is 1000 µg/kg.
Zearalenone	MLs from 20 µg/kg (infant foods) to 400 µg/kg (Refined maize oil).	Same as Regulation (EC) No 1881/2006
T-2 and HT-2	No limits in GB	MLs from 10 µg/kg (infant foods) to 1250 µg/kg (unprocessed oat grains with edible husk).
Fumonisins	MLs from 200 µg/kg (infant foods) to 4000 µg/kg (unprocessed maize).	Same as Regulation (EC) No 1881/2006
Citrinin	100 µg/kg in red yeast supplements	Same as Regulation (EC) No 1881/2006

Ergot sclerotia	0.5 g/kg	0.2 g/kg
Ergot alkaloids	No limits in GB	MLs from 20 µg/kg (infant foods) to 500 µg/kg in cereals and milling products
Tropane alkaloids	1 µg/kg each for atropine and scopolamine only applies to foods for infants	MLs for infant food align. MLs for sum atropine and scopolamine range from 0.2 µg/kg (liquid herbal infusions) to 50 µg/kg for dried anise seeds for herbal infusion.
Pyrrolizidine alkaloids	No limits in GB	MLs range from 1 µg/kg (infant liquid tea) to 1000 µg/kg in borage, lovage, marjoram and oregano.
Erucic acid	ML of 20 g/kg in vegetable oils placed on the market or used as an ingredient. Except camelina, mustard and borage oil (50 g/kg) and mustard condiment (35 g/kg)	Same as Regulation (EC) No 1881/2006
Cyanogenic glycosides	ML of 20 mg/kg in apricot kernels.	MLs from 10 mg/kg (cassava flour) to 250 mg/kg for linseed not placed on market for final consumer.

As well as mycotoxins and natural toxins there were also reports in the literature of the presence of acrylamide, a processing contaminant, in PBPP (13). Acrylamide is formed during processing that involves heat as a reaction product between some reducing sugars and amino acids. It has been commonly reported in heat treated starchy foods such as baked goods (bread, biscuits, crackers) and fried goods (potato and vegetable crisps, deep fried breadcrumbed or battered products). It’s occurrence has also been reported in a range of other foodstuffs including roasted coffee, olives, cocoa, dried fruit and extruded

snacks (14). It was agreed that all products in Phase 1 would also be analysed for acrylamide as they were all processed foods.

The analytes to be tested in each type of protein in Phase 1 were agreed in consultation with FSS taking into account budgetary and time constraints. The final product list was compiled by FSS, the protein types were selected on the basis of market share. The three main types chosen (soy, pea and wheat) are those most frequently found for sale, making them the most commonly consumed. UK market data shows soy is the largest category with 31.1% of the UK market in 2025. Pea protein was identified as the fastest growing, with projected Compound Annual Growth Rate (CAGR) of 6.4%. Market studies confirm pea protein is one of the two leading categories although the exact market share in 2025 is not reported. Wheat protein is described as present but with a smaller UK market share but there is no so information on a quantified market share. Other plant proteins, e.g. rice, hemp and potato are described as emerging niches in the UK market (15).
Additional sampling was discussed with FSA and products were prioritised based on data gaps around products such as protein powders, ready-to-eat protein shakes and protein-based cereal snack bars in addition to a range of fresh and frozen plant-based meat alternative products. It was decided to avoid products coated in breadcrumbs or other cereal based coatings as the cereal ingredients could contain mycotoxins. The product / analyte combinations agreed for Phase 1 are listed in Table 3.

Table 3. Phase 1 Analyte / product combinations.

Contaminant	Pea protein	Soy protein	Wheat gluten/ wheat protein	Mixed
Aflatoxins (B1, B2, G1, G2)	X	X	X	X
Ochratoxin A	X	X	X	X
Fumonisins	X	X	X	X
Trichothecenes (deoxynivalenol, T-2 and HT-2 toxin)	X	X	X	X
Zearalenone	X	X	X	X
Tropane alkaloids (Atropine and scopolamine)	X	X	X	X
Ergot alkaloids			X	X
Acrylamide	X	X	X	X

Ergot alkaloids were only tested in wheat based products as these contaminants specifically occur in cereals and no evidence of their occurrence in other commodities (e.g. soy) was found in the literature.

In addition, sterigmatocystin was also measured in all samples as the 11+ IAC multi-mycotoxin method was used was also able to measure this mycotoxin.

5.3 Phase 2 study design

FSS commissioned a follow on study to analyse a second set of samples. An assessment was made of recently published studies on meat alternative products to inform which additional contaminants should be included in the study. Using information from a variety of sources including Mihalache *et al.*, 2022, 2023 and 2024; Lin *et al.,* 2023; and FAO, 2020 (6-10 and 16) a table of suggested analyses was shared with FSS. In addition to the analytes in Table 3 a number of other mycotoxins and plant toxins were reported to occur in PBPP in the literature. Mycotoxins reported were *Alternaria* toxins, enniatins, beauvericin, citrinin, sterigmatocystin. Other alkaloids found were β-carboline alkaloids and pyrrolizidine alkaloids. The exposure assessment by Mihalache (9) found, based on the

model used, consumption of plant-based meat alternatives led to non-tolerable exposure to alternariol in pea and soy + wheat based products. They also noted health concerns related to liver and renal cancer for aflatoxins and OTA respectively.

The list of requested analytes for Phase 2 was more extensive than Phase 1. Following discussion with FSS it was agreed the analyses listed in Table 5 would be carried out on the samples. All samples would be analysed for all analytes except erucic acid, where only samples that contained rapeseed oil as an ingredient would be analysed. Some methods used in Phase 1 were also used for Phase 2 (11+ and ergot alkaloids), however a number of additional methods were also required to allow tests to be carried out for all the requested analytes.

6. Sampling and sample preparation

6.1 Samples – Phase 1

Food Standards Scotland (FSS) provided the detailed list of suggested products. Sample purchase was carried out by staff of FSS. Samples were purchased in Scotland from supermarkets and on-line retailers. Samples were collected, packaged appropriately to maintain chilled temperature during transport and sent by courier to the laboratory. The sampling plan requested 25 samples, but an additional sample of vegan sausages was purchased which meant a total of twenty-six samples were included in the survey.

Samples were purchased in November 2024. A small number of samples (5 out 26) were purchased from on-line retailers, the remainder were purchased from supermarkets. For most products three retail packs from one batch of each product were purchased, although in some cases either depending on availability or pack size, 2 packs or 4 packs were purchased, resulting in sample sizes from 500 g to 1000 g. A full detailed list of the samples collected has been provided separately, and a summarised list is given in Annex B (Table B1).

6.2 Samples – Phase 2

The second phase of sampling was carried out in August 2025. The samples were selected and purchased by FSS from stores in Scotland and on-line retailers. As for Phase 1 replicates of each sample were purchased to obtain a target composite sample size of 500 g to 1000 g. Samples were packaged with ice packs to maintain chilled temperature during transport and sent by courier to the laboratory.

Twenty-six samples were purchased. A full detailed list of the samples collected has been provided separately, and a summarised list is given in Annex B (Table B2).

The samples comprised the following categories – protein bars (4 samples), ready-to-eat protein shakes (3), protein powders (9) and meat alternatives (10). The products were made from soy, pea and wheat protein, or combinations of these. One sample was a mixture of rice and pea protein and one was a mixture of whey and soy protein.

This was a limited survey that only included small numbers of each type of product in each sampling phase and as such was not fully representative of the market, therefore it was not appropriate to name brands.

6.3 Sample preparation and storage

For both sample collection periods, samples were immediately logged into the laboratory information management system (LIMS) on receipt and given a unique identifying number. Storage conditions on the package were adhered to. Any samples with short expiry dates or sold as frozen products were stored in the freezer until preparation and analysed according to standard practice and in-house quality procedures to preserve sample integrity and prevent sample spoilage. These samples tended to have high moisture content so freezing also prevented any potential changes to mycotoxin contaminant levels that could have occurred if the samples became spoiled or mouldy.

Multiple packs with the same batch code / use by date or other identifier were purchased to make up each individual sample. In most cases, depending on pack size, a minimum of two units were purchased for each sample.
For all samples, all sample packs received were combined, and homogenised using laboratory equipment to produce a smooth, homogenous sample.
For meat replacements, protein bars and other solid samples a laboratory blender (GM Mill, Retsch) was used. Liquids were combined in a large container and mixed using a magnetic stirrer for approximately 30 minutes. After homogenisation the samples were split into at least 3 aliquots and were stored in the freezer after homogenisation.
All mixing equipment was thoroughly cleaned between each sample to prevent cross contamination.
For protein powders, individual packs for each sample were combined and shaken and mixed manually before dividing into aliquots for analysis. All samples were stored in the freezer after opening.

6.4 Sample analyses – Phase 1

Samples were divided into product categories with specific analytical requests for each product. These had been specified by FSS, the number of each type of sample and the analyses required are set out in Table 4. The analytes requested included Type A trichothecene mycotoxins (T-2 toxin and HT-2 toxin), Type B trichothecene (DON), as well as other *Fusarium* mycotoxins (ZON, fumonisins), and other mycotoxins of significant health concern that are regulated in other food products (aflatoxins and OTA). Samples were analysed for the tropane alkaloids atropine and scopolamine, and samples that contained wheat protein were also analysed for ergot alkaloids.

Table 4. Phase 1 product categories and requested analyses

Product	Testing Requirements	Number of samples purchased
Soy based products	Mycotoxins (T-2/HT-2, DON, ZON, Fumonisins B_1, B_2 and B_3, Aflatoxins B_1, B_2, G_1, G_2, OTA, STG) Tropane alkaloids (atropine & scopolamine)	12
Soy/wheat products	Mycotoxins (T-2/HT-2, DON, ZON, Fumonisins B_1, B_2 and B_3, Aflatoxins B_1, B_2, G_1, G_2, OTA, STG) Tropane alkaloids (atropine & scopolamine) Ergot alkaloids	4
Pea/ wheat and soy products	Mycotoxins (T-2/HT-2, DON, ZON, Fumonisins B_1, B_2 and B_3, Aflatoxins B_1, B_2, G_1, G_2, OTA, STG) Tropane alkaloids (atropine & scopolamine) Ergot alkaloids	1
Pea based products	Mycotoxins (T-2/HT-2, DON, ZON, Fumonisins B_1, B_2 and B_3, Aflatoxins B_1, B_2, G_1, G_2, OTA, STG) Tropane alkaloids (atropine & scopolamine)	9

Samples were chosen based on availability of products in each category of commodities where data gaps exist for testing for these contaminants. Samples were analysed for mycotoxins using a method based on multi-mycotoxin IAC clean-up followed by LC-MS/MS analysis giving full coverage of all mycotoxins requested by FSS as well as allowing determination of sterigmatocystin.

6.5 Sample analyses – Phase 2

The analytes requested for phase 2 and the methods used to carry out those analyses are listed in Table 5.

Table 5. List of methods and analytes for Phase 2 of the study

Method	Analytes to be included
Method 1 PAs and TAs – Fera SOP FSG 828	Pyrrolizidine alkaloids Echimidine (Em), Erucifoline (Ec), Heliotrine (Ht), Intermedine (Im), Jacobine (Jb), Lasiocarpine (Lc), Lycopsamine (Ly), Monocrotaline (Mn), Retrorsine (Rt), Senecionine (Sn), Seneciphylline (Sp) Senkirkine (Sk) Tropane alkaloids Atropine (At) and scopolamine (Sc)
Method 2 Multimycotoxin Fusarium toxins (MM2) – Fera SOP FSG 818*	3-Acetyl-deoxynivalenol 15-Acetyl-deoxynivalenol Deepoxy-deoxynivalenol Deoxynivalenol Deoxynivalenol-3-glucoside Fusarenon X HT-2 toxin Nivalenol T-2 toxin Zearalenone (and ZON-14-Glc) α-Zearalenol (and α-ZOL-14-Glc) β-Zearalenol (and β-ZOL-14-Glc)
Method 3 11+ IAC multimycotoxin IAC method -Fera SOP FSG 828	Aflatoxins (B1, B2, G1, G2) Ochratoxin A Fumonisins B1, B2 and B3 Trichothecenes (deoxynivalenol (DON), T-2 and HT-2 toxin) Zearalenone (ZON) Sterigmatocystin
Method 4 Ergot alkaloids – Fera SOP FSG 601	Ergocornine, Ergocorninine, Ergocristine, Ergocristinine Ergocryptine, Ergocryptinine Ergometrine, Ergometrinine

	Ergosine, Ergosinine Ergotamine, Ergotaminine
Method 5 – Alternaria toxins	Alternariol Alternariol monomethyl ether Altenuene Tentoxin Tenuazonic Acid
Method 8 – Erucic acid	Erucic acid – only for foods that list rapeseed oil as an ingredient
Method 9 – Other Fusarium toxins	Enniatin A, Enniatin A1, Enniatin B, Enniatin B1, Beauvericin Fusaric acid
Method 10 - Citrinin	Citrinin

* α-Zearalanol and β-Zearalanol* were requested but are not included in the method FSG 818

Fera has ISO17025 accreditation for several mycotoxin and plant toxins methods. These include a method for a suite of 17 *Fusarium* mycotoxins, by LC-MS/MS; a method for ten trichothecenes; as well as aflatoxins B_1, B_2, G_1, G_2 and OTA in a range of matrices, including cereals and cereal products using immunoaffinity column clean-up and HPLC with fluorescence detection. Ergot alkaloids and tropane alkaloids methods using LC-MS/MS are also accredited. Mycotoxin methods are required to meet, as a minimum, the performance characteristics given in Commission Regulation (EC) No 401/2006 (17). The general operation of LC-MS methods is accredited and Fera has Flexible Scope accreditation that allows accreditation to be claimed for certain analyte, matrix and instrument combinations, this was applied where the analyte matrix was not covered by the fixed scope.

7.1 Mycotoxin Analysis using 11+ Immunoaffinity column clean-up

All samples in Phase 1 and 2 were analysed for mycotoxins using an in-house method for the determination of 11+ mycotoxins by IAC clean-up and LC-MS/MS analysis (11^+Myco MS-PREP®, Art. No. RBRP128 / RBRP128B). This column was validated for the analysis of multiple mycotoxins in several matrices through the AOAC Research Institute, Certificate No. 112401. Fera was the independent laboratory that carried out some of the validation (18). The method uses immunoaffinity columns with antibodies specific to certain mycotoxins and has claimed performance for the following mycotoxins:

- Aflatoxins B_1, B_2, G_1 and G_2
- Ochratoxin A (OTA)
- Deoxynivalenol (DON)
- T-2 and HT-2 toxin
- Fumonisins B_1 and B_2
- Zearalenone (ZON)

In addition, due to cross reactivity of the antibodies the columns also cross-react with:

- Fumonisin B_3
- Sterigmatocystin

The method had previously been validated in-house for oat products, dairy alternatives, cereals and animal feed for all of the above analytes. Method optimisation and then validation for plant protein products was completed before the survey samples were analysed. Application for flexible scope accreditation was made for plant protein products for this method using the validation data generated during this study and the method is now accredited.

