Application to FSANZ for the Amendment of the Australia and New Zealand Food Standards Code to allow the use of a Beta-casein Protein Preparation Produced via Precision Fermentation

Submitting Company:
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Submitted On: 7th October 2025
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[Personal Information Removed as CCI]

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EXECUTIVE SUMMARY

Eden Brew Pty Ltd is submitting this application to amend the Australia New Zealand Food Standards Code to permit the use of a Beta-casein Protein Preparation (BCPP1) produced via precision fermentation. Approval is sought under Standard 1.5.2 (Food Produced Using Gene Technology), with the product to be included in Schedule 26, and under Standard 1.5.1 (Novel Foods) to recognise BCPP1 as a novel ingredient. In addition, a specification for identity and purity is proposed for Schedule 3 to provide enforceable compositional standards. The application has been prepared in accordance with Guidelines 3.5.1 and 3.5.2 of the FSANZ Application Handbook and is intended to be considered under the General Procedure, as no novel public health or safety issues have been identified.

The subject of the application is BCPP1, a protein preparation containing protein that includes recombinant A2 Beta-casein. The product is manufactured using a genetically modified strain of *Komagataella phaffii* (EB-ST-537) under methanol-free fermentation conditions. The remainder of the protein fraction consists of co-purified host cell proteins that are non-allergenic and non-toxic. BCPP1 is supplied as a powder and is designed for use as a nutritive and functional protein ingredient in a wide range of food applications including dairy alternatives, protein beverages, baked goods and shelf-stable formulations. The protein component is structurally and nutritionally comparable to bovine A2 Beta-casein, while the ingredient provides an animal-free, lactose-free option.

Extensive data are provided to establish the identity, composition and safety of BCPP1. Compositional analyses across multiple independent batches confirmed consistent macronutrient, amino acid, mineral and impurity profiles. Protein quality testing showed that BCPP1 has equivalent digestibility and amino acid scoring to bovine Beta-casein, confirming its nutritional adequacy.

The safety of the production organism has been addressed by demonstrating the genetic stability of the inserted sequences and the absence of unintended changes. *K. phaffii* has a long history of safe use in food biotechnology, and the modifications introduced to EB-ST-537 were limited to targeted insertions and deletions that enhance expression and stability of the protein. Bioinformatic analyses revealed no significant similarity between BCPP1 or its co-purified proteins and known allergens or toxins. Digestibility assays confirmed rapid breakdown, and toxicity studies of comparable proteins produced in *K. phaffii* further support a conclusion of safety. Analytical testing confirmed the absence of viable host cells, residual DNA, heavy metals, mycotoxins and microbial contaminants.

Dietary modelling using Australian and New Zealand consumption data showed that inclusion of BCPP1 at proposed use levels will not disturb nutritional balance or displace key dietary components. The ingredient provides high-quality protein without increasing intake of sugars, saturated fats or other nutrients of concern.

The introduction of BCPP1 has no adverse implications for public health and offers consumers access to a high-quality, animal-free protein source. For the food industry, approval would enable innovation in dairy alternatives and protein-fortified products, expanding the range of sustainable options available. For consumers, BCPP1 provides a lactose-free and animal-free protein that is nutritionally equivalent to dairy Beta-casein and meets growing demand for products aligned with dietary, ethical and environmental values. From a sustainability perspective, precision fermentation provides significant advantages over animal agriculture by reducing greenhouse gas emissions, land use and water demand, thereby supporting broader environmental goals.

The evidence presented in this application demonstrate that BCPP1 is compositionally well-characterised, consistently manufactured and safe for human consumption. Eden Brew therefore submits that BCPP1 meets the requirements for approval under the Code as both a novel food and a food produced using gene technology. Its inclusion would align Australia and New Zealand with emerging international regulatory standards for precision-fermented proteins and provide consumers with greater choice in accessing sustainable sources of nutrition.

PART 1: ADMINIST RATIVE AND GENERAL INFORMATION

This section is completed in accordance with Chapter 3.1 (General Requirements for Applications) of the Food Standards Australia New Zealand Application Handbook (FSANZ, 2024).

A. Form of the Application

This application has been prepared in English and is submitted electronically in a word-searchable format. In accordance with formatting requirements, the application includes the following components:

- An executive summary, provided as a separate electronic file
- A table of contents to assist with navigation
- Sequential page numbering on each page
- A structure with headings that clearly identify all parts of the relevant guidelines being addressed.

B. Applicant Details

(a)	Applicant's name/s	[CCI]
(b)	Company/organisation name	Eden Brew Pty Ltd
(c)	Address (street and postal)	470 St Kilda Road, Melbourne, Victoria 3004, Australia
(d)	Telephone number	[CCI]
(e)	Email address	[CCI]
(f)	Nature of the applicant's business	Precision fermentation company
(g)	Details of other individuals, companies or organisations associated with the application	[CCI]

C. Purpose of the Application

The subject of this application is a novel protein preparation, termed BCPP1, produced via precision fermentation using a genetically modified strain of *Komagataella phaffii* (designated Strain ID: EB-ST-537). The preparation is a powder with a total protein content of approximately 22 %. The A2 Beta-casein protein is the key nutritive and functional component, comprising roughly 25 % of the total protein, with the remainder consisting of co-purified host cell proteins. The application seeks approval for BCPP1 to be used as a nutritive protein ingredient in a variety of food products.

