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

Supporting document 1

Risk and technical assessment report – Application A1131

Aqualysin 1 (Protease) as a Processing Aid (Enzyme)

Executive summary

The purpose of this Application is to seek amendment of Schedule 18 – Processing Aids, of the *Australia New Zealand Food Standards Code* (the Code) to include the food enzyme aqualysin 1 protease (EC 3.4.21.111) (aqualysin 1) from *Bacillus subtilis*, containing a protease gene from *Thermus aquaticus*. The intended use and purpose of aqualysin 1 is as a processing aid in the manufacture of bakery products to influence the elasticity and plasticity of dough.

FSANZ has assessed the evidence on technological suitability and safety of aqualysin 1. The data provided with the Application are considered adequate for this assessment.

The stated purpose of the enzyme preparation, namely as a processing aid for use in the manufacture of bakery products, is clearly articulated in the Application and has been assessed as technologically justified and demonstrated to be effective. It was also concluded that the enzyme performs its technological purpose during processing and manufacture of food after which it is inactivated so does not perform any technological function in the final food. It is therefore appropriately categorised as a processing aid and not a food additive.

The enzyme also complies with the internationally accepted Joint Expert Committee on Food Additives (JECFA) specifications for chemical and microbiological purity.

There are no public health and safety concerns associated with the use of aqualysin 1 as a processing aid based on the following considerations:

- The source organism, *B. subtilis,* is not pathogenic or toxigenic, and has a well-established history of use for producing enzymes used as food processing aids.
- The enzyme was not genotoxic in vitro.
- The no observed adverse effect level (NOAEL) in a 13-week repeated dose oral toxicity study in rats was 38400 mU (units of enzyme activity)/kg bw/d, equivalent to 606 mg Total Organic Solids (TOS)/kg bw/day. This is approximately 1000-fold higher than the Theoretical Maximum Daily Intake (TMDI) based on the proposed uses in bakery products.
- Aqualysin 1 does not have the characteristics of a potential food allergen and ingestion of any residual aqualysin 1 in bakery products is unlikely to pose an allergenicity concern.

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 for aqualysin 1. A dietary exposure assessment was therefore not required.

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1 Introduction

1.1 Objectives of the assessment

Currently, there are no permissions for the enzyme aqualysin 1 sourced from *Bacillus subtilis* containing the aqualysin 1 gene from *Thermus aquaticus* in the Code. Therefore, 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 were 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 the aqualysin 1 enzyme sourced from *B. subtilis* containing the aqualysin 1 gene from *T. aquaticus* as a processing aid.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity of the enzyme

Information regarding the identity of the enzyme that was taken from the Application has been verified using an appropriate enzyme nomenclature reference (IUBMB 2016). Additional information has also been included from this reference.

Generic common name: aqualysin 1

Accepted IUBMB[1] name: aqualysin 1

IUBMB enzyme nomenclature: EC 3.4.21.111

Other names: Caldolysin

The source microorganism of the enzyme is a genetically modified *B. subtilis*. The host organism is *B. subtilis* Raα3114, with the donor organism *T. aquaticus*, strain LMG8924. More information on the source microorganism is provided in section 3.

2.1.2 Technological purpose

Enzymes, such as aqualysin, that break peptide bonds that join amino acids together in proteins are known as proteases. Proteases are defined as a group of enzymes that catalyse the hydrolytic degradation of proteins or polypeptides to smaller amino acid polymers. In flour, proteases hydrolyse large polypeptides into smaller peptides and amino acids thus decreasing the molecular weight of the proteins. Proteases are used in the baking industry to reduce the mechanical dough development by lowering the dough viscosity and increasing the extensibility of the dough. This is especially important for flour that contains unusually strong or tough gluten.

[1] International Union of Biochemistry and Molecular Biology

This specific protease, aqualysin 1, is used to retard staling of bread and bakery products such as soft rolls, bagels, donuts, Danish pastries, hamburger rolls, pizza and pita bread and cakes. The onset of staling is a quality problem for bread and bakery goods manufacturers, which limits the commercial shelf life of the products so any improvement in retarding staling is both a quality improvement and commercial benefit.