7.1.1 Mycotoxins analysis by 11+ IAC method

For all samples, an aliquot of sample (10 g) was weighed into a centrifuge bottle. Water (40 mL) was added and the sample shaken by hand to mix. A mixture of acetonitrile: methanol, (1:2), was added and the tube shaken well by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 2 hours. Following this, samples were centrifuged, and an aliquot (10 mL) of supernatant was diluted with Phosphate Buffered Saline (PBS, 110 mL). An aliquot of the diluted extract was cleaned up by passing through the Immunoaffinity Column (IAC, R-Biopharm Rhone) under gravity. The IAC was washed with water, then dried by passing air through before the analytes were eluted by passing methanol (1 mL) through the IAC into a vial, followed by water (1 mL) which was collected in the same vial to ensure maximum recovery was obtained. This solution was mixed well using a vortex mixer, and if necessary, filtered through a syringe filter before being transferred to an autosampler vial for LC-MS/MS analysis.

Quality control samples including procedural blanks, and spiked samples, were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-house reference materials, samples spiked at different concentrations were included in each batch. Limits of quantification (LOQ) for the 11+ IAC method are given in Table 6 (below). The target LOQ is defined as the lowest level at which validation using spiked samples was undertaken. The target LOQs were agreed with FSS at the start of the project. The LOQ (lowest level with acceptable signal: noise from the calibration curve) has been used when reporting results as some samples contained mycotoxins below the lowest validation concentration.

Method performance requirements for mycotoxins are given in Assimilated Commission Regulation (EC) No 401/2006 (17), no criteria are given for sterigmatocystin, however

average recovery of 70-120% is deemed to be acceptable for mycotoxins and plant toxins according to the EURL-MP Guidance doc_003 (v1.4) that sets out method performance criteria for methods of analysis (18). LC-MS analysis is covered by accreditation and quality parameters are set out in in-house document FSG 002. All criteria for method performance, i.e. calibration and confirmation of residues were met.

Table 6. Limits of Quantification (LOQ, lowest validated level) for analytes in 11+ IAC method

Compound	Target LOQ (lowest spike level) (µg/kg)	LOQ (lowest calibration point) (µg/kg)
Required compounds		
Aflatoxin B1	0.25	0.08
Aflatoxin B2	0.25	0.08
Aflatoxin G1	0.25	0.08
Aflatoxin G2	0.25	0.08
Ochratoxin A	0.25	0.08
Deoxynivalenol	5	1.56
T-2 toxin	5	1.56
HT-2 toxin	5	1.56
Zearalenone	2.5	0.78
Fumonisin B1	10	3.125
Fumonisin B2	5	1.56
Fumonisin B3	5	1.56
Sterigmatocystin	0.25	0.08

7.2 Ergot alkaloids analysis using In-house method FSG 601 - Ergot alkaloids by LC-MS/MS

Samples containing wheat protein were analysed for ergot alkaloids using In-house method FSG 601, the analytes included in the method are listed in Table 7. This method is UKAS accredited for cereals, cereal products and baby food. Additional validation for

wheat containing plant protein products was carried out to ensure the method performed satisfactorily for these products. Products containing wheat in Phase 1 and all samples in Phase 2 were analysed by this method.

Table 7. Analytes included in In-house method FSG 601.

Compound	Target LOQ (lowest spike level) µg/kg	LOQ (lowest calibration point) (µg/kg)
Ergocornine	0.5	0.25
Ergocorninine	0.5	0.25
Ergometrine	0.5	0.25
Ergometrinine	0.5	0.25
Ergocristine	0.5	0.25
Ergocristinine	0.5	0.25
Ergotamine	0.5	0.25
Ergotaminine	0.5	0.25
Ergosine	0.5	0.25
Ergosinine	0.5	0.25
a+b-Ergocryptine	0.5	0.25
Ergocryptinine	0.5	0.25

7.2.1 Ergot alkaloids method

For samples tested for ergot alkaloids, an aliquot of sample (10 g) was weighed into a sample bottle. Extraction solvent, a mixture of acetonitrile and ammonium carbonate solution (84:16, 100 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 30 minutes. Following this, samples were shaken by hand then poured through Whatman 12.5 cm hardened No. 54 ‘fast’ filter paper. The filtrate was collected in a conical flask. An aliquot (1 mL) of the filtrate was transferred into a 4 mL amber glass vial containing ca. 50 mg of Bondesil dispersive solid phase extraction (SPE) material. The mixture was filtered through a 13 mm PTFE 0.22 µm syringe filter before being transferred to an autosampler vial for LC-MS/MS analysis.

The test samples were analysed in the same analytical batch as the spiked validation samples. Samples were spiked at three different concentrations (1x target LOQ, 2 x target LOQ and 10 x target LOQ). Six replicates of each concentration and three blank samples were analysed with the test samples. Performance characteristics for mycotoxins are given in Commission Regulation (EC) No 401/2006 (17), no criteria for ergot alkaloids are given, however average recovery of 70-120% is deemed to be acceptable for all mycotoxins and plant toxins (11).

7.3 Tropane alkaloid analysis using in house method FSG 827 – LC-MS/MS analysis of tropane alkaloids (TAs)

All Phase 1 samples were analysed for tropane alkaloids atropine and scopolamine using In-house method FSG 827 LC-MS/MS analysis of tropane alkaloids (TAs) in cereals, processed cereal based foods, herbal dry tea, herbal tea infusions, and vegetable products. This method is UKAS accredited for cereal and vegetable products however a set of verification analyses were carried out for the products included in the survey to ensure the method performance was acceptable.

7.3.1 Tropane alkaloids method

For all samples, an aliquot of sample (4 g) was weighed into a polypropylene tube. Internal standard solution containing deuterium labelled atropine and scopolamine was added to all samples. Extraction solvent (methanol/water/formic acid solution (75/25/0.4, 40 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 30 minutes. Following this, samples were centrifuged, and an aliquot (10 mL) of supernatant was taken for SPE clean-up using cation exchange. After conditioning the cartridge, the sample extract was loaded. The SPE cartridge was washed with a solution of formic acid in methanol and water, and the cartridge dried by vacuum. The cartridges were eluted with a solution of 0.5% ammonia in methanol. The collected eluate was evaporated to dryness under a nitrogen atmosphere in a water bath at 50°C using a Turbovap. The dry residue was redissolved in 500 µL of sample resuspension solvent (10% methanol in water) before being transferred to a filter vial and closed. Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-

house reference materials, samples spiked at three different concentrations (1 µg/kg, 2 µg/kg and 10 µg/kg) were included in each batch. The limit of quantification (LOQ) for both analytes was 0.1 µg/kg. Performance characteristics for plant toxins are given in EURL-MP Guidance doc_003 (v1.4) (19), average recovery of 70-120% is deemed to be acceptable, however average recoveries can be acceptable within the range 50-130% when precision criteria for RSD_r are met.

7.4 Acrylamide analysis using in house method FSG 262 – GC-MS analysis of acrylamide

All Phase 1 samples were analysed for acrylamide using In-house method FSG 262 GC-MS analysis of acrylamide in foods. This method is UKAS accredited for foods and beverages. It is based on the PD CEN/TS 17083:2017 method for determination in of acrylamide (20) and has been used extensively for many years in range of foods, and therefore no further validation or verification was required.

7.4.1 Acrylamide method

For all samples, an aliquot of sample (5 g) was weighed into a bottle. Internal standard solution was added to all samples. Freshly boiled hot water (100 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for at least one hour. Following this, samples were removed from the shaker and allowed to cool. An aliquot (approximately 40 mL) of extract was transferred to a centrifuge tube. Carrez solution I (1 mL) and Carrez II (1 mL) were added with shaking after each addition. The tubes were centrifuged and a portion of supernatant (25 mL) transferred to a clean centrifuge tube. This was shaken with n-hexane (approximately 15 mL) and centrifuged again. An aliquot of the aqueous extract (15 mL) was derivatised by bromination by addition of 10% sulphuric acid solution (600 µL), 0.1M potassium bromate solution (1 mL) and 0.4mg/ml potassium bromide solution (6.25 mL). The tube was swirled to mix the sample after each addition, capped securely and shaken well to mix. Bromination took place in the dark in a refrigerator (<10°C) for at least 1 hour. After incubation, 1M sodium thiosulfate solution (200 µL) was added until the yellow colour disappeared after shaking. This extract was shaken with ethyl acetate and salt, after which the ethyl acetate was removed by pipette and then evaporated to a volume of ca. 0.5 mL. Triethylamine (50 µL) was added, the vial capped securely and shaken to dissolve the

residue. If required the extract was filtered through a 0.45µm PTFE syringe filter. All extracts were analysed by GC-MS.
Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. The limit of quantification (LOQ) was 30 µg/kg.

7.5 Pyrrolizidine alkaloids and tropane alkaloids FSG 828– LC-MS/MS analysis

This method is similar to the method for tropane alkaloids described in section 7.3, it uses acidified extraction followed by cation exchange SPE clean-up. It allows the analysis of up to 41 pyrrolizidine alkaloids and atropine and scopolamine in a single analysis. The method has undergone in-house validation for a range of foodstuffs. Flexible scope application is in progress for these matrices. All Phase 2 samples were analysed with this method. The analytes requested by FSS are listed in Table 5. It should be noted that lycopsamine does not separate chromatographically from another pyrrolizidine alkaloid, indicine. Results for lycopsamine are therefore reported as a combined value for lycopsamine and indicine.

7.5.1 PA and TAs method

For all samples, an aliquot of sample (4 g) was weighed into a polypropylene tube. Internal standard solution was added to all samples. Extraction solvent, (methanol/water/formic acid solution (60/40/0.4, 40 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 60 minutes. Following this, samples were centrifuged, and an aliquot (5 mL) of supernatant was taken for SPE clean-up using cation exchange. After conditioning the cartridge, the sample extract was loaded. The SPE cartridge was washed with a solution of formic acid in methanol and water, and the cartridge dried by vacuum. The cartridges were eluted with a solution of 5% ammonia in methanol. The collected eluate was evaporated to dryness under a nitrogen atmosphere in a water bath at 50°C using a Turbovap. The dry residue was redissolved in 100 µL of methanol followed by 900 µL water before being transferred to a filter vial and closed.
Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-house reference materials, samples spiked 10 µg/kg (before extraction and immediately

before analysis) were included in each batch. The limit of quantification (LOQ) for pyrrolizidine alkaloid analytes was 1 µg/kg and was 0.25 µg/kg for atropine and scopolamine. As lycopsamine and indicine are reported together higher reporting limit (LOQ) of 2 µg/kg has been used for these analytes.

7.6 Fusarium multimycotoxin analysis FSG/818 – LC-MS/MS analysis

The method is accredited for the analysis of 17 Fusarium mycotoxins in cereals and animal feed. All Phase 2 samples were analysed using this method.

7.6.1 Fusarium mycotoxins analysis FSG/818

For all samples, an aliquot of sample (5 g) was weighed into a polypropylene tube. Internal standard solution was added to all samples. Extraction solvent, acetonitrile/water (84/16, 20 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 120 minutes. Following this, samples were centrifuged, and an aliquot (10 mL) of supernatant was taken for SPE clean-up using Oasis Prime HLB cartridges. The cartridge is conditioned with a small volume of sample, then 1 mL of the sample extract was loaded. The sample extract is allowed to pass through the cartridge and collected in a glass tube. An aliquot (0.5 mL) of cleaned extract was evaporated to dryness under a nitrogen atmosphere in a water bath at 50°C using a Turbovap. The dry residue was redissolved in 500 µl of sample resuspension solvent (100 µl of internal standard solution and 400 µl water) before being filtered into an autosampler vial.

Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-house reference materials, samples spiked at three different concentrations (1 x LOQ, 2 x LOQ and 10 x LOQ) were included in each batch. The limits of quantification (LOQ) for the 17 analytes in the method ranged from 2.5 – 50 µg/kg.

7.7 Alternaria toxins – LC-MS/MS analysis

The method used is based on CEN method EN 17521:2020 – Determination of Alternaria toxins in tomato, wheat and sunflower seeds by SPE clean-up and HPLC-MS/MS. This method is not UKAS accredited. Fera participated in the interlaboratory method validation

study for this method. A set of verification analyses were carried out to ensure the method performance was acceptable. The method includes altenuene (ALT), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and tenuazonic acid (TEA) and isotopically labelled standards are used to internally standardise the method. All Phase 2 samples were analysed by this method.

7.7.1 Alternaria toxins method

A test portion (2 g) of the sample was spiked with the isotopically-labelled internal standards. The sample was extracted with a methanol, water and acetic acid solution. The sample/extraction solvent mixture was centrifuged and an aliquot of the supernatant collected. The extract was diluted with an equal volume of 1 % aqueous acetic acid solution, and concentrated on a polymeric solid-phase extraction (SPE) cartridge (Phenomenex Strata X2, 6mL, 200 mg sorbent). The extract was eluted from the SPE column with methanol and ethyl acetate solution (75/25, v/v, 7 mL). The eluate was then evaporated under a gentle stream of nitrogen and reconstituted, filtered through a polytetrafluoroethylene (PTFE) syringe filter and analysed by HPLC-MS/MS.
The LOQs were 1 µg/kg each for altenuene (ALT), alternariol (AOH), alternariol monomethyl ether (AME), and 5 µg/kg for tentoxin (TEN) and tenuazonic acid (TEA).

A method verification exercise was carried out for this method before it was used to analyse the survey samples. Replicate spiked samples (n=6) were prepared at three spiking levels, equivalent to 1 x, 2 x and 5 x LOQ for all 5 analytes. These were analysed by LC-MS/MS and the results are presented in Table C31.

During the analysis of the survey samples, spiked samples at the LOQs were carried out both before extraction and immediately before analysis to determine recovery and assess any effects from matrix suppression or enhancement.

7.8 Fusarium toxins – LC-MS/MS analysis

This method is based on a multi-mycotoxin ‘dilute and shoot’ method that uses LC-MS/MS analysis. The analytes included were Enniatin A, Enniatin A1, Enniatin B, Enniatin B1, Beauvericin and Fusaric acid. The analytes were extracted in the one extraction, however the LC-MS/MS analysis was carried out using 2 sets of LC-MS/MS conditions as fusaric

acid required a different LC column to obtain satisfactory chromatography and peak shape.

7.8.1 Fusarium toxins analysis

For all samples, an aliquot of sample (5 g) was weighed into a polypropylene tube. Extraction solvent, acetonitrile/water/acetic acid (79/20/1, 20 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 120 minutes. An aliquot (0.5 mL) of extract was diluted with 0.5 mL of dilution solution (acetonitrile/water/acetic acid (20/79/1). This was well mixed then filtered through a syringe filter into an autosampler vial.

Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-house reference materials, samples spiked at 2.5 µg/kg for enniatins and beauvericin and 10 µg/kg for fusaric acid (before extraction and immediately before analysis) were included in each batch. The limits of quantification (LOQ) for enniatins and beauvericin were 2.5 µg/kg each and was 10 µg/kg for fusaric acid.

