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Supporting document 1

Risk and technical assessment – Application A1342

Beta-casein protein preparation from GM *Komagataella phaffii*

Executive summary

FSANZ has assessed an application from Eden Brew Pty Ltd seeking approval for the use of a beta-casein protein preparation (BCPP1) from a genetically modified (GM) yeast strain *Komagataella phaffii* (EB-ST-537) expressing the gene for bovine beta-casein A2 variant protein. BCPP1 is intended as an ingredient providing a source of protein in a wide range of foods such as dairy analogues, protein beverages, baked goods and shelf-stable formulations.

BCPP1 is a white to off-white powder consisting of approximately 22% protein, about 25% of which is beta-casein. The remaining protein fraction comprises host cell proteins. Carbohydrate, consisting primarily of common dietary polysaccharides, makes up approximately 68% of the total preparation. During its production, the product is subject to heat treatment prior to ultrafiltration, diafiltration and spray drying. Stability studies confirm that BCPP1 can be added to a range of food matrices and remain stable under the relevant storage conditions for those foods. Stability studies indicate BCPP1 is stable during storage and can be uniformly incorporated into a range of different food products.

Although the preparation exhibits functions such as emulsification, foaming, gelation and water-binding, FSANZ considers these functions are secondary to the primary purpose of its addition to food as a protein source. The intended use of BCPP1 is therefore not as a food additive as defined in the Code.

There is no single method of detection for the preparation itself, due to its mixed composition. The identity and concentration of beta-casein in BCPP1 can be confirmed using standard analytical methods for beta casein.

A new specification for BCPP1 has been prepared, based on information from the applicant, and includes additional microbial parameters which are broadly consistent with existing microbiological criteria for low moisture, powdered ingredients and with specifications in Schedule 3.

The production organism *K. phaffii* is a well-characterised, non-pathogenic and non-toxigenic yeast with a long history of safe use. Molecular analyses confirmed the introduced genetic elements are present as intended, do not include antibiotic resistance marker genes and are stably maintained.

The beta-casein in BCPP1 was shown to be equivalent to that naturally present in bovine

milk and is therefore expected to have the same allergenic potential as beta-casein present in conventional milk. Bioinformatic analyses of the most abundant host cell proteins present in BCPP1 did not identify any proteins with homology to known toxins or allergens.

Except for the allergenicity risk for individuals with milk allergy, BCPP1 does not raise safety concerns under the intended conditions of use. The preparation is susceptible to pepsin digestion, and the carbohydrate fraction in BCPP1 is expected to consist predominantly of common dietary polysaccharides, which are not of toxicological concern. Supporting studies on related *K. phaffii* protein preparations showed no genotoxicity and no adverse effects in 28-day and 90-day oral toxicity studies in rats.

The nutrition assessment found that using BCPP1 as a substitutional protein ingredient does not raise nutritional concerns for Australian or New Zealand consumers. Mean and high-percentile intakes were estimated to contribute only small amounts of additional protein (approximately 2.9–3.9 g/day), mainly from bakery products and soy beverages, and are unlikely to pose a risk.

FSANZ concludes there are no public health and safety concerns for the general population associated with the addition of BCPP1 to food. However, as beta-casein is a known milk allergen, foods containing BCPP1 would present an allergenicity risk for individuals allergic to milk proteins.

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List of Abbreviations

Abbreviation	Description
ABS	Australian Bureau of Statistics
AI	adequate intake
AMR	antimicrobial resistance
BCPP1	Beta-casein protein preparation
bp	base pair
CCI	confidential commercial information
CFU	colony forming units
DNA	deoxyribonucleic acid
EAR	estimated average requirement
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organisation
FSA	UK Food Standards Agency
FSANZ	Food Standards Australia New Zealand
g	gram
GM	genetically modified
kDa	kilodalton
kg	kilogram
LC-MS/MS	liquid chromatography-tandem mass spectrometry
mg	milligram
MPC	milk protein concentrate
NA	not available
ng	nanogram
NNPAS	National Nutrition and Physical Activity Survey
NNS	National Nutrition Survey
NOAEL	no observed adverse effect level
NRV	nutrient reference value
ORF	open reading frame
PCR	polymerase chain reaction
PDCAAS	protein digestibility corrected amino acid score
QPS	qualified presumption of safety
RP-HPLC	reversed-phase high-performance liquid chromatography
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
µg	microgram

Abbreviation	Description
UL	upper level of intake
WGS	whole genome sequencing
w/w	weight/weight

1 Introduction

FSANZ has received an application from Eden Brew Pty Ltd seeking approval for the use of a preparation (BCPP1) containing beta-casein as an ingredient in a wide range of foods (e.g. dairy analogues, protein beverages, baked goods and shelf-stable formulations).

The objectives of this technical and risk assessment were to:

- confirm the proposed use is not solely for a technological purpose
- evaluate potential public health and safety issues that may arise from the addition of BCPP1 to foods as proposed.

2 Technical assessment

2.1 Product overview and production method

BCPP1 is derived from a genetically modified (GM) yeast strain, *Komagataella phaffii* (EB-ST-537), containing the bovine (*Bos taurus*) *CSN2* gene encoding the beta-casein A2 protein.

BCPP1 is produced as a white to off-white powder using the following process.

K. phaffii EB-ST-537 from an established working cell bank is used to establish a seed culture which is transferred to a production fermenter for biomass generation and subsequent protein expression. The expressed beta-casein protein is secreted into the fermentation medium in soluble form. Fermentation medium containing the beta-casein protein is separated from the yeast biomass by centrifugation and then subjected to micro-filtration, pH adjustment and heat treatment prior to concentration by ultrafiltration, and diafiltration to exchange the fermentation medium for water. In the final step, the pH is adjusted and the preparation dried.

2.2 Composition

BCPP1 is approximately 22% protein, about 25% of which is beta-casein, which equates to approximately 5.5% of the preparation. The remaining protein is host cell proteins from the production organism, *K. phaffii*. Carbohydrate makes up approximately 68% of the total preparation. Individual sugars (glucose, lactose, fructose, sucrose, maltose) are each present at less than 0.2 g/100 g. See section 5.4.4 for further information about the carbohydrate content.

2.3 Technological function

The applicant states the preparation exhibits the following functions when added to food: emulsification, foaming, gelation and water-binding.

Although these functions are consistent with the technological purposes of food additives (listed in S14—2), FSANZ does not consider the preparation to be *used as a food additive* as defined in the Code. Rather, it can perform these functions due to its protein and/or carbohydrate content, in a similar way to any other food ingredient with a similar protein and/or carbohydrate content e.g. egg white which enables aeration, flour which contributes to viscosity. Additionally, it is proposed to be added at levels not normally consistent with the use of a food additive. FSANZ considers the listed functions are therefore secondary to the primary purpose of addition, which is as a source of protein.

2.4 Method of detection

In food products, beta casein can be detected and quantified using standard immunoassay and mass spectrometry-based methods. These include standard analytical methods such as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), reversed-phase high-performance liquid chromatography (RP-HPLC), and liquid chromatography–tandem mass spectrometry (LC-MS/MS).

2.5 Stability

The applicant has performed stability studies on BCPP1 with the details provided as Confidential Commercial Information (CCI) under Section 114 of the Act. The food matrices studied were dairy analogues, protein beverages, fermented products, baked goods, extruded snacks, dry mixes and shelf-stable powders. These studies confirm that BCPP1 can be added to such products and remain stable under the relevant storage conditions for the shelf life of those foods.

Based on this information, and the water activity of the powder, FSANZ has no concerns regarding the stability of the product during storage.

2.6 Microbiological considerations

FSANZ highlights the importance of process hygiene criteria in the Compendium of Microbiological Criteria for Food (FSANZ 2025a). Process hygiene criteria are microbiological criteria applied to confirm that hygiene measures or process controls are operating as intended. These criteria are typically based on indicator organisms and are applied at specified points, or multiple points, throughout the manufacturing process. Common indicators include total aerobic plate counts, Enterobacteriaceae, yeasts and moulds, and spore-forming microorganisms.

