FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk and Technical Assessment – Application A1240

Polygalacturonase from GM *Aspergillus oryzae* as a processing aid

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Executive summary

AB Enzymes applied to Food Standards Australia New Zealand (FSANZ) to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include polygalacturonase (EC 3.2.1.15), a pectinase, as a processing aid. It is produced from a genetically modified (GM) strain of *Aspergillus oryzae* (*A. oryzae*) containing the polygalacturonase gene from *Aspergillus tubingensis* (*A. tubingensis*). The specific name for the production organism is *A. oryzae* strain AR-183. The proposed use of polygalacturonase is as a processing aid in the manufacture and/or processing of fruit and vegetable juices/products; and in the production of coffee, flavouring substances and wine.

FSANZ has undertaken an assessment to determine whether the enzyme achieves its technological purpose in the quantity and form proposed to be used and to evaluate public health and safety concerns that may arise from the use of this enzyme.

FSANZ concludes that the proposed use of polygalacturonase as an enzyme processing aid in the manufacture and/or processing of fruit and vegetable juices/products; and in the production of coffee, flavouring substances and wine, is consistent with its typical function of hydrolysing pectin in those foods. Analysis of the evidence provides adequate assurance that the use of the enzyme, in the form and requested amount, is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

Polygalacturonase performs its technological purpose during the production of foods by breaking down plant cell walls, and is not performing a technological purpose in the final food, therefore functioning as a processing aid as defined in the Code. There are relevant identity and purity specifications for the enzyme in the Code.

No public health and safety concerns were identified in the assessment of polygalacturonase from a modified strain of *A. oryzae* under the proposed conditions of use.

The host strain is neither pathogenic nor toxigenic. No food safety hazard was identified in the isolation and use of the polygalacturonase gene from *A. tubingensis*. Analysis of the production strain (*A. oryzae* AR-183) confirmed the presence and stability of the introduced DNA.

Toxicity testing of the enzyme showed no evidence of genotoxicity *in vitro.* The no observed

adverse effect level (NOAEL) in a 90-day oral gavage study in rats was 1000 mg TOS[1]/kg bw/day, which was the highest dose tested. The theoretical maximum daily intake (TMDI) was calculated by FSANZ to be 0.105 mg TOS/kg bw/day. Comparison of the NOAEL and the TMDI gives a margin of exposure (MOE) of approximately 9500.

Bioinformatic analysis indicated that the enzyme shows no significant homology with any known toxins or venoms. A degree of homology between the enzyme and several pollen allergens was found. Taking into account that none of these allergens is a food allergen and that only low levels of the enzyme processing aid are expected to be present in final food products, the risk of food allergy from the proposed uses of the enzyme is likely to be low.

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

[1] TOS: Total organic solids.

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1. Introduction

The applicant, AB Enzymes, is seeking permission for polygalacturonase (EC 3.2.1.15) for use as a processing aid. The enzyme is produced from a genetically modified (GM) strain of *Aspergillus oryzae* (*A. oryzae)* containing the polygalacturonase gene from *Aspergillus tubingensis* (*A. tubingensis*)*.* The specific name for the production strain is *A. oryzae* strain AR-183*.*

Polygalacturonase is a pectinase suitable for hydrolysing pectin and can be used in the manufacture and/or processing of fruit and vegetable juices/products; and in the production of coffee, flavouring substances and wine.

Marketed via an enzyme preparation, if approved, the enzyme is to be used in combination with pectinesterase being assessed under FSANZ Application A1241 (A1241 was submitted simultaneously with A1240). The polygalacturonase/pectinesterase enzyme blend is sold by the applicant under the proprietary name ROHAPECT® MA Plus. It will be used as a processing aid at low levels and is either not present in the final food or present in insignificant quantities, having no technical function in the final food.