7.9 Citrinin – LC-MS/MS analysis

All Phase 2 samples were analysed for citrinin using an in-house method using IAC clean-up and LC-MS/MS analysis. The method also uses an isotopically labelled citrinin standard to internally standardise the method.

7.9.1 Citrinin analysis

For all samples, an aliquot of sample (5 g) was weighed into a polypropylene tube. Isotopically labelled citrinin was added to all samples as internal standard. Extraction solvent, methanol/water (75/25, 250 mL) was added and the sample shaken by hand to mix. Samples were placed on a rotary shaker and extracted by shaking for 120 minutes. The sample was sonicated then filtered through Whatman 113V filter paper. An aliquot (2 mL) of extract was diluted with PBS, and 10 ml of the diluted extract was applied to a citrinin IAC. The IAC was washed with 10mM phosphoric acid (10 mL), and the cartridge dried by pushing through 10 mL of air.

The IAC was eluted with 2 x 1 mL portions of methanol, water (2 mL) was added and the solution mixed before being filtered through a syringe filter into an autosampler vial.

Quality control samples including procedural blanks, and spiked samples were included in the analytical batches to check accuracy (recovery), and blank control, i.e. no contribution from reagents or laboratory environment. In the absence of suitable matrix matched in-house reference materials, samples spiked at 25 µg/kg citrinin (before extraction and immediately before analysis) were included in each batch. The limit of quantification (LOQ) for citrinin was 2.5 µg/kg

7.8 Erucic acid – GC-FID analysis

Firstly, the samples are freeze dried. Fat was extracted from the samples using hexane. The extracts were dissolved in toluene before a methylation reagent was added. The vial was sealed and heated overnight to convert into fatty acid methyl esters (FAME's). These fatty acids were then extracted using hexane and analysed by Gas Chromatography-Flame Ionisation Detection (GC-FID). Fatty acid peaks were identified by comparison to the retention time of the 37-component FAME Standard peaks. The peak areas observed in the samples were used to create a profile of the fatty acid composition of the extracted fat.

The results are reported showing the amount of fat extracted from 2 g lyophilised sample, and the % fatty acid composition of the extracted fat expressed as erucic acid methyl ester. These results are converted to values in g/kg, erucic acid in fat to allow comparison with the MLs in Commission Regulation EC (No) 1881/2006 (2).

8.1 Mycotoxins 11+ IAC method validation

Initial method development work was carried out to determine the best extraction solvents and sample loading volume and sample mass equivalent for the IACs. The aim was to maximise the amount loaded on the IAC to ensure the target LOQs were achieved. The initial test used 80% methanol as extraction solvent, with the addition of salt. The extract was diluted with PBS and a relatively large volume (50 mL) was loaded on the IAC. This was equivalent to 0.625 g sample loaded. The approach resulted in acceptable recoveries for aflatoxins, HT-2, T-2 and zearalenone, but recoveries for other analytes were lower than the acceptable minimum of 70%.

A series of different combinations of solvent mixes (ratios and solvent combinations) and different sample loading amounts were assessed. The results (not shown) found that the combination of acetonitrile / methanol / water as extraction solvent and a sample loading equivalent to 0.25 g sample resulted in the most consistent, acceptable recovery of all analytes across all three concentrations (1x, 2x and 10x target LOQ), while still allowing the target LOQs to be achieved. This method was used in a validation batch consisting of three blank samples, and six spikes each at 1x, 2x and 10x target LOQ as required by Fera in-house procedure for Application of Flexible Scope accreditation.

The results of the in-house validation are presented in Annex C, Table C1. Average recoveries are between 70-120% for all mycotoxins at all concentrations, and for the overall average with one exception. This was for the 1xLOQ spike average for ochratoxin A where the average recovery was 68.8%. The repeatability was also high at this concentration with an RSD_r (CV%) at 28.2%. The sample used as the blank material contained an average concentration of 0.33 µg/kg OTA, therefore the background level was greater than the spike addition level, which can cause variability and lower recovery results. The higher concentration spikes both gave average recovery values greater than 70% and the overall average was 73.2% with acceptable precision. One result was excluded from the data set for the 10xLOQ replicates for ochratoxin A. This samples contained a lower concentration than the blank, unspiked sample, so it ether was not injected properly on the LC-MS, or more likely was not spiked prior to extraction.

In all other cases the target LOQ spikes gave acceptable recovery and precision. Therefore, the method was deemed to be acceptable. The calibration range used for each analyte extended below the target LOQ, to allow spiked samples with recovery less than 100% to be accurately quantified. In all cases two calibration solutions lower than the target LOQ were used in the calibration curves, a total of 9 calibration points were used. In all cases calibration curves met QC requirements for linearity and residuals from in-house document FSG 002. This has allowed results in survey samples below the target LOQ to be reported where they also met other reporting criteria, i.e. confirmation by ion ratio.

8.2 Mycotoxins 11+ results Phase 1

The results for the mycotoxins in protein products from Phase 1 are given in Table C2 to Table C5.

Table presents results for the six protein powder samples. Most results (69 of 78 data points, 88%) were below the LOQ for the method. Two samples contained no detectable residues of mycotoxins. Sterigmatocystin, DON, HT-2, T-2 and fumonisins B_1, B_2 and B_3 were not detected in any protein powder samples. Three samples contained OTA, one pea protein powder at 9.33 µg/kg (S24-069134), and two samples of soy protein powder contained 4.15 and 3.22 µg/kg (S24-069137 and S24-069135). For samples S24-069134 and S24-069135, a pea and soy respectively, OTA was the only mycotoxin detected. Sample S24-069137 (soy) also contained aflatoxins B_1, B_2, G_1 and G_2 and zearalenone. The aflatoxin B_1 level was 2.24 µg/kg, and the sum of the four aflatoxins was 4.94 µg/kg. The sample also contained 1.13 µg/kg zearalenone.
There are GB MLs for aflatoxins and ochratoxin A in Commission Regulation (EC) No 1881/2006 in some foods but there are no MLs for mycotoxins currently in force for soy, pea or other plant proteins (1, 2) but there are MLs in force in GB for a range of products such as cereals (3 µg/kg) and dried vine fruit (10 µg/kg). There is also a ML for wheat gluten not placed on the market for the final consumer of 8 µg/kg (1, 2). There is an ML in force in the EU (Commission Regulation (EU) 2023/915) for soybeans (as well as sunflower seeds, pumpkin seeds and, watermelon seeds) of 5 µg/kg (4). The levels of aflatoxins (AFB1 and total) and OTA found in sample S24-069134 exceed the GB MLs for other foods such as cereals and wheat gluten. They also exceed the EU ML for soybean. The OTA level in S24-069137 and S24-069135 exceed GB MLs for other foods such as cereals for direct consumption.

Table C3 presents results for soy protein products, these included tofu and meat substitutes. Three of the nine products contained at least one mycotoxin, while the other six contained no mycotoxins above the LOQ. The three samples that contained mycotoxins, all soy mince products, contained different mycotoxins, but at low concentrations. S24-069322 contained low levels of AFB1 and OTA, S24-0698890 contained 1.85 µg/kg OTA, and sample S24-069321 contained FB1, FB2 and FB3, 31.8 µg/kg in total.

Table C4 presents results for pea protein based products, these were all meat substitute products. As with the other samples there was a low incidence of low concentrations of a small number of mycotoxins, only 4 residues of mycotoxins were found above the LOQ. These were detected in four of the six samples, two samples did not contain any mycotoxins. Three samples contained OTA at levels of 0.26 µg/kg (S24-069888), 0.53 µg/kg (S24-069886) and 0.54 µg/kg (S24-069889). One sample (S24-069885) contained 1.75 µg/kg ZON. None of these concentrations are close to or above any MLs.

Table C5 presents the results for the other samples that were based on combinations of soy, pea and wheat proteins. One sample contained no detectable mycotoxins, the other four samples contained at least one mycotoxin. DON was most frequently detected, it was found in four out five samples at levels from 2.85 to 7.35 µg/kg (all of these samples contained wheat protein). For one of the soy and wheat mixed products (S24-069325) DON was the only mycotoxin detected. Sample S24-069891 also contained OTA and S24-069328 also contained ZON. Sample S24-069326, a pea, wheat and soy mixed product contained DON, OTA and STG at low concentrations. This was the only sample that contained STG.

Table C6 summarises the QC data that was obtained during the analysis of the Phase 1 survey samples. All QC criteria for LC-MS analysis were met, and the results show acceptable recovery across three concentrations for all the different types of products tested. The lowest recovery was found for the 1xLOQ OTA spike in protein powder due to the background concentration in this sample as described above.

8.3 Mycotoxins 11+ results Phase 2

The results for the mycotoxins in protein products from Phase 2 are given in Table C7 to Table C9.

Table C7 presents the results of the nine protein powder products analysed in Phase 2. As in Phase 1 OTA was the most frequently detected mycotoxin, it was detected in 8 of the 9 samples at concentrations ranging from 0.37 to 2.27 µg/kg. This was lower than the maximum level found in Phase 1. The two highest levels were found in pea protein powders. The next most frequently detected mycotoxin was AFB1, 4 samples contained concentrations from 0.11 to 0.63 µg/kg. None of these levels were at or above GB MLs set for OTA or AFB1 in other foods such as wheat gluten, nuts or cereals and are well below the EU ML for OTA in soybeans.
Three samples contained ZON, levels were 1.06 to 2.80 µg/kg, all three samples contained pea protein. One of these samples also contained fumonisin B1 and B2 at 14.91 and 5.89 µg/kg.
Sterigmatocystin was detected in 2 samples (0.20 and 1.55 µg/kg), the higher level was found in the sample that contained pea and rice protein.

Table C8 presents the results of the meat replacement products. Again OTA was most frequently detected, it was found in 4 out of 10 samples. The levels measured were all below 0.5 µg/kg (range 0.13 to 0.32 µg/kg). One wheat and pea based product contained DON at 11.22 µg/kg. ZON was detected in two samples, one at 1.88 µg/kg, the other residue of 1.28 µg/kg was not confirmed, the uncorrected value was just at the LOQ of the method. Both products were soy based.

Table C9 presents the results of ready to drink protein shakes and protein and cereal bars. DON was the most frequently detected mycotoxin, it was found in 2 out of 3 shakes and 2 out of 4 protein bars. The levels measured ranged from 2.32 to 14.55 µg/kg. Three samples contained OTA (all protein and cereal bars), levels were all below 1 µg/kg. Three samples contained ZON, one shake (2.08 µg/kg) and 2 protein bars (1.67 and 4.85 µg/kg).

The results of the QC for these analyses are presented in Table C10. Spike recoveries met the requirements for Commission Regulation EC (No) 401/2006 for all analytes in all products (19). Sterigmatocystin had slightly lower average recoveries of 60-66%, but these are still within the range of 50-130% deemed acceptable in some cases, e.g. where low

concentrations or no residues are measured or the mycotoxins are not regulated (19, 21, 22).

8.4 Ergot alkaloid analyses – method verification

In-house validation according to the protocol Application for Flexible scope was carried out for mixed plant protein products. The results are given in Table C11. No analytical standard is available for β-ergocryptine, therefore there are no results for this during validation. However, this compound occurs in naturally contaminated samples and is separated from α-ergocryptine, where present it is quantified using α-ergocryptine and is included in the sum value with the other 11 epimers to calculate the sum of ergot alkaloids. The validation data show acceptable recovery and precision for all analytes included in the sum parameter at all three spiking levels. Samples were spiked at 1x, 2x and 10x target LOQ. In this case the target LOQ was 0.5 µg/kg for each epimer.

8.5 Ergot alkaloids – Phase 1 analyses

Five samples were analysed for ergot alkaloids, these were the samples that included wheat protein as an ingredient (Table C12). Two samples were found to contain ergot alkaloids, one contained nine epimers giving a sum of ergot alkaloids of 6.90 µg/kg, eight epimers were detected in the second sample giving a sum of ergot alkaloids of 5.71 µg/kg. The reporting limit for individual compounds was reduced to 0.25 µg/kg, as three compounds were detected between this level (the lowest calibration point) and the validated LOQ (0.5 µg/kg). The residues of ergocorninine, ergocristine and α- and β-ergocryptinine were at concentrations between 0.25 and 0.5 µg/kg, all three residues were confirmed by ion ratio so were accepted as quantitative results.

8.6 Ergot alkaloids – Phase 2 analyses

All samples from Phase 2 were analysed for ergot alkaloids.

Table C13 presents the results for the ready to drink shakes and protein and cereal bars. No residues of ergot alkaloids were detected in any of the three ready to drink shakes analysed.

Two of the four protein bars contained ergot alkaloids, one containing a sum total of 3.41 µg/kg and the other 11.01 µg/kg. Both products contained wheat (flour and or gluten) as ingredients, so it is expected the ergot alkaloid content originated from that.

Table C14 contains the results of the plant protein powder products. Of the nine samples tested only one sample, S25-037301, contained detectable ergot alkaloids. Trace levels (between the lowest calibration standard and the lowest validated level, or reporting level) were found for 2 ergot alkaloids (ergocristine and ergosine). The sum ergot alkaloids (sum of 12 compounds) value for this sample was 0.56 µg/kg. This is below the normal RL but the residues were confirmed by mass spectrometry and so the result has been reported.

Table C15 contains the results of the meat replacement products. One of the ten samples analysed contained detectable levels of 6 ergot alkaloids, 2 of these were above the reporting level (RL), and 4 were between the RL and the lowest calibration standard. The sum value for ergot alkaloids found in this sample was 2.81 µg/kg. This product's ingredients listed vegetable protein (17%) (wheat, pea) as the second ingredient, wheat gluten is also listed in the ingredients this. Again the inclusion of wheat is the most likely source of the ergot alkaloids.

There are no MLs for ergot alkaloids in GB. There are MLs in force in the EU (4), which also apply in Northern Ireland, but there are no specific MLs not for plant protein products. The lowest ML in force is 20 µg/kg for processed cereal based food for infants and young children. The levels found in the products in this study were well below this.

8.7 Tropane alkaloid method FSG 827 analyses – verification analyses

The in-house method FSG 827 for tropane alkaloids is accredited for cereals, herbal teas and vegetable products. However, as the samples to be tested in the survey were different to the vegetable mixes tested during initial validation some verification analyses were carried out to ensure the method was suitable. The results of these analyses are given in Table C16. A variety of sample types were analysed. For each sample type two blank (unspiked portions) and a sample spiked before extraction at 10 µg/kg were extracted and cleaned up. One of the blank portions was spiked at a level equivalent to 10 µg/kg after clean-up, this was labelled 'tissue standard', the sample spiked before analysis was the 'spike'. The use of the tissue standard allows any effects (e.g. signal enhancement or

suppression) due to that matrix to be observed. A calculation of the ratio of the recovery measured in the ‘spike’ sample to the recovery in the ’tissue standard’ can be made to assess true recovery. The results in Table C16 showed that there was some signal enhancement in the tissue standards, however these recovery results were generally within the acceptable range of 70-120% and none were greater than 130% which can be deemed acceptable (22). The method uses isotopically labelled internal standards for both atropine and scopolamine which corrects for any losses or enhancement. Therefore, during analysis of the survey samples only spiked samples were used for QC.