As BCPP1 is derived from a new microbial source not traditionally used for this purpose in Australia and New Zealand, and is produced using gene technology, this application seeks approval under a dual-assessment pathway. It addresses the requirements for both a novel food ingredient (as per Guideline 3.5.2) and a food produced using gene technology (as per Guideline 3.5.1).

C.1 Food regulatory measure(s) to be varied

The application seeks a variation to the Australia New Zealand Food Standards Code, specifically:

- **Standard 1.5.2–Food produced using gene technology:** Approval is sought to include BCPP1 in Schedule 26–Food produced using gene technology, thereby permitting its sale and use as a food produced using gene technology
- **Standard 1.5.1–Novel Foods:** Approval is sought to permit the sale of BCPP1 as a novel food.
- **Schedule 3–Identity and Purity:** A specification will be established for BCPP1 to ensure its identity and purity are clearly defined and enforceable.

These are the primary regulatory measures that must be varied to enable the lawful sale and use of BCPP1 in the food supply of Australia and New Zealand.

C.2 Purpose relevant to specific application guidelines

This application relates to matters dealt with under Chapter 3 of the FSANZ Application Handbook (2024), specifically Guidelines 3.5.1 and 3.5.2.

Purpose Relevant to Guideline 3.5.1–Foods produced using gene technology

The purpose of this application, in relation to Guideline 3.5.1, is to provide FSANZ with the comprehensive technical information required to conduct a thorough safety assessment of BCPP1 produced using the genetically modified strain *Komagataella phaffii* (EB-ST-537). This includes:

- detailed information on the host organism and donor sequences
- a description of the genetic modification process and molecular characterisation of the inserted sequences
- data on the stability of the genetic modification
- characterisation and safety assessment of the expressed Beta-casein and other proteins present in the preparation (including bioinformatics analyses for toxicity and allergenicity)
- compositional analysis comparing BCPP1 to conventional animal-derived Beta-casein; and
- assessment of any potential impurities or residues from the fermentation and downstream processing.

This information will enable FSANZ to evaluate the safety of BCPP1 as a food produced using gene technology and its suitability for inclusion in Schedule 26.

Purpose relevant to Guideline 3.5.2–Novel foods

The purpose of this application, in relation to Guideline 3.5.2, is to provide FSANZ with the information required to assess BCPP1 as a novel food ingredient derived from a new source. BCPP1 is produced using a genetically modified microorganism not previously used for this purpose in the Australian or New Zealand food supply. Accordingly, the application includes:

- detailed information on the identity, composition, physical and chemical properties, and manufacturing process of BCPP1

- specifications for identity and purity, supported by certificates of analysis for multiple production batches
- compositional data and protein quality analyses to demonstrate that BCPP1 is functionally and nutritionally comparable to bovine Beta-casein
- information on dietary intake and exposure, including proposed food categories, use levels, and estimated consumption
- nutritional impact data to demonstrate that the inclusion of BCPP1 in the food supply will not cause dietary imbalance; and
- safety data, including consideration of the safety of the production organism, potential allergenicity, absence of toxins, and evidence supporting the safe use of the preparation as a novel food.

This information will enable FSANZ to determine the safety and appropriateness of BCPP1 as a novel food and to consider its inclusion in the Code via Standard 1.5.2 and Schedule 26.

D. Justification for the Application

D.1 The need for the proposed change

Schedule 26 of Standard 1.5.2 currently lists permitted foods produced using gene technology but does not include Beta-casein protein preparations. Approval is therefore required to allow the lawful sale and use of BCPP1 in Australia and New Zealand.

BCPP1 provides a functionally and nutritionally equivalent alternative to bovine Beta-casein protein, produced without reliance on animal agriculture. Its inclusion in the Code will support innovation in the food supply, enabling the development of animal-free foods that meet consumer demand for sustainable, ethical, and high-quality protein sources.

Because BCPP1 is produced using a genetically modified microorganism not previously used for this purpose, it must be assessed as a novel food in addition to being considered a food produced using gene technology. The proposed change is therefore necessary to ensure BCPP1 is legally recognised and appropriately regulated under the Code.

D.2 The advantages of the proposed change over the *status quo*

Food production contributes approximately one-third of global greenhouse gas emissions (GHG) and is a major driver of environmental degradation, surpassing the impact of any other industry (IPBES, 2019; IPCC, 2020). Livestock alone is linked to roughly half of GHG emissions (Poore and Nemecek 2018). This realisation has sparked a notable shift in both food production practices and the dietary choices of socially conscious consumers.

