

**22 February 2018**  
**[39-18]**

Approval report – Application A1136

## Protein Glutaminase as a Processing Aid (Enzyme)

---

Food Standards Australia New Zealand (FSANZ) has assessed an application made by Amano Enzyme Inc. to permit the use of protein-glutaminase from *Chryseobacterium proteolyticum* strain AE-PG as a processing aid to enhance protein solubility in various products.

On 21 September 2017, FSANZ sought submissions on a draft variation and published an associated report. FSANZ received seven submissions.

FSANZ approved the draft variation on 7 February 2018. The Australia and New Zealand Ministerial Forum on Food Regulation was notified of FSANZ's decision on 20 February 2018.

This Report is provided pursuant to paragraph 33(1)(b) of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act).

# Table of contents

<b>EXECUTIVE SUMMARY .....</b>	<b>3</b>
<b>1 INTRODUCTION .....</b>	<b>4</b>
1.1 THE APPLICANT .....	4
1.2 THE APPLICATION .....	4
1.3 THE CURRENT STANDARD .....	4
1.3.1 <i>International Standards</i> .....	4
1.4 REASONS FOR ACCEPTING APPLICATION .....	4
1.5 PROCEDURE FOR ASSESSMENT.....	5
1.6 DECISION .....	5
<b>2 SUMMARY OF THE FINDINGS.....</b>	<b>5</b>
2.1 SUMMARY OF ISSUES RAISED IN SUBMISSIONS .....	5
2.1.1 <i>Specificity of protein glutaminase</i> .....	10
2.1.2 <i>Protein glutaminase and coeliac disease</i> .....	10
2.2 RISK ASSESSMENT .....	11
2.3 RISK MANAGEMENT .....	12
2.3.1 <i>Levels of addition</i> .....	12
2.3.2 <i>Specifications</i> .....	12
2.3.3 <i>Labelling</i> .....	12
2.4 RISK COMMUNICATION .....	13
2.4.1 <i>Consultation</i> .....	13
2.5 FSANZ ACT ASSESSMENT REQUIREMENTS .....	13
2.5.1 <i>Section 29</i> .....	13
2.5.2 <i>Subsection 18(1)</i> .....	14
2.5.3 <i>Subsection 18(2) considerations</i> .....	15
<b>3 REFERENCES.....</b>	<b>16</b>
ATTACHMENT A – DRAFT VARIATION TO THE <i>AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE</i> .....	18
ATTACHMENT B – DRAFT EXPLANATORY STATEMENT .....	20

## Supporting document

The [following document](#)<sup>1</sup> which informed the assessment of this Application is available on the FSANZ website:

SD1 Risk and technical assessment report

---

<sup>1</sup> [Application A1136](#)

## Executive summary

Amano Enzyme Inc. (Amano) submitted an Application seeking permission for the enzyme protein glutaminase sourced from *Chryseobacterium proteolyticum* strain AE-PG, to be added to the list of permitted processing aids in the *Australia New Zealand Food Standards Code* (the Code).

Protein glutaminase enhances protein solubility in the manufacture and/or processing of baked products and pasta/noodle making, milk and dairy processing, meat and fish processing, grain processing, yeast products and egg based products. The technological purpose is to improve emulsification, foam stabilisation and gelling in these proteinaceous foods. It also decreases the formation of 'off flavours' associated with flavour-protein interactions compared to alternative technologies such as thermal or chemical modification methods.

The safety data submitted by Amano was only for protein glutaminase sourced from one particular non-genetically modified strain of *C. proteolyticum* (i.e. *Chryseobacterium proteolyticum* strain AE-PG). FSANZ therefore only assessed protein glutaminase sourced from that particular strain.

FSANZ has concluded there are no public health and safety issues associated with using protein glutaminase sourced from *C. proteolyticum* strain AE-PG as a processing aid. The enzyme also complies with the internationally accepted Joint Expert Committee on Food Additives (JECFA) specifications for chemical and microbiological purity.

FSANZ was satisfied that the enzyme's use as a processing aid in the manner specified in the Application was technologically justified. FSANZ also concluded that, as the enzyme performs its technological purpose during processing and manufacture of food only, it is appropriately categorised as a processing aid rather than a food additive. Enzymes used to produce and manufacture food are considered processing aids and the relevant permissions are listed in Schedule 18 of the *Australia New Zealand Food Standards Code* (the Code). Enzymes of microbial origin permitted to be used as processing aids are also listed in Schedule 18.

FSANZ has therefore prepared a draft variation to the Code to permit the use as a processing aid of protein glutaminase (EC 3.5.1.44) sourced from *C. proteolyticum* strain AE-PG, for the particular technological purpose of deamidating proteins during the manufacture and/or processing of the following types of food: baked products; pasta; noodles; milk; other dairy products; meat; fish; grains; yeast; and egg based products. The draft variation will require that the amount used must be consistent with good manufacturing practice (GMP).

# 1 Introduction

## 1.1 The Applicant

The Applicant is Amano Enzyme Inc. (Amano). It produces specialty enzymes for the food industry, pharmaceuticals and diagnostic medicines.

## 1.2 The Application

The Application sought permission to use an enzyme, protein glutaminase sourced from *Chryseobacterium proteolyticum* (*C. proteolyticum*), strain AE-PG as a processing aid to manufacture specified food products.

Protein glutaminase benefits food manufacturers by enhancing protein solubility in various applications.