This specific protease is a thermophilic (growing optimally at high temperatures) alkaline protease, which is less active and so more readily controlled during process conditions than other neutral proteases commonly used in baking processes. Therefore there are advantages in using this enzyme to have better control of dough strength and elasticity during the baking process.

The advantages aqualysin 1 provides include:

- faster dough development upon mixing
- better dough machinability
- reduced dough rigidness, which provides better processing tolerance
- improved dough structure and extensibility during the shaping and moulding process
- improved uniformity of final baked good shape, which might otherwise be impaired by processing of the dough
- consistent batter viscosity, important for production of waffles, pancakes and biscuit
- improved short-bite[2] of certain products like hamburger breads.

The enzyme is inactivated by heat during the baking processes, and so has no technological purpose in the final bakery products.

2.2 Manufacturing process

2.2.1 Production of the enzyme

The production of the aqualysin 1 enzyme preparation occurs by standard enzyme fermentation processes using the source microorganism genetically modified *B. subtilis* containing the gene for aqualysin 1 from *T. aquaticus*. Once the fermentation has been completed the broth containing the enzyme undergoes a number of separation and concentration steps to produce the final commercial enzyme preparation. It is then spray dried onto a solid carrier (wheat maltodextrin), taking the form of a powder. The preparation is finally standardised to ensure the appropriate concentration of the enzyme is in the preparation.

The production of the enzyme preparation is represented by the schematic in Figure 1 taken from the Application.

[2] Short bite, also sometimes referred to as opposite to chewiness and/or to toughness, is used to designate the force and total work needed to break a sample of bakery product and/or the number of chews needed to masticate a similar sample until a consistency that makes it ready to swallow (taken from Patent number US 20010097440 A1 by Puratos N.V. “Method and composition to improve short bite of bakery products”)

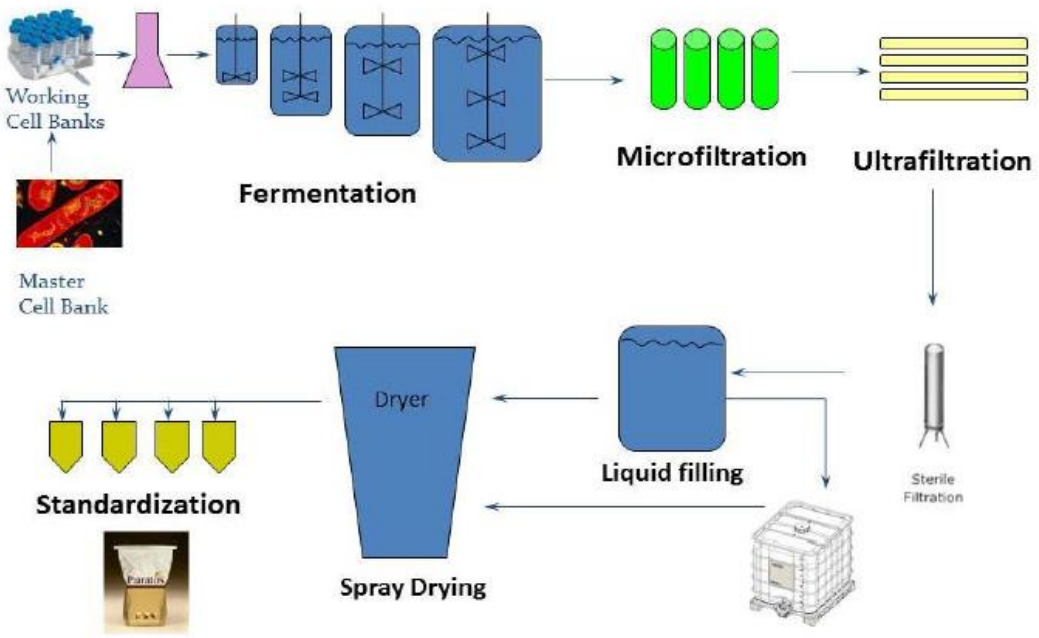

***Figure 1**: Schematic of the production process of the enzyme preparation*

2.2.2 Potential presence of allergens

The carrier for the enzyme preparation is wheat-derived maltodextrin. The enzyme preparation will be added to flour used to produce bread and other baked products, therefore wheat or other cereals containing gluten will be the main ingredients in these baked goods. The presence of wheat or products derived from wheat such as maltodextrin would be of concern to people with wheat allergies or intolerances.

