Supporting document 1

Risk and technical assessment report – Application A1099

Serine Protease (Trypsin) as a Processing Aid (Enzyme)

Executive summary

Application A1099 seeks approval for the use of a serine protease (trypsin) isolated from a genetically modified strain of *Fusarium venenatum* as a processing aid. The stated purpose of this enzyme, namely the production of peptides and small proteins with different functionalities via hydrolysis of animal or vegetable proteins, is clearly articulated in the Application. The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified to be effective in achieving its stated purpose. The enzyme preparation meets international purity specifications for enzymes used in the production of food.

There are no public health and safety issues associated with the use of the enzyme preparation, containing serine protease (trypsin) produced by genetically modified (GM) *F. venenatum* containing the gene for trypsin derived from the fungus *F. oxysporum,* as a food processing aid on the basis of the following considerations:

- The production organism is not toxigenic or pathogenic and is absent in the final enzyme preparation proposed to be used as a food processing aid.

- Residual enzyme may be present in the final food but would be inactive.

- Bioinformatic analysis indicated that the enzyme has no biologically relevant homology to known protein allergens or toxins.

- The enzyme caused no observable effects at the highest tested doses in rat oral gavage studies. The NOAEL was 3,605 mg Total Organic Solids (TOS) per kg body weight per day, the highest dose tested.

- The enzyme preparation was not genotoxic *in vitro*.

Based on the reviewed toxicological data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate. A dietary exposure assessment was therefore not required.

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1 Introduction

FSANZ received an application from Novozymes Australia Pty Ltd seeking approval for the enzyme serine protease (trypsin specificity, EC 3.4.21.4) as a processing aid to be used in the production of food. The enzyme is produced from a genetically modified (GM) strain of *Fusarium venenatum* expressing a serine protease (trypsin) gene from *F. oxysporum*.

The Applicant proposes to use serine protease (trypsin) to produce smaller proteins and peptides from various animal and vegetable proteins. The Applicant claims that the smaller proteins and peptides can be used as ingredients in a variety of food and beverage products for different functionalities (e.g. protein fortification, reduced allergenicity, improved emulsifying capacity etc.).

1.1 Objectives of the Assessment

As there are no permissions for the enzyme trypsin from microbial sources currently in the *Australia New Zealand Food Standards Code* (the Code), any application to amend the Code to permit the use of this enzyme as a food processing aid requires a pre-market assessment.

The objectives of this risk assessment are to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid

- evaluate any potential public health and safety concerns that may arise from the use of serine protease (trypsin) as a processing aid.

2 Food Technology Assessment

2.1 Characterisation of serine protease (trypsin)

2.1.1 Identity of the enzyme

Information regarding the identity of the enzyme that was taken from the Application has been verified using enzyme nomenclature references (JECFA 2012; IUBMB 2014). Additional information has also been included from these references.

Generic common name	Serine protease
Accepted IUBMB[1] name:	Trypsin
IUBMB enzyme nomenclature:	EC 3.4.21.4
C.A.S. number:	9004-07-7
Other names:	α-trypsin; β-trypsin; cocoonase; parenzyme; parenzymol; tryptar; trypure; pseudotrypsin; tryptase; tripcellim; sperm receptor hydrolase

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[1] International Union of Biochemistry and Molecular Biology

Reaction:	Protease with preferential cleavage at Arginine and Lysine
Commercial name:	TL1 conc BG

2.1.2 Enzymatic properties

Trypsin belongs to the serine protease superfamily. Its catalytic characteristic is that it cleaves on the carboxyl end (C-terminal) arginine (Arg) and lysine (Lys) on peptide chains. In the past, trypsin for food uses has been obtained from purified extracts of bovine or porcine pancreatic tissue (see the Table to clause 15 in Standard 1.3.3 at http://www.comlaw.gov.au/Series/F2008B00616). Trypsin-like serine proteases are highly represented in eukaryotes and also found in prokaryotes, archae and viruses (Bazan and Fletterick 1988; Laskar et al. 2012) and along with chymotrypsin are grouped into the clan PA proteases (Di Cera 2009).