1.1 Objectives of the assessment

The objectives of this technical and safety assessment were to:

- determine whether the proposed purpose is a solely technological purpose (function) and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used, and

- evaluate potential public health and safety issues that may arise from the use of this enzyme, produced by a genetically modified organism, as a processing aid, specifically by considering the:
  - history of use of the host and gene donor organisms
  - characterisation of the genetic modification(s)
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity of the enzyme

The production microorganism of the enzyme is a GM strain of *A. oryzae*. The donor microorganism of the polygalacturonase gene is *A. tubingensis* (further details contained in section 3). The applicant provided relevant information regarding the identity of the polygalacturonase enzyme. FSANZ verified this using the IUBMB[2] enzyme nomenclature database (McDonald et al 2009). Details of the identity of the enzyme are provided in Table 1.

[2] International Union of Biochemistry and Molecular Biology

Table 1 Identity

Generic common name:	Polygalacturonase
Accepted IUBMB name:	Endo-polygalacturonase
Systematic name:	(1→4)-α-D-galacturonan glycanohydrolase (endo-cleaving)
Other names:	Endopolygalacturonase, Endogalacturonase; Endo-D-galacturonase;
EC number:	3.2.1.15
Reaction:	(1,4-α-D-galacturonosyl)$_{n+m}$ + H_2O = (1,4-α-D-galacturonosyl)$_n$ + (1,4-α-D-galacturonosyl)$_m$

For a graphical representation of the hydrolysis reaction catalysed by polygalacturonase, refer to its record in the enzyme database BRENDA[3] (Chang et al 2021).

2.2 Manufacturing process

2.2.1 Production of the enzyme

AB Enzyme's polygalacturonase is produced by submerged fermentation of the genetically modified strain of *A. oryzae*. The main fermentation steps are, inoculum, seed fermentation, main fermentation followed by the recovery stage involving primary and liquid separation, concentration to achieve the desired enzyme activity, polish and germ filtration to provide a concentrated enzyme solution free of the production strain and insoluble substances. This is followed by formulation of the enzyme into an enzyme preparation.[4] AB Enzymes polygalacturonase enzyme preparation is sold mainly as a liquid product consisting of glycerol, sodium chloride and water. A manufacturing flow-chart was provided as an Appendix with the application. The production is manufactured in accordance with current Good Manufacturing Practice for Food[5] and the principles of Hazard Analysis and Critical Control Point (HACCP).

The application states that all raw materials used in the fermentation and recovery processes are standard ingredients of food grade quality that meet predefined quality standards. The raw materials conform to either specifications set out in the Food Chemical Codex, 12th edition, 2020 or regulations applying in the European Union. The applicant has advised that a wheat-based material is used during fermentation and is wholly consumed during fermentation. FSANZ considers that it is therefore likely that the wheat-based material in the fermentation media is unlikely to be present in the final commercial enzyme preparation. The Product Data Sheet states absence of cereals containing gluten (i.e. wheat, rye, barley, oats spelt, kamut).

Details on the manufacturing process, raw materials and ingredients used in the production of the polygalacturonase enzyme preparation were provided in the application or as

[3] www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.15
[4] Enzymes are generally sold as enzyme preparations, which consist of the enzyme(s) and other ingredients, to facilitate their storage, sale, standardisation, dilution or dissolution.
[5] known as cGMP, as distinct from GMP (which refers to the level of use of the enzyme)

Confidential Commercial Information.

2.2.2 Specifications

There are international specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO/WHO 2006) and in the Food Chemicals Codex (FCC 2008). These specifications are included in earlier publications of the primary sources listed in section S3—2 of Schedule 3 of the Code and enzymes used as a processing aid must meet either of these specifications. The applicant states that the final enzyme preparation complies with the requirements in both of these specifications. Schedule 3 of the Code also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant provided the results of analysis of three different batches of the polygalacturonase preparation. Table 2 provides a comparison of the analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, and commercial-in-confidence information provided by the applicant, the enzyme preparation met all relevant specifications for arsenic and metals and the microbiological criteria.