2.2.3 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 (JECFA 2016) and in the Food Chemicals Codex (Food Chemicals Codex 2014). These primary sources of specifications are listed in the table to Section S3—2 in Schedule 3 – Identity and Purity. Enzyme preparations need to meet these enzyme specifications. Schedule 3 also includes specifications for heavy metals (section S3—4) if they are not specified within specifications in sections S3—2 and S3—3.

The enzyme preparation also meets the French purity criteria of enzymes (the order of 19 October 2006 on the use of processing aids in the manufacture of certain foodstuffs).

Table 1 provides a comparison of the product specifications with the international specifications established by JECFA as well as those detailed in the Code (as applicable).

Table 1: Product specifications for commercial enzyme preparation compared to JECFA and the Code specifications for enzymes

Analysis	Specifications		
	Product	JECFA	Australia New Zealand Food Standards Code (metals) (section S3—4)
Lead (mg/kg)	<0.10	≤ 5	≤2
Arsenic (mg/kg)	<0.10	-	≤1
Mercury (mg/kg)	<0.010	-	≤1
Cadmium (mg/kg)	<0.010	-	≤1
Antimicrobial activity	Not detected	Not detected	-
Coliforms (cfu/g)	<10	≤30	-
Salmonella (in 25 g)	Absent	Absent	-
E. coli (in 25 g)	Absent	Absent	-

Certificate of Analyses (CoA) were provided on three samples of the enzyme preparation which indicated compliance with the specifications. Based on the CoA against the above specifications, the final enzyme preparation meets international and Code specifications for enzyme preparations used in the production of food.

2.2.4 Stability

The aqualysin 1 enzyme has optimal activity from pH 7, with the peak at pH 9.5. Its optimum activity is achieved at approximately 70°C, within the range of 30–80°C. The enzyme has high thermostability but is inactivated at 90°C. The enzyme activity was stable in the pH range of 7–10 and the temperature of 70°C.

Analyses provided in the Application confirmed that commercial powdered enzyme preparations are stable for up to 12 months.

2.3 Food technology conclusion

FSANZ concludes that the stated purpose of this enzyme preparation; namely, for use as a processing aid in the manufacture of bakery products 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 stated purpose is to reduce the mechanical dough development by lowering the dough viscosity and increasing the extensibility of the dough. The enzyme performs its technological purpose during processing and manufacture of food after which it is inactivated so does not perform any technological function in the final food. It is therefore appropriately categorised as a processing aid and not a food additive. The enzyme preparation meets international purity specifications.

3 Hazard assessment

3.1 Background

3.1.1 Chemistry

Aqualysin 1 is an alkaline serine protease from the extreme thermophile *T. aquaticus*. Relevant physicochemical and enzymatic properties of aqualysin 1, and product specifications, are in the food technology assessment (Section 2).

3.1.2 Description of the genetic modification

The genetic modification involved the integration of the protease gene (*aqul)* from *T. aquaticus* into *B. subtilis* parent strain TD1100 to give a production strain designated Raα3114.

An expression cassette was constructed comprising:

- part of a promoter from *B. subtilis*
- a signal peptide from *B. subtilis*
- the coding sequence of the *aqul* gene (GenBank accession no. D90108) from *T. aquaticus* strain LMG8924
- a terminator sequence from *T. aquaticus*

The construction of the expression cassette and its integration into a linear vector was achieved through a series of polymerase chain reactions (PCR). In addition to the expression cassette, the vector contained a chloramphenicol resistance gene (to allow for subsequent selection of putative transformants) and two fragments (5' A and 3' B) that have the same 5' and 3' sequence as a gene normally present in *B. subtilis* and are used to ensure the vector is actually integrated into the T1100 chromosome at either the 5' or 3' corresponding sequence sites.