The serine protease enzyme that is the subject of this Application has trypsin specificity and catalyses the hydrolysis of peptide bonds in proteins with preferential cleavage at the arginine and lysine amino acids to produce smaller proteins and peptides of variable lengths. The enzyme is used for partial or extensive hydrolysis of both animal and vegetable proteins (e.g. whey, casein, gluten and proteins from soy, corn, rice, peas, lentils, meat and fish). The hydrolysed proteins are then used as ingredients in a variety of food and beverage products for different functionalities (e.g. protein fortification, reduced allergenicity, improved flavour of hydrolysed protein etc.).

The enzyme preparation (granulated powder) is active during the processing of the protein-containing food/food ingredient, with deactivation of the enzyme occurring when it is denatured by high temperatures used in the manufacturing of the final food product. The enzyme has no action or function in the final food product.

2.1.3 Physical properties

The commercial enzyme preparation is supplied as a yellow to light brown granulate with approximately 95% Total Organic Solids (TOS).

2.2 Production of the enzyme

The enzyme is produced by a submerged fed-batch pure culture fermentation process which is common for the production of many food enzymes.

The production steps can be summarised as a fermentation process, a purification process, formulation of the final commercial enzyme preparation, and a quality control process. The raw materials used are food grade. More detail of the individual steps is provided in the Application.

The fermentation process involves two steps, the initial inoculum fermentations to produce enough of the microorganism for the production fermentation and then the main fermentation. The production organism secretes the enzyme into the fermentation broth.

The downstream processing steps taken after the main fermentation to produce the enzyme consist of: removal of the production strain and other solids, ultrafiltration to concentrate and further purify the enzyme, filtration, and finally further concentration and granulation.

2.3　Specifications

There are international specifications for enzyme preparations used in the production of food that have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2001) and the Food Chemicals Codex (U.S. Pharmacopeial Convention 2014). The Applicant states that the serine protease (trypsin) preparation meets the JECFA purity specifications for food grade enzymes. See Table 1 for a comparison of the product specifications and the JECFA specifications. The specification monographs listed above are primary references for specifications in Standard 1.3.4 – Identity and Purity.

Table 1: Product specifications for commercial serine protease (trypsin) preparation compared to JECFA specifications for enzymes

Analysis	Product specification	JECFA specification
Lead (mg/kg)	≤ 5	≤ 5
Arsenic (mg/kg)	≤ 3	
Mercury (mg/kg)	≤ 0.5	
Cadmium (mg/kg)	≤ 0.5	
Standard plate count (cfu/g)	≤ 10,000	
Coliforms (cfu/g)	≤ 30	≤30
Salmonella (in 25 g)	Not detected	Absent
E. coli (in 25 g)	Not detected	Absent

The Application states that the serine protease (trypsin) preparation contains no detectable antibiotic activity. Absence of antimicrobial activity is a requirement of the JECFA specifications for enzymes used in food processing.

The final enzyme preparation meets international specifications for enzyme preparations used in the production of food.

The enzyme preparation does not contain any allergenic substances that would require mandatory labelling declarations.

2.4　Technological function of the enzyme

The enzyme is used as a processing aid for partial or extensive hydrolysis of proteins from both animal and vegetable sources (e.g. casein, whey, gluten, and proteins from meat, fish, corn, soy, rice, peas and lentils). Based on the information provided by the company, the serine protease (trypsin) has benefits that include higher yields of soluble proteins and peptides, milder process conditions, reduced amounts of salts (compared with acid hydrolysed protein) and protein hydrolysates with controlled peptide profile due the specificity of the enzyme.

2.5　Food technology conclusion

The stated purpose for this enzyme, namely for use as a processing aid in the production of protein hydrolysates, is clearly articulated in the Application. The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme preparation meets international purity specifications.

3 Hazard Assessment

3.1 Background

3.1.1 Chemistry

Details of the chemistry of the serine protease (trypsin) (henceforth referred to as SP-T[2]) produced by *F. venenatum* including relevant physicochemical and enzymatic properties, and product specifications, are provided in the Food Technology Assessment (Section 2).

