FOOD STANDARDS
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Te Mana Kounga Kai - Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment – Application A1321

Acetolactate decarboxylase from *Bacillus licheniformis* (gene donor: *Brevibacillus brevis*) for use as a processing aid

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Executive summary

Novozymes Australia Pty Ltd (now trading as Novonesis Australia Pty Ltd) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme acetolactate decarboxylase (EC 4.1.1.5), from *Bacillus licheniformis* containing the acetolactate decarboxylase gene from *Brevibacillus brevis*. The enzyme is proposed to be used as a processing aid in the manufacture of beer, wine and other yeast fermented beverages to reduce the formation of diacetyl, a compound that imparts an off-flavour in the food product.

The proposed use of acetolactate decarboxylase from *B. licheniformis* as a processing aid is technologically justified. Acetolactate decarboxylase performs its primary technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code.

No public health or safety concerns were identified with the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

There is a history of use of acetolactate decarboxylase from *B. licheniformis* overseas, including in Denmark, France and Mexico. No significant homology between the enzyme and known toxins or allergens was identified. Acetolactate decarboxylase from *B. licheniformis* was not genotoxic *in vitro*, and no adverse effects were observed in a 13-week dietary toxicity study in rats. The no observed adverse effect level (NOAEL) in this study was 644 mg total organic solids (TOS)/kg bw/day, the highest dose tested.

The theoretical maximum daily intake (TMDI) of this acetolactate decarboxylase was calculated to be 0.02 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of approximately 32,000.

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. FSANZ concludes there are no public health and safety concerns with the proposed use of acetolactate decarboxylase from *B. licheniformis*.

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1 Introduction

Novozymes Australia Pty Ltd[1] has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of acetolactate decarboxylase (EC 4.1.1.5) as a processing aid in brewing processes, other cereal based beverage processes and beverage alcohol (distilling) processes. The applicant subsequently confirmed that the application's intent is to permit the enzyme's use in the manufacture of beer, wine and other yeast fermented beverages to reduce the formation of diacetyl (a compound that imparts an off-flavour) during yeast fermentation. The enzyme is produced by *Bacillus licheniformis* containing the gene for acetolactate decarboxylase from *Brevibacillus brevis*.

It will be utilised at the minimum level required to achieve the intended technological purpose, in accordance with Good Manufacturing Practice (GMP).[2]

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely a technological function and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety concerns that may arise from the use of this enzyme as a processing aid by considering the:
  - safety and history of use of the production microorganism
  - safety of the enzyme preparation.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided information regarding the identity of the enzyme, which has been verified using the IUBMB[3] enzyme nomenclature reference database (McDonald et al. 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	acetolactate decarboxylase
Systematic name:	(2*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase [(3*R*)-3-hydroxybutan-2-one-forming]
Other names/common names:	α-acetolactate decarboxylase; (*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase; (*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase [(*R*)-2-acetoin-forming]

[1] Now trading as Novonesis Australia Pty Ltd

[2] GMP is defined in section 1.1.2—2 of the Code as follows: ***GMP*** *or* ***Good Manufacturing Practice****, with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*

(a) limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and

(b) to the extent reasonably possible, reducing the amount of the substance or its derivatives that:

*(i) remains as a *component of the food as a result of its use in the manufacture, processing or packaging; and*

(ii) is not intended to accomplish any physical or other technical effect in the food itself;

(c) preparing and handling the substance in the same way as a food ingredient.

[3] International Union of Biochemistry and Molecular Biology.

IUBMB enzyme nomenclature: EC 4.1.1.5

CAS number: 9025-02-9

Reaction: acetolactate decarboxylase catalyses the below chemical reaction (see Figure 1)

(2*S*)-2-hydroxy-2-methyl-3-oxobutanoate (2-acetolactate) = (3*R*)-3-hydroxybutan-2-one (acetoin) + CO_2

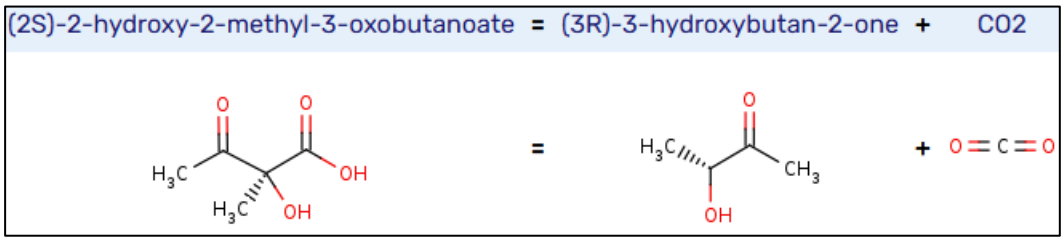

Figure 1: Reaction catalysed by acetolactate decarboxylase (source: BRENDA)

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps.