8.8 Tropane alkaloid analyses results – Phase 1

The results of the survey samples are presented in Table C17. Four samples contained tropane alkaloids above the individual LOQs of 0.1 µg/kg. The LOQ for method FSG 827 is lower than the combined method used in Phase 2 as the final sample extract injected on the LC-MS/MS is more concentrated.
Two samples contained only atropine (at 0.19 and 0.2 µg/kg, S24-069325 and S24-069323 respectively), while two samples contained both atropine and scopolamine. One sample (S24-069322) contained 0.4 µg/kg atropine and 0.14 µg/kg scopolamine. The other sample (S24-069883) contained 1.23 µg/kg atropine and 0.48 µg/kg scopolamine. All four samples were soy based products, the sample with the highest concentration (S24-069883) was a tofu sample. Sample S24-069322 was a soy meat free mince, sample S24-069323 was soy based vegan ‘chicken’ pieces. Sample S24-069325 was a soy & wheat product described as a vegetarian shredded duck.
There are no MLs for tropane alkaloids in plant protein products, the only MLs in force in GB are for baby food and processed cereal based food for infants and young children, where MLs are set at 1.0 µg/kg each for atropine and scopolamine (2).

The results of QC samples analysed with the Phase 1 survey samples are given in Table C18. The individual recovery values were all within the acceptable range of 70-120% apart from the lowest concentration spikes for the pea/soy/wheat mixed product. All other QC, including blanks, and LC-MS parameters (linearity, residuals, ion ratios and IS response) met criteria specified in FSG 002 Quality Control for LC-MS.

8.9 Acrylamide analyses results – Phase 1 samples

Table C19 presents the results of the acrylamide analysis of the Phase 1 products. Of the 26 samples analysed only two contained acrylamide above the LOQ of 30 µg/kg. Both were samples of meat replacement products. One sample (S24-069324) was a burger style product based on pea protein that contained 36.8 µg/kg. The other product was a vegan sausage product (S24-069891) made from soy and wheat, it contained 41.7 µg/kg. There are no MLs for acrylamide but there are Benchmark levels (BMLs), these are designed to help food business operators judge the success of any mitigation measures and are not intended to be used for enforcement. The BMLs range from 40 µg/kg for baby foods, processed cereal based for infants and young children to 4000 µg/kg for chicory based coffee substitutes (5).

The two samples that contained residues both contained acrylamide just below and just above the BML of 40 µg/kg for baby food. The majority of samples (24 out of 26) did not contain acrylamide above the LOQ of 30 µg/kg.

8.10 Fusarium mycotoxins results from method MM2 (FSG 818)

Samples were analysed for Fusarium mycotoxins using method FSG 818. The samples are extracted by a common extraction and clean-up method and analysed by LC-MS/MS. Due to the chemical differences in the analytes the samples have to be run on LC-MS/MS twice using both positive and negative ionisation mode to ensure the best analytical performance for all the analytes.

The results for plant protein powders and protein bars are presented in Table C20 and Table C21. Zearalenone was the only mycotoxin that was confirmed in any of these samples. Two protein powders (S25-037300 and S25-037454) contained ZON at a level just below the LOQ (both contained 2.3 µg/kg). Two protein bars also contained residues of ZON that were confirmed by ion ratio, one at 1.7 µg/kg (S25-037304) and the other at 4.6 µg/kg (S25-037453).

These results are very similar to the ZON results found for the same samples using the 11+ IAC method (Section 8.3). The results found using the IAC method for protein powder samples S25-037300 and S25-37454 were 2.8 and 2.56 µg/kg respectively. While for the protein bars S25-037304 and S25-037453 the ZON levels measured by the IAC method were 1.67 and 4.85 µg/kg respectively. These results show good agreement and give confidence in the levels measured.

One residue of fusarenon X (22.5 µg/kg) was found in sample S25-037304 (a vegan protein bar) but the ion ratio did not confirm the identity of the peak so this result is indicative only.

The results for the ready to drink shakes and meat replacements are given in Table C22 and Table C23. The only mycotoxin residue detected in these samples was DON at 12.6 µg/kg, in sample S25-037319, a sample of plant based steak strips. Again a similar result was found using the IAC method, 11.2 µg/kg DON was found in the same sample by that method.

None of the samples contained any Fusarium mycotoxins at levels close to MLs in force for other foodstuffs.

8.11 Enniatins, beauvericin and fusaric acid results

Results for enniatins, beauvericin and fusaric acid are presented in Table C24 to Table C26. None of these mycotoxins were detected in the ready to drink shakes.
All four of the protein bar samples contained fusaric acid, although the concentration in 3 of the samples was below the LOQ of 10 µg/kg. The concentrations found were 4.8, 6.1 and 8.9 µg/kg. These levels were above the lowest calibration standard and confirmed by ion ratio so have been reported for information. The sample that contained the highest concentration of fusaric acid (S25-037303) at 26.9 µg/kg, did not contain any of the other compounds. The other 3 protein bar samples contained different combinations of beauvericin and the enniatins. Sample S25-037453 contained beauvericin, enniatin A1, B and B1 as well as fusaric acid.

Table C25 contains the results of the plant protein powders. One sample contained no detectable residues of the mycotoxins, one sample contained only a low level of enniatin B (below the LOQ). One sample contained beauvericin (below LOQ) and enniatin B1, however this did not confirm by ion ratio. The other 6 samples each contained at least 3 of the 6 mycotoxins tested by this method. The majority of the residues found for enniatins and beauvericin were at or below the LOQ. The identity of most of the mycotoxins detected was confirmed by ion ratio. However, for four residues of enniatin B1 the analyte response was not confirmed and the results are reported as indicative. Spiked samples, that contained a known added amount of the analyte showed a similar effect where the

peak response was not confirmed by ion ratio. It is possible there was an underlying interference for enniatin B1 for this matrix that affected the ion ratio response.
One sample (S25-037454) contained a large residue of fusaric acid that was above the maximum of the calibration line (250 µg/kg) and therefore could not be quantified accurately. The analytical recovery for fusaric acid for protein powders was also low (average 36.6 %). When this correction factor was applied to the results this gave a value of >1000 µg/kg. This is an indicative result. There are no MLs or guidance values for fusaric acid and very little information regarding the toxicological relevance of this toxin and therefore whether this concentration is significant. For interest it may be worthwhile testing this sample further, using step wise dilutions and matrix matched calibration to allow accurate quantification of the fusaric acid concentration. This would allow accurate quantification and address the issues of low recovery and matrix suppression that were observed during the initial analysis.

Table C26 contains the results of the meat replacement products. These results were similar to other products tested. There were several samples that contained low levels of beauvericin, enniatins and fusaric acid at concentrations just below the LOQ. In all cases the identity of the compounds was confirmed by ion ratio. Two samples contained enniatin B1, at levels of 2.6 and 6 µg/kg that did not confirm by ion ratio. All ten samples contained a residue of at least one mycotoxin, four samples contained fusaric acid only. One sample contained fusaric acid at 118.4 µg/kg. This result was confirmed by ion ratio. Again, in the absence of MLs in any products that could be used as a comparator it is not possible to comment on the significance of this result.

8.12 Pyrrolizidine and tropane alkaloids results

Table C27 contains the pyrrolizidine alkaloid (PA) results for the meat replacement and ready to drink shake samples. No residues of PAs were found above the LOQ of 1 µg/kg for the analytes reported.

Table C28 contains the PA results for the protein powders and protein bars. No residues of PAs were found above the LOQ of 1 µg/kg for the analytes reported.

Some recovery values were low for some analytes, particularly for seneciphylline, and outside the normal range deemed acceptable (50 – 130%) (22). However, the lower end of

the calibration range used for the analysis is much lower than the reporting limit (LOQ). This give confidence that despite the low recovery if residues were present at or above 1 µg/kg they would have been detected. The EU Regulation on method performance (18) stipulates that a method used for PAs in dry products should have an LOQ of ≤ 10 µg/kg, so the method used complied with this.

Table C29 presents the tropane alkaloid results for the meat replacement and ready to drink shake samples. Atropine and scopolamine were not detected in the shake samples. Two meat replacement samples, S25-037318 and S25-037339, contained atropine at 0.52 and 1.15 µg/kg respectively. The sample that contained the lower concentration also contained 0.25 µg/kg scopolamine, although this was not confirmed by ion ratio.

The tropane alkaloid results for protein powders and protein bars are presented in Table C30. One protein bar, S25-037453, contained 0.79 µg/kg atropine and 0.38 µg/kg scopolamine, the other three samples did not contain tropane alkaloids above the LOQ. Three protein powders contain scopolamine at 0.25, 0.45 and 0.5 µg/kg, although none of these residues were confirmed by ion ratio.

The LOQ for tropane alkaloids was slightly higher for this method than the method used in Phase 1 (0.25 µg/kg compared to 0.1 µg/kg). This is because they were analysed in a combined method with the PAs. For this method, a smaller amount of sample is taken through clean-up, and the final volume for analysis by LC-MS/MS is larger as a compromise to improve the method performance and reduce suppression and interferences for the PAs that are also in the method. However, the method still meets the LOQ requirements for tropane alkaloids of 1 µg/kg each for atropine and scopolamine as set out in Regulation (EU) 2023/2783 (22).

8.13 Alternaria toxins method verification

The results of the method verification for the Alternaria toxin method are summarised in Table C31. For all 5 toxins in the method, altenuene, alternariol, alternariol monomethylether, tentoxin and tenuazonic acid average recoveries were in the range 96.7 – 109.8% across three spiking levels. The only exception was for the LOQ spikes for altenuene where the average recovery was 126.2%, this is still within the range of 50 - 130% that can be deemed acceptable (21).

The relative standard deviation values (RSDr or cv) were all in the range 1.3 to 9.1%, well below the value of 20% stipulated in the EURL-MP guidance document (19) and the recently implemented EU Regulation (EU) 2023/2782 that lays down methods for sampling and analysis for mycotoxins (21). This Regulation does not apply in GB, but it formalises method performance criteria in the EURL-MP guidance document (19) and is generally recognised as setting out good practice for laboratory performance.

8.14 Alternaria toxins results

The results of the analysis for Alternaria toxins are presented in Table C32.

Table Low concentrations of tenuazonic acid (from 3.8 to 6.5 µg/kg) were found in all three protein shake samples. Two of the three results found were below the validated LOQ for the method, but the concentration was above the lowest calibration solution and the ion ratio confirmed so the results have been reported for information.
No other Alternaria toxins were detected above the LOQ in any of the shake samples.

Two of the four protein bars contained tenuazonic acid at levels of 11.4 µg/kg (S25-037303) and 18 µg/kg (S25-037453). No other Alternaria toxins were detected.

Three of nine protein powders contained no detectable Alternaria mycotoxins. Of the other six samples one contained a low level of tenuazonic acid below the LOQ. One sample contained a level of alternariol just above the LOQ, at 1.3 µg/kg (S25-037455), but this was not confirmed by ion ratio. One sample contained a similar unconfirmed level of alternariol and 5.4 µg/kg tenuazonic acid (S25-037454). Two samples contained both alternariol and alternariol monomethylether at 5.6 and 3.4 µg/kg (S25-037299) and 13.5 and 6.1 µg/kg (S25-037298) respectively. The final sample contained alternariol (4.1 µg/kg), alternariol monomethyl ether (2.3 µg/kg) and tenuazonic acid (5.6 µg/kg).
Of the meat replacement products, 3 out of 10 samples did not contain Alternaria toxins. Six samples contained tenuazonic acid at levels from 5.2 to 62.7 µg/kg, five of these samples did not contain other Alternaria toxins, but one sample that contained 22.3 µg/kg tenuazonic acid also contained 1.1 µg/kg alternariol (S25-037320). Sample S25-037318 that contained the highest concentration of tenuazonic acid (62.7 µg/kg) was a pea based plant burger. One sample contained 5.0 µg/kg alternariol and 2.1 µg/kg alternariol monomethylether (S25-037341).
There are no regulations in force for Alternaria toxins, so there are no MLs to compare these results to. However, these findings are similar to results reported in other published studies (6-10).

8.15 Citrinin analysis results

Table C33 presents the results of the citrinin analysis of the Phase 2 protein products. Citrinin was not detected in any of the samples above the LOQ of 2.5 µg/kg. The survey samples were extracted in two analytical batches: Batch PC25-04884 – protein powders and protein bars and Batch PC25-04988 – meat replacement and ready to drink shakes.

Quality control samples matched to the products being analysed were included in each batch. The mean analytical recovery for each batch was 99.3 % and 100.9 %.

8.16 Erucic acid results

The results of the erucic acid analysis are presented in Table C34.
Regulation EC (No) 1881/2006 sets MLs for erucic acid content of oil (2). In this case the ML of 20 g/kg in vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food would apply. The samples selected for analysis of erucic acid all contained rapeseed oil as ingredient as listed on the sample label. Ten samples were analysed, the levels of erucic acid found ranged from 0 to 6 g/kg of the fat / oil. These are all well below the ML of 20 g/kg for vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food.

9. Summary and Conclusions

9.1. A survey of plant protein products was carried out in two phases. Samples were purchased in Scotland or on-line at two time points. Twenty-six samples were purchased each time, these consisted of products such as plant protein powders, vegan sausages and burgers, shakes and protein bars made from different plant proteins. Samples in Phase 1 were analysed for regulated mycotoxins, tropane alkaloids and acrylamide, a subset were analysed for ergot alkaloids. Phase 2 samples were analysed for regulated mycotoxins, a broader range of Fusarium mycotoxins, ergot alkaloids, citrinin, Alternaria toxins, plant alkaloids (tropanes and pyrrolizidine), and a subset were analysed for erucic acid.

9.2. All analyses were carried out using accredited or in-house validated methods that met required quality control parameters. In Phase 1 one sample of soy powder contained a total aflatoxin level of 4.94 µg/kg. This sample also contained OTA (4.15 µg/kg). Another sample of pea powder contained 9.33 µg/kg OTA. While there are no MLs for protein products specifically, the levels of OTA and aflatoxins are at or above MLs in place for comparable foods.

9.3. Alternaria toxins were found in over half the samples tested. There are no regulations in force for Alternaria toxins, however, these findings are similar to results reported in other published studies (6-10). Residues of other Fusarium toxins were found at low levels, except fusaric acid, which was found in 16 out of 26 samples. The highest levels reported were 118.4 µg/kg and >1000 µg/kg although the latter was an indicative result. This mycotoxin is difficult to analyse for; the analytical recovery was low which added to the uncertainty about the result. There are no MLs for fusaric acid and little information about its toxicological relevance. Therefore, it is not possible to make comparisons or comment on the significance of these 2 higher values.