Microbial contamination from the production environment should be controlled and monitored in both the fermentation and processing phases, this is especially the case for heat-resistant and spore-forming microorganisms (Sturme et al. 2025).

As described in section 2.1, BCPP1 is produced via fermentation of a genetically modified *K. phaffii* strain, followed by separation of biomass and a series of downstream processing steps including microfiltration, pH adjustment, heat treatment, ultrafiltration/diafiltration and drying.

The combined pH adjustment and heat treatment step would be expected to reduce viable microorganisms present in the fermentation medium. It was noted data were not available to directly assess the extent of microbial reduction under the specified conditions, or characterise microbial presence following this step, including during subsequent processing operations.

Test results were available for *Salmonella* spp., *Listeria* spp., and *Escherichia coli*, as well as mycotoxins in three independent BCPP1 production batches. These results confirmed the absence of the tested pathogens and mycotoxins in these batches.

Effective microbiological control of the BCPP1 ingredient during processing is required to prevent contamination being carried through to the final food. Process hygiene indicator data and validated evidence of process performance should be implemented by the manufacturer to demonstrate that microbial contamination is consistently controlled prior to the ingredient entering food systems that can support microbial growth.

In addition to the microbial specifications for *Escherichia coli*, *Salmonella spp*. and *Listeria spp*. proposed by the applicant (page 42 of the application), FSANZ is proposing the following additional microbiological specifications for BCPP1:

- Aerobic mesophilic bacteria (total count): not more than 500 CFU/g
- Enterobacteriaceae: not more than 10 CFU/g
- Yeasts and moulds: not more than 100 CFU/g.

Test results were not available for total aerobic counts, yeasts, moulds and coliforms in the processing phase to inform the proposed limits for microbiological specifications. The proposed specifications are, however, broadly consistent with criteria for low-moisture, powdered ingredients and with previous specifications included in Schedule 3.

2.7 Specifications

The applicant proposed a specification for BCPP1 based on analytical results, product function and production system controls – see Table 1.

The analytical results for 4 batches of BCPP1 representative of the final preparation indicate the preparation can meet the comparable specification parameters under composition and metals in Table 1. The analytical results for 3 batches of BCPP1 representative of the final preparation indicate the preparation can meet the specification parameter for purity (beta-casein as a percentage of protein present in the preparation). FSANZ has proposed a narrower range for total protein than that requested by the applicant, to more accurately reflect the composition of the product assessed. Specifying both minimum and maximum values limits variability in the composition of the preparation, noting that other compositional parameters are expressed as maximum limits.

Residual host DNA in three independent batches of BCPP1 was assessed using quantitative PCR assays targeting *K. phaffii*-specific genomic sequences. All tested batches contained residual host DNA levels below internationally established limits for food ingredients produced via microbial fermentation, with concentrations under 10 ng/g.

In relation to microbiological parameters, the specifications proposed by the applicant were based on limited end-product testing. As such, FSANZ cannot confirm if the proposed microbiological parameters are technically achievable under routine manufacturing conditions.

The proposed specification for BCPP1 is listed in Table 1 below. The limits proposed by the applicant are provided for comparison.

Table 1 Proposed specification for BCPP1

Parameter	**Limit proposed by applicant**	**FSANZ proposed specification**
(a) Appearance	White to off-white powder	White to off-white powder
(b) Composition:		
(i) Total Protein (%)	no less than 20	20-24
(ii) Carbohydrates (%)	no more than 70	no more than 70
(iii) Fat (%)	no more than 3	no more than 3
(iv) Ash (%)	no more than 3	no more than 3
(v) Moisture (%)	no more than 6	no more than 6
(vi) Total Sugars (%)	no more than 1	no more than 1

(c) Purity:		
(i) Beta-casein protein	more than 20 (% of total protein)	>4% of BCPP1
(d) Metals		
(i) Lead (mg/Kg)	no more than 0.05	no more than 0.05
(ii) Arsenic (mg/Kg)	no more than 0.05	no more than 0.05
(iii) Mercury (mg/Kg)	no more than 0.05	no more than 0.05
(iv) Cadmium (mg/Kg)	no more than 0.05	no more than 0.05
(e) Microbiological		
(i) *Escherichia coli*	negative to test	absent in 10 g
(ii) *Salmonella* spp.	negative to test	absent in 25 g
(iii) *Listeria* spp.	negative to test.	absent in 25 g
(iv) Aerobic mesophilic bacteria total count	No limit proposed	not more than 500 CFU/g
(v) *Enterobacteriaceae*	No limit proposed	not more than 10 CFU/g
(vi) Yeasts and moulds	No limit proposed	not more than 100 CFU/g

2.8 Conclusion from the technical assessment

Beta-casein Protein Preparation, termed BCPP1 by the applicant, is a preparation produced via fermentation using a GM strain of *Komagataella phaffii* (EB-ST-537). It is a white to off-white powder consisting of approximately 22% protein, about 25% of which is beta-casein. The remaining protein is host cell proteins from *K. phaffii*. Carbohydrate content is approximately 68%.

During its production, the product is subject to a heat treatment prior to ultrafiltration, diafiltration and spay drying.

Although the preparation exhibits functions such as enabling aeration and increasing viscosity, FSANZ considers these functions are secondary to the primary purpose of addition, which is as a protein source. The preparation would therefore not be '*used as a food additive*' as defined in the Code.

There is no one method of detection for the preparation itself, due to its mixed composition. The identity and concentration of beta-casein in BCPP1 can be confirmed using standard analytical methods for beta casein.

The applicant has performed studies on the stability of BCPP1 itself with the details provided as CCI. Based on that information and the water activity of the powder, FSANZ has no concerns regarding the stability of the product during storage. Stability studies indicate BCPP1 is stable during storage and can be uniformly incorporated into a range of different food products. Its use in certain foods would not be technically feasible or desirable.

There is no relevant specification in the Code for BCPP1, therefore a new specification has been prepared for inclusion in the Code based on that proposed by the applicant.

3 History of use

3.1 Host organism

Komagataella phaffii, formerly known as *Pichia pastoris*, has undergone several taxonomic revisions. Although *P. pastoris* was historically treated as a single species, based on isolates first obtained in Europe and later from North America, genomic analyses demonstrated that these strains represent two distinct species (Zahrl et al. 2017). Kurtzman (2005) subsequently reassigned these taxa as *K. phaffii* and *Komagataella pastoris* based on sequencing of 26S ribosomal DNA. Following this formal taxonomic split and clarification of species boundaries, the use of the updated identifier *Komagataella phaffii* is considered the most accurate and scientifically appropriate nomenclature. The applicant used *K. phaffii* strain NRRL Y-7556 for their production organism and provided CCI information confirming its identity as *K. phaffii*.

K. phaffii was originally isolated from natural environments such as tree exudates (including oak tree sap) and soil, where it exists as a saprophytic yeast (Bernauer et al. 2021, Heistinger et al. 2020). The species is not associated with pathogenicity in humans, animals or plants. The organism is naturally adapted to specific ecological niches and displays a restricted growth profile, with an optimal growth temperature of approximately 30 °C and poor survival at temperatures approaching mammalian body temperature (Carneiro et al. 2022). These biological characteristics limit its ability to persist or proliferate in host organisms or in the wider environment outside controlled fermentation conditions.

K. phaffii is not considered pathogenic or toxigenic to humans or animals. The only published clinical report, identified by FSANZ, is a case where *K. phaffii* DNA was detected, via sequencing, in a patient's surgically removed intestinal tissue (Liu et al. 2023). However, this finding likely represents secondary colonisation or environmental contamination in a highly compromised host and does not establish *K. phaffii* as a causative agent of disease.