Table 2 *Comparison of manufacturer's polygalacturonase preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

Analysis	Analysis provided by manufacturer*	Specifications		
		JECFA (2006)	*Food Chemicals Codex (FCC, 2020)*	*Australia New Zealand Food Standards Code (section S3—4)*
Lead (mg/kg)	<0.05	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	<0.5	-	-	≤1
Cadmium (mg/kg)	<0.05	-	-	≤1
Mercury (mg/kg)	<0.05	-	-	≤1
Coliforms (cfu/g)	<30	≤30	≤30	-
***Salmonella* (in 25 g)**	Not detected	Absent	Negative	-
***E. coli* (in 25 g)**	Not detected	Absent	-	-
Antibiotic activity	Not detected	Absent	-	-

* across three samples

Whilst the manufacturing processes ensure the production microorganism is removed from the final enzyme preparation, the food enzyme is a biological isolate of variable composition, containing the enzyme protein, as well as organic and inorganic material derived from the microorganism and fermentation process. Refer to section 3.4 below for the total organic solids (TOS) value.

2.3 Technological purpose of the enzyme

Under the current application, polygalacturonase is intended for use to in the production of a number of specific products (refer to section 3.2.1 below).

Polygalacturonase is a pectin-degrading enzyme (along with pectate and pectin lyases, and pectinesterase). These enzymes are often collectively called ‘pectinases’ and are typically found in plants and microorganisms (especially fungi). They are a mixture of enzymes that act on pectic substances (plant polysaccharides that maintain the integrity of the cell wall or middle lamella).

As identified by the IUMBM (IUMBM 2022), polygalacturonase catalyses the random hydrolysis of (1→4)-α-D-galactosiduronic linkages in pectate and other galacturonans. This results in depolymerisation of pectin. As stated in the application, the practical outcome of such activity is that intercellular barriers (middle lamella) are broken down.

The application describes the two classes of pectinases – namely pectin depolymerases (further classified as polygalacturonases and pectin lyases) and pectinesterases. Polygalacturonase is a pectin depolymerase (it splits the main chain), whereas pectinesterase (under A1241) has the ability to de-esterify pectin by the removal of methoxy residues. Polygalacturonase causes a reduction in viscosity of a pectic substrate. As explained in the application, several enzymes are sometimes used simultaneously, to achieve complete pectin degradation.

The stated technological purpose of the polygalacturonase enzyme is supported by scientific literature (e.g. Damodaran et al, 2008; Nagodawithana and Reed,1993).

The applicant provided information on the physical and chemical properties of the enzyme preparation. Table 3 summarises this information.

Table 3 *Physical and chemical properties of polygalacturonase enzyme preparation*

Physical and chemical properties of commercial enzyme preparation	
Enzyme activity	Minimum polygalacturonase activity 75,000 PGU/mg* (from three batches)
Appearance	Brown coloured liquid
Storage conditions	Store below 10°C.
Storage stability	Three year shelf life at 10°C
Density	1.1 g/ml

*Assay of polygalacturonase activity (AB Enzymes internal method)

Polygalacturonase (from three non-GM sources) is approved for use in the manufacture of foods since it is listed in subsection S18—4(5).

2.4 Technological justification

As outlined above, the technological need of the enzymatic conversion of pectin with the help of polygalacturonase can be described as enabling the degradation of pectin. Pectin causes technical difficulties during food production due to its high viscosity and gelling properties in processing raw materials that contain this component.

The enzyme performs its function of catalysing the hydrolysis of pectic substances during the production of plant foods. It is therefore performing as a processing aid as defined by the Code.

The Code already permits polygalacturonase and pectinesterase (from other sources) to be used in the manufacture of foods, the two enzymes the applicant combines in its commercial product (ROHAPECT® MA Plus. The specific benefits of the action of polygalacturonase in the manufacture and/or processing of fruit and vegetable juices/products; and in the production of coffee, flavouring substances and wine, as summarised from the application, are described below.

The Codex guideline, *Guidelines on Substances used as Processing Aids* (CAC/GL 75-2010) sets out general principles for the safe use of substances used as processing aids. The Guideline states that substances used as processing aids shall be used under conditions of good manufacturing practice (GMP). Therefore use of commercial enzyme preparations should follow GMP, where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction. The applicant requested use of the enzyme at GMP levels.