9.4. Overall, the results are reassuring, for the majority of samples tested, mycotoxins and plant toxins were not detected, despite the fact that low LOQs were achieved. For the mycotoxins found, the concentrations measured were below comparable MLs, except for two samples. The study provides valuable results to address a data gap about the occurrence of mycotoxins and plant toxins in vegetarian and plant based foods purchased in Scotland and can be used for risk and exposure assessments for plant based foods.

10. Acknowledgements

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Table A1. Comparison of UK/EU Maximum Level (ML) Ranges and Survey Results at or above LOQs (Phase 1)

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
OTA	0.5	Infant food	80	Liquorice extract

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069134	Pea powder	OTA	9.33
S24-069135	Soy powder	OTA	3.22
S24-069137	Soy powder	OTA	4.15
S24-069322	Soy based mince	OTA	0.39
S24-069890	Soy based mince	OTA	1.85
S24-069886	Pea based ‘meatballs’	OTA	0.53
S24-069888	Pea based vegan burgers	OTA	0.26
S24-069889	Pea based vegan sausages	OTA	0.54
S24-069326	Pea, wheat & soy ‘chicken’ pieces	OTA	0.32
S24-069891	Pea & wheat based vegan sausage	OTA	0.11

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Aflatoxin B1	0.1	Infant food	12.0	Almonds, pistachios & apricot kernels to be sorted
Total aflatoxins	4.0	Dried fruit, nuts, cereals ready to eat	15.0	Nuts to be sorted

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069136	Pea powder	AFB1	0.15
S24-069137	Soy powder	AFB1 AFB2 AFG1 AFG2 Total AF	2.24 0.12 2.46 0.12 4.94
S24-069322	Soy based mince	AFB1	0.22

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
ZON	20	Infant food	400	Refined maize oil

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069137	Soy powder	ZON	1.13
S24-069885	Pea based sausage	ZON	1.75
S24-069328	Soy & wheat vegan ‘chicken’ breast	ZON	1.1

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
DON	200 (GB) 150 (EU incl NI)	Infant food	1750	Unprocessed durum wheat, oats maize (GB) Unprocessed oats (EU incl NI)

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069325	Soy & wheat vegetarian ‘shredded duck’	DON	7.35
S24-069326	Pea, wheat & soy ‘chicken’ pieces	DON	3.76
S24-069328	Soy & wheat vegan ‘chicken’ breast	DON	3.07
S24-069891	Pea & wheat based vegan sausage	DON	2.85

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Sum FB1 and FB2	200	Infant food	4000	Unprocessed maize

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069321	Soy based Vegemince	FB1 & FB2	28.44

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Sum ergot alkaloids	20 (EU inc. NI)	Infant food	500 (EU inc. NI)	Rye milling products

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S24-069326	Pea, wheat & soy ‘chicken’ pieces	Sum ergots	5.71
S24-069891	Pea & wheat based vegan sausage	Sum ergots	6.90

Tropane Alkaloids	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Atropine Scopolamine	1.0 1.0	Baby food	1.0 1.0	Baby food
Sum of Atropine and Scopolamine	0.2 (EU inc. NI) 5 µg/kg (EU inc. NI)	Liquid herbal infusions Cereals	50 (EU inc. NI)	Dry herbal infusions

Survey Results			
Sample Number	Commodity/Product Type	Tropane alkaloids	Result at or above LOQ, µg/kg
S24-069322	Soy based mince	Sum	0.54
S24-069323	Soy based vegan ‘chicken’ pieces	At	0.2
S24-069883	Organic tofu (soy)	Sum	1.71
S24-069325	Soy & wheat vegetarian ‘shredded duck’	At	0.19

Table A2. Comparison of UK/EU Maximum Level (ML) Ranges and Survey Results at or above LOQs (Phase 2)

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
OTA	0.5	Infant food	80	Liquorice extract

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S25-037297	Soy protein powder	OTA	0.44
S25-037298	Pea protein powder	OTA	2.11
S25-037299	Pea & fava bean protein powder	OTA	0.92
S25-037300	Pea & soy protein powder	OTA	0.41
S25-037301	Vegan plant blend protein powder	OTA	0.46
S25-037454	Whey & soy protein powder	OTA	1.70
S25-037455	Pea protein powder shake	OTA	2.27
S25-037459	Pea & rice protein powder	OTA	0.37
S25-037317	Soy & pea based bacon	OTA	0.32
S25-037318	Pea based plant burger	OTA	0.22
S25-037319	Wheat and pea based steak	OTA	0.13
S25-037341	Pea based vegan burger	OTA	0.23
S25-037303	Soy protein & oat bars	OTA	0.55
S25-037304	Soy protein & oat bars	OTA	0.39
S25-037453	Protein blend bars	OTA	0.39

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Aflatoxin B1	0.1	Infant food	12.0	Almonds, pistachios & apricot kernels to be sorted
Total aflatoxins	4.0	Dried fruit, nuts, cereals ready to eat	15.0	Nuts to be sorted

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S25-037299	Pea & fava bean protein powder	AFB1	0.34
S25-037300	Pea & soy protein powder	AFB1	2.24
S25-037454	Whey & soy protein powder	AFB1 AFG1 Total AF	0.63 0.29 1.21
S25-037459	Pea & rice protein powder	AFB1	0.44
S25-037341	Pea based vegan burger	AFB1	0.12
S25-037457	Pea based ready to drink shake	AFB1	0.23
S25-037303	Soy protein & oat bars	AFB1	0.15

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
ZON	20	Infant food	400	Refined maize oil

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg IAC / MM2*
S25-037300	Pea & soy protein powder	ZON	2.80 / 2.3
S25-037454	Whey & soy protein powder	ZON	2.56 / 2.3
S25-037459	Pea & rice protein powder	ZON	1.06 / <2.5
S25-037315	Organic tofu (soy)	ZON	1.28i / <2.5
S25-037339	Organic tofu (soy)	ZON	1.88 / <2.5
S25-037457	Pea based ready to drink shake	ZON	2.08 / <2.5
S25-037304	Soy protein & oat bars	ZON	1.67 / 1.7
S25-037453	Protein blend bars	ZON	4.85 / 4.6

*Results from 2 different methods for comparison.

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
DON	200 (GB) / 150 (EU incl NI)	Infant food	1750	Unprocessed durum wheat, oats maize (GB) / Unprocessed oats (EU incl NI)

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg IAC / MM2*
S25-037455	Pea protein powder shake	DON	3.43
S25-037319	Wheat and pea based steak	DON	11.22 / 12.6
S25-037457	Pea based ready to drink shake	DON	14.55
S25-037458	Pea based ready to drink shake	DON	2.32
S25-037304	Soy protein & oat bars	DON	2.44
S25-037453	Protein blend bars	DON	8.32

*Results from 2 methods for comparison, unless stated result from MM2 method was <10 µg/kg

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Sum FB1 and FB2	200	Infant food	4000	Unprocessed maize

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S25-037454	Whey & soy protein powder	FB1 & FB2	20.8

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Sum T-2 and HT-2 toxins	10 (EU inc. NI)	Infant food	1250 (EU inc. NI)	Unprocessed oat grains

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S25-037455	Pea protein shake powder	HT-2	2.45
S25-037302	Protein & cereal bars	HT-2	4.16

Mycotoxin	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML (µg/kg)	Commodity
Sum ergot alkaloids	20 (EU inc. NI)	Infant food	500 (EU inc. NI)	Rye milling products

Survey Results			
Sample Number	Commodity/Product Type	Mycotoxin	Result at or above LOQ, µg/kg
S25-037304	Soy protein & oat bars	Sum ergots	3.41
S25-037453	Protein blend bars	Sum ergots	11.01
S25-037301	Vegan plant blend protein powder	Sum ergots	0.56
S25-037319	Wheat and pea based steak	Sum ergots	2.81

Tropane Alkaloids	Lowest GB/EU ML (µg/kg)	Commodity	Highest GB/EU ML	Commodity
Atropine Scopolamine	1.0 1.0	Baby food	1.0 1.0	Baby food
Sum of Atropine and Scopolamine	0.2 (EU inc. NI) 5 µg/kg (EU inc. NI)	Liquid herbal infusions Cereals	50 (EU inc. NI)	Dry herbal infusions

Survey Results			
Sample Number	Commodity/Product Type	Tropane alkaloids	Result at or above LOQ, µg/kg
S25-037301	Vegan plant blend protein powder	Sc	0.45i
S25-037454	Whey & soy protein powder	Sc	0.50i
S25-037459	Pea & rice protein powder	Sc	0.25i
S25-037453	Protein blend bars	Sum	1.17
S25-037318	Pea based plant burger	Sum	0.77
S25-037339	Organic tofu (soy)	At	1.15

Table B1. Summarised sample information – Phase 1

Fera Sample No.	Product Category	General Product Description
S24-069134	Pea Protein Powder	Vegan Pea Protein
S24-069135	Soy Protein Powder	Vegan Soy Protein
S24-069136	Pea Protein Powder	Pea Protein
S24-069137	Soy Protein Powder	Soy Protein
S24-069138	Soy Protein Powder	Plant Based Soya Protein
S24-069318	Pea Protein Powder	Pea Protein
S24-069319	Soy based product	Tofu
S24-069320	Soy based product	Tofu
S24-069321	Soy based product	Vege Mince
S24-069322	Soy based product	Meat Free Mince
S24-069323	Soy based product	Vegan Chicken Pieces – Roast flavour
S24-069324	Pea based product	Plant Based Burger
S24-069325	Soy & wheat product	Vegetarian Shredded Hoisin Duck
S24-069326	Pea, wheat and soy product	Ready To Eat Tikka Chicken Style Pieces
S24-069327	Soy & wheat product	Plant Chicken Breast
S24-069328	Soy & wheat product	Vegan Chicken Breast
S24-069882	Soy based product	Vegan organic Tofu
S24-069883	Soy based product	Organic Tofu
S24-069884	Soy based product	Plant based chicken pieces
S24-069885	Pea based product	Sausage
S24-069886	Pea based product	Plant based meatballs
S24-069887	Pea based product	Mince
S24-069888	Pea based product	Vegan burgers
S24-069889	Pea based product	Vegan Lincolnshire sausage
S24-069890	Soy based product	Plant Based Mince
S24-069891	Soy & wheat product	Sausage

Table B2. Summarised sample information – Phase 2

Fera Sample No.	Product Category	General Product Description
S25-037296	Pea Protein Powder	Pea Protein Isolate
S25-037297	Soy Protein Powder	Vegan Soy Protein Isolate
S25-037298	Pea Protein Powder	Vegan Pea Protein Isolate
S25-037299	Protein Blend Powder	Vegan Pea and Fava Bean Isolate Blend
S25-037300	Protein Blend Powder	Vegan Pea Protein and Soya Protein
S25-037301	Bulk Vegan Protein Powder	Vegan Protein Powder Blend (Pea, Soya, Pumpkin Seed, Quinoa Flour, Flaxseed)
S25-037454	Protein Blend Powder	Whey Protein and Soy Protein
S25-037455	Protein Superfood Shake	Pea Protein Powder Vegan Shake
S25-037459	Plant Protein Blend Powder	Pea Protein and Rice Protein
S25-037302	Protein Flapjacks Cocoa Oat	Soya Protein & Oat Bars
S25-037303	Protein Flapjacks	Soya Protein & Oat Bars
S25-037304	Oat Square Salted Caramel	Vegan Protein Blend Bars
S25-037453	Complete Nutrition Bar Peanut Caramel	Protein Blend Bars
S25-037315	Soy based product	Organic Tofu
S25-037316	Pea based product	Plant based ham
S25-037317	Soy and Pea based product	Plant based bacon
S25-037318	Pea based product	Plant based burger
S25-037319	Vegetable protein based product (Wheat and pea)	Plant based steak
S25-037320	Soy based product	Plant based meatballs
S25-037339	Soy based product	Organic Tofu
S25-037340	Soy based product	Plant based Beef Style Mince
S25-037341	Pea based product	Vegan burger
S25-037342	Pea based product	Plant based sausages
S25-037456	Soy based product	Plant Protein Chocolate Flavour Drink
S25-037457	Pea based product	Plant based ready to drink shake
S25-037458	Pea based product	Plant based ready to drink shake
S25-037459	Pea & rice based product	Plant based protein powder

Table C1. Validation data for plant protein products – 11+ IAC Multimycotoxin IAC method

		AFB1	**AFB2**	**AFG1**	**AFG2**	**OTA**	**STG**
Spike level (n=6)	LOQ	0.25	0.25	0.25	0.25	0.25	0.25
1xLOQ	Average recovery (%)	81.5	90.0	85.4	90.9	68.8	74.6
	s.d.	3.9	3.1	5.8	5.6	19.4	2.6
	cv (%)	4.8	3.5	6.7	6.2	28.2	3.5
2xLOQ	Average recovery (%)	85.8	90.8	88.6	95.4	73.3	75.8
	s.d.	3.3	1.7	3.1	2.3	10.6	2.4
	cv (%)	3.8	1.8	3.5	2.4	14.4	3.2
10xLOQ	Average recovery (%)	86.5	90.4	88.7	93.2	*77.5**	75.6
	s.d.	3.6	2.7	2.7	3.6	*9.1**	3.0
	cv (%)	4.1	3.0	3.1	3.9	*11.8**	4.0
	Overall average recovery	**84.6**	**90.4**	**87.6**	**93.2**	***73.2***	**75.3**

*one outlying result excluded from this set, sample likely not spiked prior to extraction n=5

Table C1 contd. Validation data for plant protein products – 11+ IAC Multimycotoxin IAC method

		DON	HT2	T2	ZON	FB1	FB2	FB3
Spike level (n=6)	LOQ	5	5	5	2.5	10	5	5
1xLOQ	Average recovery (%)	99.6	112.3	99.4	89.0	78.7	72.2	76.2
	s.d.	4.9	17.3	2.1	3.3	4.4	3.4	3.4
	cv (%)	4.9	15.4	2.1	3.7	5.6	4.7	4.4
2xLOQ	Average recovery (%)	108.3	99.8	100.3	87.8	82.6	79.8	79.9
	s.d.	15.7	9.1	1.9	4.5	2.7	2.6	4.2
	cv (%)	14.5	9.1	1.9	5.1	3.3	3.3	5.3
10xLOQ	Average recovery (%)	104.9	96.4	97.8	85.0	86.5	80.5	84.5
	s.d.	5.7	3.4	3.5	3.0	3.1	2.3	1.7
	cv (%)	5.4	3.5	3.5	3.5	3.5	2.9	2.0
	Overall average recovery	**104.3**	**102.9**	**99.2**	**87.3**	**82.6**	**77.5**	**80.2**

Table C2. Mycotoxin results for Phase 1 plant protein powders, using 11+ IAC method. Results in µg/kg, corrected for recovery.