Competent cells of TD1100 were incubated in a solution containing the vector (now circularised) and putative transformants were selected for their ability to grow on a medium containing chloramphenicol. In this system, omission of chloramphenicol then resulted in homologous recombination at either the A or B sites and corresponding loss or retention respectively of the expression cassette. Analysis by Southern blotting allowed the selection of those strains that contained only the gene of interest and no chloramphenicol resistance gene. Protease production was evaluated by the ability of the strains to produce a halo of hydrolysis around the colonies on milk-containing solid medium.

By essentially repeating this procedure a number of times using different target A and B gene sequences, it was possible to produce a final production strain (RAα3114) containing several copies of the *aqul* gene. Southern blotting was also used to confirm the absence in RAα3114 of two other antibiotic resistance genes used during the development of TD1100 and in the production of the vector.

To test the stability of the insert in the production strain, Southern blot analysis, using a probe derived from the introduced *aqul* gene, was done on total DNA taken from colonies (three replicates) sampled over at least 10 successive subcultures (corresponding to more than 100 generations) and was compared to reference genomic DNA of the production strain. The band patterns (number and size) obtained for all the samples corresponded 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 aqualysin 1 was evaluated by considering the:

- hazard of the production organism, including history of safe use in food production processes
- toxicity studies on the enzyme preparation intended for commercial use
- bioinformatic analysis of the enzyme for potential allergenicity.

3.2 Hazard of the production organism

The parental strain (TD1100) has been derived from a type strain of *B. subtilis* through a series of targeted steps designed to inactivate the gene essential for sporulation, remove resident xylanase activity, and permit better expression of the introduced gene. These modifications are designed to improve efficiency and safety and do not raise any safety concerns. Following the transformation process and selection, 16S analysis was performed to make a phylogenetic comparison between TD1100 and RAα3114. This showed 100% identity between the two and confirmed that RAα3114 is indeed *B. subtilis*.

B. subtilis is not pathogenic or toxigenic (de Boer and Diderichsen, 1991), and has been recommended for a qualified presumption of safety (QPS) status by the Scientific Committee of the European Food Safety Authority (EFSA, 2007). FSANZ has previously assessed the safety of *B. subtilis* as the production organism for a number of enzymatic processing aids, as specified in Schedule 18 (Processing Aids) of the Code.

3.3 Hazard of the enzyme

3.3.1 Use of the enzyme as a food processing aid in other countries

Aqualysin 1 has been evaluated by the French agency ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail; National Agency for Food, Environmental and Occupational Health and Safety) and by Health Canada. ANSES concluded in 2012 that it found no health risk factors for consumers from the use of the enzyme. The Applicant was advised by Health Canada in 2014 that the enzyme will be added to the *List of Permitted Food Enzymes*. Aqualysin 1 has also been the subject of self-determination of GRAS status in the USA.

3.4 Evaluation of toxicity studies of the enzyme product

3.4.1 Genotoxicity

Reports of genotoxicity assays submitted by the Applicant include a bacterial reverse mutation assay (Ames test) and a chromosomal aberration test in Chinese Hamster Ovary (CHO) cells. The enzyme preparations used in the genotoxicity assays were representative of the food enzyme intended for use in bakery products.

Bacterial reverse mutation assay –Nelson Laboratories study report No. 421021 (2008)

The study was conducted in compliance with US FDA regulations 21 CFR Part 58. The test material was described as thermostable protease Ra *T. aquaticus* dried on maltodextrin. For the purpose of the assay, it was dissolved and diluted in sterile water. All assays were performed in triplicate. The tester strains of *Salmonella typhimurium* were TA97a, TA98, TA100, TA102 and TA1535, and the tests were conducted with and without addition of S9 fraction for metabolic activation. The test material was assayed using the plate incorporation method and the spot test method. Test article concentrations used were 0.05, 0.16, 0.5, 1.6 and 5 mg/plate for tester strains TA97a, TA98, TA102, TA1535 and the initial assay with TA100. The plate incorporation assay was repeated for TA100 with test article concentrations of 0.0005, 0.0016, 0.005, 0.016, 0.05 and 0.16 mg/plate. Positive control substances used in the absence of S9 fraction were sodium azide, 4-nitro-o-phenylenediamine, 2-aminofluorene, Mitomycin-C and 2-aminoanthracene, while those used in the presence of S9 fraction were 2-aminoanthracene and 2-aminofluorene. The positive controls were tested without S9 fraction and were tested only by the plate incorporation method. A solvent control was also tested in all assays. The criteria for a positive response