3.1.2 Description of the genetic modification

SP-T is produced by a GM strain of *F. venenatum,* [formerly identified as *F. graminearum* (Wiebe 2002) whose sexual stage is known as *Gibberella zeae*] derived from host strain WTY842-1-11, which expresses a trypsin-like serine protease gene (henceforth referred to as *sp-t*). Since *F. venenatum* can produce mycotoxins including trichothecenes that are harmful to humans (Boenisch and Schäfer 2011), the gene encoding trichodiene synthase (*tri5*) was deleted by site-directed gene disruption in which the *tri5* gene was replaced by the acetamidase (*amdS*) gene from *Aspergillus nidulans*. The disruption of *tri5* was confirmed by Southern blot analysis and the absence of type A trichothecenes (e.g. diacetoxyscirpenol) in WTY842-1-11 was confirmed by liquid chromatography-diode array detection-electrospray ionisation-mass spectrometry (LC/DAD/MS).

The genetic material was introduced into the host strain (WTY842-1-11) by incubating protoplasts with a linearised fragment of plasmid pJRoy75. This fragment contained two expression cassettes:

- An *sp-t* expression cassette comprising:

  - a promoter from the *F. venenatum* glucoamylase (*glaA*) gene
  - the *sp-t* gene derived from the fungus *F. oxysporum*, strain DSM2672
  - a terminator from the *F. oxysporum* serine protease gene

- A *bar* cassette containing the *bar* gene from *Streptomyces hygroscopicus* that confers tolerance to the chemical phosphinothricin. The purpose of this trait is to act as a selectable marker by allowing transformed cells (which would also contain the serine protease gene) to grow on a medium containing phosphinothricin.

The genetic material from the cassettes was incorporated into the production strain (WTY939-8-3) genome by random (non-homologous) integration. Southern blot analysis indicated that the *sp-t* gene is present, and a digital PCR method was used to determine that there are multiple copies of each of the *bar* and *sp-t* genes. The number of integrations did not permit determination of either the integration site(s) or accurate mapping of each copy. Southern blot analysis showed there are no other coding sequences from the plasmid present in the final production strain; this means, in particular, there are no antibiotic resistance genes present.

To test the stability of the insert in the production strain, Southern blot analysis, using a probe derived from the *sp-t* gene, was done on DNA from cells from three separate end-of-production batches and was compared to reference genomic DNA of the production strain.

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[2] The Applicant has requested the identity of the serine protease gene and protein be considered confidential commercial information. FSANZ has agreed with this request and therefore chosen an abbreviation for use in this document that is entirely artificial and does not reflect any official nomenclature.

The band patterns (number and size) obtained for all the production batches were identical to the band pattern of the reference production strain, thus indicating stability of the insert.

3.1.3 Scope of the hazard assessment

The hazard of SP-T derived from the *F. venenatum* production strain was evaluated by considering the:

- hazard of the production organism, including any history of safe use in food production processes

- hazard of the encoded protein, including potential allergenicity

- toxicity studies on the enzyme preparation intended for commercial use.

3.2 Hazard of the production organism – *F. venenatum*

The production strain, containing multiple *sp-t* genes, is genetically engineered from strain A3/5, a natural isolate. *F. venenatum* is well adapted to large scale fermentation and has been developed as a host for the production of industrial and food enzymes (Berka et al 2004).

It is an ascomycete plant pathogen which causes fusarium head blight in cereals, especially wheat and barley, and contamination of the grain with mycotoxins that can be harmful to humans and livestock
(http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx).
As indicated in Section 3.1.2, while wild type *F. venenatum* may be able to produce mycotoxins of concern, there is no possibility that the production strain is able to produce trichothecenes. Strain WTY842-1-11 was also tested by LC/DAD/MS for the minor mycotoxins fusarin C (FUC) and 'butenolide' (BUT) and these were not detected.

The biomass of *F. venenatum,* largely comprising mycoprotein, has been marketed as a low-calorie, high-fibre food in various countries as the product Quorn® (Trinci 1994; Wiebe 2002). There have been possible allergen concerns with this organism (Jacobsen 2003) and FSANZ has a statement on its website about the product.
(http://www.foodstandards.gov.au/consumer/generalissues/quorn/Pages/default.aspx). There is some indication that acidic ribosomal protein P2 may be an allergen of concern (Hoff et al. 2003).