Fruit and vegetable manufacture and/or processing (fruit juices/products and vegetable juices/products)
Polygalacturonase assists in the degradation of pectin in processing of these products. While fruits and vegetables naturally contain polygalacturonase (hence the ripening of fruit and vegetables), the concentration is too variable and the specificity of the naturally occurring enzyme may not be optimal for the desired production process.

Coffee production
Pectinases, including polygalacturonase, are used during coffee processing for processing improvement such as to assist with separation of the bean from the outer layers and a shorter fermentation time.

Flavouring substances production
Flavouring substances used to flavour foods can be produced using enzymes (such as cellulases and pectinases). Enzymatic pre-treatment for the extraction of flavour components from various plant materials enhance aroma recovery.

Wine production
Grapes have a high pectin content, and pectinases are already used in wine making. They are used in a number of stages in the wine making process, as described in the application. For example, when polygalacturonase is used in combination with pectinesterase in red wine making, the visual aspects (e.g. colour, stability) are improved compared to untreated wines.

2.5 Food technology conclusion

FSANZ concludes that the proposed use of this polygalacturonase in the production of a number of foods, is consistent with its typical function as a pectinase. FSANZ concludes that the evidence presented to support the proposed use provides adequate assurance that the use of the enzyme, in the form and requested amount (i.e. at a level consistent with GMP) is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

Polygalacturonase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

3 Safety assessment

Some information relevant to this section is Confidential Commercial Information (CCI), so full details have not been provided in this public report.

3.1 Source microorganisms

3.1.1 Host organism

The enzyme production strain, *A. oryzae* AR-183, is derived from a parental strain of *A. oryzae* collected in South America in 1984.

While *A. oryzae* has been implicated in illness in severely ill and/or immunocompromised individuals, it is not considered to be pathogenic in healthy humans (Barbesgaard et al 1992). It is listed as a Risk Group 1 microorganism by the German Federal Office of Consumer Protection and Food Safety.[6] Evidence was also provided that the enzyme production strain was not detected in the liquid enzyme concentrate.

A. oryzae is generally considered to be a domesticated form of *A. flavus*—a species which includes many aflatoxin-producing strains—and it can be difficult to differentiate between the two (Frisvad et al 2018). The applicant provided commercial-in-confidence evidence that adequately demonstrated that the production strain was correctly identified as *A. oryzae*. Data was provided to show that it did not produce toxicologically significant amounts of mycotoxins. Three different batches of liquid enzyme concentrate—ie concentrated, filtered fermentation culture supernatant—showed levels of aflatoxins below 0.1 ug/kg.

Taking into consideration the evidence provided, and the long history of safe use of *A. oryzae* for food and enzyme production, it is concluded that the enzyme production strain *A. oryzae* AR-183 is non-pathogenic and non-toxigenic, and does not present an unacceptable food safety risk.

3.1.2 Gene donor organism

The gene for the polygalacturonase enzyme was isolated from *A. tubingensis*, a filamentous fungus belonging to *Aspergillus* section Nigri (the black aspergilli; Samson et al. 2006). *A. tubingensis* has been implicated in skin, ear and respiratory infections in humans (Gautier et al. 2016). However, as the polygalacturonase gene has been manipulated through standard DNA cloning methods, and extraneous material from *A. tubingensis* would not be carried across to the enzyme production organism, no food safety hazard is identified.

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of the DNA to be introduced and method of transformation

An expression cassette containing the polygalacturonase gene was introduced into a spontaneous mutant host strain of *A. oryzae,* producing the AR-183 production strain. The polygalacturonase gene is derived from *A. tubingensis* and is under the control of a promoter and terminator. Data provided by the applicant and analysed by FSANZ confirmed the identity of the polygalacturonase enzyme.