Genome sequencing analyses of *K. phaffii* have not identified genes encoding known toxin pathways, including genes associated with common mycotoxins or virulence factors characteristic of pathogenic fungi. While numerous *K. phaffii* strains have been sequenced, full proteomic characterisation has not been completed for all strains, including strain NRRL Y-7556 (Claes et al. 2024; Valli et al. 2020). Nevertheless, there is no reported evidence that *K. phaffii* produces toxic secondary metabolites or expresses proteins associated with pathogenicity. There is also no evidence that *K. phaffii* harbours endogenous antimicrobial resistance (AMR) genes or produces antimicrobial compounds.

To further assess the presence of endogenous AMR genes and other sequences such as virulence factors, the applicant screened the production organism's genome against several publicly available online databases specific to bacterial sources. No endogenous AMR genes or virulence factors were identified.

K. phaffii has predominantly been used as a production organism of highly purified products with minimal host cell components, the majority of which are secreted proteins. In contrast, preparations containing higher amounts of host cell proteins and other cellular components such as carbohydrates have been less commonly assessed. The safety of the host cell components is considered in section 5. Safety assessment of BCPP1.

K. phaffii has been used for more than four decades in the manufacture of pharmaceutical compounds, animal feeds, food enzymes and proteins (Anaya et al. 2024, Barone et al. 2023). Multiple regulatory authorities, including the European Food Safety Authority (EFSA), have evaluated the organism and concluded that it is suitable for use as a production

organism. EFSA has granted *K. phaffii* Qualified Presumption of Safety (QPS) status for production purposes, subject to the condition that no viable cells are present in the final product (EFSA 2018, EFSA 2024). This qualification is consistent with the species' primary use as a production host rather than a food microorganism (EFSA 2018). Similarly, food ingredients and processing aids produced using *K. phaffii* have been authorised in the United States, Australia, New Zealand and other jurisdictions following case-by-case safety assessments that identified no public health or safety concerns associated with the source organism.

To demonstrate the absence of viable cells from the production organism in the final product, the applicant provided culture-based testing data for three independent production lots. The data demonstrated the production organism was not detected in 1 g samples of final BCPP1 preparation.

In conclusion, FSANZ's assessment identified no microbiological safety concerns associated with the use of *K. phaffii* NRRL Y-7556 as a production organism for BCPP1.

3.2 Donor organisms

Bos taurus

The *CSN2* gene was synthesised *in vitro* based on the amino acid sequence of the beta-casein A2 variant naturally present in *B. taurus* (bovine) milk. *B. taurus* has a long history of safe use as food, with its meat, milk, and milk products consumed worldwide for thousands of years. However, allergenicity is associated with specific milk proteins, such as caseins and whey proteins. A more detailed assessment of allergenicity concerns is addressed in section 5.3.1.

Saccharomyces cerevisiae

A helper gene from *Saccharomyces cerevisiae* was introduced into the host organism. *S. cerevisiae* has an extensive and well-documented history of safe use in food and beverage production, dating back more than 5,000 years. It is widely utilised in baking, brewing, and winemaking, and is commonly referred to as baker's and brewer's yeast.

Other organisms

Two synthetic transcription modulator genes were introduced into the host organism. These genes encode engineered fusion proteins composed of functional domains derived from sequences sourced from multiple non-pathogenic organisms.

A range of non-coding genetic elements, including promoters, regulatory sequences, and transcription termination signals have been used to regulate the expression of the inserted genes. These genetic elements are either synthetic or derived from *K. phaffii*.

4 Characterisation of the production strain

4.1 Molecular characterisation

Molecular characterisation is necessary to provide an understanding of the genetic material introduced into the host genome and helps to frame the subsequent parts of the safety assessment. The molecular characterisation addresses three main aspects:

- the transformation method together with a detailed description of the DNA sequences introduced to the host genome
- a characterisation of the inserted DNA, including any rearrangements that may have occurred as a result of the transformation
- the genetic stability of the inserted DNA and any accompanying expressed traits.

4.1.2 Transformation method

Details of the methodology used in the development of the production organism were provided as CCI.

To create the production organism, the host organism, parental *K. phafii* strain Y-7556, was modified using standard molecular biology techniques.

Antibiotic-resistance marker genes were used during the development of the production organism to facilitate the selection of positive transformants but were subsequently excised using the Cre/lox recombination system[1], leaving behind a lox72 sequence at the deletion site.

4.1.3 Description of the inserted DNA

The production organism contains two expression cassettes (Figure 1):

- ***Cassette 1*** - contains the helper gene from *S. cerevisiae* under the control of a synthetic promoter and coupled with the synthetic transcription modulator gene 1.

- ***Cassette 2*** - Contains the bovine *CSN2* gene under the control of a synthetic promoter and coupled with the synthetic transcription modulator gene 2.

The helper gene encodes a transcription factor that enhances the secretion of beta-casein, while the two synthetic transcription modulators function to promote the expression of the helper and *CSN2* gene.

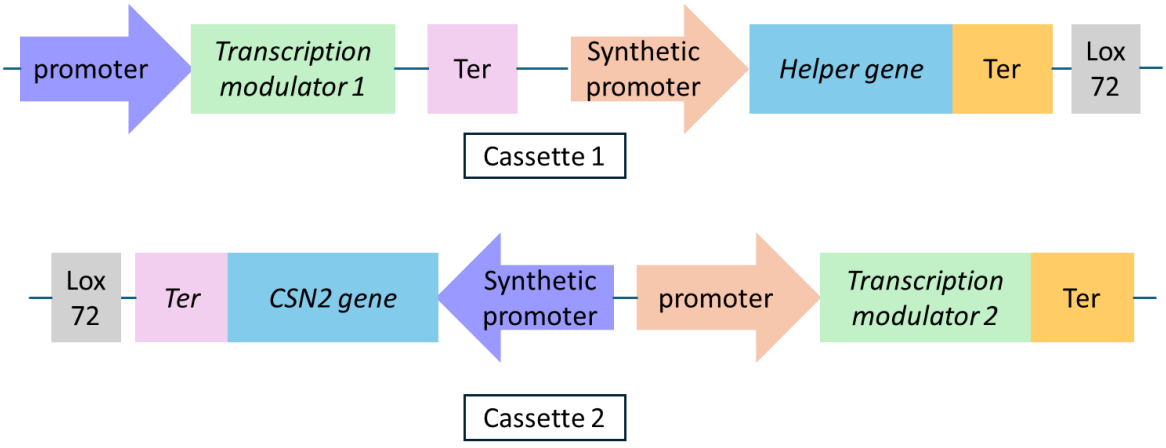

Figure 1. *Expression cassettes contained in the production organism.*

[1] The Cre/lox system enables site-specific recombination between loxP sites mediated by Cre recombinase, allowing precise removal of selectable markers without introducing unintended genetic changes.

4.2 Characterisation of the inserted DNA and site(s) of insertion

A range of analyses were undertaken to characterise the genetic modification in the final production organism. These analyses focused on the nature and stability of the insertion and whether any unintended re-arrangements or products may have occurred as a result of the transformation process.

4.2.1 Site of integration and insert integrity

The production organism was sequenced using Whole Genome Sequencing (WGS) using a hybrid approach combining Oxford Nanopore long-read and Illumina short-read technologies.

Sequence analysis confirmed that four distinct loci within the production organism were modified as follows:

- ***Loci 1 and 2***: Deletion of the two endogenous protease genes and insertion of the lox72 site at locus 2.
- ***Locus 3***: Integration of a single copy of cassette 1.
- ***Locus 4***: Integration of a single copy of cassette 2.

Sequence integrity of cassette 1 and 2 was confirmed, with no mutations or rearrangements detected.

Flanking regions at all insertion sites were verified to contain the expected regulatory sequences and no unintended genomic changes were observed. All modifications were consistent with the construct designs provided by the applicant and independently assessed by FSANZ.

4.2.2 Absence of selective marker genes

WGS was used to assess the presence of antibiotic-resistance marker genes used to select positive transformants. Results obtained from the final production organism showed absence of all the antibiotic resistance marker genes used. These analyses confirmed that the Cre/lox excision process was successful.