Notwithstanding the above points, data submitted with the application indicate that the *F. venenatum* production strain is not detectable in the final enzyme preparation to be used as a food processing aid. The organism is removed during a multi-step recovery of the purified enzyme following submerged fed-batch pure culture fermentation. A germ filtration stage in the manufacturing process involves filtrations at defined pH and temperature intervals that result in an enzyme concentrate solution free of the production strain.

3.3 Hazard of the encoded protein – SP-T

A BLASTP[3] sequence comparison of the SP-T protein with human, bovine and porcine protein sequences (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/) indicates that there is less than 50% identity.

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[3] the BLASTP (Basic Local Alignment Search Tool Protein) algorithm (Altschul et al. 1997) is used to compare a protein sequence with a database of protein sequences.

The SP-T produced by *F. venenatum* has preferential cleavage at Arg and Lys. The safety of this enzyme has been assessed by JECFA (Choudhuri et al. 2012) and allocated an Acceptable Daily Intake (ADI) of 'not specified'.

The intention is that the enzyme preparation (designated TL1 conc BG and comprising water and SP-T, formulated to achieve the desired enzyme activity – see Table 2) is used as a processing aid for the production of partly or extensively hydrolysed proteins of vegetable and animal origin. The applicant claims the enzyme is particularly useful when a moderate, controlled hydrolysis is desirable. The enzyme is expected to be inactivated by high temperature once the desired degree of hydrolysis is obtained, and therefore any residual enzyme in the final food would be present as denatured protein and undergo normal proteolytic digestion in the gastrointestinal tract.

Bioinformatic analyses were performed to assess the similarity to known allergens and toxins of the trypsin.

3.3.1 Bioinformatic analyses for potential allergenicity

The *in silico* analyses compared the SP-T sequence with known allergens in two databases:

- the FARRP (Food Allergy Research and Resource Program) dataset within AllergenOnline (University of Nebraska; http://www.allergenonline.org/).

- the dataset within the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee. (http://www.allergen.org/).

Three types of analysis were used to search the databases:

a) the FASTA algorithm (Pearson and Lipman 1988) version 3.4, using the BLOSUM50 scoring matrix (Henikoff and Henikoff 1992). The E-score[4] generated by this indicates whether there are any alignments that meet or exceed the Codex Alimentarius (Codex 2003) FASTA alignment threshold (35% identity over 80 amino acids) for potential allergenicity. This threshold aims to detect potential conformational IgE-epitopes.

b) The same as a) but with scaling enabled in order to find any matches that may have high identity over windows shorter than 80 amino acids.

c) The Needleman-Wunsch global alignment algorithm (Needleman and Wunsch 1970) in the program package EMBOSS (http://www.ebi.ac.uk/Tools/emboss/). This explores all possible alignments and chooses the best one (the optimal global alignment). Hence it can identify those hits with more than 35% identity over the full length of the sequences.

In all three types of analysis across the two databases, a total of 16 allergens were identified that show greater than 35% identity with SP-T. These comprised thirteen mite-related allergens (from the species *Blomia, Dermatophagoides, Tyrophagus* and *Euroglyphus*), three insect-related allergens (from the species *Bombus* [bumblebee], *Apis* [honey bee] and *Polistes* [paper wasp]), and one dog-related allergen (from *Canis familiaris*), the normal route of exposure for all of which would be respiratory.

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[4] Comparisons between highly homologous proteins yield E-values approaching zero, indicating the very low probability that such matches would occur by chance. A larger E-value indicates a lower degree of similarity. Commonly, for protein-based searches, hits with E-values of 10^{-3} or less and sequence identity of 25% or more are considered significant although any conclusions reached need to be tempered by an investigation of the biology behind the putative homology (Baxevanis 2005).

A different search (using the same protein as a query) undertaken by JECFA (referenced in Choudhuri et al. 2012) using the Allermatch database (http://www.allermatch.org/) and Structural Database of Allergenic Proteins (http://fermi.utmb.edu/SDAP/) found six of these allergens and noted that they were all serine proteases and would therefore be expected to have some degree of amino acid similarity with trypsin. The JECFA consideration also looked at the occurrence of six contiguous amino acid sequences of SP-T that are shared by allergenic proteins and noted that many of these sequences are also shared by human trypsin and are most likely not part of any allergenic epitopes. The use of six contiguous amino acid sequences was recommended by the FAO/WHO (FAO/WHO 2001) as one means of identifying allergenic potential but has now been largely dismissed because of the unacceptably high rate of false positives (Goodman 2006).