The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020b).

Acetolactate decarboxylase is produced by submerged fed-batch fermentation of a *B. licheniformis* strain carrying the gene for acetolactate decarboxylase from *B. brevis*. The fermentation process includes inoculating pure stock culture into fermentation media, followed by seed and main fermentation stages. Samples are taken at consistent intervals during the fermentation process to confirm the absence of microbial contamination. The fermentation is terminated if contamination develops.

When fermentation is complete, a multi-step recovery process is undertaken to separate and purify the enzyme from the *B. licheniformis* biomass. The separation of solids from the enzyme-containing media involves pre-treatment with flocculants, primary separation by drum filtration or centrifugation methods, and germ filtration to remove fine microbial particles. The resulting product is concentrated by ultrafiltration and/or evaporation to increase the activity/dry matter ratio. Sucrose and/or glucose are added to the enzyme concentrate to aid with preservation and stabilisation.

The typical composition of the liquid enzyme preparation is shown in Table 1.

Table 1: Typical composition of acetolactate decarboxylase preparation

Component	Approximate % w/w
Enzyme solids (total organic solids)	3
Glycerol	54.55
Water	42
Lactic acid	0.20
Potassium sorbate	0.20
Sodium benzoate	0.05

The applicant has stated the enzyme is manufactured in accordance with GMP and provided documentation regarding the compliance of their quality management system used in the manufacturing process with ISO 9001:2015 (Appendix 4.1–4.2). The resultant product meets the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) (see also section 2.2.2 of this report).

Information on the raw materials used in the production and recovery of the enzyme preparation is deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) and has been evaluated by FSANZ but cannot be disclosed in this report.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by JECFA in its Compendium of Food Additive Specifications (FAO JECFA Monographs 34 (2025)), explicitly FAO/WHO (2006) and in the FCC (14th edition, 2024), referenced in section S3—2 of Schedule 3 of the Code. Enzymes used as processing aids need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3.

Schedule 3 of the Code 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 a certificate of analysis for three independent batches of the acetolactate decarboxylase enzyme preparation (section A.5 and Appendix 3.1 of the application). Table 2 provides a comparison of the summary results of those analyses with the international specifications established by JECFA and the FCC, as well as those in the Code. Based on those results, the enzyme met all relevant specifications.

In addition, the specification for identity and purity of the enzyme product provided by the applicant indicates an absence of the production strain.

Table 2: Analysis of three representative batches of the enzyme preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Test parameters	Test results	Specifications		
		JECFA	Food Chemicals Codex	The Code – section S3—4
Lead (mg/kg)	ND (LOD <0.5)	≤5	≤5	≤2
Arsenic (mg/kg)	ND (LOD <0.3)	-	-	≤1
Cadmium (mg/kg)	ND (LOD <0.05)	-	-	≤1
Mercury (mg/kg)	ND (LOD <0.05)	-	-	≤1
Coliforms (cfu/g)	<10	≤30	≤30	-
***Salmonella* (in 25 g)**	ND	Absent	Negative	-
***Escherichia coli* (in 25 g)**	ND	Absent	-	-
Antimicrobial activity	ND	Absent	-	-

ND = not detected; LOD = limit of detection; cfu = colony forming units

2.3 Technological purpose

The acetolactate decarboxylase enzyme preparation is intended for use as a processing aid in the manufacture of beer, wine and other yeast fermented beverages. The applicant requested use of the enzyme at GMP levels, with use levels up to 0.02 g of enzyme preparation per kilogram of fermenting wort.

Acetolactate decarboxylase catalyses the conversion of 2-acetolactate into acetoin. When used during yeast fermentation, the enzyme reduces the formation of diacetyl, a by-product of yeast amino acid metabolism that arises from the non-enzymatic decarboxylation of 2-acetolactate. Diacetyl imparts an off-flavour in brewed beverages and wine at concentrations above approximately 0.1 ppm (Krogerus and Gibson 2013; Bartowsky and Henschke 2004).