## 1.3 The current Standard

Enzymes used in processing and manufacturing food are considered processing aids.

Paragraph 1.1.1—10(6)(c) of the *Australia New Zealand Food Standards Code* (the Code) provides that a food for sale must not have, as an ingredient or a component, a substance that is used as a processing aid, unless expressly permitted.

Standard 1.3.3 and Schedule 18 of the Code list the permitted processing aids. Enzymes of microbial origin permitted to be used as processing aids are listed in the table to subsection S18—4(5) or in the table to subsection S18—9(3).

Section 1.1.2—13 of the Code defines the expression ‘used as a processing aid’. That definition imposes certain conditions on substances permitted by Standard 1.3.3 and Schedule 18 to be used as a processing aid, such that it does not perform a technological function in the final food for sale.

Protein glutaminase sourced from *C. proteolyticum* strain AE-PG is not listed in Schedule 18 and is not permitted to be used as a processing aid.

### 1.3.1 International Standards

The enzyme preparation has been approved for use in food production in France and the USA.

The Codex Alimentarius does not establish Standards for processing aids or for enzymes. Individual countries regulate the use of enzymes differently to the Code.

However, there are internationally recognised specifications for enzymes. These enzyme specifications are established by JECFA (2006) and the Food Chemicals Codex (Food Chemicals Codex 2014).

## 1.4 Reasons for accepting Application

The Application was accepted for assessment because:

- it complied with the procedural requirements under subsection 22(2) of the FSANZ Act; and
- it related to a matter that warranted the variation of a food regulatory measure.

## 1.5 Procedure for assessment

The Application was assessed under the General Procedure.

## 1.6 Decision

The draft variation as proposed following assessment was approved without amendment.

Protein glutaminase (EC 3.5.1.44) sourced from *C. proteolyticum* strain AE-PG, will be listed in the table to subsection S18—9(3), permitting its use for the specific technological purpose stated below with the condition that the amount used must be consistent with good manufacturing practice (GMP).

The enzyme will be listed in the table to subsection S18—9(3), rather than the table to subsection S18—4(5) as requested by the Applicant. This is because the enzyme will be permitted to be used only for a specific technological purpose—to deamidate proteins during the manufacture and/or processing of the following foods: baked products, pasta, noodles, milk, other dairy products, meat, fish, grains, yeast, and egg based products.<sup>2</sup>

The approved draft variation is at Attachment A. The variation takes effect on the date of gazettal.

The related explanatory statement is at Attachment B. An explanatory statement is required to accompany an instrument that is lodged on the Federal Register of Legislation.

## 2 Summary of the findings

### 2.1 Summary of issues raised in submissions

FSANZ called for submissions on a proposed draft variation to Schedule 18 on 21 September 2017. Seven submissions were received. The Victorian Departments of Health and Human Services, and Economic Development, Jobs, Transport and Resources; the New Zealand Food and Grocery Council and the New Zealand Ministry for Primary Industries supported the application. The Food Intolerance Network, South Australia Health and the New Zealand Institute for Plant & Food Research raised concerns with aspects of the Application or the draft variation. The Food Intolerance Network also submitted a copy of an online petition signed by over 11,000 people, via Change.Org, requesting FSANZ 'reject hiding MSG in foods'.

Issues raised in submissions and FSANZ's responses are detailed in Table 1.

---

<sup>2</sup> Deamidation is a chemical reaction in which an amide functional group in the side chain of the amino acids asparagine or glutamine is removed or converted to another functional group.

Table 1 summary of submissions

Issue	Submitter	Response
<p><b>Protein glutaminase is a food additive and not a processing aid and therefore is required to be shown in the statement of ingredients.</b></p> <p><b>The products of the enzyme protein glutaminase perform a ‘technological function’ in the food for sale. This function is flavour enhancement. This enzyme is designed to, and will, produce free glutamates which are flavour enhancers.</b></p> <p><b>The Application states that the use of enzyme <i>also</i> “decreases flavour fade or ‘off flavour’ problems associated with flavour-protein interactions”.</b></p>	<p>Food Intolerance Network</p> <p>Petition</p>	<p>The enzyme is categorised as a processing aid pursuant to the Code.</p> <p>Food enzymes perform their technological purpose during the manufacture or processing of food but play no such function in the final food. Protein glutaminase is inactivated by either an increase in heat or a reduction in pH so does not perform a technological purpose in the final product. As such, it is considered a processing aid in accordance with section 1.1.2—13 of the Code.</p> <p>The technological purpose of protein glutaminase is to alter the structure and properties of protein in foods to increase the protein’s solubility. Proteins remain intact during this reaction and no free amino acids (including glutamic acid and salts such as monosodium glutamate (MSG)) are produced, meaning flavour enhancement does not occur.</p> <p>The permission granted by the approved draft variation will not permit the enzyme’s use as a food additive, including as a flavour enhancer.</p> <p>The mention of reduction in “off-flavours” in the earlier Call for Submissions report related to alternative production methods to using enzymes for protein deamination, such as chemical reactions or using heat processes, which cause more off-flavours to develop. The report has been amended to clarify this point.</p>
<p><b>The presence of the enzyme must be declared in labelling due to the public health risks. Some consumers have been scientifically proven to react to the products created by this enzyme (eg, due to the elevated levels of glutamates) and so must avoid these products in foods. They will not be able to do so if permission is given to use this enzyme as a processing aid.</b></p>	<p>Food Intolerance Network</p> <p>Petition</p>	<p>FSANZ’s risk assessment concluded that the use of the enzyme as a processing aid for the prescribed technological purpose will not pose a risk to public health or safety.</p> <p>As demonstrated in FSANZ’s risk assessment (SD1), protein glutaminase from <i>C. proteolyticum</i> strain AE-PG does not have the characteristics of a potential food allergen and ingestion of any residual trace levels of protein glutaminase in food products is unlikely to pose an allergenicity concern. That assessment is based on the best available scientific evidence.</p> <p>Claims that the enzyme will result in elevated levels of glutamates and be a hidden form of MSG are not correct.</p>