4.2.3 Stability of inserted DNA

Genetic stability

Genetic stability of the inserted genes was confirmed by analysis of cultures at the end of a 96-hour fermentation run (10 - 11 generations). Droplet digital PCR analysis targeting all the inserted genes showed that the copy number ratios of all the inserted genes remained stable throughout fermentation with only minor fluctuations that are not statistically significant. These data confirmed that the inserted genes are expressed over multiple generations and are stable.

Phenotypic stability

The applicant provided analytical data for 3 independent batches of the final product demonstrating consistent expression of the beta-casein protein across batches. Protein composition and yield were comparable, supporting stable production of the target protein.

4.2.4 Open reading frame (ORF) analysis

A bioinformatic analysis of the inserted DNA, as well as the flanking DNA regions, was undertaken to identify any novel ORFs which had been created in the production organism as a result of the genetic modification at loci 1-4, and whether any of these putative peptides have potential for allergenicity or toxicity.

The DNA sequence spanning the right and left insert-flank junctions of inserted DNA were translated *in silico* from start-to-stop codon in all six reading frames. Potential ORFs were identified.

Toxicity and allergenicity analyses of putative peptides from the identified ORFs did not identify any proteins with homology to known toxins, and did not identify any proteins with allergenic potential, except for beta-casein itself.

These analyses are theoretical only. In the absence of functional regulatory elements such as promoters and terminators it is highly unlikely that any of the identified ORFs would be expressed in the production organism.

4.3 Conclusion

WGS sequencing data showed that the two expression cassettes were each integrated as single copies at specific loci within the genome of the production organism. The inserted expression cassettes were confirmed to have the expected sequence and organisation, and no antibiotic-resistance marker genes used during the development of the production organism remained in the genome. The inserted sequences were shown to be stably integrated and inherited over several generations. None of the potential ORFs created by the insertions raised allergenicity or toxicity concerns.

5 Safety assessment of BCPP1

5.1 Characterisation of novel proteins

A large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects. Only a small number of dietary proteins have the potential to impact health, because of anti-nutrient properties or triggering an allergic reaction in sensitive individuals (Delaney et al. 2008).

The production organism expresses four novel proteins; the bovine beta-casein A2 protein, the helper protein and two synthetic transcription modulators. The helper protein and the transcription modulators are retained intracellularly and are not secreted during fermentation; accordingly, they are not expected to be present in the final product. LC-MS/MS analysis of three independent batches of BCPP1 did not detect these proteins in the final preparation.

BCPP1 therefore contains the secreted beta-casein A2 protein along with residual host cell proteins derived from the production organism. The safety of the secreted beta-casein and the residual host cell proteins is assessed below.

5.2 Bovine beta-casein

Beta-casein is a major protein component in bovine milk, representing approximately 30% of the total milk protein content (Pal et al 2015). It consists of a single polypeptide chain of 209 amino acids with a molecular mass of approximately 24 kDa (Duarte-Vázquez et al 2017). Evidence from dental calculus of ancient human remains indicates that adult humans have consumed milk from other species since at least 3000 BCE (Warriner et al 2014).

Bovine beta-casein plays key roles in calcium and phosphate transport, casein micelle stability, and curd formation, making it essential for cheese production and milk processing. It is a complete protein, providing all essential amino acids and supporting sustained nutrient release due to its slow digestion rate.

The protein is encoded by the *CSN2* gene, which exhibits a high degree of polymorphism and results in multiple variants (Sebastiani et al 2020). Among its naturally occurring variants, A1 and A2 are most common and differ by a single amino acid at position 67 (Cieślińska et al 2022). From a technological perspective, beta-casein variants can influence milk processing characteristics, with the A2 variant commonly associated with improved coagulation properties and firmer curd formation, which are advantages in cheese-making (Caroli et al 2009; Mishra et al 2009; Faggion et al 2025). These properties make beta-casein important for both nutritional quality and industrial functionality.

The beta-casein within BCPP1 is produced by a GM strain of *K. phaffii* containing the *CSN2* gene, encoding the beta-casein A2 protein (P02666)[2]. The introduced gene encodes a protein of 209 amino acids with a molecular weight of 24kDa.

5.2.1 N-terminal sequencing

To confirm the identity of the beta-casein component in BCPP1, three independent batches of BCPP1 were analysed by LC-MS/MS with Isobaric Tags for Relative and Absolute Quantitation (iTRAQ)[3]. Two N-terminal forms were detected:

[2] UniProt database entry for bovine beta-casein - https://www.uniprot.org/uniprotkb/P02666/entry

[3] iTRAQ - Mass-spectrometry technique that compares protein levels across samples by chemically tagging peptides with isobaric labels. These tags share the same mass but release distinct reporter ions during MS/MS, allowing simultaneous quantification of multiple samples in one experiment.

i) a predominant form containing a four–amino-acid extension (EAEA) relative to the predicted start site, and
ii) a minor truncated variant lacking the initial arginine.

To verify these results, Edman sequencing was performed on all three batches. The sequencing consistently identified the extended N-terminus containing the four amino acid extension as the primary form, confirming that the truncated species is present only at low abundance. Together, these findings demonstrate that the beta-casein component of the preparation is a highly homogeneous protein with a consistent N-terminal sequence arising from predictable signal peptide cleavage.

All the identified N-terminal variants of beta-casein were assessed *in silico* for assessment of potential allergenicity or toxicity. Simulated digestion with pepsin, trypsin and chymotrypsin was performed and resulting peptides were screened against the AllergenOnline[4] and UniProtKB toxin[5] databases. No significant homology to known allergens or toxins were identified, indicating no new safety concerns associated with the N-terminal variations.

5.2.2 Glycosylation analysis

Most secreted and cell membrane proteins in mammals undergo post-translational modifications such as glycosylation. Glycosylation is a key structural feature that often differentiates allergenic proteins from non-allergenic ones (Altman 2007). While glycosylation is common in milk proteins, bovine beta-casein naturally lacks glycosylation sites, and studies confirm no evidence of glycosylation in its native form (Dingess et al 2021). However, recombinant expression in *K. phaffii* can introduce glycosylation, accounting for up to 30% of the protein (Choi and Jiménez-Flores 2001).

To confirm the glycosylation level of beta-casein in BCPP1, three independent batches of BCPP1 were analysed using LC-MS/MS analysis. Results showed only a limited number of simple mannose-based glycosylation, consistent with typical host cell glycosylation patterns observed in *K. phaffii*.

5.3 Safety of the novel proteins

5.3.1 Potential allergenicity of the expressed beta-casein protein

Beta-casein is a recognised bovine milk allergen and is one of several milk proteins capable of eliciting IgE-mediated and non-IgE-mediated allergic reactions (Docena et al 1996; Lam et al 2008; El-Agamy 2007; Natale et al 2004). Reviews of milk allergens and associated immune mechanisms provide detailed evidence of the allergenic potential of beta-casein within the broader context of cow's milk allergy (Jensen et al 2022).

Recent population-based data indicate a 1.3% prevalence of IgE-mediated cow's milk allergy among 1-year-old Australian infants, with most affected children outgrowing the allergy in early childhood (Soriano et al. 2023). Despite interest in non-bovine milks as alternative protein sources, studies consistently show they do not reliably reduce allergenicity for individuals sensitised to cow's milk (Restani et al. 1999; Infante Pina et al. 2003; Muñoz Martín et al. 2004).

The beta-casein expressed in BCPP1 is identical in amino acid sequence to the naturally occurring bovine beta-casein A2 variant and is therefore expected to have the same

[4] Allergen protein database - http://www.allergenonline.org/
[5] UniProtKB toxin database - https://www.uniprot.org/help?query=toxins

allergenic potential as beta-casein present in conventional milk.

5.3.2 Potential toxicity of the expressed beta-casein protein

Studies assessing the safety of the expressed beta-casein protein, including pepsin digestibility and heat stability, were provided by the applicant and are presented in Section 5.4.