were a two-fold increase in the reversion rate over that of the negative control, and demonstration of a dose-response effect. All positive control substances gave the expected mutagenic response, but no evidence of mutagenicity was observed for the test material by either the plate incorporation method or the spot test. It was concluded that the test material did not meet the criteria for a potential mutagen.

Chromosomal aberration test in cultured Chinese Hamster Ovary (CHO) cells –TNO Study Report 8402/02 (2009)

The study was conducted in compliance with OECD Principles of Good Laboratory Practice and with OECD guideline 473 Genetic Toxicology: in vitro mammalian chromosome aberration test (adopted 21 July 1997). Test material was examined for its potential to induce structural chromosomal aberrations in CHO cells in both the absence and presence of S9 fraction. The solvent and negative control was culture medium. The positive control substances were Mitomycin C in the absence of S9 fraction and cyclophosphamide in the presence of S9 fraction. All assays were conducted in duplicate.

In the first test, both with and without S9 fraction, cells were pulse-treated for 4 hours and harvested 18 hours after onset of treatment.

In the first test with S9 fraction, the concentrations of test material were 10, 100, 150, 300, 500, 1000 1500, 2000, 3500 and 5000 µg/ml. The test material was cytotoxic to all cells at ≥ 2000 µg/ml, and to > 50% of cells at 1500 µg/ml. Approximately 30% of cells were rounded at 1000 µg/ml. Cells were examined for chromosomal aberrations at doses of 100, 150, 200, 300, 500, 1000 and 1500 µg/ml, and results compared to negative and positive controls.

In the first test in the absence of S9 fraction, the concentrations of test material were 75, 100, 150, 200, 300, 500,1000, 1500, 2000, 3500 and 5000 µg/ml. All cells were dead at ≥ 1000 µg/ml, and approximately 50% of cells were rounded at 500 µg/ml. Cells were examined for chromosomal aberrations at 75, 100, 150, 300 and 500 µg/ml, and compared to negative and positive controls.

In the second test in the presence of S9 fraction, again with treatment/harvesting times of 4/18 hours, the concentrations tested were 100, 200, 300, 500, 750, 1000, 1300, 1500, 1800, 2000, 2500, 3000, 3500, and 4000 µg/ml. All cells were dead at ≥ 2500 µg/ml. Approximately 40% of cells were rounded at 1800 and 2000 µg/ml, and cells were described as ‘slightly affected’ at 1300 and 1500 µg/ml. Cells were examined for chromosomal aberrations at 500, 750, 1000, 1300, 1500, 1800 and 2000 µg/ml, and compared to negative and positive controls.

In the second test in the absence of S9 fraction, the test material was assayed by continuous treatment (treatment/harvesting times of 18/18). Dose levels were 6.25, 12.5, 25, 50, 100, 200, 300, 500, 750, 1000, 1300, 1500, 1800 and 2000 µg/ml. Cytotoxicity was not observed at any concentration, but cell growth was impaired at ≥ 1500 µg/ml. Cells were examined for chromosomal aberrations at 300, 500, 750, 1000, 1300, 1500, 1800 and 2000 µg/ml, as well as in the negative and positive control cultures.

The test material did not induce a statistically significant increase in the number of cells with structural chromosomal aberrations at any of the concentrations or exposure regimes examined, as compared to the negative control assays. In the presence of metabolic activation, there was a statistically significant increase in the number of cells with numerical aberrations, in the form of endoreduplicated cells. This increase was considered to be dose-related in the first test, and suggested that the test material may interfere with chromosomal segregation during cell division.

The numbers of cells with structural chromosomal aberrations were within historical control range for all negative control assays, and the positive control substances induced the expected increases in the incidence of structural aberrations, confirming the validity of the study.