Issue	Submitter	Response
		<p>The enzyme does not contain or produce MSG. MSG is a single salt composed of the single amino acid, glutamic acid.</p> <p>The technological purpose of protein glutaminase is to alter the structure and properties of protein in foods to increase the protein's solubility. Proteins remain intact during this reaction and no free amino acids (including glutamic acid and salts such as MSG) are produced, meaning flavour enhancement will not occur. The enzyme acts on glutaminyl residues bound to the large protein molecule to produce the same large protein molecule but now with a bound glutamyl residue; it does not produce small simple molecules such as free glutamate (see full explanation in SD1, specifically sections 1.1, 2.1 and 2.3).</p>
<p><b>Approving protein glutaminase's use as a processing aid will provide a new way of hiding MSG in foods.</b></p>	<p>Private Individual</p> <p>Petition</p>	<p>See responses above.</p>
<p><b>The presence of the enzyme must be declared in labelling as consumers have a right to know what is in their food.</b></p> <p><b>Food manufacturers are hiding ingredients in foods, by categorising them as processing aids, thereby blatantly misleading consumers.</b></p> <p><b>Declaring protein glutaminase as a processing aid is in breach of Object (c) of the Food Standards Australia New Zealand Act 1991: <i>the provision of adequate information relating to food to enable consumers to make informed choices</i></b></p>	<p>Food Intolerance Network</p> <p>Petition</p>	<p>FSANZ's risk assessment is that the use of the enzyme as a processing aid for the prescribed technological purpose will not pose a risk to public health or safety. That assessment is based on the best available scientific evidence.</p> <p>The issue of whether processing aids should be declared in the statement of ingredients was considered in 1997 as part of Proposal P143 – Assessment of provisions for the statement of ingredients<sup>3</sup>. The decision was taken to not require processing aids to be declared in the statement of ingredients. This was considered a pragmatic approach taking into account the costs to the food industry of additional labelling and possible benefits to consumers.</p> <p>Not declaring processing aids in the statement of ingredients is consistent with labelling requirements internationally, including within Codex Alimentarius.</p> <p>Food manufacturers can voluntarily add labelling information to indicate their use despite the labelling exemption.</p> <p>See also the responses above.</p>

<sup>3</sup> Copy available upon request to [standards.management@foodstandards.gov.au](mailto:standards.management@foodstandards.gov.au)

Issue	Submitter	Response
<p><b>Protein glutaminase is a protease as currently listed in the Australia New Zealand Food Standards Code and so is legally required to be shown in the Statement of Ingredients.</b></p> <p><b>Protein glutaminase should be listed in Schedule 8 under 1101 proteases (and wherever else required) so that it appears in the statement of ingredients on foods</b></p>	<p>Food Intolerance Network</p>	<p>As described above, protein glutaminase is a processing aid for the purpose for this Application.</p> <p>Schedule 8 of the Code lists the food additive names and code numbers for the purposes of subsection 1.2.4—7(1) of the Code. That section only requires the declaration of substances used as a food additive. It does not apply to substances that are used as processing aids.</p> <p>FSANZ’s risk assessment concluded that the use of the enzyme as a processing aid in the manner proposed poses no risk to public health and safety. Therefore, no additional labelling requirements are required. The general exemption from processing aids being declared in the statement of ingredients will therefore apply.</p>
<p><b>Concern over and opposition to the recent proposal to delete amylases, proteases and lipases from the list of additives that are required to be shown in the Statement of Ingredients, as detailed in Annex A of the Food Intolerance Network submission.</b></p> <p><b>Attachment A is a copy of the joint FAO/WHO food standards programme Codex Committee on Food Additives proposed draft revision to the international numbering system (INS) for food additives (CAC/GL 36-1989)</b></p>	<p>Food Intolerance Network</p>	<p>No such proposal exists. Annex A of the submission does not relate to any proposal by FSANZ to delete the three enzymes mentioned in Schedule 8. The report of the 49th Session of the CCFA (REP17/FA) (paragraph 112) that considered the document, CX/FA 17/49/12 (from which the extract for Annex A of the submission was taken), makes no reference to information regarding amylases (INS 1100 i, ii, iii, iv, v, vi), proteases (INS 1101 i, ii, iii, iv, v, vi) and lipases (INS 1104), other than to note that the proposed deletion of these substances from Class Names and the International Numbering System (INS) for Food Additives (CAC/GL 36-1989) is outside the mandate of the working group established to consider such matters.</p>
<p><b>Some food category terms used in the draft variation referring to the use of the enzyme are not defined by the Code. These terms are “bakery products” and “other dairy products”. The proliferation of food terms that are not defined in the Code (Standard 1.1.2) makes interpretation and enforcement difficult.</b></p>	<p>South Australia Health</p>	<p>FSANZ does not consider there is a need to provide a prescriptive, all-inclusive definition for “bakery products”, or “other dairy products”. These terms are already present and undefined in the Code (see, for example, Standard 2.2.1 and Schedules 10, 15, 17, and 22). Where definitions are provided (e.g. definition of “dairy products”), these definitions are only illustrative (i.e. “dairy products” includes ...) and are not prescriptive. In the absence of a definition, these terms generally have their accepted and ordinary meaning. FSANZ is not aware of any evidence of a problem with this approach to date.</p>
<p><b>There is a possibility that the glutamine deamidation activity of protein glutaminase may result in the formation of elevated levels of epitopes (small peptides) responsible for coeliac disease in bakery products. This could potentially trigger fully developed coeliac disease in pre-disposed individuals.</b></p>	<p>New Zealand Institute for Plant &amp; Food Research</p>	<p>Microbial glutaminase has a long history of safe use in food processing. FSANZ is not aware of any scientific studies demonstrating an association between use of protein-glutaminase and increased levels of coeliac disease epitopes, or an increased risk of coeliac disease.</p> <p>FSANZ notes a study by Gerrard and Sutton (2005) which hypothesised that</p>