5.3.3 Safety of host cell proteins present in BCPP1

The identity of proteins present in BCPP1 was determined using mass spectrometry, with samples from three independent batches digested with trypsin and analysed by LC-MS/MS.

The applicant has provided the identities and mean quantities (as % of total protein) of the 20 most abundant proteins in BCPP1 other than beta-casein. These proteins are endogenous to *K. phaffii* and make up approximately 75% of the total protein content of BCPP1, although each is present at a low level compared to beta-casein. The 20 most abundant host derived proteins consist of common cellular enzymes, elongation factors, and chaperones in addition to a few proteins that have not been fully characterised.[6]

The amino acid sequences of the top 20 proteins were subjected to bioinformatic analyses, looking for similarity to known toxins and allergens. The data indicates there are no similarities between these proteins and known toxins or allergens.

Bioinformatic data concerning homology of potential ORFs with known toxins and allergens are addressed in Section 4.2.4.

5.4 Toxicological assessment of BCPP1

5.4.1 Protein digestibility

Pepsin digestibility of three independent fermentation batches of BCPP1 was assessed, with bovine beta-casein used as a control substance. BCPP1 samples were analysed with and without prior treatment with α-mannosidase, to determine whether glycosylation had any effect on susceptibility to proteolysis. Samples were collected at timepoints of 1, 5, 10, 30, 60, 120, and 240 min during the digestion assay, and proteins separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were stained with Coomassie blue, and Western blots of gels were labelled with anti-beta-casein antibody. Intact BCPP1 and bovine beta-casein were visualised as a major protein band at approximately 28 kDa, which was rapidly degraded upon exposure to pepsin. The protein band was substantially diminished within 5 minutes of digestion and was not detectable by 60 minutes. There was no significant difference in degradation between BCPP1 and bovine beta-casein.

Deglycosylation of BCPP1 samples by treatment with α-mannosidase prior to pepsin digestion led to a shift in molecular weight consistent with removal of glycan moieties but did not alter the rate or extent of pepsin-mediated digestion.

Labelling of proteins on Western blots with an anti-beta-casein antibody revealed rapid loss of immunoreactive signal for both BCPP1 and bovine beta-casein as a result of pepsin digestion. In all samples, the beta-casein signal was markedly reduced by 10 minutes and absent by 60 minutes. Negative control samples that had not been digested with pepsin

[6] Refer to Table 13 of the Application dossier (page 48) for a complete list and UniProt accession numbers.

retained strong beta-casein signals throughout the 240-minute incubation period.

5.4.2 Heat stability

Heat stability of BCPP1 samples was compared to that of bovine beta-casein using a similar general design to the pepsin digestion, i.e. treatment of samples from three different batches of BCPP1 and a sample of bovine beta-casein, followed by SDS-PAGE and Western blotting to separate and immunolabel proteins. Heat treatment at 80°C for 10 minutes was selected to exceed typical pasteurisation conditions. As for the pepsin digestion experiment, BCPP1 samples were treated both with and without pre-treatment with α-mannosidase. Heat treatment had negligible effect on BCPP1 samples.

5.4.3 Animal toxicity studies

FSANZ considered that toxicological testing for the BCPP1 preparation was not required because no hazard was identified for bovine beta-casein A2 or the BCPP1 preparation as part of the compositional and bioinformatic analysis.

FSANZ notes that viable *K. pfaffii* fed to mice for 20 days at a dietary concentration of 7 log CFU.g−1 was not associated with any adverse effects and had some antibacterial effect in vivo against a challenge with pathogenic *Salmonella enteriditis* var. Typhimurium (França et al. 2015). In addition, live K. *pfaffii* have been administered intravenously to mice (1 x 10^6 cfu in 200 µl of physiological saline) without adverse effects, and with clearance within 48 hours (Becerril-García et al. 2022).

FSANZ has previously assessed a 28-day dietary study in rats with a soy leghemoglobin protein preparation produced by a *K. phaffii* strain (MXY0541) closely related to NRRL Y-11340 which reported no adverse effects and identified a NOAEL of 750 mg/kg bw/day (Fraser et al. 2018).

Toxicity data from protein preparations produced in *K. phaffii* were provided, including several 90-day oral toxicity studies in rats. These studies consistently showed no treatment-related adverse effects at any tested dose, with the no observed adverse effect level (NOAEL) generally corresponding to the highest dose tested, indicating a low order of toxicity.

Additional supporting data was provided from shorter-term studies. These findings are consistent with the 90-day study outcomes and support the conclusion that purified protein preparations produced in *K. phaffii* do not exhibit systemic toxicity, even at high dietary exposure levels.

The applicant also provided genotoxicity studies including bacterial reverse mutation assays and *in vitro* mammalian cell assays assessing chromosomal damage (chromosome aberration and micronucleus assays). The results of these studies, including those on soy leghemoglobin (Fraser et al. 2018; Reyes et al. 2023), oubli sweet protein (brazzein) (Meetro et al. 2025), and recombinant collagen (Dai et al. 2025), showed no evidence of mutagenicity or clastogenicity.

There is no evidence that protein preparations produced by *K. phaffii*, including those that contain host cell proteins, raise concerns for systemic toxicity or genotoxicity.

5.4.4 Toxicity evaluation of the carbohydrate component in BCPP1

BCPP1 contains a significant level of carbohydrate fraction (68%) derived from the production organism, *K. phaffii*. The carbohydrate fraction is presumed to be primarily

composed of polysaccharides that are normal dietary components, such as mannans, beta-glucans and chitin fragments, and there are no toxicological concerns associated with these polysaccharides. Carbohydrates as a class are very unlikely to be toxic and are quickly broken down to sugars in the gastrointestinal tract.

5.5 Conclusion from the safety assessment

BCPP1 is comprised of the bovine beta-casein protein along with residual host cell proteins. The helper protein and the transcription modulators are absent from the final preparation.

Biochemical analysis of the bovine beta-casein in BCPP1 showed the expressed protein to be highly homogeneous with simple mannose-based glycosylation consistent with expression in *K. phaffii*. Bioinformatic analyses of the 20 most abundant host cell proteins present in BCPP1 did not identify any proteins with homology to known toxins, and did not identify any proteins with allergenic potential, except for the beta-casein itself.

BCPP1 shows comparable susceptibility to pepsin digestion, and comparable heat stability, to beta-casein extracted from bovine milk, and the glycosylation present in beta-casein expressed by *K. phaffi*i had no effect on these properties. The carbohydrate fraction in BCPP1 is presumed to consist mainly of common dietary polysaccharides which do not raise toxicological concerns.

Studies with protein preparations produced by related *K. phaffii* production strains, including those with residual host proteins, found no evidence of genotoxicity *in vitro* or *in vivo* and no adverse effects were observed in a 28-day and 90-day oral toxicity study in rats corresponding to the highest dose tested.

6 Nutrition assessment

6.1 Approach for assessment

BCPP1 is a dry powder containing approximately 22% protein, of which approximately 25% of the protein fraction is beta-casein. Proposed maximum use levels range from 3% to 15% (see Table 6 for proposed food categories and concentrations).

The applicant states that BCPP1 will be used as a protein ingredient in foods as a substitute for dairy and plant derived proteins, to support the development of innovative products that meet growing consumer demand for animal-free, lactose-free and more sustainable food options.

Unless otherwise specified, the nutrition assessment relates to the BCPP1 preparation as a whole. Protein quality was assessed for the protein fraction in BCPP1 using an i*n vitro* Protein Digestibility Corrected Amino Acid Score (PDCAAS) method, with purified bovine beta-casein used as the reference comparator.

The application does not request, or provide information on, the use of BCPP1 in infant formula products, infant foods, foods for special medical purposes, formulated supplementary sports foods, or formulated supplementary foods for young children. The scope of this assessment therefore does not include these special purpose foods.