The conclusion from these bioinformatic analyses is that, despite matches with several allergens, any oral intake of SP-T is unlikely to pose an allergenicity concern.

3.3.2 Bioinformatic analyses for potential toxicity

The SP-T sequence was compared to sequences present in the UniProt database (http://www.uniprot.org/) containing entries from Swiss-Prot and TrEMBL and using the term 'toxin' to refine the search. The comparison method used a ClustalW 2.0.10 sequence alignment program (http://www.clustal.org/clustal2/) (Larkin et al. 2007) to align each sequence from the database with the chymotrypsin sequence.

The greatest homology found was 26.3%, which indicates that SP-T is unlikely to be toxic.

3.4 Evaluation of unpublished toxicity studies

Report submitted

Lund, T. Ø.; Berg, N.W. (2013). Summary of toxicity data. SP387/TL1. File ID # 2013-15851, Novozymes A/S (unpublished).

Unpublished toxicity studies on a representative SP-T preparation were submitted by the Applicant and independently evaluated by FSANZ. These studies comprised two genotoxicity tests – an Ames test conducted in accordance with OECD test Guideline 471 (OECD 1997a) and an *in vitro* chromosome aberration test conducted in accordance with OECD test Guideline 473 (OECD 1997b) – and two oral gavage studies – a 13 week study in rats conducted in accordance with OECD Test Guideline 408 (OECD 1998) and a 25 day rat study carried out in accordance with OECD Test Guideline 407 (OECD 1995).

The test substance was SP-T preparation (designated as batch PPF26813 or PPF32126) prepared to the manufacturer's specifications. Batch PPF26813 is a mixture of three identically produced fermentation sub-batches recovered by purification/concentration according to the same procedure as used for production of TL1 conc BG; PPF32126 was prepared by concentrating PPF26813 by evaporation. The test substance was supplied in liquid form (dissolved in water) and differed from the actual product, TL1 conc BG, which would be supplied in granulate form comprising 95% (w/w) SP-T (TOS). A comparison of PPF26813, PPF32126 and TL1 con BG conc BG is given in Table 2.

Table 2: Comparison of PPF26813, PPF32126 and TL1 conc BG

Characteristic	PPF26813	PPF32126	TL1 conc BG
Activity (KMTU/g)[1]	117	340	1,500
Water (% w/w)	86.7	62.5	5
Ash (% w/w)	2.3	6.9	-
Total Organic Solids (TOS) (% w/w)	11.0	30.6	95

[1] KMTU =Kilo Microbial Trypsin Units (calculation method provided by Applicant)

3.4.1 Genotoxicity

Individual studies

Pedersen, P.B. (2007). SP 387/TL1, batch PPF 26813: Test for mutagenic activity with strains of *Salmonella typhimurium* and *Escherichia coli*. Study ID # 20078045, Novozymes A/S (unpublished).

Whitwell, J. (2007). SP 387/TL1: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Ref # 20076031, Covance (unpublished).

The results of these *in vitro* studies are summarised in Table 3. Negative (water vehicle) and positive controls were tested in each study and gave expected results. The chromosomal aberration study comprised two experiments that involved two different exposure times and different dose concentrations. It is concluded that the SP-T preparation did not induce chromosome aberrations or gene mutations when tested up to 5,000 µg/ml (an acceptable maximum concentration for such studies).

Table 3: Summary of genotoxicity test results

Test	Test system	Test article	Concentration or dose range	Result
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* strains TA 98, 100, 1535 & 1537 *Escherichia coli* strain WP2 *uvrA*	SP-T obtained from *F. venenatum* (Batch No.PPF26813) Water vehicle	156–5000 µg test substance (in solution) per millilitre ± S9[1]	Negative
***In vitro* chromosome aberration assay**	Human lymphocytes prepared from the pooled blood of 3 female donors	As above	1st experiment: 2813, 3750 and 5000 µg/ml ± S9 2nd experiment: 3200, 4000 and 5000 µg/ml −S9 and 3200, 4000 and 5000 µg/ml +S9	Negative

S-9 = metabolic activation system comprising liver preparation from rats induced with Aroclor 1254

3.4.2   Subchronic toxicity studies

Individual studies

Glerup, P. (2008). SP 387/TL1: 3-month toxicity study in rats. Study # 65860, LAB Research Scantox (unpublished).