The benefits of acetolactate decarboxylase, as stated in the application, include:

- reduced formation of diacetyl during yeast fermentation and thereby a reduction of the off-flavours caused by this compound
- a faster maturation process and therefore shorter production time.

The technological purpose stated by the applicant is consistent with the typical function of acetolactate decarboxylase. This is supported by scientific literature, which verify the enzyme function and demonstrate the contribution of the enzyme to reduced diacetyl formation and shorter maturation times in yeast fermented beverages, such as beer and wine (Godtfredsen and Ottesen 1982; Spaans et al. 2026; Blomqvist et al. 1991; Li et al. 2018).

Acetolactate decarboxylase performs its primary function during food manufacturing and has no function in the final food. The applicant stated that the enzyme is inactivated by processes that use high temperature or else removed during certain processing steps. Thus, the enzyme meets the definition of a processing aid and does not function in the final food. Information on the physical and chemical properties of the enzyme, as provided by the applicant, are summarised in Table 3.

Table 3: Acetolactate decarboxylase enzyme preparation physical and chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	2500 ADU(L)/g[a]
Appearance	Liquid, light brown colour
Temperature optimum	Optimum activity within range 30–55°C, with peak activity at 40°C
Thermostability	Enzyme activity starts to decrease when exposed to temperatures above 60°C, with minimal activity at 80°C
pH range and optimum	Optimum activity within pH range 5–7, with peak activity at pH 6

[a]ADU(L) = enzyme activity units, where it is understood that 1 ADU is the amount of enzyme that produces 1 micromole of acetoin per minute under specified conditions (temperature, pH, substrate type).

2.4 Allergen considerations

FSANZ has evaluated the raw materials used in the manufacture of the enzyme preparation (CCI, see section 2.2.1 of this report) and noted that the applicant conducts regular testing to confirm the absence of detectable allergens in the final enzyme product. The applicant provided a product data sheet (Appendix 2.1 of the application) that states that the enzyme preparation does not contain known food allergens.

2.5 Food technology conclusion

The use of acetolactate decarboxylase as a processing aid to convert 2-acetolactate into acetoin in the processing of beer, wine and other yeast fermented beverages is consistent with the typical function of the enzyme. The stated benefits include reduced formation of diacetyl during yeast fermentation steps and shorter maturation times. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Acetolactate decarboxylase fulfils its technological purpose during the manufacture of the nominated food products. Processes such as enzyme inactivation with high temperature and/or removal during certain processing steps ensure that acetolactate decarboxylase does not perform a technological purpose in the final food. It therefore functions as a processing aid for the purposes of the Code.

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

3 Safety assessment

This safety assessment aims to evaluate potential public health and safety concerns arising from using the acetolactate decarboxylase enzyme associated with a genetically modified strain of *Bacillus licheniformis* as a processing aid.

Some information relevant to this section is CCI, so full details cannot be disclosed under the Act.

3.1 Source microorganism

B. licheniformis has a long history of safe industrial use, particularly in the production of

enzymes for food processing, dating back to 1972 (de Boer et al. 1994; Sewalt et al. 2018; Muras et al. 2021). *B. licheniformis* is a gram-positive, endospore-forming, mesophilic and facultative anaerobic bacterium, present in soil and marine environments. Its optimal growth temperature is around 50°C and it can survive at much higher temperatures. Taxonomically, *B. licheniformis* is classified under the genus *Bacillus*, family *Bacillaceae*, order Bacillales, class Bacilli, and phylum Bacillota (formally Firmicutes) (Whitman 2009).

FSANZ has previously assessed the safety of *B. licheniformis* as the source organism for processing aids. Schedule 18 of Standard 1.3.3 of the Code permits the use of the following enzymes produced by *B. licheniformis*, including: alpha-amylase, chymotrypsin, endo-1,4-beta-xylanase, beta-galactosidase, glycerophospholipid cholesterol acyltransferase, maltotetraohydrolase, pullulanase, and serine proteinase.