6.2 Objectives

The objectives of the nutrition assessment were to

- Determine whether there are any nutritional concerns regarding the use of BCPP1 preparation as a source of protein in the proposed foods and at the proposed use levels.
- Compare the nutritional profile of BCPP1 with other commonly used dairy and plant derived protein sources.
- Determine whether BCPP1 will affect the absorption of other nutrients.

6.3 Macronutrient content

Macronutrient compositional data for BCPP1 provided by the applicant were compared with protein ingredients currently used in the food supply, including milk protein concentrate (MPC), soy protein concentrate and pea protein (Table 2).

Table 2 Composition of BCPP1 and comparator protein sources (per 100 g)

	BCPP1 Mean ± SD	MPC>70	Pea protein concentrate	Soy protein concentrate
Energy (kJ)	1602 ± 4.7	1568	1630	1579
Protein (g)	22.45 ± 0.3	85	75.8	89
Fat (g)	1.65 ± 0.2	2.2	8.0	1.7
Saturated fat (g)	0.28 ± 0.05	1.23	1.8	0.5
Trans fat (g)	< 0.1	0.03	NA	NA
Carbohydrate (g)	68.3 ± 0.48	1.6	6.8	3.9

Sugars (g)	< 1	1.3	0.5	0.2
Sodium (mg)	475.5 ± 33.63	200	835	1577

1. Sourced from the Australian Food Composition Database for: F007493: Protein powder, whey based, protein >70%, unfortified.
2. Pea protein concentrate: mean values from nutrition information panel data from 3 products available online in Australia and New Zealand.
3. Soy protein concentrate: mean values from nutrition information panel from 3 products available online in Australia and New Zealand.

6.3.1 Energy

BCPP1 is proposed for use at levels of up to 15% in foods as a substitutional protein ingredient and is not intended to increase overall dietary energy intake. The energy content of BCPP1 per 100 g is comparable to that of MPC and pea and soy protein concentrates (Table 2). At the proposed use levels, its contribution to dietary energy intake does not raise a nutritional concern.

6.3.2 Carbohydrate

BCPP1 contains a substantially higher proportion of carbohydrate compared to comparator protein ingredients (Table 2). The carbohydrate fraction of BCPP1 is 68% by weight and consists primarily of non-sugar polysaccharides, which the applicant states are presumed to be fermentation-derived mannans, beta-glucans and chitin fragments originating from the host organism's cell wall. The carbohydrate fraction of BCPP1 does not meet the FSANZ definition of dietary fibre and therefore is not considered to contribute to fibre intake. Sugar content is minimal.

As the carbohydrate fraction of BCPP1 is composed of non-sugar polysaccharides that are not intended to contribute to dietary energy or fibre intake, and there are no nutrient reference values (NRV) for total carbohydrate intake, the carbohydrate content of BCPP1 does not raise a nutritional concern.

6.3.3 Fat

The total and saturated fat content of BCPP1 is low and comparable to that of the comparator protein ingredients (Table 2). Trans fat was not detected (limit of detection 0.1 g/100 g). Given the low fat content of BCPP1, its contribution to intakes of total fat, saturated fat and trans fat does not raise a nutritional concern.

6.3.4 Protein

BCPP1 has a total protein content of approximately 22%, of which beta-casein constitutes around 25% of total protein, equivalent to 5.62% of the dry weight. The remaining protein fraction consists of host cell proteins derived from the production organism, *K. phaffii.*

The protein content of BCPP1 is lower than that of comparator protein ingredients of milk protein concentrate (85%), soy protein concentrate (87%) and pea protein concentrate (76.5%) (Table 2).

6.3.4.1 Protein quality

Protein quality reflects the ability of a dietary protein to provide adequate essential amino acids and is determined by both the amino acid composition and digestibility of the protein. Protein quality can be quantified using the Protein Digestibility Corrected Amino Acid Score (PDCAAS).

BCPP1 contains all essential amino acids, as shown by the amino acid profile presented in Table 3.

Table 3 Amino acid content (g/100 g) of BCPP1

Amino acid	Concentration g/100 g
Alanine	6.49
Arginine	2.11
Aspartic acid	11.47
Cysteine	0.41
Glutamic acid	17.96
Glycine	3.08
Histidine*	1.14
Isoleucine*	4.7
Leucine*	9.04
Lysine*	5.79
Methionine*	1.38
Phenylalanine*	5.33
Proline	8.11
Serine	7.42
Threonine*	7.18
Tryptophan*	0.65
Tyrosine	2.35
Valine*	5.33

*Essential amino acids

The applicant provided an *in vitro* PDCAAS assessment, which incorporates both amino acid scoring and protein digestibility. Table 4 shows the amino acid score, digestibility and PDCAAS values for 4 batches and 2 blended samples of BCPP1, with purified bovine beta-casein included as the reference protein and soy protein and casein included as controls.

Table 4 Protein quality testing for BCPP1 batches, reference and control proteins

Preparation	Amino acid score	Digestibility (%)	PDCAAS (%)
BCPP1 Batch 1	0.56	85	48
BCPP1 Batch 2	0.46	85	39
BCPP1 Batch 3	0.61	86	52
BCPP1 Batch 5	0.59	84	50
BCPP1 Blend B1/B2	0.51	85	43
BCPP1 Blend B3/B5	0.61	85	52
Bovine Beta casein (reference)	0.46	100	46
Soy Protein (control)	1.16	100	100[1]
Casein (kit control)	0.95	99	94

1. The PDCAAS score for soy protein isolate was truncated to 100 from a calculated value of 116, in accordance

with international guidelines.

The mean PDCAAS score for BCPP1 (47.3 ± 2.89) was not significantly different from that of the bovine beta casein reference (46.0 ± 0; *p*=0.68), indicating comparable protein quality when assessed using the same methodology. The PDCAAS value for BCPP1 is based on a protein digestibility of approximately 85%, indicating that the majority of the protein is digested under simulated gastrointestinal conditions.

The PDCAAS value for purified bovine beta-casein reflects assessment of an isolated protein fraction rather than a composite milk protein mixture. As a single protein fraction, beta-casein may be limiting in one or more indispensable amino acids when assessed against the reference pattern, resulting in a lower PDCAAS than protein mixtures with complementary amino acid profiles.

FSANZ notes that high-quality animal-derived proteins (for example, bovine milk protein, meat and hen egg protein) typically have PDCAAS values at or close to 100. Soy protein isolate has a PDCAAS of approximately 96–100, while pea protein has reported PDCAAS values of approximately 71–78 (Calvez et al. 2024). In comparison, the PDCAAS value for BCPP1 is lower than these high-quality reference proteins but similar to that of isolated beta-casein.

Although BCPP1 has a lower protein concentration and a PDCAAS value below that of high-quality reference proteins, these characteristics must be considered in the context of intended use and dietary patterns. BCPP1 is not intended to serve as a sole source of protein in foods, and foods containing BCPP1 will be consumed as part of a varied diet alongside other protein sources. National nutrition survey data from Australia and New Zealand indicate that most of the population consume adequate protein (ABS 2026a; University of Otago and Ministry of Health 2011). In this context, the protein quality and concentration of BCPP1 do not raise a nutritional concern.

6.4 Micronutrient content

The applicant provided analytical data on the micronutrient composition of BCPP1, which were assessed by comparison with MPC. These data are presented in Table 5. Micronutrient data were not available for soy protein concentrate or pea protein concentrate, with the exception of sodium (see Table 2).

Estimated dietary intakes were compared with NRVs, including Estimated Average Requirements (EARs), Adequate Intakes (AIs) and Upper Levels of Intake (ULs) (refer to Table 5 above). EAR and UL are to be used for comparisons with usual (long term) intakes from the whole diet, and AI are based on population intakes. Therefore, any comparisons of nutrient intakes from single portions of a food are outside the context of intakes from the whole diet and are for indicative or comparative purposes only, not to assess adequacy or excess.