Kaaber, K. (2014). SP387/Tl1: A 25-day oral (gavage) toxicity study in rats. Study # 73488, CiToxLab Scantox (unpublished)

3.4.2.1 3-month toxicity study

The SP-T preparation (Batch No. PPF26813) was administered by gavage to four groups of 10 SPF Sprague Dawley (strain Ntac:SD) rats/sex at doses of 0, 58, 192 or 581 mg TOS/kg bw/d for 92 days (water vehicle). Rats were sourced from Taconic Europe A/S (Ejby, Denmark), were approximately 5 weeks old and had body weights within a range of ± 20 g for each sex at the commencement of dosing. Rats were housed under standard conditions, with food and water available *ad libitum*. Standard gross toxicological endpoints were recorded during the treatment period (deaths, clinical signs, bodyweight and food consumption). Ophthalmoscopy was performed prior to treatment and on day 92. Pre-treatment and before termination, the animals were examined with respect to motor activity (open field test) and reactivity to different types of stimuli. Blood was collected on day 92 for the analysis of standard haematology and clinical chemistry parameters and a bone marrow smear was taken at necropsy. Following sacrifice, rats were necropsied, organ weights recorded and standard tissues prepared for histopathology.

Two animals, one male and one female, receiving the 192 TOS/kg bw/d treatment died on days 35 and 69 respectively but in neither case could a clear cause of death be established. There were no deaths, clinical signs, motor activity abnormalities or ophthalmic abnormalities attributable to administration of the test substance. Bodyweight gain and food consumption were comparable across all groups. There was no treatment-related effect on any haematology or clinical chemistry parameter. There were no treatment-related macroscopic abnormalities, differences in organ weights or histopathological findings. The NOAEL (No Observed Adverse Effect Level) for both males and females was 581 mg TOS/kg bw/d, the highest dose tested.

3.4.2.2 25-day oral gavage

The objective of this study was to establish a higher NOAEL than that established in the 3-month study. The SP-T preparation (Batch No. PPF32126) was administered by gavage to four groups of 10 SPF Sprague Dawley (strain Ntac:SD) rats/sex at doses of 0, 360, 1190 or 3605 mg TOS/kg bw/d for 25 days (water vehicle). Findings of lower food consumption, decreased body weight gain and increased water consumption in mice receiving the highest dose were, in the absence of other indications, concluded to be due to the unpalatable nature of the high protein level *per se* rather than to any direct effect of SP-T itself. Therefore, the NOAEL was 3,605 mg TOS/kg bw/d, corresponding to an enzyme activity of 4,005 KMTU/kg bw/d.

3.5   Hazard assessment conclusions

There are no public health and safety issues associated with the use of TL1 conc BG, containing serine protease (trypsin) produced by GM *F. venenatum,* as a food processing aid on the basis of the following considerations:

- The production organism is not toxigenic or pathogenic and is absent in the final enzyme preparation proposed to be used as a food processing aid.

- Residual enzyme may be present in the final food but would be inactive.

- Bioinformatic analysis indicated that the enzyme has no biologically relevant homology to known protein allergens or toxins.

- The enzyme caused no observable effects at the highest tested doses in rat oral gavage studies. The NOAEL was 3,605 mg TOS/kg bw per day, the highest dose tested.

- An enzyme preparation was not genotoxic *in vitro*.

Based on the reviewed toxicological data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate. A dietary exposure assessment is therefore not required.

4   Conclusion

This risk assessment considered the technological suitability, potential hazard of the production microorganism and the potential hazard of serine protease (trypsin).

It was concluded that the proposed use of the enzyme was technologically justified in the form and prescribed amounts, and was demonstrated to be effective. The evidence presented was sufficient to determine that no safety concerns with production microorganisms or the enzyme exist. Thus serine protease (trypsin) derived from a genetically modified strain of *F. venenatum* containing the gene for trypsin from *F. oxysporum,* is unlikely to pose any health risk when used as a food processing aid.

5   References

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