It was concluded that under the conditions of the study, the test material was not clastogenic to CHO cells.

3.4.2 Animal studies

13-week repeat-dose oral gavage study in Sprague-Dawley rats –CIT Study Report 37412 TCR (2012).

This study was conducted in compliance with the Principles of Good Laboratory Practice of the OECD and of the EU, and the study design was based on OECD Guideline 408, Repeated Dose 90-Day Oral Toxicity Study in Rodents. The enzyme preparations used in the study were representative of the food enzyme intended for the use in bakery products.

The test material was RA Protease aqualysin 1 from *T. aquaticus.* The batch of aqualysin, Lot #1101, was concentrated to have a higher enzymatic activity and Total Organic Solids (TOS) than the commercial enzyme. The batch used on study had a TOS of 23.86% and enzymatic activity of 31095 mU/mL. The mean TOS of the commercial product is 4.99% and the mean enzymatic activity is 3144 mU/mL, giving a mU/mg TOS of 63.33.

The study was conducted using Sprague-Dawley rats, with10/sex/group. Rats were acclimatised for 7 days prior to the first day of treatment, at which they were approximately 6 weeks old. They were pair-housed in suspended wire cages with *ad libitum* access to food and water. Animals were maintained under standard laboratory husbandry conditions. Rats were gavaged daily with 0, 12800, 25600 or 38400 mU/kg bw/day test material at constant dose volumes of 1.23, 0.41, 0.82 and 1.23 mL/kg bw/day respectively. The concentration of test material in the treated groups was constant at 31095 mU/mL. The vehicle and control article was deionised water. Endpoints during the in-life phase of the study included survival, clinical observations, bodyweights, food consumption, ophthalmology, and performance in a Functional Observational Battery (FOB) conducted in Week 11. Blood was collected prior to scheduled termination for haematology and clinical chemistry. Animals were killed by exsanguination under anaesthesia. A complete gross necropsy was conducted on all rats and organ weights were determined for adrenals, brain, heart, kidneys, liver, spleen, thymus, gonads, epididymides of males and uteri of females. Bone marrow smears were prepared from femoral marrow. Tissues were preserved and processed for histopathological examination.

No treatment-related effects were observed on survival, food intake, ophthalmological findings, performance on FOB, haematology, organ weights, gross findings on necropsy, or histopathological findings. Ptyalism (hypersalivation) and discolouration of fur was observed in all groups but was increased, both in the proportion of animals affected and in the duration of the observations, in mid- and high-dose groups when compared to controls. These clinical observations were not considered to be adverse. Although mean body weights of males were not affected by treatment, treated female rats showed moderate increases in group mean bodyweight, relative to that of female controls, although there was no clear dose-response relationship. This difference was not considered to be adverse. Male rats treated with ≥ 25600 mU/kg bw/d test material had slightly higher group mean AST levels compared to male controls, but females in all treated groups had lower group mean AST levels than that of female controls, and the differences were in all cases very slight and not considered to be toxicologically relevant. The group mean serum phosphorus level of the high-dose (38400 mU/kg bw/d) females was significantly lower than that of female controls, but there was no

corresponding change in group mean serum phosphorus in males. Group mean serum creatinine was minimally elevated in females treated with ≥25600 mU/kg bw/d test material, relative to female controls, but there was no corresponding change in males. The highest dose of RA Protease aqualysin 1 used on the study, 38400 mU/kg bw/d, was identified as the No Observed Adverse Effect Level (NOAEL).

The NOAEL of 38400 mU/kg bw/d is equivalent to 606 mg TOS/kg bw/day.

Other studies

Other studies included in the Application were GLP studies of respiratory toxicity in rats, acute dermal irritation in rabbits and acute ocular irritation in rabbits. These studies are relevant to the safety of workers handling the enzyme preparation in bakeries and other food industry settings, but are not relevant to the safety of the consumer of products in which aqualysin 1 has been used as a processing aid. The studies are therefore not reviewed in detail in this hazard assessment. It is noted that the LC_{50} for inhaled aqualysin 1 in the rat is in excess of 5.07 mg/L air; that aqualysin 1 is classified as a mild irritant to rabbit skin according to the Draize classification scheme; and that aqualysin 1 is classified as a minimal irritant to the rabbit eye according to a modified Kay and Calandra classification scheme.