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6 www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/register_datenbanken/organismenliste_xls.html;jsessionid=F8D7A39D1FED4DD1FA972AA6A8FEC7F6.1_cid363

The acetamidase gene (*amdS)* from *Aspergillus nidulans* (*A. nidulans*) is also found in the expression cassette (Kelly and Hynes, 1985). The *amdS* gene allows for selection of transformants on acetamide-containing media. Acetamide has been widely used as a selection marker in fungal transformations. The inclusion of this selection marker is standard in filamentous fungi systems producing a variety of recombinant gene products (Gryshyna *et al.,* 2016) and there are no safety concerns with its use.

The expression cassette was integrated into the genome of the host strain through standard transformation techniques using protoplasts. While a plasmid was used in the construction of the expression cassette, no plasmid derived sequences were integrated.

3.2.2 Characterisation of inserted DNA

Southern blot analysis was used to characterise the inserted DNA in the AR-183 production strain. The results indicate the presence of the expression cassette in the genome of AR-183.

Due to the transformation method, no antibiotic resistance genes were introduced into the AR-183 production strain.

No recombinant DNA was detected in the liquid enzyme concentrate.

3.2.3 Genetic stability of the inserted gene

The assessment confirmed the inserted DNA is integrated into the production organism's genome and does not have the ability to replicate autonomously. The inserted gene is therefore considered to be genetically stable.

To provide further evidence of the stability of the introduced polygalacturonase gene the applicant provided phenotypic data demonstrating that the enzyme is expressed over successive generations.

The applicant examined the activity level of the polygalacturonase enzyme in a number of fermentation batches. These data confirmed that the polygalacturonase gene is expressed over multiple generations and is stable.

3.3 Safety of the polygalacturonase enzyme

3.3.1 History of safe use of polygalacturonase

The enzyme has been approved for use in Denmark and France, and confidential information was provided by the applicant to show that substantial quantities are sold commercially. FSANZ notes that the enzyme is also sold commercially in countries where there is a general approval of enzymes for food manufacture.

3.3.2 Bioinformatics concerning potential for toxicity

A recent (August 2020) homology search, using the amino acid sequence of the polygalacturonase as the query sequence, was performed on proteins marked as toxins in the NCBI Identical Protein Groups (IPG) database using BLAST-P, which is a basic local alignment search tool. Search results were provided to FSANZ to show that the polygalacturonase enzyme does not show significant homology to any protein sequence identified as a toxin.

3.3.3 Toxicology data

Toxicology studies conducted with the polygalacturonase that is the subject of this application include a 13-week repeat-dose oral gavage study in rats, and two genotoxicity studies; a bacterial reverse mutation assay (Ames test) and an *in vitro* micronucleus assay.

3.3.3.1 Short term study in animals

Thirteen-week repeat-dose oral gavage study of polygalacturonase in Wistar rats (Schmid et al 2020; unpublished study). Regulatory status: GLP; in compliance with OECD test guideline 408

The control article and vehicle for this study was sterile water for injection. Stability and homogeneity of the test article in this vehicle were verified. Rats, 10/sex/group, were group housed under standard laboratory conditions of environment and husbandry. They were gavaged daily, for 90 days, with 0, 100, 300 or 1000 mg TOS[7]/kg bw/day. Parameters measured included survival, clinical observations, bodyweight changes, food consumption, performance on a functional observational battery, clinical pathology parameters (endocrine, haematology, coagulation, clinical chemistry, urinalysis), gross necropsy findings, weights of selected organs, and histopathology of a comprehensive list of organs and tissues.

All rats survived to the end of the in-life phase. There were no test article-related effects on any parameters measured. Salivation in one 1000 mg TOS/kg bw/day male and one 1000 mg TOS/kg bw/day female immediately after dose administration on several days, was interpreted as a response to the discomfort of the gavage procedure and not related to the test article itself. There were no other treatment-related clinical observations.

It was concluded that the No Observed Adverse Effect Level (NOAEL) of polygalacturonase synthesized by GM *A. oryzae* is 1000 mg TOS/kg bw/day, the highest dose tested.

3.3.3.2 Genotoxicity studies

Bacterial reverse mutation assay (Schreib et al 2019; unpublished study). Regulatory status: GLP; in compliance with OECD Guideline 471

The test systems used for this study were *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA 1535, TA1537 and TA102. The solvent and negative control article was distilled water. Appropriate positive control articles, as recommended in the OECD guideline, were used.