Fera Sample No.	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S24-069134	Pea Powder	<0.08	<0.08	<0.08	<0.08	**9.33**	<0.08
S24-069135	Soy Powder	<0.08	<0.08	<0.08	<0.08	**3.22**	<0.08
S24-069136	Pea Powder	**0.15**	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069137	Soy Powder	**2.24**	**0.12**	**2.46**	**0.12**	**4.15**	<0.08
S24-069138	Soy Powder	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069318	Pea Powder	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08

Fera Sample No.	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S24-069134	Pea Powder	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069135	Soy Powder	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069136	Pea Powder	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069137	Soy Powder	<1.56	<1.56	<1.56	**1.13**	<3.125	<1.56	<1.56
S24-069138	Soy Powder	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069318	Pea Powder	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56

Table C3. Mycotoxin results for Phase 1 Soy based products, using 11+ IAC method. Results in µg/kg, corrected for recovery.

Fera Sample No.	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S24-069319	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069320	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069321	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069322	Soy based product	**0.22**	<0.08	<0.08	<0.08	**0.39**	<0.08
S24-069323	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069882	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069883	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069884	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069890	Soy based product	<0.08	<0.08	<0.08	<0.08	**1.85**	<0.08

Fera Sample No.	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S24-069319	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069320	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069321	Soy based product	<1.56	<1.56	<1.56	<0.78	**24.70**	**3.74**	**3.31**
S24-069322	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069323	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069882	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069883	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069884	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069890	Soy based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56

Table C4. Mycotoxin results for Phase 1 Pea based products, using 11+ IAC method. Results in µg/kg, corrected for recovery.

Fera Sample No.	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S24-069324	Pea based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069885	Pea based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069886	Pea based product	<0.08	<0.08	<0.08	<0.08	**0.53**	<0.08
S24-069887	Pea based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069888	Pea based product	<0.08	<0.08	<0.08	<0.08	**0.26**	<0.08
S24-069889	Pea based product	<0.08	<0.08	<0.08	<0.08	**0.54**	<0.08

Fera Sample No.	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S24-069324	Pea based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069885	Pea based product	<1.56	<1.56	<1.56	**1.75**	<3.125	<1.56	<1.56
S24-069886	Pea based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069887	Pea based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069888	Pea based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069889	Pea based product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56

Table C5. Mycotoxin results for Phase 1 mixed plant protein products, using 11+ IAC method. Results in µg/kg, corrected for recovery.

Fera Sample No.	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S24-069325	Soy & wheat product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069326	Pea, wheat and soy product	<0.08	<0.08	<0.08	<0.08	**0.32**	**0.25**
S24-069327	Soy & wheat product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069328	Soy & wheat product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S24-069891	Soy & wheat product	<0.08	<0.08	<0.08	<0.08	**0.11**	<0.08

Fera Sample No.	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S24-069325	Soy & wheat product	**7.35**	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069326	Pea, wheat and soy product	**3.76**	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069327	Soy & wheat product	<1.56	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56
S24-069328	Soy & wheat product	**3.07**	<1.56	<1.56	**1.1**	<3.125	<1.56	<1.56
S24-069891	Soy & wheat product	**2.85**	<1.56	<1.56	<0.78	<3.125	<1.56	<1.56

Table C6. Quality control data for Phase 1 11+ IAC mycotoxin method analysis. Values reported are % Recovery.

	AFB1	AFB2	AFG1	AFG2	OTA	STG
Target LOQ (µg/kg)	0.25	0.25	0.25	0.25	0.25	0.25
	Recovery (%)					
Sp 1x LOQ Powder	72.3	82.8	78.0	86.2	50.2	63.5
Sp 2x LOQ Powder	86.9	94.5	87.9	90.8	83.0	75.3
Sp 10x LOQ Powder	87.5	93.2	90.5	93.9	86.9	73.9
Average Powder Sp	82.2	90.2	85.5	90.3	73.4	70.9
Sp 1x LOQ Soy Tofu	84.1	79.7	83.1	81.1	84.6	73.9
Sp 2x LOQ Soy Tofu	88.1	92.0	90.6	95.0	78.7	75.5
Sp 10x LOQ Soy Tofu	88.4	94.1	93.2	97.3	81.6	75.9
Average Soy Sp	86.9	88.6	89.0	91.2	81.6	75.1
Sp 1x LOQ Pea product	81.1	93.2	79.9	82.0	74.6	72.6
Sp 2x LOQ Pea product	92.6	96.4	96.3	94.8	70.5	77.7
Sp 10x LOQ Pea product	89.6	97.0	93.3	97.3	82.2	77.4
Average Pea product Sp	87.7	95.6	89.8	91.4	75.8	75.9
Sp 1x LOQ Mixed	92.5	92.0	90.8	94.3	87.1	80.4
Sp 2x LOQ Mixed	90.0	93.2	88.3	97.1	86.0	80.1
Sp 10x LOQ Mixed	90.0	92.0	90.4	90.6	79.1	80.5
Average Mixed Sp	90.8	92.4	89.8	94.0	84.1	80.4

Table C6. contd. Quality control data for 11+ IAC mycotoxin method analysis. Values reported are % recovery.

	DON	HT2	T2	ZON	FB1	FB2	FB3
Target LOQ (µg/kg)	5	5	5	2.5	10	5	5
Recovery (%)							
Sp 1x LOQ Powder	89.9	90.1	88.5	78.6	79.4	75.0	75.1
Sp 2x LOQ Powder	107.1	98.0	98.1	88.7	89.3	85.8	89.8
Sp 10x LOQ Powder	97.6	100.1	99.8	82.6	92.2	86.0	89.1
Average Powder Recovery	98.2	96.1	95.5	83.3	87.0	82.3	84.7
Sp 1x LOQ Soy Tofu	85.5	91.5	91.2	77.7	58.9	54.8	57.1
Sp 2x LOQ Soy Tofu	99.5	85.8	94.8	91.0	63.0	58.4	63.1
Sp 10x LOQ Soy Tofu	94.8	94.5	95.5	87.3	81.7	74.0	73.9
Average Soy Recovery	93.3	90.6	93.8	85.3	67.9	62.4	64.7
Sp 1x LOQ Pea product	98.1	81.4	92.1	92.4	52.8	56.0	74.2
Sp 2x LOQ Pea product	101.4	88.2	104.9	97.5	60.4	60.7	71.2
Sp 10x LOQ Pea product	102.9	95.6	102.5	93.0	78.0	72.9	75.3
Average Pea product Recovery	100.8	88.4	99.8	94.3	63.7	63.2	73.5
Sp 1x LOQ Mixed	104.6	85.7	101.8	96.4	86.5	75.2	72.7
Sp 2x LOQ Mixed	115.2	97.3	98.6	88.3	84.5	79.2	76.4
Sp 10x LOQ Mixed	94.1	90.1	94.9	87.4	80.3	74.7	81.1
Average Mixed Recovery	104.6	91.0	98.4	90.7	83.8	76.4	76.7

Table C7. Mycotoxin results for Phase 2 plant protein powders, using 11+ IAC method. Results in µg/kg, corrected for recovery

Analytical Batch PC25-04914

Fera Sample Number	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S25-037296	Pea protein powder	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037297	Soy protein powder	<0.08	<0.08	<0.08	<0.08	**0.44**	<0.08
S25-037298	Pea protein powder	<0.08	<0.08	<0.08	<0.08	**2.11**	<0.08
S25-037299	Pea & fava protein powder	**0.34**	<0.08	<0.08	<0.08	**0.92**	**0.20**
S25-037300	Pea & soy protein powder	**0.11**	<0.08	<0.08	<0.08	**0.41**	<0.08
S25-037301	Vegan blend protein powder	<0.08	<0.08	<0.08	<0.08	**0.46**	<0.08
S25-037454	Whey & soy protein powder	**0.63**	<0.08	**0.29**	<0.08	**1.70**	<0.08
S25-037455	Pea protein shake powder	<0.08	<0.08	<0.08	<0.08	**2.27**	<0.08
S25-037459	Pea & rice protein powder	**0.44**	<0.08	<0.08	<0.08	**0.37**	**1.55**

Fera Sample Number	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S25-037296	Pea protein powder	<1.56	<1.56	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037297	Soy protein powder	<1.56	<1.56	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037298	Pea protein powder	<1.56	<1.56	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037299	Pea & fava protein powder	<1.56	<1.56	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037300	Pea & soy protein powder	<1.56	<1.56	<1.56	**2.80**	<3.13	<1.56	<1.56
S25-037301	Vegan blend protein powder	<1.56	<1.56	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037454	Whey & soy protein powder	<1.56	<1.56	<1.56	**2.56**	**14.91**	**5.89**	<1.56
S25-037455	Pea protein shake powder	**3.43**	**2.45**	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037459	Pea & rice protein powder	<1.56	<1.56	<1.56	**1.06**	<3.13	<1.56	<1.56

Table C8. Mycotoxin results for Phase 2 meat replacement products, using 11+ IAC method. Results in µg/kg, corrected for recovery

Analytical Batch PC25-04661

Fera Sample Number	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S25-037315	Soy product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037316	Pea based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037317	Soy and pea based product	<0.08	<0.08	<0.08	<0.08	**0.32**	<0.08
S25-037318	Pea based product	<0.08	<0.08	<0.08	<0.08	**0.22**	<0.08
S25-037319	Wheat and pea based product	<0.08	<0.08	<0.08	<0.08	**0.13**	**0.33**
S25-037320	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037339	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037340	Soy based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037341	Pea based product	**0.12**	<0.08	<0.08	<0.08	**0.23**	<0.08
S25-037342	Pea based product	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08

Fera Sample Number	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S25-037315	Soy product	<1.56	<3.13	<1.56	**1.28i**	<3.13	<1.56	<1.56
S25-037316	Pea based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037317	Soy and pea based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037318	Pea based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037319	Wheat and pea based product	**11.22**	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037320	Soy based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037339	Soy based product	<1.56	<3.13	<1.56	**1.88**	<3.13	<1.56	<1.56
S25-037340	Soy based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037341	Pea based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037342	Pea based product	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56

i indicates result did not confirm by ion ratio, uncorrected concentration was at the LOQ

Table C9. Mycotoxin results for Phase 2 ready to drink protein shake products and protein bars, using 11+ IAC method. Results in µg/kg, corrected for recovery

Analytical Batch PC25-04661

Fera Sample Number	Product type	AFB1	AFB2	AFG1	AFG2	OTA	STG
S25-037456	RTD protein shake	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037457	RTD protein shake	**0.23**	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037458	RTD protein shake	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037302	Protein & cereal bar	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
S25-037303	Protein & cereal bar	**0.15**	<0.08	<0.08	<0.08	**0.55**	<0.08
S25-037304	Protein & cereal bar	<0.08	<0.08	<0.08	<0.08	**0.39**	**0.22**
S25-037453	Protein & cereal bar	<0.08	<0.08	<0.08	<0.08	**0.39**	**0.54**

Fera Sample Number	Product type	DON	HT2	T2	ZON	FB1	FB2	FB3
S25-037456	RTD protein shake	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037457	RTD protein shake	**14.55**	<3.13	<1.56	**2.08**	<3.13	<1.56	<1.56
S25-037458	RTD protein shake	**2.32**	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037302	Protein & cereal bar	<1.56	**4.16**	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037303	Protein & cereal bar	<1.56	<3.13	<1.56	<0.78	<3.13	<1.56	<1.56
S25-037304	Protein & cereal bar	**2.44**	<3.13	<1.56	**1.67**	<3.13	<1.56	<1.56
S25-037453	Protein & cereal bar	**8.32**	<3.13	<1.56	**4.85**	<3.13	<1.56	<1.56

Table C10. Quality control data for Phase 2 11+ mycotoxin method analysis.

Batch PC25-04914 – protein powders

Recovery (%)	AFB1	AFB2	AFG1	AFG2	DON	FB1	FB2	FB3	HT2	OTA	STG	T2	ZON
Spike level (µg/kg)	2.5	2.5	2.5	2.5	50	100	50	50	50	2.5	2.5	50	25
Sp1	76.6	85.7	81.4	83.3	106.5	80.0	75.4	77.5	89.2	84.1	64.0	93.5	73.7
Sp2	79.3	85.8	84.0	85.8	104.2	84.6	80.3	82.4	87.8	82.1	63.4	95.9	74.9
Sp3	88.1	91.4	91.2	92.1	85.5	86.9	87.6	87.8	87.4	86.0	74.7	97.3	78.5
Sp4	71.7	83.2	80.5	88.6	101.4	84.3	81.0	89.2	94.1	80.9	37.7	104.8	67.6
Average	**78.9**	**86.5**	**84.3**	**87.4**	**99.4**	**83.9**	**81.1**	**84.2**	**89.6**	**83.3**	**60.0**	**97.9**	**73.7**

Batch PC25-04661 – vegan meat replacement products, protein shakes and cereal & protein bars

Recovery (%)	AFB1	AFB2	AFG1	AFG2	DON	FB1	FB2	FB3	HT2	OTA	STG	T2	ZON
Spike level (µg/kg)	2.5	2.5	2.5	2.5	50	100	50	50	50	2.5	2.5	50	25
Vegan meat Sp1	88.6	88.8	89.6	87.9	90.9	86.7	81.2	83.2	91.8	82.9	59.2	97.0	77.2
Vegan meat Sp2	87.1	85.5	86.9	87.9	95.5	84.8	79.7	82.0	91.4	89.6	62.3	97.2	79.3
Average	**87.8**	**87.1**	**88.2**	**87.9**	**93.2**	**85.7**	**80.4**	**82.6**	**91.6**	**86.3**	**60.8**	**97.1**	**78.3**
Vegan milk Sp1	80.0	79.7	75.6	79.6	87.5	83.5	88.3	84.4	86.3	83.8	53.6	94.5	77.6
Vegan milk Sp2	78.3	85.8	87.8	84.1	74.3	85.0	82.8	86.7	98.6	92.7	69.1	95.9	75.1
Vegan milk Sp3	93.7	93.2	92.0	93.9	89.2	104.1	101.9	102.8	98.2	84.4	74.5	104.3	89.7
Average	**84.0**	**86.2**	**85.1**	**85.9**	**83.6**	**90.8**	**91.0**	**91.3**	**94.4**	**87.0**	**65.7**	**98.2**	**80.8**
Cereal Sp1	87.0	94.7	94.5	90.0	93.5	100.9	102.0	100.0	102.6	73.4	65.9	110.5	88.4
Cereal Sp2	87.6	88.2	88.5	90.5	92.1	97.4	92.8	94.0	100.5	90.8	62.8	104.9	76.7
Cereal Sp3	85.0	86.3	87.8	88.2	95.1	92.0	94.6	93.5	95.8	94.0	66.9	99.3	82.7
Cereal Sp4	82.9	79.1	82.7	81.8	96.9	74.4	78.5	79.4	92.1	74.3	58.1	96.0	79.8
Average	**85.6**	**87.1**	**88.4**	**87.6**	**94.4**	**91.2**	**91.9**	**91.7**	**97.7**	**83.1**	**63.4**	**102.7**	**81.9**

Table C11. Validation data for ergot alkaloids

Spike level*		**Ergocornine**	**Ergocorninine**	**Ergocristine**	**Ergocristinine**	**a-Ergocryptine**	**b-Ergocryptine#**	**a+b Ergocryptinine**
1xLOQ (n=6)	Average recovery (%)	76.1	84.8	79.5	100.9	76.6	-	100.3
	s.d.	3.0	2.8	3.4	1.9	4.1	-	3.3
	cv (%)	4.0	3.3	4.2	1.9	5.4	-	3.2
2xLOQ (n=5)	Average recovery (%)	75.1	78.1	73.8	89.5	74.9	-	91.9
	s.d.	1.9	0.6	1.7	1.8	3.1	-	2.0
	cv (%)	2.5	0.8	2.2	2.0	4.1	-	2.2
10xLOQ (n=6)	Average recovery (%)	76.3	78.1	76.6	86.5	77.0	-	90.3
	s.d.	1.7	2.8	2.2	2.3	2.0	-	1.6
	cv (%)	2.3	3.6	2.8	2.7	2.6	-	1.8
	Overall Average recovery	**75.9**	**80.5**	**76.8**	**92.5**	**76.2**	**-**	**94.3**

*LOQ for individual ergot alkaloids was 0.5 µg/kg each.
no analytical standard available for b-Ergocryptine so cannot be added as spike. Results in samples quantified from a-Ergocryptine.