Issue	Submitter	Response
<p><b>Further research needs to be performed to ensure that a clear consensus on the risks around its use in bakery products can be formed.</b></p>		<p>pre-treatment of cereal products with another food enzyme, microbial transglutaminase, could result in the formation of epitopes associated with coeliac disease. In addition, a small number of earlier studies have shown a potential for microbial transglutaminase treatment of bread to deamidate gluten-related peptides which are then immunogenic when incubated with serum from coeliac disease patients (Cabrera-Chávez et al 2008; Cabrera-Chávez et al 2009). However these studies have used much higher doses of transglutaminase than those used in normal bakery practices and are not relevant for regulatory purposes. In contrast, a recent study (Heil et al 2017) has shown that wheat bread prepared with standard bakery levels of microbial transglutaminase does not result in the formation of deamidated gliadins that are detectable by sera from celiac disease patients.</p> <p>Individuals with coeliac disease or gluten intolerance would be advised to avoid gluten-containing foods irrespective of whether they contain protein glutaminase. This would be expected to manage any potential concerns that this enzyme might increase the levels of coeliac disease epitopes in gluten containing foods.</p>

### **2.1.1 Specificity of protein glutaminase**

In its submission to A1136, the Food Intolerance Network (FIN) claimed that the protein glutaminase is a hidden form of MSG and would vastly increase MSG levels in foods. Furthermore, FIN also claimed that the use of protein glutaminase will mislead the consumer. An online campaign was launched on the change.org website, titled “Reject a new way of hiding MSG in foods”, attracted over 11,600 consumers in support of it.

FSANZ sets food standards based on risk analysis using the best available scientific evidence consistent with requirements in the Food Standards Australia New Zealand Act 1991. This evidence not only includes the safety of a substance proposed to be added to, or used to produce, food, but also the purpose of that substance in food.

Protein glutaminase is a protein-deamidating enzyme, which catalyses the deamidation of protein bound glutamyl residues to protein bound glutamyl residues (Yamaguchi et al 2001). The specificity of this enzyme is for carbon-nitrogen bonds rather than peptide bonds (Kikuchi et al) and on this basis it does not specifically cleave the amino acid backbone of the protein chain. Importantly, the enzyme is ineffective on free glutamine (Yamaguchi et al 1991, Hashizume et al 2011).

The generation of negatively charged bound glutamyl residues on the protein chain triggers the unfolding of the protein thereby increasing solubility. This results in the enhanced functionality of the protein in relation to emulsification, foam stabilisation and gelling.

Based on this mechanism of action, it is not plausible that free glutamates, other amino acids or MSG would be produced by the action of this enzyme.

### **2.1.2 Protein glutaminase and coeliac disease**

In its submission to A1136, the New Zealand Institute for Plant & Food Research suggested protein glutaminase could result in the formation of elevated levels of epitopes (small peptides) responsible for coeliac disease in bakery products. The group had previously hypothesised that this effect could arise from the use of microbial transglutaminase in cereal products (Gerrard and Sutton 2005). They state that because the level of glutamine deamidation activity is likely to be higher in protein glutaminase than in microbial transglutaminase it is likely that higher levels of the epitopes responsible for coeliac disease may be generated. It suggested these epitopes could potentially trigger fully developed coeliac disease in pre-disposed individuals. They therefore recommended that protein glutaminase is not approved for use in bakery products until further research is done to ensure a clear consensus on the risks associated with its use in bakery products.

In considering changes to the Food Standards Code, FSANZ uses an internationally accepted risk analysis framework to ensure food regulatory measures are based on the best available scientific evidence. By applying this framework we ensure the effectiveness and appropriateness of food regulatory measures.

FSANZ has reviewed the literature and has not identified any evidence of an association between use of protein glutaminase and increased levels of coeliac disease epitopes in foods, or an increased risk of coeliac disease.

Microbial transglutaminase is an approved processing aid in Schedule 18—4(5) of the Code and has a long history of safe use in food processing. Currently there are no clinical or epidemiological data identifying a relationship between coeliac disease and the use of microbial transglutaminase in baked foods.

Some investigators have shown that microbial transglutaminase deamidates gluten-related peptides (Skovbjerg et al 2004; Dekking et al 2008) and that microbial transglutaminase-treated gluten proteins and other wheat proteins are immunogenic when incubated with serum from coeliac disease patients (Cabrera-Chávez et al 2008; Dekking et al 2008; Matthias et al 2016).