A conservative high-intake scenario of 32 g/day BCPP1 was used, representing the highest 90th percentile estimated intake of BCPP1 across Australian and New Zealand dietary intake assessments (refer Table 7 below). For NRV comparison, children aged 1–3 years were used as the reference population, as this age group is included within the Australian dietary intake assessment (2+ years) and has the lowest nutrient requirements and upper level compared with older children and adults. This provides a conservative basis for assessing the risk of excessive intake.

Table 5 Micronutrient content of BCPP1 and milk protein concentrates (MPC), and comparison with NRV for children aged 1–3 years at high intake of BCPP1 (32 g/day)

Vitamin/mineral	MPC>70[1] (per 100 g)	BCPP1 (per 100 g)	EAR (per day)	% EAR at high intake	UL (per day)	%UL at high intake
Phosphorus (mg)	480	539	380	45	3000	5.7
Sodium (mg)	200	476	200-400	51	1,000	15.2
Potassium (mg)	480	342	2,000 (AI)	5.5	Not set	-
Iron (mg)	0.49	1.53	4	12.2	20	2.4
Copper (mg)	0.054	0.04	0.7 (AI)	1.7	1	1.2
Magnesium (mg)	78	3.98	65	2.0	65	2.0
Retinol (ug retinol equivalents)	9	<5	210	<0.8	600	<0.3
Molybdenum (ug)	43	183	13	450	300	19.5
Calcium (mg)	420	3.65	360	0.3	2,500	0.05
Manganese (mg)	0.006	0.02	2 (AI)	0.3	Not set	-
Zinc (mg)	0.25	0.32	2.5	4.1	7	1.5
Vitamin C (mg)	0	<1	25	<1.3	Not set	-
Niacin (mg)	0	1.53	5	9.8	10	4.9
Thiamin (mg)	0.1	<0.02	0.4	<1.6	Not set	-
Riboflavin (mg)	0.17	0.08	0.4	6.4	Not set	-
Pyridoxine (mg)	0.11	0.03	0.4	2.6	15	0.1
Pantothenic acid (mg)	0.06	0.16	3.5	1.5	Not set	-

1. Sourced from the Australian Food Composition Database for: F007493: Protein powder, whey based, protein >70%, unfortified

At an estimated high dietary intake of 32 g/day BCPP1, the contribution of most vitamins and minerals to daily requirements was low, generally contributing less than 10% of the EAR or AI for children aged 1–3 years. For nutrients with an established UL, estimated intakes were well below these levels.

Sodium and phosphorus made the largest proportional contributions at high intake, contributing approximately 51% and 45% of the EAR, respectively. However, intakes of both nutrients remained well below their respective UL, indicating no risk of excessive intake at the 90th percentile intake scenario. Although the sodium concentration of BCPP1 was higher than that of the MPC comparator, it was lower than that for soy and pea protein concentrates (see Table 2).

Estimated molybdenum intake at high consumption exceeded the EAR for young children; however, intake remained well below the UL (approximately 20%), indicating no risk of excessive intake.

Compared with MPC, BCPP1 contains lower levels of some micronutrients per 100 g, including calcium and magnesium (Table 5). However, protein ingredients added to foods are not expected to be major contributors to overall micronutrient intakes in the Australian or New Zealand diet. Therefore, these differences are unlikely to affect overall micronutrient adequacy.

At an estimated high dietary intake of 32 g/day BCPP1, contributions of vitamins and minerals were low relative to NRV and remained well below established ULs. On this basis, FSANZ considers the vitamin and mineral content of BCPP1 is unlikely to result in excessive intakes for Australian or New Zealand consumers and does not raise a nutritional concern.

6.5 Effect of BCPP1 on absorption of other nutrients

FSANZ considered whether BCPP1 could interfere with the absorption of other nutrients. The applicant stated that BCPP1 does not contain anti-nutritional factors such as protease inhibitors, lectins, phytates or oxalates as they are not present in yeast cells and are not introduced during the fermentation process.

FSANZ undertook a literature search in Pubmed on 15 December 2025[7] to identify any relevant literature; however no studies were identified. FSANZ notes that anti-nutritional factors are generally associated with foods from plant material (Gemede and Ratta 2014) and therefore does not consider that BCPP1 is likely to affect absorption of other nutrients.

6.6 Conclusion from the nutrition assessment

The nutrition assessment evaluated the macronutrient composition, protein quality, micronutrient content and potential effects on nutrient absorption associated with the proposed use of BCPP1 as a substitutional protein ingredient in foods at use levels of up to 15%.

Although BCPP1 has a lower protein concentration and PDCAAS value than high-quality reference proteins, it contains all essential amino acids and has a protein quality comparable to purified bovine beta-casein.

At a conservative high dietary intake of 32 g/day BCPP1, contributions of most vitamins and minerals were low relative to NRV and remained well below established ULs and therefore did not indicate a risk of excessive intake. BCPP1 does not contain anti-nutritional factors associated with impaired nutrient absorption, and no evidence was identified to suggest it would interfere with the absorption of other nutrients.

FSANZ concludes that the proposed use of BCPP1 as a protein ingredient in foods does not raise nutritional concerns for Australian or New Zealand consumers.

[7] Search terms: ("antinutrient" or "antinutritional" or "anti-nutrient" or "anti-nutritional") and (“beta casein”)

7 Dietary intake assessment

7.1 Introduction and purpose

This application outlined the proposed use of BCPP1 in a variety of different food categories at maximum levels ranging from 3 to 15% (w/w) as a nutritive and functional protein ingredient including use in dairy alternatives, protein beverages, baked goods, nutritional products, and mixed foods such as soups, sauces and dressings. BCPP1 is not intended to be included as an ingredient in whole, unprocessed foods, clear and high acid beverages, foods with strict compositional requirements such as butter, foods that do not normally contain protein and where its addition has no functional purpose.

The purpose of the dietary intake assessment was to estimate the level of chronic dietary intake of BCPP1 for the Australian and New Zealand populations and identify the major food group contributors to dietary intake. Chronic dietary intake estimates are used to represent the long term, usually life-long, dietary intake for the population from the range of foods containing the chemical of interest.

7.2 Methodology

Dietary intake assessments require data on the concentration of the chemical of interest in the food requested and consumption data for the foods that have been collected through a national nutrition survey.

The dietary intake assessment for BCPP1 was undertaken using FSANZ's dietary modelling computer program Harvest. A summary of the general FSANZ approach to conducting the dietary intake assessment for this application is on the FSANZ website. A detailed discussion of the FSANZ methodology and approach to conducting dietary intake assessments is set out in Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes (FSANZ, 2024).

7.2.1 Food consumption data used and population groups assessed

As permissions in the Code apply to both Australia and New Zealand, dietary intake assessments were undertaken for both countries.

The hazard assessment did not identify any population sub-groups or at-risk groups for which there were specific safety considerations or where separate dietary exposure estimates were required. In addition, the food classes requested in the application for the addition of BCPP1 are consumed by all age groups of the Australian and New Zealand populations. Therefore, the whole survey population from each of the nutrition surveys were used for the dietary intake assessment.

The food consumption data used for dietary intake assessments were:

- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), one 24-hour food recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS, 2015).

- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS), one 24-hour food recall covering 3,275 New Zealand school children aged 5-14 years, with 25% of respondents also completing a second 24-hour recall (Ministry of Health 2005).

- 2008–09 New Zealand Adult Nutrition Survey (2008 NZ ANS): a 24-hour recall of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. (Ministry of Health 2011a; Ministry of Health 2011b).

The design of these nutrition surveys and the key attributes, including survey limitations, are set out on the FSANZ website.

In this assessment, dietary intakes were estimated for 'consumers only' (e.g. consumers of foods containing BCPP1). Nutrition survey respondents who had no consumption of these foods were not included in the results presented. All results were weighted to make them representative of the respective populations.

For Australia two days of consumption data were averaged for this assessment as it reflects longer term food consumption patterns and therefore provides a better estimate of longer-term intake compared to a single 24-hour recall. Consumption amounts for New Zealand were based on a single day of nutrition survey data.