Table C11. Contd. Validation data for ergot alkaloids

Spike level*		**Ergometrine**	**Ergometrinine**	**Ergosine**	**Ergosinine**	**Ergotamine**	**Ergotaminine**
1xLOQ (n=6)	Average recovery (%)	95.8	86.5	83.7	87.9	92.7	76.7
	s.d.	3.0	3.0	2.8	3.8	3.0	2.5
	cv (%)	3.1	3.5	3.4	4.3	3.3	3.2
2xLOQ (n=5)	Average recovery (%)	95.0	85.7	78.1	86.9	85.9	74.7
	s.d.	2.1	0.9	2.0	0.9	2.6	1.1
	cv (%)	2.3	1.1	2.6	1.0	3.0	1.5
10xLOQ (n=6)	Average recovery (%)	97.5	85.2	77.5	87.7	84.4	76.2
	s.d.	2.1	0.8	2.4	2.0	2.7	2.4
	cv (%)	2.2	0.9	3.0	2.3	3.2	3.1
	Overall Average recovery	**96.2**	**85.8**	**79.9**	**87.5**	**87.8**	**75.9**

*LOQ for individual ergot alkaloids was 0.5 µg/kg each.

Table C12. Ergot alkaloid results for Phase 1 plant protein products containing wheat protein. Results in µg/kg, corrected for recovery

Fera sample number	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	a-Ergocryptine	b-Ergocryptine#	a+b-Ergocryptinine
S24-069325	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S24-069326	<0.5	**0.32***	**0.44***	**0.58**	<0.5	<0.5	**0.40***
S24-069327	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S24-069328	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S24-069891	**0.25***	**0.56**	**0.80**	**1.47**	<0.5	<0.5	**0.55**

Fera sample number	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Sum ergot alkaloids
S24-069325	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S24-069326	<0.5	<0.5	**0.60**	**0.62**	**1.50**	**1.24**	**5.71**
S24-069327	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S24-069328	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S24-069891	<0.5	<0.5	**1.24**	**0.77**	**0.83**	**0.42***	**6.90**

#results quantified with a-Ergocryptine
Reporting limit (LOQ) set for lowest validated concentration was 0.5 µg/kg. *residues reported that are greater than lowest calibration standard (0.25 µg/kg) where ion ratio was confirmed.

Table C13. Ergot alkaloid results for Phase 2 ready to drink shakes and protein bars. Results in µg/kg, corrected for recovery

Fera sample number	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	a-Ergocryptine	b-Ergocryptine#	a+b-Ergocryptinine
S25-037456	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037457	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037458	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037302	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037303	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037304	<0.5	<0.5	<0.5 **(0.25*)**	0.55	<0.5	<0.5	<0.5
S25-037453	<0.5 **(0.36*)**	**0.7**	**0.75**	**1.06**	<0.5 **(0.32*)**	<0.5	**0.69**

Fera sample number	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Sum ergot alkaloids
S25-037456	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037457	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037458	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037302	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037303	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037304	<0.5	<0.5	**0.75**	<0.5 **(0.36*)**	**1.07**	<0.5 **(0.43*)**	**3.41**
S25-037453	<0.5	<0.5	**2.23**	**0.82**	**3.36**	**1.01**	**11.01**

#results quantified with a-Ergocryptine

Reporting limit set equivalent to lowest validated concentration (0.5 µg/kg). Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.

Table C14. Ergot alkaloid results for Phase 2 plant protein powder products. Results in µg/kg, corrected for recovery

Fera sample number	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	a-Ergocryptine	b-Ergocryptine#	a+b-Ergocryptinine
S25-037296	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037297	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037298	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037299	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037300	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037301	<0.5	<0.5	<0.5 (0.27*)	<0.5	<0.5	<0.5	<0.5
S25-037454	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037455	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037459	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

Fera sample number	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Sum ergot alkaloids
S25-037296	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037297	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037298	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037299	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037300	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037301	<0.5	<0.5	<0.5 (0.29*)	<0.5	<0.5	<0.5	**0.56**
S25-037454	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037455	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037459	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0

#results quantified with a-Ergocryptine. Reporting limit (LOQ) set equivalent to lowest validated concentration (0.5 µg/kg). Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.

Table C15. Ergot alkaloid results for Phase 2 plant protein meat replacement products. Results in µg/kg, corrected for recovery

Fera sample number	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	a-Ergocryptine	b-Ergocryptine#	a+b-Ergocryptinine
S25-037315	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037316	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037317	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037318	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037319	<0.5	<0.5	<0.5 (0.30*)	<0.5 (0.37*)	<0.5	<0.5	<0.5
S25-037320	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037339	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037340	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037341	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
S25-037342	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

Fera sample number	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Sum ergot alkaloids
S25-037315	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037316	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037317	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037318	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037319	<0.5	<0.5	**0.65**	<0.5 (0.35*)	**0.84**	<0.5 (0.30*)	**2.81**
S25-037320	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037339	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037340	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037341	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0
S25-037342	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<6.0

#results quantified with a-Ergocryptine. Reporting limit (LOQ) set equivalent to lowest validated concentration (0.5 µg/kg). Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.

Table C16. Method verification for tropane alkaloids in plant protein products

Sample Number	Sample Name	Recovery (%)	At	Sc
S24-065037	Meat free bacon	Spike	95.3	96.3
	(Soy/wheat)	Tissue Standard	126.6	132.7
		True Recovery	75.3	72.5
S24-065038	Organic Tofu	Spike	98.0	101.8
	(Soy)	Tissue Standard	110.3	108.4
		True Recovery	88.9	93.9
S24-065042	Vegetarian Hoisin duck	Spike	88.3	95.5
	(Soy/wheat)	Tissue Standard	103.0	123.2
		True Recovery	85.7	77.5
S24-065045	Meatballs	Spike	93.6	98.3
	(Pea)	Tissue Standard	101.9	108.2
		True Recovery	91.8	90.8
S24-065044	Veggie Mince	Spike	92.1	98.7
	(Soy)	Tissue Standard	103.7	128.2
		True Recovery	88.8	77.0
S24-065041	Soya powder	Spike	96.2	98.3
	(Soy)	Tissue Standard	106.9	123.9
		True Recovery	90.0	79.3
S24-082197	Pea Powder	Spike	94.3	101.3
	(Pea)	Tissue Standard	97.0	104.1
		True Recovery	97.3	97.3
	Average True recovery (n=7)		88.3	84.0
	s.d.		6.73	9.71
	cv (%)		7.6	11.6

Table C17. Tropane alkaloid (atropine and scopolamine) results in Phase 1 plant protein products. Results in µg/kg corrected for recovery.

Sample	Product	At	Sc
S24-069135	Soy Protein Powder	<0.1	<0.1
S24-069137	Soy Protein Powder	<0.1	<0.1
S24-069138	Soy Protein Powder	<0.1	<0.1
S24-069319	Soy Protein Powder	<0.1	<0.1
S24-069320	Soy based product	<0.1	<0.1
S24-069321	Soy based product	<0.1	<0.1
S24-069322	Soy based product	**0.40**	**0.14**
S24-069323	Soy based product	**0.20**	<0.1
S24-069882	Soy based product	<0.1	<0.1
S24-069883	Soy based product	**1.23**	**0.48**
S24-069884	Soy based product	<0.1	<0.1
S24-069890	Soy based product	<0.1	<0.1
S24-069134	Pea Protein Powder	<0.1	<0.1
S24-069136	Pea Protein Powder	<0.1	<0.1
S24-069318	Pea Protein Powder	<0.1	<0.1
S24-069324	Pea based product	<0.1	<0.1
S24-069325	Soy & wheat product	**0.19**	<0.1
S24-069326	Pea, wheat and soy product	<0.1	<0.1
S24-069327	Soy & wheat product	<0.1	<0.1
S24-069328	Soy & wheat product	<0.1	<0.1
S24-069885	Pea based product	<0.1	<0.1
S24-069886	Pea based product	<0.1	<0.1
S24-069887	Pea based product	<0.1	<0.1
S24-069888	Pea based product	<0.1	<0.1
S24-069889	Pea based product	<0.1	<0.1
S24-069891	Soy & wheat product	<0.1	<0.1

Table C18. Quality control results for tropane alkaloids.

Spike level	Product type	Recovery (%)	
		At	**Sc**
Sp 1µg/kg	Tofu (soy)	102.3	101.0
Sp 2µg/kg	Tofu (soy)	100.2	99.1
Sp 10µg/kg	Tofu (soy)	101.3	101.0
	Tofu Average	101.3	100.4
Sp 1µg/kg	Pea product	113.5	106.2
Sp 2µg/kg	Pea product	118.4	118.9
Sp 10µg/kg	Pea product	109.2	109.1
	Pea Average	113.7	111.4
Sp 1µg/kg	Wheat/pea/soy	125.3	124.6
Sp 2µg/kg	Wheat/pea/soy	119.6	120.0
Sp 10µg/kg	Wheat/pea/soy	117.8	116.6
	Average	120.9	120.4

Table C19. Acrylamide results for Phase 1 plant protein products. Results in µg/kg, corrected for recovery

Batch PC25-01440

Fera sample number	Acrylamide concentration, µg/kg
S24-069134	<30
S24-069135	<30
S24-069136	<30
S24-069137	<30
S24-069138	<30
S24-069318	<30
S24-069319	<30
S24-069320	<30
S24-069321	<30
S24-069322	<30
S24-069323	<30
S24-069324	**36.8**
S24-069325	<30
S24-069326	<30
S24-069327	<30
S24-069328	<30
S24-069882	<30
S24-069883	<30
S24-069884	<30
S24-069885	<30
S24-069886	<30
S24-069887	<30
S24-069888	<30
S24-069889	<30
S24-069890	<30
S24-069891	**41.7**

Table C20. Mycotoxin results for Phase 2 plant protein powders and protein bar products, MM2 Fusarium toxin method FSG 818 (positive mode). Results in µg/kg, corrected for recovery

Batch PC25-04855

Fera sample number	3-AcDON	15-AcDON	DON	DAS	FUSX	HT-2	NEO	NIV	T-2	T-2 a3Glc
S25-037296	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037297	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037298	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037299	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037300	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037301	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037454	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037455	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037459	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037302	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037303	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037304	<10	<20	<10	<10	22.5i	<10	<10	<50	<10	<10
S25-037453	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10

Values flagged ‘i’ fail ion ratio so identity was not confirmed.

Table C21. Mycotoxin results for Phase 2 plant protein powders and protein bar products, MM2 Fusarium toxin method FSG 818 (negative mode). Results in µg/kg, corrected for recovery

Batch PC25-04855

Fera sample number	DON-3Glc	a-ZOL	b-ZOL	a-ZOL-14-Glc	b-ZOL-14-Glc	ZON	ZON-14-Glc
S25-037296	<10	<5	<5	<5	<5	<2.5	<5
S25-037297	<10	<5	<5	<5	<5	<2.5	<5
S25-037298	<10	<5	<5	<5	<5	<2.5	<5
S25-037299	<10	<5	<5	<5	<5	<2.5	<5
S25-037300	<10	<5	<5	<5	<5	<2.5 (2.3*)	<5
S25-037301	<10	<5	<5	<5	<5	<2.5	<5
S25-037454	<10	<5	<5	<5	<5	<2.5 (2.3*)	<5
S25-037455	<10	<5	<5	<5	<5	<2.5	<5
S25-037459	<10	<5	<5	<5	<5	<2.5	<5
S25-037302	<10	<5	<5	<5	<5	<2.5	<5
S25-037303	<10	<5	<5	<5	<5	<2.5	<5
S25-037304	<10	<5	<5	<5	<5	<2.5 (1.7*)	<5
S25-037453	<10	<5	<5	<5	<5	4.6	<5

Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.

Table C22. Mycotoxin results for Phase 2 plant protein products, meat replacements and ready to drink shakes MM2 Fusarium toxin method (positive mode). Results in µg/kg, corrected for recovery

Batch PC25-05000

Fera sample number	3-AcDON	15-AcDON	DON	DAS	FUSX	HT-2	NEO	NIV	T-2	T-2 a3Glc
S25-037315	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037316	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037317	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037318	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037319	<10	<20	12.6	<10	<10	<10	<10	<50	<10	<10
S25-037320	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037339	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037340	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037341	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037342	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037456	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037457	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10
S25-037458	<10	<20	<10	<10	<10	<10	<10	<50	<10	<10

Table C23. Mycotoxin results for Phase 2 plant protein products, meat replacements and ready to drink shakes MM2 Fusarium toxin method (negative mode). Results in µg/kg, corrected for recovery

Batch PC25-05000

Fera sample number	DON-3Glc	a-ZOL	b-ZOL	a-ZOL-14-Glc	b-ZOL-14-Glc	ZON	ZON-14-Glc
S25-037315	<10	<5	<5	<5	<5	<2.5	<5
S25-037316	<10	<5	<5	<5	<5	<2.5	<5
S25-037317	<10	<5	<5	<5	<5	<2.5	<5
S25-037318	<10	<5	<5	<5	<5	<2.5	<5
S25-037319	<10	<5	<5	<5	<5	<2.5	<5
S25-037320	<10	<5	<5	<5	<5	<2.5	<5
S25-037339	<10	<5	<5	<5	<5	<2.5	<5
S25-037340	<10	<5	<5	<5	<5	<2.5	<5
S25-037341	<10	<5	<5	<5	<5	<2.5	<5
S25-037342	<10	<5	<5	<5	<5	<2.5	<5
S25-037456	<10	<5	<5	<5	<5	<2.5	<5
S25-037457	<10	<5	<5	<5	<5	<2.5	<5
S25-037458	<10	<5	<5	<5	<5	<2.5	<5

Table C24. Enniatins, beauvericin and fusaric acid results for Phase 2 plant protein shakes and protein bars. Results in µg/kg, corrected for recovery.