B. licheniformis is generally considered non-pathogenic due to the absence of invasive traits (Muras et al. 2021). However, isolates have been identified as the cause of foodborne illness associated with cooked meats, ice cream, cheese, raw milk, infant feed, and prawns (Salkinoja-Salonen et al. 1999). Such incidences of human infection and pathogenicity are rare and typically limited to immunocompromised individuals (Haydushka et al. 2012).

The US Environmental Protection Agency (EPA) has assessed *B. licheniformis* in fermentation facilities as low risk to human health and the environment (EPA 1997) while the European Food Safety Authority (EFSA Panel on Biological Hazards (BIOHAZ) et al. 2022) has classified of *B. licheniformis* as having qualified presumption of safety (QPS) status. The US Food and Drug Administration (FDA) has affirmed that carbohydrase and protease enzyme products derived from *Bacillus licheniformis* are generally recognized as safe (GRAS) for use in the production of alcoholic beverages, candy, nutritive sweeteners, and protein hydrolysates (Federal Register 1999; FDA 1983).

The *B. licheniformis* production strain referred to in this application was derived from *B. licheniformis* Si3 strain, which was developed from the natural isolate *B. licheniformis* Ca63. The production organism is a non-pathogenic, non-toxigenic, and genetically modified strain. The production strain is marker-free, and it does not produce secondary metabolites of toxicological concern to humans.

The donor organism of the gene encoding acetolactate decarboxylase is *Brevibacillus brevis*. In the application, the donor was initially referred to as *Bacillus brevis* and has subsequently been updated to *Brevibacillus brevis* in accordance with accepted international taxonomic conventions.

The applicant provided data that adequately demonstrates the production organism's identity as *B. licheniformis.* Applying the safe strain concept (Pariza and Johnson 2001), the applicant provided information highlighting the risk of toxin production by the production organism is very low. Microbiological testing certificates were also provided to FSANZ, confirming the absence of the production organism in the final enzyme preparation.

The microbiological assessment undertaken by FSANZ did not identify any public health and safety concerns related to the use of genetically modified *B. licheniformis* as a production organism for acetolactate decarboxylase enzyme.

3.2 Characterisation of the genetic modification to the production strain

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

The gene encoding the acetolactate decarboxylase enzyme is derived from *B. brevis*. Data provided by the applicant and analysed by FSANZ confirmed the identity of the acetolactate decarboxylase gene.

The gene was inserted into the genome of the host *B. licheniformis* as part of an expression cassette containing a hybrid promoter and a terminator from *B. licheniformis*. The expression cassette was integrated at specific integration sites present in the host *B. licheniformis* genome using a vector based on standard *Staphylococcus aureus* vectors (Gryczan et al. 1978; Horinouchi and Weisblum 1982). The final production strain was selected based on rapid growth and high acetolactate decarboxylase activity.

3.2.2 Characterisation of the inserted DNA

Genome sequencing data provided by the applicant confirmed the presence of the inserted DNA at the specific integration sites in the genome of the production strain. The data also confirmed the absence of antibiotic resistance genes, marker genes and other vector-derived DNA in the final production strain.

3.2.3 Stability of the inserted DNA

The applicant has stated the inserted DNA does not have the ability to replicate autonomously or be transferred to other organisms by conjugation.

To provide further evidence of the stability of the inserted acetolactate decarboxylase gene, the applicant provided phenotypic data from large-scale fermentation of the production strain, which confirmed the gene is expressed stably over multiple generations.

3.3 Safety of the enzyme

3.3.1 History of safe use

The applicant's acetolactate decarboxylase enzyme has received regulatory approval in Denmark, France and Mexico.

Acetolactate decarboxylase enzymes have a long history of safe use in food production, and several acetolactate decarboxylase preparations derived from other sources have been approved for use as processing aids by FSANZ and included in Section 18 of the Code. Acetolactate decarboxylases from other sources are also approved in other countries such as Brazil, Canada, China, Denmark, France, Japan and Mexico.

3.3.2 Bioinformatic assessment of homology with known toxins

The applicant conducted a search for sequence homology of acetolactate decarboxylase from *B. licheniformis* to known toxins in the NCBI Identical Protein Groups resource[4] in October 2024. No hits with an E-value < 0.00001 were identified, indicating the enzyme is not significantly similar to known toxins.