However, the experimental conditions in many of these studies are of uncertain relevance to the use of microbial transglutaminase in food production. For example, several studies have involved the incubation of the enzyme with synthetic gluten peptides or the use of gluten peptides experimentally bound to transglutaminase, rather than using food production conditions (Skovbjerg et al 2004; Gianfrani et al 2007; Dekking et al 2008; Matthias et al 2016). In experiments where microbial transglutaminase was used in bakery products, the concentrations were much higher than those used in standard bakery production (Cabrera-Chávez et al 2008, 2009).

In contrast, other studies have reported no difference in the immunoreactivity of gliadin (a gluten protein) extracted from pasta with or without microbial transglutaminase treatment (Ruh et al 2014), and that microbial transglutaminase treatment of flour or gliadins may inhibit the immunogenic response to gliadin through its cross-linking activity (Gianfrani et al 2008; Lombardi et al 2013; Zhou et al 2017). In addition, Heil et al (2017) showed that wheat bread prepared with typical microbial transglutaminase concentrations used in standard bakery processes (approximately 1 – 3 units per kg of flour) does not lead to immunodetectable amounts of coeliac disease immunotoxic deamidated gliadins.

Overall, there is currently no evidence that protein glutaminase or microbial transglutaminase used according to permitted uses in the Food Standards Code are associated with increased levels of coeliac disease epitopes in gluten-containing food or an increased risk of coeliac disease. Individuals with coeliac disease or gluten intolerance would be advised to completely avoid gluten-containing foods irrespective of whether they are manufactured using protein glutaminase. This would be expected to manage any potential concerns that this enzyme might increase the levels of coeliac disease epitopes in gluten-containing foods and lead to a significantly increased risk of coeliac disease.

FSANZ will continue to monitor any new studies on the use of protein glutaminase and potential links to an increased risk of coeliac disease.

Based on FSANZ's assessment of the application, the FSANZ Board recommends the approval of this application.

## **2.2 Risk assessment**

FSANZ conducted a risk assessment on the proposed use of the enzyme [see supporting document 1 (SD1)].

In summary, the technological purpose of this enzyme, namely, for use as a processing aid to manufacture various proteinaceous foods, was clearly articulated in the Application. The evidence presented provided adequate assurance that the enzyme, in the form and prescribed amounts, was technologically justified in achieving its technological purpose. Also, it performs its technological purpose during processing and manufacture of food and does not perform a technological purpose 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.

The technological purpose of protein glutaminase is to alter the structure and properties of protein in foods to increase the protein's solubility. Proteins remain intact during this reaction

and no free amino acids (including glutamic acid and salts such as MSG) are produced, meaning flavour enhancement will not occur. The enzyme acts on proteins, being the glutamyl residue bound to the large protein molecule to produce the same large protein molecule but now with a bound glutamyl residue; it does not produce small simple molecules like free glutamate (see full explanation in SD1, specifically sections 1.1, 2.1 and 2.3).

After undertaking a risk assessment, FSANZ concluded there are no public health and safety issues associated with using protein glutaminase (EC number of 3.5.1.44) sourced from *C. proteolyticum* strain AE-PG as a food processing aid for the particular technological purpose of the Application. Protein glutaminase was not genotoxic *in vitro* and caused no adverse effects in a sub-chronic toxicity study in rats. In the absence of any identifiable hazard an Acceptable Daily Intake 'not specified' is appropriate. The enzyme does not have the characteristics of a potential food allergen.

## **2.3 Risk management**

The risk assessment concluded there are no public health and safety concerns associated with using this enzyme as intended. The food technology aspect of the risk assessment concluded that the enzyme meets its stated technological purpose for use as a processing aid in the manufacture of certain proteinaceous products, and not as a food additive. The main risk management options available to FSANZ were to approve or reject the request to amend the Code, imposing any appropriate conditions. Other risk management issues for this Application are related to labelling and enzyme nomenclature, which are discussed below. The consideration of costs and benefits summarised in section 2.5.1.1 takes account of the safety of the enzyme preparation.

The Application requested listing the permission for the enzyme in section S18—4(5), however this would have permitted the enzyme's use for any technological purpose. This was not consistent with the risk assessment, which assessed the enzyme only for the technological purpose stated in the Application. For this reason the permission for the enzyme will be listed in subsection S18—9(3), limiting its technological purpose to deamidate proteins in the manufacture and/or processing of baked products, pasta; noodles; milk; other dairy products; meat; fish; grains; yeast and egg based products. The Applicant has confirmed that it had no objection to this approach.

### **2.3.1 Levels of addition**

In the absence of any public health or safety concerns identified by the risk assessment, there was no reason to limit the levels of addition apart from the requirement to use in accordance with GMP.

### **2.3.2 Specifications**

The Codex Alimentarius does not establish standards for processing aids or for enzymes, however there are internationally recognised specifications for enzymes. These enzyme specifications are established by JECFA (2006) and the Food Chemicals Codex (2014).

Individual countries regulate the use of enzymes differently to the Code. The enzyme preparation has been approved for use in food production in France and the USA.

### **2.3.3 Labelling**

As a general rule, processing aids, including those in subsection in S18—9(3), are exempt from the requirement to be declared in the statement of ingredients in accordance with paragraphs 1.2.4—3(2)(d) and (e) in Standard 1.2.4.