Batch PC25-05800

Fera sample number	Beauvericin	Enniatin A	Enniatin A1	Enniatin B	Enniatin B1	Fusaric acid
S25-037456	<2.5	<2.5	<2.5	<2.5	<2.5	<10
S25-037457	<2.5	<2.5	<2.5	<2.5	<2.5	<10
S25-037458	<2.5	<2.5	<2.5	<2.5	<2.5	<10
Average recovery (%)	78.1	76.4	82.8	78.3	80.1	102.0
S25-037302	<2.5	<2.5	<2.5	**<2.5 (0.6*)**	**<2.5 (1.2*)**	**<10 (8.9*)**
S25-037303	<2.5	<2.5	<2.5	<2.5	<2.5	**26.9**
S25-037304	<2.5	**<2.5 (0.6*)**	**<2.5 (1.5*)**	**<2.5 (2.4*)**	**4.8**	**<10 (6.1*)**
S25-037453	**<2.5 (0.9*)**	<2.5	**<2.5 (1.0*)**	**7.3**	**7.1**	**<10 (4.8*)**
Average recovery (%)	93.0	89.2	104.5	94.7	102.9	55.1

Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.

Table C25. Enniatins, beauvericin and fusaric acid results for Phase 2 plant protein powder products. Results in µg/kg, corrected for recovery

Batch PC25-05800

Fera sample number	Beauvericin	Enniatin A	Enniatin A1	Enniatin B	Enniatin B1	Fusaric acid
S25-037296	<2.5	<2.5	<2.5	<2.5	<2.5	<10
S25-037297	<2.5	<2.5	<2.5	<2.5 **(0.6*)**	<2.5	<10
S25-037298	<2.5	<2.5 **(1.4*)**	**5.5**	**9.2**	**21.6**	<10
S25-037299	<2.5 **(0.9*)**	<2.5 **(1.2*)**	**2.5**	**11.8**	**18.2i**	**25.3**
S25-037300	<2.5 **(1.0*)**	<2.5 **(0.7*)**	<2.5	<2.5 **(0.8*)**	**3.0i**	**22.9**
S25-037301	<2.5	<2.5	<2.5 **(0.8*)**	<2.5 **(2.3*)**	**16.0i**	**43.4**
S25-037454	**13.5**	<2.5	<2.5	<2.5 **(1.4*)**	<2.5	**>1000q**
S25-037455	<2.5 **(0.8*)**	<2.5 **(1.2*)**	**6.6**	**9.6**	**29.1**	<10
S25-037459	<2.5 **(1.0*)**	<2.5	<2.5	<2.5	**3.0i**	<10
Average recovery (%)	83.0	73.1	85.3	89.4	85.3	83.0

Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio. Values flagged 'i' fail ion ratio so identity was not confirmed. Values flagged 'q' exceeded the calibration range and are indicative for concentration.

Table C26. Enniatins, beauvericin and fusaric acid results for Phase 2 plant protein meat replacement products. Results in µg/kg, corrected for recovery

Batch PC25-05800

Fera sample number	Beauvericin	Enniatin A	Enniatin A1	Enniatin B	Enniatin B1	Fusaric acid
S25-037315	<2.5	<2.5	<2.5	<2.5 **(0.7*)**	6.0i	<10
S25-037316	<2.5 **(1.8*)**	<2.5	<2.5	<2.5	<2.5	**118.4**
S25-037317	<2.5	<2.5	<2.5	<2.5	<2.5	<10 **(4.1*)**
S25-037318	<2.5	<2.5	<2.5	<2.5 **(0.7*)**	<2.5	**13.1**
S25-037319	<2.5 **(0.9*)**	<2.5 **(1.2*)**	<2.5 **(2.4*)**	**6.4**	**9.8**	<10 **(8.0*)**
S25-037320	<2.5	<2.5	<2.5	<2.5	<2.5 **(0.7*)**	<10 **(6.9*)**
S25-037339	<2.5	<2.5	<2.5	<2.5 **(0.8*)**	2.6i	<10
S25-037340	<2.5	<2.5	<2.5	<2.5	<2.5	<10 **(9.8*)**
S25-037341	<2.5	<2.5	<2.5	<2.5	<2.5	<10 **(6.7*)**
S25-037342	<2.5	<2.5	<2.5	<2.5	<2.5	<10 **(4.9*)**
Average recovery (%)	69.9	68.0	77.6	76.9	75.6	94.1

Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio. Values flagged 'i' fail ion ratio so identity was not confirmed.

Table C27. Pyrrolizidine alkaloid results for Phase 2 plant protein meat replacement and protein shake products. Results in µg/kg, corrected for recovery

Batch PC25-05189

Fera sample number	Em	Er	Ht	Im	Jb	Lc	Id/Ly*	Mc	Rt	Sn	Sp	Sk
S25-037315	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037316	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037317	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037318	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037319	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037320	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037339	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037340	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037341	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037342	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
Spike 1	72.3	69.0	59.6	74.8	125.1	79.0	78.1	73.5	69.7	68.6	30.0	77.9
Tissue Standard 1	79.6	76.5	63.4	81.5	76.8	86.0	83.3	80.2	78.8	75.3	75.4	84.1
True recovery Sp1	90.8	90.1	94.1	91.8	162.9	91.8	93.7	91.6	88.5	91.1	39.8	92.6
S25-037456	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037457	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037458	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
Spike 2	52.3	58.0	48.8	54.0	108.8	56.9	58.0	60.4	56.6	48.5	10.2	78.1
Tissue Standard 2	73.7	78.4	64.1	78.2	85.8	74.3	75.7	81.4	76.8	66.4	61.8	93.5
True Recovery Sp2	71.0	74.0	76.2	69.1	126.9	76.5	76.6	74.2	73.7	73.1	16.4	83.6

*reporting level increased as 2 compounds co-elute

Table C28. Pyrrolizidine alkaloid results for Phase 2 plant protein powder and protein bar products. Results in µg/kg, corrected for recovery.

Batch PC25-05061

Fera sample number	Em	Er	Ht	Im	Jb	Lc	Id/Ly*	Mc	Rt	Sn	Sp	Sk
S25-037296	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037297	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037298	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037299	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037300	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037301	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037454	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037455	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037459	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
Spike 1	54.7	60.1	67.3	74.6	114.3	75.5	77.6	63.5	66.8	62.0	10.4	84.3
Tissue Standard Sp1	59.7	69.0	65.6	85.9	69.1	66.1	82.4	69.1	64.8	64.8	60.2	88.3
True recovery Sp1	91.6	87.1	102.5	86.9	165.4	114.2	94.1	91.9	103.0	95.7	17.2	95.5
S25-037302	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037303	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037304	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
S25-037453	<1	<1	<1	<1	<1	<1	<2	<1	<1	<1	<1	<1
Spike Sp2	46.9	28.1	38.8	34.3	39.8	55.6	30.1	24.4	32.1	37.2	35.4	42.1
Tissue Standard Sp2	51.5	30.2	42.0	38.9	31.8	66.7	31.4	26.6	34.2	41.5	42.1	46.2
True recovery Sp2	91.0	93.0	92.2	88.1	124.9	83.3	95.8	91.7	93.7	89.7	84.0	91.2

*reporting level increased as 2 compounds co-elute

Table C29. Tropane alkaloid results for Phase 2 plant protein meat replacements and ready to drink shakes. Results in µg/kg, corrected for recovery.

Batch PC25-05189

Fera sample number	Atropine (At)	Scopolamine (Sc)
S25-037315	<0.25	<0.25
S25-037316	<0.25	<0.25
S25-037317	<0.25	<0.25
S25-037318	**0.52**	**0.25i**
S25-037319	<0.25	<0.25
S25-037320	<0.25	<0.25
S25-037339	**1.15**	<0.25
S25-037340	<0.25	<0.25
S25-037341	<0.25	<0.25
S25-037342	<0.25	<0.25
Spike 1 (Recovery, %)	112.6	126.5
Tissue Standard Sp1	128.5	151.7
True recovery Sp1	87.6	83.4
S25-037456	<0.25	<0.25
S25-037457	<0.25	<0.25
S25-037458	<0.25	<0.25
Spike 2 (Recovery, %)	104.6	122.4
Tissue Standard Sp2	135.8	162.3
True Recovery Sp2	77.1	75.4

Values flagged ‘i’ fail ion ratio so identity was not confirmed.

Table C30. Tropane alkaloid results for Phase 2 plant protein powder and protein bar products. Results in µg/kg, corrected for recovery.

Batch PC25-05061

Fera sample number	Atropine (At)	Scopolamine (Sc)
S25-037296	<0.25	<0.25
S25-037297	<0.25	<0.25
S25-037298	<0.25	<0.25
S25-037299	<0.25	<0.25
S25-037300	<0.25	<0.25
S25-037301	<0.25	0.45i
S25-037454	<0.25	0.50i
S25-037455	<0.25	<0.25
S25-037459	<0.25	0.25i
Spike 1 (Recovery, %)	131.4	153.1
Tissue Standard Sp1	131.2	155.8
True recovery Sp1	100.1	98.3
S25-037302	<0.25	<0.25
S25-037303	<0.25	<0.25
S25-037304	<0.25	<0.25
S25-037453	**0.79**	**0.38**
Spike 2 (Recovery, %)	111.2	131.7
Tissue Standard Sp2	116.2	139.6
True Recovery Sp2	95.8	94.3

Values flagged ‘i’ fail ion ratio so identity was not confirmed

Table C31. Method verification results for Alternaria toxins method

	Altenuene	Alternariol	Alternariol Monomethyl Ether	Tentoxin	Tenuazonic Acid
Spike level (µg/kg)	1	1	1	5	5
1xLOQ mean recovery (%)	126.2	109.8	105.5	96.7	101.9
sd	10.4	10.0	3.7	5.4	5.0
cv (%)	8.2	9.1	3.5	5.6	4.9
Spike level (µg/kg)	2	2	2	10	10
2xLOQ mean recovery (%)	107.6	104.7	102.8	99.1	96.9
sd	5.3	6.3	1.4	4.1	2.3
cv (%)	4.9	6.0	1.3	4.1	2.4
Spike level (µg/kg)	5.0	5.0	5.0	25.0	25.0
5xLOQ mean recovery (%)	104.8	104.7	103.1	102.8	98.1
sd	4.5	1.5	1.3	2.8	2.4
cv (%)	4.3	1.4	1.3	2.7	2.5

Table C32. Alternaria toxin results for Phase 2 plant protein products. Results in µg/kg, corrected for recovery

Batch PC25-05683

Fera sample number	Altenuene	Alternariol	Alternariol Monomethyl Ether	Tentoxin	Tenuazonic Acid
S25-037456	<1	<1	<1	<5	**<5 (3.8*)**
S25-037457	<1	<1	<1	<5	**<5 (4.3*)**
S25-037458	<1	<1	<1	<5	**6.5**
Average recovery, %	99.4	117.8	104.0	100.8	99.3
S25-037302	<1	<1	<1	<5	<5
S25-037303	<1	<1	<1	<5	**11.4**
S25-037304	<1	<1	<1	<5	<5
S25-037453	<1	<1	<1	<5	**18.0**
Average recovery, %	82.9	88.5	104.3	105.8	91.4
S25-037296	<1	<1	<1	<5	<5
S25-037297	<1	<1	<1	<5	<5
S25-037298	<1	**13.5**	**6.1**	<5	<5
S25-037299	<1	**5.6**	**3.4**	<5	<5
S25-037300	<1	<1	<1	<5	**<5 (3.8*)**
S25-037301	<1	**4.1**	**2.3**	<5	**5.6**
S25-037454	<1	**1.1i**	<1	<5	**5.4**
S25-037455	<1	**1.3i**	<1	<5	<5
S25-037459	<1	<1	<1	<5	<5
Average recovery, %	126.1	100.8	87.1	111.9	115.2
S25-037315	<1	<1	<1	<5	**6.6**
S25-037316	<1	<1	<1	<5	<5
S25-037317	<1	<1	<1	<5	**10.8**
S25-037318	<1	<1	<1	<5	**62.7**
S25-037319	<1	<1	<1	<5	**5.2**
S25-037320	<1	**1.1**	<1	<5	**22.3**
S25-037339	<1	<1	<1	<5	**11.5**
S25-037340	<1	<1	<1	<5	<5
S25-037341	<1	**5.0**	**2.1**	<5	<5
S25-037342	<1	<1	<1	<5	<5
Average recovery, %	99.4	104.9	106.7	104.4	106.4

Values in parentheses flagged '*' fell within the range of the lowest calibration standard and the LOQ, and identity was confirmed by ion ratio.
Values flagged 'i' fail ion ratio so identity was not confirmed.

Table C33. Citrinin results for Phase 2 samples, Results in µg/kg, corrected for recovery.

Batch PC25-04884 – protein powders and protein bars

Fera sample number	Product type	Citrinin (µg/kg)
S25-037296	Pea protein powder	<2.5
S25-037297	Soy protein powder	<2.5
S25-037298	Pea protein powder	<2.5
S25-037299	Pea & fava protein powder	<2.5
S25-037300	Pea & soy protein powder	<2.5
S25-037301	Vegan blend protein powder	<2.5
S25-037454	Whey & soy protein powder	<2.5
S25-037455	Pea protein shake powder	<2.5
S25-037459	Pea & rice protein powder	<2.5
S25-037302	Protein and cereal bar	<2.5
S25-037303	Protein and cereal bar	<2.5
S25-037304	Protein and cereal bar	<2.5
S25-037453	Protein and cereal bar	<2.5

Average recovery, %, for Batch PC25-04988 (n=3) = 99.3%

Batch PC25-04988 – meat replacement and protein shake products

Fera sample number	Product type	Citrinin (µg/kg)
S25-037315	Soy product	<2.5
S25-037316	Pea based product	<2.5
S25-037317	Soy and pea based product	<2.5
S25-037318	Pea based product	<2.5
S25-037319	Wheat and pea based product	<2.5
S25-037320	Soy based product	<2.5
S25-037339	Soy based product	<2.5
S25-037340	Soy based product	<2.5
S25-037341	Pea based product	<2.5
S25-037342	Pea based product	<2.5
S25-037456	Ready to drink shake	<2.5
S25-037457	Ready to drink shake	<2.5
S25-037458	Ready to drink shake	<2.5

Average recovery, %, for Batch PC25-04884 (n=4) = 100.9%

Table C34. Erucic acid analysis results for plant protein products that contained rapeseed oil as an ingredient

Fera sample number	Extracted Weight fat/oil (g)	Weight fat/oil (mg)	% fatty acid composition of extracted fat Erucic acid methyl ester	Erucic acid content of fat g/kg
S25-037458	0.508	508	0.26	2.6
S25-037457	0.480	480	0.28	2.8
S25-037342	0.3322	332.2	0.26	2.6
S25-037341	0.3539	353.9	0.20	2.0
S25-037340	0.2844	284.4	0.31	3.1
S25-037319	0.258	258	0.60	6.0
S25-037318	0.4103	410.3	0.24	2.4
S25-037317	0.1017	101.7	0.00	0.0
S25-037303	0.2524	252.4	0.59	5.9
S25-037302	0.2963	296.3	0.14	1.4

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