Two experiments were conducted; using the plate incorporation method (experiment I) and the pre-incubation method (experiment II). All tests were conducted in triplicate, and with or without S9 mix for metabolic activation. Concentrations of test article in both experiments were 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. No precipitation or toxicity was observed at any concentration of the test article.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed at any concentration level, with or without S9 mix, in either experiment I and II. The expected increases in revertant colony numbers were observed with all the positive control articles used, confirming the validity of the assay.

It was concluded that polygalacturonase produced with *A. oryzae* was not mutagenic under the conditions of the assay.

[7] TOS = Total Organic Solids

In vitro *micronuclei assay (Donath et al 2019; unpublished study). Regulatory status: GLP; in compliance with OECD Guideline 473*

The test system for this study comprised human lymphocytes harvested from the peripheral blood of healthy non-smoking donors of unspecified sex.

Test article concentrations for the definitive experiments, based on results of the dose-range-finding experiment, were 400, 500 and 600 µg/mL without S9 mix and 250, 500 and 750 µg/mL with S9 mix in the 4-hour exposure protocol (Experiment I), and 25, 50 and 100 µg/mL without S9 mix in the 44-hour exposure protocol (Experiment II). Appropriate positive control articles, as recommended by the Guideline, and negative control (culture medium) were also assayed.

No precipitation of the test article was observed at any concentration, but some evidence of cytotoxicity was observed. In Experiment I, increased cytostasis was observed at 600 µg/mL without S9 and at 750 µg/mL with S9. In experiment II an increase in cytostasis was observed at ≥50 µg/mL.

No biologically relevant increase of the micronucleus frequency was noted after treatment with the test article in either experiment, with or without S9 mix for metabolic activation. The positive control articles induced the expected statistically significant increases in the frequencies of micronuclei, confirming the validity of the assay.

It was concluded that polygalacturonase produced by GM *A. oryzae* did not induce structural and/or numerical chromosomal damage in human lymphocytes, and is considered to be non-mutagenic in the *in vitro* mammalian cell micronucleus test.

3.3.4 Potential for allergenicity

The results of recent (2020) searches of the FARRP[8] and SDAP[9] databases for homology with the amino acid sequence of the polygalacturonase were provided. Searches were for alignment of the entire amino acid sequence and alignment of sliding 80-amino acid window of the protein to known protein allergens. The identity percentages of all the hits from both FARRP and SDAP were below the set 35 % identity limit and the three hits having the best E-values[10] were all different in the different databases. Using the 80 amino acid sliding window search, the enzyme sequence was found to have degrees of identity from 35.8 % to 46.3 % with pollen allergens of different plant species, including maize, Bahia grass, Japanese cedar, and London Plane trees. These pollen allergens are respiratory allergens rather than food allergens, and there is good evidence that respiratory allergens do not represent an allergic hazard when consumed (Bindslev-Jensen et al 2006). The predictive value of the 80 amino acid sliding window search has been called in to question by Ladics et al. (2007) and Goodman and Tetteh (2011), with the latter authors recommending that degree of identity less than 50% should not be considered relevant. As noted in Section 2.2.1 above, wheat-based products are used in the fermentation media, however they are likely to be consumed during the fermentation process. Given the absence of homology of the enzyme with known food allergens, and that allergens from wheat are unlikely to be present in the commercial preparation, FSANZ concludes that the commercial enzyme preparation is unlikely to pose a risk of food allergy.

[8] http://allergenonline.org
[9] https://fermi.utmb.edu/
[10] The E-value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E-value approaches zero, the less likely an alignment could have been produced by chance.

3.3.5 Assessments by other regulatory agencies

No safety assessments by other regulatory agencies are available.

3.4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worse-case scenario' approach to estimating likely levels of dietary exposure assuming all added polygalacturonase enzyme from GM *A. oryzae* remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass *et al.,* 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive. The TMDI can then be compared to an acceptable daily intake (ADI) or a NOAEL to estimate a margin of exposure for risk characterisation purposes.