3.3.3 Toxicology data

The applicant submitted reports of two genotoxicity studies of acetolactate decarboxylase from *B. licheniformis* as well as a 13-week oral toxicity study in rats. The test items used in these studies were representative batches of the enzyme concentrate produced as described in Section 2.2.1, without the final stabilisation and standardisation steps. The total organic solids (TOS) content of the test item used in the 13-week toxicity study was 92.9%.

[4] https://www.ncbi.nlm.nih.gov/ipg/

Short-term toxicity

13-week dietary toxicity study in rats (Broadmeadow 1990) Regulatory status: GLP; conducted according to US FDA (1982) guidelines for safety assessment of direct food additives

Acetolactate decarboxylase (batch number PPD2936) was administered in the diet to groups of 20 male and 20 female CD rats for 13 weeks at concentrations of 0, 200, 140 or 10,000 ppm (equal to 0, 13, 93 or 644 mg TOS/kg bw/day for males and 0, 16, 109 or 756 mg/kg bw/day for females[5]). Clinical signs, food consumption and body weights were recorded throughout the study. Ophthalmology was performed before study initiation on all animals and on study week 12 on controls and high dose animals. Blood samples were collected for haematology and clinical chemistry during study week 13. Coagulation analyses were not performed. Samples for urinalysis were collected from 10 rats/sex/group during study week 12. At the end of the study animals were killed and subjected to gross necropsy. Organ weights were recorded and tissues from the control and high dose groups were examined microscopically.

There were no deaths following treatment with the test items, but one control animal died during the blood collection in week 13 of treatment. Food consumption, body weight, body weight gain and food conversion ratios were unaffected by treatment. No treatment-related ocular lesions were observed. There were no treatment-related adverse effects on haematology, clinical chemistry or urinalysis parameters. No treatment-related organ weight changes were observed, and there were no treatment-related macroscopic or histopathological changes.

The no observed adverse effect level (NOAEL) in this study was 10,000 ppm (equal to 644 mg TOS/kg bw/day), the highest dose tested.

Genotoxicity

Genotoxicity studies with acetolactate decarboxylase from *B. licheniformis* comprised a bacterial reverse mutation test and an *in vitro* micronucleus assay. The test item was batch number PPD36621. These studies were GLP compliant and conducted according to OECD Test Guidelines. The positive controls in these studies produced the expected responses.

As summarised in Table 4, acetolactate decarboxylase showed no evidence of mutagenicity, clastogenicity or aneugenicity in these studies.

Table 4: Genotoxicity studies with acetolactate decarboxylase from *B. licheniformis*

Test	Test object	Concentration	Results	Reference
Bacterial reverse mutation test (OECD TG 471); treat and plate	Salmonella typhimurium strains TA1535, TA100, TA1537 & TA98; Escherichia coli	0, 156, 313, 625, 1250, 2500 or 5000 µg TOS/mL	Negative ± S9	Pedersen 2015

[5] The study report states the achieved doses were 0, 14.16, 100.3 and 693 mg/kg bw/day (males) and 0, 16.9, 116.1 and 814 mg/kg bw/day (females) for the 13-week dietary toxicity study (see Appendix 5.4), however the doses were not adjusted for the TOS content of the test item, which was not provided in the study report or the original dossier. During the assessment period, the applicant provided information on the TOS content (92.9%), and the doses were converted to the TOS values provided in the text. These values were used in FSANZ's assessment to determine the no observed adverse effect level (NOAEL) and margin of exposure (MOE).

Test	Test object	Concentration	Results	Reference
method	WP2uvrApKM101			
In vitro micronucleus assay (OECD TG 487)	Human peripheral blood lymphocytes	3h exposure: 0, 3000, 4000 or 5000 µg TOS/mL; 24h exposure: 0, 100, 500, 1000 or 3000 µg TOS/mL[a,b]	Negative ± S9	Whitwell 2015

[a] the highest concentration evaluated following 24h exposure was associated with 50-60% cytotoxicity.
[b] the protocol for the 24h exposure (treatment for 24h -S9 followed by 24h recovery in the presence of cytochalasin B) is not in line with the preferred protocol in the current version of OECD TG 487 (treatment for 24h -S9 in the presence of cytochalasin B; harvest at the end of treatment) but was compliant with the version of OECD TG 487 current at the time the study was conducted. This deviation is not considered to affect the validity of the results.