The risk assessment concluded that the use of the enzyme preparation as a processing aid poses no risk to public health and safety and that it will perform the specified technological purpose as a processing aid. Therefore, no additional labelling requirements are required in this case. The general exemption for processing aids will apply to foods produced using this enzyme as a processing aid.

See also the examination of labelling issues in Table 1.

## **2.4 Risk communication**

### **2.4.1 Consultation**

Consultation is a key part of FSANZ's standards development process. Public submissions on a proposed draft variation to Schedule 18 were called on 21 September 2017 and closed 2 November 2017.

The call for submissions was notified through a media release, Food Standards News and FSANZ's social media channels. FSANZ has communicated directly with some submitters on issues raised relating to coeliac disease and MSG. A [web page was also developed](#) to address concerns about MSG.

Seven submissions were received from various stakeholders and considered by the FSANZ Board. FSANZ acknowledges the valuable contribution by these organisations to the rigour of our assessment.

## **2.5 FSANZ Act assessment requirements**

### **2.5.1 Section 29**

#### **2.5.1.1 Consideration of costs and benefits**

The Office of Best Practice Regulation (OBPR) granted FSANZ a standing exemption from the requirement to develop a Regulatory Impact Statement for the approval of additional processing aids (OBPR correspondence dated 24 November 2010, reference 12065). This standing exemption was provided as permitting additional processing aids is a minor, deregulatory change and their use is voluntary. This standing exemption relates to the introduction of a food to the food supply that has been determined to be safe.

However, notwithstanding that exemption, the FSANZ Act requires FSANZ to have regard to whether costs that would arise from the proposed measure outweigh the direct and indirect benefits to the community, government or industry that would arise from the proposed measure.

FSANZ's assessment is that the direct and indirect benefits that would arise from permitting the use of protein glutaminase as a processing aid outweigh the costs arising from that permission being granted.

The use of the enzyme as a processing aid in the manner proposed will not pose a health or safety risk for consumers (see above). Consumers may benefit from: foods having improved texture and reduced off flavours; the choice of an additional range of food products that become available due to the use of enzyme by Australian and New Zealand manufacturers; and access to products manufactured using enzyme that are currently manufactured overseas. If permission to use the enzyme as a processing aid is not granted, consumers may have access to a smaller range of products.

Protein glutaminase benefits food manufacturers by enhancing protein solubility in various applications such as baking, pasta/noodle making, milk, dairy, meat, fish, grain processing and manufacture of yeast products and egg-based products. The functionality that protein glutaminase provides is an improvement in emulsification, foam stabilisation and gelling. It can also decrease 'off flavour' problems associated with flavour-protein interactions, found in alternative technologies such as chemical and thermal treatments. Its use by industry is voluntary, therefore it will only be used where industry believe a net benefit exists above using existing manufacturing processes.

The enzyme has already been permitted for use overseas (see above). Therefore, if permission to use the enzyme as a processing aid is not granted, Australia/New Zealand food manufacturers will be unable to access and use a product assessed as safe that is available to their overseas competitors. There is also a potential cost to overseas food manufacturers and importers, in that they will be unable to expand the international trade of products made using this enzyme preparation, respectively, to Australia/New Zealand.

Approval of the draft variation may be result in a cost to government in terms of monitoring and compliance.

#### **2.5.1.2 Other measures**

There are no other measures (whether available to FSANZ or not) that would be more cost-effective than a food regulatory measure developed or varied as a result of the Application.

#### **2.5.1.3 Any relevant New Zealand standards**

Standards 1.1.1, 1.1.2 and 1.3.3 and Schedule 18 apply in both Australia and New Zealand. There are no relevant New Zealand only standards.

#### **2.5.1.4 Any other relevant matters**

Other relevant matters are considered below.

### **2.5.2 Subsection 18(1)**

FSANZ has also had regard to the three objectives in subsection 18(1) of the FSANZ Act during the assessment.

#### **2.5.2.1 Protection of public health and safety**

FSANZ undertook a safety assessment (SD1), summarised in section 2.2 and concluded there are no public health and safety concerns relating to permitting the enzyme, protein glutaminase sourced from *C. proteolyticum* strain AE-PG, as an enzyme processing aid for the purposes stated in the draft variation.

#### **2.5.2.2 The provision of adequate information relating to food to enable consumers to make informed choices**

The risk assessment concluded that the use of the enzyme preparation poses no risk to public health and safety and it performs its technological purpose as a processing aid. Therefore, the generic exemption from declaration of processing aids in the statement of ingredients will apply to foods produced using this enzyme as a processing aid and no additional labelling requirements are proposed.

See also the consideration of labelling issues in Table 1 and in Section 2.3.3 above.

### **2.5.2.3 The prevention of misleading or deceptive conduct**

This issue was considered in response to submitter concerns and comments (see FSANZ's response in Table 1 above).

### **2.5.3 Subsection 18(2) considerations**

FSANZ has also had regard to:

- **the need for standards to be based on risk analysis using the best available scientific evidence**

FSANZ has used the best available scientific evidence to conduct the risk analysis, which is provided in SD1. The Applicant submitted a dossier of scientific studies as part of its Application. Other technical information, including scientific literature, was also used to assess the Application.

- **the promotion of consistency between domestic and international food standards**

There are no Codex Alimentarius Standards for enzymes. However, this enzyme is permitted for use in France and the USA. It also meets international specifications for enzyme preparations, being the JECFA Compendium of Food Additive Specifications and the Food Chemicals Codex.