7.2.2 Concentration data used

The concentration data used in the dietary intake assessment for the added BCPP1 scenario were sourced from the application where they were presented as 'maximum use level % (w/w)'. A summary of the concentrations used in estimating the dietary intake is shown in Table 6.

For the purposes of the dietary intake assessment, concentrations of BCPP1 must be expressed for foods as consumed. The applicant did not specify BCPP1 concentrations for powdered product formats such as soups and gravy. FSANZ recalculated the BCPP1 content of dry powder formulations to account for dilution during preparation, consistent with the label data preparation instructions.

As a result, the calculated BCPP1 concentrations in the dry powders are higher than those proposed by the applicant, reflecting dilution upon reconstitution rather than any difference in the intended concentration of BCPP1 in the final food as consumed.

Table 6 Applicant proposed food categories and concentrations used in FSANZ dietary intake assessment for the added BCPP1 scenario

Food Category	Example product type	Maximum Use Level (%w/w)	Harvest food classifications code	Harvest food classification name	Harvest Maximum Use Level (g/kg)
Dairy Analogues	Milk alternatives (e.g. oat, soy, almond)	3%	14.1.7 14.1.8 14.7.9	Soy beverage Cereal beverages Nut- or seed-based beverages	30
	Yoghurt-style products	5%	4.3.8.5	Vegetarian yoghurt, soy based	50
	Cheese analogues	5%	12.6.1	Vegan cheese	50
Protein Beverages#	Ready-to-drink protein shakes	10%	13.3.3	Ready to drink protein shakes	100
	Powdered beverage mixes	15%	13.3.4	Powdered beverages	150
Nutritional Products	Formulated meal replacements	15%	13.3.1 13.3.2	Solid meal replacement & formulated supplementary foods Liquid meal replacement & formulated supplementary foods (other than those in 13.3.3 and 13.3.4)	150
Bakery Products	Protein-enriched breads, biscuit and snack bars	8%	7 20.2.2.3	Breads and bakery products Cereal products, bars	80
Mixed Foods	Soups, sauces and dressings	3%	20.2.6.1 20.2.6.2 20.2.7 20.2.8	Gravy, sauces, & condiments Sauces and syrups, sweet Mayonnaise & salad dressing Soups	30
Mixed Foods			20.2.8.4.1	Soups, dry mix, not reconstituted	360^
Mixed Foods			20.2.6.2.7.1	Gravy, sauces & condiments, dry mix, not reconstituted	200^

^Maximum use level calculated by FSANZ to reflect dilution.
#Protein Beverages are not considered under Standard 2.9.4 Formulated supplementary sports foods and are relative to Standards 2.6.2 Non-alcoholic beverages and brewed soft drinks and 2.9.3 Formulated meal replacements and formulated supplementary foods in the Code.

7.2.3 Assumptions and limitations of the dietary intake assessment

The aim of the dietary intake assessment was to make the most realistic estimation of dietary intake of BCPP1 as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary intake was not underestimated.

Assumptions made in the dietary intake assessment included:

- All foods in classes used in the model contain BCPP1 at the maximum proposed level.
- Where a food has a specified BCPP1 concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient e.g. bread in sandwiches.
- There are no changes in BCPP1 concentrations due to cooking e.g. baking bread and biscuits.
- Where multiple food categories are assessed, it is assumed that consumers may be exposed to BCPP1 from all relevant food groups on the same day.
- The assessment assumes that foods are prepared and consumed as reported in the surveys, and that any dilution, reconstitution or processing factors are consistent with typical household preparation practices.
- There are no other contributions to BCPP1 intake, for example through the use of dietary supplements or medicines.

Limitations of this dietary exposure assessment include:

- Food consumption data from the most recent available national nutrition surveys for Australia and New Zealand were used and are assumed to be representative of current dietary patterns for the population groups assessed. The most recently published Australian national nutrition survey data used in Harvest is the 2011-12 NNPAS, since then, dairy analogue products have seen considerable growth in the market. Dairy and meat substitutes purchased from Australian supermarkets and retailers increased by 14 per cent in 2020-21 following a 14 per cent increase between 2018-19 and 2019-20 (ABS, 2022). This is also reflected in the FSANZ Branded Food Database and supporting research (FSANZ, 2025b).
- As only one day of food consumption data are available from New Zealand nutrition surveys, the estimated dietary intakes are likely to be overestimated (particularly at the high percentile of intake) given that two days of survey data is more appropriate for estimating longer term consumption and therefore dietary intake.

FSANZ acknowledges that the Australian NNS data are 15 years old. Conducting dietary modelling based on 2011-12 NNS food consumption data provides the best estimate currently available of actual consumption of a food and the resulting estimated exposure to a food chemical. It should be noted that while the NNS may not include information regarding food products that are now available in the market, for staple foods such as breads, cereals and milk etc the data derived from the 2011-12 NNS is still adequate. Whilst data from the 2023 NNPAS has been released by the ABS, only summary consumption statistics at the food group level have been published to date. At the time of this assessment the raw data from the survey that allows extraction of consumption for specific foods was not available to FSANZ, therefore the 2023 NNPAS data were not able to be used for this assessment.

In addition to the specific assumptions made and limitations identified in relation to this dietary intake assessment, there are several limitations associated with the nutrition surveys per se. A discussion of these limitations is included in the Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes (FSANZ, 2024).

7.3 Estimated dietary intakes of BCPP1

Estimated mean and 90th percentile dietary intakes for consumers of BCPP1 based on maximum use levels were calculated in g/day as shown in Table 7.

Table 7 Estimated dietary intake to BCPP1

Country	Age group	Proportion of consumers to respondents (%)	Estimated dietary intake of BCPP1 (g/ day)	
			Mean	P90
Australia*	2 years and above	99.7	13	23
New Zealand#	5-14 years	99.3	18	32
	15 years and above	97.6	17	31

*Based on food consumption data from Day 1 and 2. Average of intakes across the two days presented.
#Based on food consumption data from Day 1 only.

According to Usual Intake data from the 2023 NNPAS, the mean intake of protein for Australians aged 2 years is 100 g/day for males and 76 g/day for females (ABS 2026b). In this assessment the mean estimated dietary intake of BCPP1 across the population groups assessed ranged from approximately 13-18 g/day (Table 7). Given that BCPP1 contains approximately 22% protein, this equates to a mean estimated dietary protein intake of approximately 2.9 – 3.9 g/per day from BCPP1, with its proposed use as a substitutional protein ingredient in food formulations.

7.3.1 Major contributing foods to dietary exposures

The food categories that contributed ≥5% to the estimated dietary intake of BCPP1 are summarised in Appendix 1, Table A1.1. ‘Bread & bakery products’ and ‘Gravy, sauces & condiments’ (either dry or reconstituted) are the major contributing food categories common for all three population groups assessed. ‘Soy beverages’ is a major contributor for New Zealand consumers.

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Appendix 1

Table A1.1 A summary of major contributing (≥5%) food categories to chronic dietary intake of BCPP1

Country	Age group	Food category (as proposed by the applicant)	Harvest classification		% Contribution estimated dietary intake
			Harvest classification code	Harvest classification name	
Australia*	2 years and above	Bakery Products	7	Breads & bakery products	74
		Mixed Foods	20.2.6.2	Gravy, sauces & condiments	8
New Zealand#	5-14 years	Bakery Products	7	Breads & bakery products	78
		Dairy Analogues	14.1.7	Soy beverage	5
		Mixed Foods	20.2.6.2.7.1	Gravy, sauces and condiments, dry mix, not reconstituted	5
	15 years and above	Bakery Products	7	Breads & bakery products	76
		Dairy Analogues	14.1.7	Soy beverage	5
		Mixed Foods	20.2.6.2	Gravy, sauces & condiments	7

*Based on food consumption data from Day 1 and 2. Average of intakes across the two days presented.
#Based on food consumption data from Day 1 only.