3.3.4 Potential for allergenicity

The potential allergenicity of the applicant's acetolactate decarboxylase was assessed by comparing its amino acid sequence with those of known allergens. The applicant conducted the following searches in January 2025 using the COMprehensive Protein Allergen REsource (COMPARE[6]):

- 35% identity over 80 amino acids
- 35% identity over 80 amino acids with scaling enabled
- Full length alignment
- 100% identity over 8 contiguous amino acids

No matches with known allergens were found. A literature search also found no reports of food allergy to acetolactate decarboxylase enzymes.

Based on the available information, acetolactate decarboxylase from *B. licheniformis* is unlikely to pose an allergenicity concern.

3.3.5 Assessments by other regulatory agencies

The Danish Veterinary and Food Administration has approved the use of acetolactate decarboxylase from *B. licheniformis* for use in brewing processes and other cereal based beverage processes. The Danish safety assessment is not available to FSANZ but information provided by the applicant indicates this approval was based on a safety assessment made in accordance with guidelines from the European Food Safety Authority (EFSA) on submission of a dossier on food enzymes.

The enzyme has also been approved in France and Mexico, but safety assessments from these agencies are not available to FSANZ.

4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the 'budget method' calculation presented by the applicant as a 'worst-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the acetolactate

[6] https://comparedatabase.org/

decarboxylase enzyme preparation 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 (MOE) for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020a). The method is used by overseas regulatory bodies and JECFA (FAO/WHO 2021) for dietary exposure assessments for processing aids.

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

- there is no intention to use the enzyme in the processing of solid foods, so solid foods are not included in the exposure calculation
- the maximum mean estimated consumption of beer and beer-like beverages from the EFSA Comprehensive European Food Consumption Database was used to represent consumption for the exposure calculation (EFSA et al 2024), as opposed to the traditional consumption amounts used in the budget method based on physiological requirements
- all beer and beer-like beverages contain the highest use level of 0.6 mg TOS/kg in the raw material (fermenting wort)
- the density of fermenting wort is 1 kg/L
- 1 kg of fermenting wort gives 1 kg beer
- the highest use level of acetolactate decarboxylase enzyme preparation is used in 100% of beer and beer-like beverages consumed, representing a worst-case scenario
- the enzyme is intended to be used in beverage alcohol (distilling) processes, but as no TOS will remain in the final distilled beverage spirits due to the distillation process, the TMDI contribution is assumed to be zero.
- all of the TOS from the enzyme preparation remains in the final food for all uses other than distilled spirits.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.006522 mg TOS/kg bw/day.

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

- the maximum physiological requirement for liquid is 100 mL/kg body weight/day (the standard level used in a budget method calculation for non-milk beverages)
- FSANZ would generally assume 25% of non-milk beverages 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).

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations for non-milk beverages is 0.02 mg TOS/kg bw/day.

Both FSANZ's and the applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method or deterministic calculations undertaken. This includes that it was assumed that all of the TOS from the enzyme preparation remains in the final beverages whereas the applicant has stated that it is

denatured by heat during processing or removed by down-stream processes.

5 Discussion

The use of acetolactate decarboxylase from *B. licheniformis* containing the acetolactate decarboxylase gene from *B. brevis* as a processing aid in the manufacture of beer, wine and other yeast fermented beverages is consistent with the typical function of the enzyme.

The enzyme is intended to be used to reduce the formation of diacetyl during yeast fermentation, where it may confer benefits including a reduction of off-flavours in the final food and a faster maturation process.

Acetolactate decarboxylase is functioning as a processing aid for the purposes of the Code and does not perform a technological purpose in the food for sale. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

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

No public health or safety concerns were identified with the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

There is a history of use of acetolactate decarboxylase from *B. licheniformis* overseas, including in Denmark, France and Mexico. No significant homology between the enzyme and known toxins or allergens was identified. Acetolactate decarboxylase from *B. licheniformis* was not genotoxic *in vitro*, and no adverse effects were observed in a 13-week dietary toxicity study in rats. The NOAEL was 644 mg TOS/kg bw/day, the highest dose tested.

The TMDI was calculated by FSANZ to be 0.02 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a MOE of approximately 32,000.

In the absence of any identifiable hazard an ADI ‘not specified’ is appropriate for acetolactate decarboxylase.

FSANZ concludes there are no public health and safety concerns with the proposed use of acetolactate decarboxylase from *B. licheniformis*.

6 References

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