In their budget method calculation, the applicant made the following assumptions:

- the maximum physiological requirement of solid foods (including milk) is 25 g/kg body weight/day
- the maximum physiological requirement for non-milk beverages is 100 mL/kg body weight/day (the standard level used in a budget method calculation)
- 50% of solid foods and 25% of non-milk beverages contain polygalacturonase
- the maximum polygalacturonase level in final solid foods was 1.0 mg TOS/kg food and for non-milk beverages was 3.2 mg TOS/kg food (i.e. the highest use level from all uses within each group)
- all of the enzyme remains in the final food.

Based on these assumptions, the applicant calculated the TMDI of polygalacturonase to be 0.093 mg TOS/kg body weight/day.

As assumptions made by the applicant differ to those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI using the following different assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

- the maximum physiological requirement for solid foods (including milk) is 50 g/kg body weight/day. This is the standard level used in a budget method calculation where there is potential for the enzyme to be in baby foods or general purpose foods that would be consumed by infants (Hansen, 1966), which for this enzyme would be from processed fruits and vegetables (e.g. canned fruits, canned vegetables and jams), soups, sauces, bouillons, dressings, condiments, snack foods, meat-derived foods and breads/crackers.

- FSANZ would generally assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion as a worst case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI based on FSANZ's calculations for solid foods and non-milk beverages were 0.025 mg TOS/kg body weight/day and 0.080 mg TOS/kg body weight/day respectively, resulting in a total of 0.105 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that the enzyme remains in the final foods and beverages. The applicant has stated that the enzyme is inactivated by heat or removed during further processing steps and does not have a function in the final food.

4 Discussion

AB Enzyme's polygalacturonase is produced by a genetically modified strain of *A. oryzae*. It is intended for use as a processing aid in the manufacture and/or processing of fruit and vegetable juices/products; and in the production of coffee, flavouring substances and wine.

The production strain contains an expression cassette—containing the polygalacturonase gene from *A. tubingensis* and the acetamidase gene from *A. nidulans* as a selectable marker—integrated into its genome.

The enzyme is produced by submerged fermentation of the production strain, followed by recovery and clean-up from the fermentation medium in accordance with principles of cGMP and HACCP.

Evidence provided confirms that the production strain is neither pathogenic nor toxigenic, and analysis of the activity level of the polygalacturonase enzyme in a number of fermentation batches over multiple generations confirmed the presence and stability of the introduced DNA.

Toxicity testing of the enzyme showed no evidence of genotoxicity *in vitro.* The NOAEL in a 13-week oral gavage study in rats was 1000 mg TOS/kg bw/day, which was the highest dose tested. The TMDI was calculated by FSANZ to be 0.105 mg TOS/kg bw/day. Comparison of the NOAEL and the TMDI gives a margin of exposure (MOE) of approximately 9500.

Bioinformatic analysis indicated that the enzyme shows no significant homology with any known toxins or venoms. A degree of homology between the enzyme and several pollen allergens was found. Taking into account that none of these allergens is a food allergen and that only low levels of the enzyme processing aid are expected to be present in final food products, the risk of food allergy from the proposed uses of the enzyme is likely to be low.

5 Conclusion

FSANZ concludes that the proposed use of this polygalacturonase is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The applicant requested use at GMP levels.

Polygalacturonase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is, therefore, appropriately categorised as a processing aid as defined in the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

The enzyme production strain *A. oryzae* AR-183 is non-pathogenic and non-toxigenic, and does not present an unacceptable food safety risk. No food safety hazard was identified in the isolation and use of the polygalacturonase gene from *A. tubingensis*, and there are no safety concerns with the use of the acetamidase selectable marker gene from *A. nidulans*.

No public health and safety concerns were identified in the assessment of enzyme under the proposed use conditions. Based on the reviewed data it is concluded that, in the absence of any identifiable hazard, an ADI 'not specified' is appropriate.

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