- **the desirability of an efficient and internationally competitive food industry**

Granting permission for this enzyme provides food manufacturers with an improvement in functionality when processing proteinaceous foods.

- **the promotion of fair trading in food**

FSANZ did not identify any relevant issues relating to this consideration.

- **any written policy guidelines formulated by the Forum on Food Regulation**

FSANZ had regard to the [Policy Guideline for the Addition to Food of Substances other than Vitamins and Minerals](#),<sup>4</sup> which includes specific order policy principles for substances added to achieve a solely technological function, such as processing aids. These specific order policy principles state that permission should be granted where:

- the purpose for adding the substance can be articulated clearly by the manufacturer as achieving a solely technological function (i.e. the 'stated purpose')
- the addition of the substance to food is safe for human consumption
- the amounts added are consistent with achieving the technological function
- the substance is added in a quantity and a form which is consistent with delivering the stated purpose
- no nutrition, health or related claims are to be made in regard to the substance.

---

<sup>4</sup> [Policy Guidelines](#)

### 3 References

Cabrera-Chávez F, Rouzaud-Sández O, Sotelo-Cruz N, Calderón de la Barca AM (2008) Transglutaminase treatment of wheat and maize prolamins of bread increases the serum IgA reactivity of celiac disease patients. *Journal of Agricultural and Food Chemistry* 56(4):1387-91.

Cabrera-Chávez F, Rouzaud-Sández O, Sotelo-Cruz N, Calderón de la Barca AM (2009) Bovine milk caseins and transglutaminase-treated cereal prolamins are differentially recognized by IgA of celiac disease patients according to their age. *Journal of Agricultural and Food Chemistry* 57(9):3754-9.

Dekking EHA, Van Veelen PA, de Ru A, Kooy-Winkelaar EMC, Gröneveld, T, Nieuwenhuizen WF, Koning, F (2008) Microbial transglutaminases generate T cell stimulatory epitopes involved in celiac disease. *Journal of Cereal Science* 47(2):339-346.

[Food Chemicals Codex 9<sup>th</sup> Edition \(2014\)](#), The United States Pharmacopeia, United States Pharmacopeial Convention, Rockville, MD.

Gerrard JA, Sutton KH (2005) Addition of Transglutaminase to Cereal Products May Generate the Epitope Responsible for Coeliac Disease. *Trends in Food Science & Technology* 16:510-512.

Gianfrani C, Siciliano RA, Facchiano AM, Camarca A, Mazzeo MF, Costantini S, Salvati VM, Maurano F, Mazzarella G, Iaquinto G, Bergamo P, Rossi M (2007) Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease. *Gastroenterology* 133(3):780-9.

Hashizume et al (2011) - [Crystal Structures of Protein Glutaminase and Its Pro Forms Converted into Enzyme-Substrate Complex](#). Accessed 19 Feb 2018.

Heil A, Ohsam J, van Genugten B, Diez O, Yokoyama K, Kumazawa Y, Pasternack R, Hills M (2017) Microbial Transglutaminase Used in Bread Preparation at Standard Bakery Concentrations Does Not Increase Immunodetectable Amounts of Deamidated Gliadin. *Journal of Agricultural and Food Chemistry* 65(32):6982-6990.

[International Union of Biochemistry and Molecular Biology \(IUBMB\) Enzyme Nomenclature](#). Accessed 29 Nov 2017.

JECFA (2006) [General specifications and considerations for enzyme preparations used in food processing](#).

Kikuchi et al (1971) - [Peptidoglutaminase. Enzymes for selective deamidation of  \$\gamma\$ -amide of peptide-bound glutamine](#). Accessed 19 Feb 2018.

Lombardi E, Bergamo P, Maurano F, Bozzella G, Luongo D, Mazzarella G, Rotondi Aufiero V, Iaquinto G, Rossi M (2013) Selective inhibition of the gliadin-specific, cell-mediated immune response by transamidation with microbial transglutaminase. *Journal of Leukocyte Biology* 93(4):479-88.

Matthias T, Jeremias P, Neidhöfer S, Lerner A (2016) The industrial food additive, microbial transglutaminase, mimics tissue transglutaminase and is immunogenic in celiac disease patients. *Autoimmunity Reviews* 15(12):1111-1119.



Ruh T, Ohsam J, Pasternack R, Yokoyama K, Kumazawa Y, Hils M (2014) Microbial transglutaminase treatment in pasta-production does not affect the immunoreactivity of gliadin with celiac disease patients' sera. *Journal of Agricultural and Food Chemistry* 62(30):7604-11.

Skovbjerg H, Koch C, Anthonsen D, Sjöström H (2004) Deamidation and cross-linking of gliadin peptides by transglutaminases and the relation to celiac disease. *Biochimica et Biophysica Acta* 1690(3):220-30.

Yamaguchi et al (1991) - [Crystal Structures of Protein Glutaminase and Its Pro Forms Converted into Enzyme-Substrate Complex](#). Accessed 19 Feb 2018.

Yamaguchi, S., Jeenes, D. J. and Archer, D. B. (2001), [Protein-glutaminase from \*Chryseobacterium proteolyticum\*, an enzyme that deamidates glutaminyl residues in proteins.](#) *European Journal of Biochemistry*. Accessed 19 Feb 2018.