3.4.3 Bioinformatic analysis for potential allergenicity

An *in silico* analysis was used to compare the amino acid sequence, not including the signal peptide, of aqualysin 1 enzyme protein from *B. subtilis* with that of known allergens in the FARRP (Food Allergy Research and Resource Program) dataset available at http://www.allergenonline.org . The analyses were:

- The full FASTA search, which provides per cent identity and an E-score to indicate whether there are any alignments between the query protein and sequences within the allergen database.

- The sliding 80mer sliding window search in order to find any matches that meet or exceed the Codex Alimentarius (Codex 2003) FASTA alignment threshold (at least 35% identity over 80 amino acids) for potential allergenicity. This threshold aims to detect potential conformational IgE-epitopes.

- An 8mer exact match, which may be indicative of cross-reactive proteins.

Twenty-three homologies were found, of which 20 are linked to respiratory allergies and three are linked to dermal allergies. Many are proteases, and all are associated with bacterial or fungal genera, including *Rhodotorula, Penicillium, Cladosporium*, *Aspergillus, Arthroderma, Davidiella, Trichophyton,* and *Bacillus*. No matches were found with any known food allergens. It was concluded that aqualysin 1 does not have the characteristics of a potential food allergen and ingestion of any residual aqualysin 1 in bakery products is unlikely to pose an allergenicity concern. It is noted that no clinical allergenicity has been identified during the research and development work, during pre-industrial trials and industrial up-scaling, or during downstream processing. The only allergen present in the commercial enzyme preparation is the food-grade wheat maltodextrin carrier. However this is not an issue because the enzyme is exclusively intended to be used in bakery products, in which the main ingredient is wheat flour or other gluten-containing cereals.

3.5 Risk assessment discussion and conclusions

B. subtilis is not pathogenic or toxigenic, and has a well-established history of use for production of enzymes used as food processing aids. Aqualysin 1 is in use as a food processing aid in France, Canada and the USA.

There is no evidence that aqualysin 1 is genotoxic, and it was well-tolerated by rats in a 13-week repeat-dose oral gavage study. The NOAEL in that study was 38400 mU (units of enzyme activity)/kg bw/d, equivalent to 606 mg TOS/kg bw/day. In contrast, the Theoretical Maximum Daily Intake (TMDI) of aqualysin by a European consumer of very large amounts of bread (90 kg/year) is calculated to be 0.6229 mg TOS/kg bw/day, almost 1000-fold less. Australian consumers generally eat less bread than Europeans, with a mean intake of 32.3 kg/year for Australian adults as compared to a mean intake of 50 kg/year for European adults. Thus the margin of safety for aqualysin is >1000 fold for Australian consumers. FSANZ notes that the quantity of aqualysin 1 used in bakery products is limited by the fact that if it is used in excess, it causes poor structure of the bread.

It was concluded that aqualysin 1 does not have the characteristics of a potential food allergen and ingestion of any residual aqualysin 1 in bakery products is unlikely to pose an allergenicity concern.

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 for aqualysin 1. A dietary exposure assessment was therefore not required.

4 Conclusion

This risk and technical assessment considered the technological suitability, and potential hazard of the enzyme and source microorganism, including potential allergenicity.

FSANZ concluded that the proposed use of the enzyme is technologically justified in the form and prescribed amounts as a processing aid and has been demonstrated to be effective to perform the stated purpose. The evidence presented is sufficient to determine that no safety concerns with the source organism or the enzyme exist. Aqualysin 1 is intended for use in the manufacture of bakery products and is inactivated by heat, so although residual enzyme may be present in the final food, it would be inactive and susceptible to digestion like other dietary proteins. Thus the aqualysin 1 enzyme sourced from *B. subtilis* containing the aqualysin 1 gene from *T. aquaticus* is unlikely to pose any health risk when used as a food processing aid.

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