Zhou L, Kooy-Winkelaar YMC, Cordfunke RA, Dragan I, Thompson A, Drijfhout JW, van Veelen PA, Chen H, Koning F (2017) Abrogation of Immunogenic Properties of Gliadin Peptides through Transamidation by Microbial Transglutaminase Is Acyl-Acceptor Dependent. *Journal of Agricultural and Food Chemistry* 65(34):7542-7552.

## Attachments

- A. Approved draft variation to the *Australia New Zealand Food Standards Code*
- B. Explanatory Statement

## Attachment A – Draft variation to the *Australia New Zealand Food Standards Code*



### Food Standards (Application A1136 – Protein Glutaminase as a Processing Aid (Enzyme)) Variation

---

The Board of Food Standards Australia New Zealand gives notice of the making of this variation under section 92 of the *Food Standards Australia New Zealand Act 1991*. The variation commences on the date specified in clause 3 of this variation.

Dated [to be completed by Delegate]

Glen Neal  
General Manager, Risk Management & Intelligence  
Delegate of the Board of Food Standards Australia New Zealand

**Note:**

This variation will be published in the Commonwealth of Australia Gazette No. FSC XX on XX Month 20XX. This means that this date is the gazettal date for the purposes of clause 3 of the variation.

**1 Name**

This instrument is the *Food Standards (Application A1136 – Protein Glutaminase as a Processing Aid (Enzyme)) Variation*.

**2 Variation to a standard in the *Australia New Zealand Food Standards Code***

The Schedule varies a Standard in the *Australia New Zealand Food Standards Code*.

**3 Commencement**

The variation commences on the date of gazettal.

**Schedule**

**[1] Schedule 18** is varied by inserting into the table to subsection S18—9(3) in alphabetical order

Protein glutaminase (EC 3.5.1.44)  
sourced from *Chryseobacterium*  
*proteolyticum* strain AE-PG

To deamidate proteins during the manufacture and/or processing of the following types of food: GMP

- (a) baked products;
- (b) pasta;
- (c) noodles;
- (d) milk;
- (e) other dairy products;
- (f) meat;
- (g) fish;
- (h) grains;
- (i) yeast; and
- (j) egg based products.

## **Attachment B – Draft Explanatory Statement**

### **1. Authority**

Section 13 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) provides that the functions of Food Standards Australia New Zealand (the Authority) include the development of standards and variations of standards for inclusion in the *Australia New Zealand Food Standards Code* (the Code).

Division 1 of Part 3 of the FSANZ Act specifies that the Authority may accept applications for the development or variation of food regulatory measures, including standards. This Division also stipulates the procedure for considering an application for the development or variation of food regulatory measures.

FSANZ accepted Application A1136. It sought an amendment to the Code to permit the use of protein glutaminase from *Chryseobacterium proteolyticum* (*C. proteolyticum*) strain AE-PG as a processing aid to deamidate proteins during the manufacture and/or processing of baked products, and pasta/noodle making, milk and dairy processing, meat and fish processing, grain processing, yeast products and egg based products. The Authority considered the Application in accordance with Division 1 of Part 3 and has prepared a draft variation to the Code.

Following consideration by the Australia and New Zealand Ministerial Forum on Food Regulation, section 92 of the FSANZ Act stipulates that the Authority must publish a notice about the draft variation of a standard.

Section 94 of the FSANZ Act specifies that a variation of a standard, in relation to which a notice is published under section 92 is a legislative instrument, but is not subject to parliamentary disallowance or sunseting under the *Legislation Act 2003*.

### **2. Purpose**

The purpose of this legislative instrument is to amend the Code to permit the use of the enzyme, protein glutaminase (EC 3.5.1.44) sourced from *C. proteolyticum* strain AE-PG as a processing aid to deamidate proteins during the manufacture and/or processing of foods such as baked products, pasta, noodles, milk, other dairy products, meat, fish, grains, yeast, and egg based products. This requires an addition to the table to subsection S18—9(3) in Schedule 18.

### **3. Documents incorporated by reference**

The variations to food regulatory measures do not incorporate any documents by reference.

### **4. Consultation**

In accordance with the procedure in Division 1 of Part 3 of the FSANZ Act, the Authority's consideration of Application A1136 included one round of public consultation followed by an assessment and the preparation of a draft variation and associated assessment summary.

The Office of Best Practice Regulation (OBPR) granted FSANZ a standing exemption from needing to develop a Regulatory Impact Statement for the approval of additional processing aids, in a letter dated 24 November 2010 (reference 12065). This standing exemption was provided as permitting additional processing aids is a minor, deregulatory change and their use is voluntary. This standing exemption relates to the introduction of a food to the food supply that has been determined to be safe.

## 5. Statement of compatibility with human rights

This instrument is exempt from the requirements for a statement of compatibility with human rights as it is a non-disallowable instrument under section 94 of the FSANZ Act.

## 6. Variation

Item [1] of the variation inserts a new entry into the table to subsection S18—9(3) in Schedule 18.

The new entry will, in effect, permit the enzyme, protein glutaminase (EC number 3.5.1.44), sourced from *C. proteolyticum* strain AE-PG, to be used as a processing aid in food for the particular technological purpose of deamidating proteins during the manufacture and/or processing of the following types of food, with the condition that the amount that may be used must be consistent with good manufacturing practice:

- (a) baked products;
- (b) pasta;
- (c) noodles;
- (d) milk;
- (e) other dairy products;
- (f) meat;
- (g) fish;
- (h) grains;
- (i) yeast; and
- (j) egg based products.