The growth of animal-free protein production has rapidly expanded, primarily pushed by political agendas seeking sustainable, ethical, and environmentally friendly solutions to climate challenges (for example, United Nations Climate Change Conference COP26). Plant-based products, such as plant milk alternatives and plant-based meats, have gained significant traction. Recent research indicates that plant-based proteins could necessitate 38–91 % less land, 53–95 % less water, and 69–92 % fewer carbon emissions compared to their meat counterparts (Lee and Eastman 2022). Integrating alternative proteins into diets has the potential to reduce global warming impact, water usage, and land utilisation by over 80 %, offering a pivotal role in ecosystem restoration and lowering the global carbon footprint of agrifood systems (Mazac et al., 2022). The plant-based food market is forecasted to grow from $29.4 billion in 2020 to $162 billion in 2030 (Bloomberg 2021), with an estimated market size of $3.1 billion in Australia (Admassu et al., 2020).

A less-explored facet within the alternative protein domain is precision fermentation. This process utilises microorganisms like yeast to convert sugars and minerals into complex food ingredients, including proteins and flavour compounds. Advances in gene technology have enabled rapid reprogramming of microorganisms for cost-effective and sustainable production of specific food protein ingredients.

The methylotrophic yeast *Pichia pastoris*, now reclassified as *Komagataella phaffii* (Kurtzman 2005), has a history of safe use for producing pharmaceuticals and industrial chemicals (Ahmad et al., 2014; Spohner et al., 2015). Several products derived from *K. phaffii* have been approved by regulatory bodies like by European Food Safety Authority (EFSA), U.S Food and Drug Administration (FDA) and Food Standards Australia New Zealand (FSANZ). For example, designated as Generally Recognized As Safe (GRAS) by the FDA, *K. phaffii* has approval for producing various recombinant proteins, including soy leghemoglobin used by Impossible Foods Inc. (GRN No. 737). FSANZ also recently sanctioned *K. phaffii* for soy leghemoglobin production (branded LegH Prep) as a meat analogue additive (see FSANZ A1186).

Eden Brew acknowledges the multifaceted aspects of alternative protein production, encompassing challenges, risks, benefits, and opportunities as highlighted to Codex (FAO/WHO 2021). Consequently, Eden Brew has diligently assessed EB-ST-537 and presents this information to FSANZ for consideration under Standard 1.5.2 Food Produced using Gene Technology.

D.3 Regulatory impact

D.3.1 Costs and benefits of the application

The inclusion of BCPP1 from strain EB-ST-537 in Schedule 26 will not have an adverse impact on consumers, the food industry or government. For consumers and the industry, amendment of the Code will provide access to an alternative non-animal derived source of Beta-casein protein, allowing them to make informed choices and decisions aligned with their health, ethical, religious, and/or environmental values. For the government, the burden is limited to necessary activities for a variation of Schedule 26—Food Produced Using Gene Technology and Schedule 3–Identity and Purity.

D.3.2 Impact on international trade

The inclusion of BCCP1 from strain EB-ST-537 in Schedule 26 is unlikely to have any impacts on international trade. Milk proteins from precision fermentation are already being marketed internationally. For example, the United States, Singapore, Hong Kong and India have all approved products containing whey protein derived from precision fermentation. Following assessment by FSANZ, Eden Brew intends to seek market authorisations in other jurisdictions, facilitating the international trade of this product.

E. Information to Support the Application

This application has been prepared in accordance with the *Food Standards Australia New Zealand Handbook* (as at, 1 July 2024), Guideline 5.5.1 and Guideline 3.5.2. These Guidelines collectively ensure that FSANZ has sufficient information to evaluate both the safety of the genetically modified production system and the suitability of BCPP1 as a novel food ingredient.

Collectively, results support this application for amendment to the *Australia New Zealand Food Standards Code* to allow inclusion of the Beta-casein protein preparation in accordance with Standard 1.5.2-Food Produced Using Gene Technology.

E.1 Data Requirements

In preparing this submission, Eden Brew has followed the general data requirements in Guideline 3.1.1 of the Handbook. All data are fully referenced with details of the source, author and year of production, and studies have been conducted using validated or standardised methods with documented sensitivity and specificity. Where population-relevant data were required, Australian and New Zealand populations were used as the reference. Statistical analyses were applied appropriately, and electronic copies of all references, studies and reports have been included.

E.2 Information relevant to Guideline 3.5.1–Foods produced using gene technology

Information has been provided on the identity of the host organism (*Komagataella phaffii)* and the donor sequences used in the genetic modification, along with the history of safe use of *K. phaffii* in food and biotechnology applications. The dossier describes the molecular characterisation of the inserted sequences, demonstrates their stability, and confirms the absence of unintended changes.

BCPP1 has been characterised in terms of the proteins expressed, with bioinformatics analyses, allergenicity assessment and toxicity evaluations included. Compositional analyses compare BCPP1 to conventional bovine Beta-casein across relevant nutrients. The nutritional impact assessment demonstrates that BCPP1 delivers protein of comparable quality to bovine Beta-casein and does not adversely affect dietary balance.

E.3 Information relevant to Guideline 3.5.2–Novel foods

This application seeks exclusive permission for the sale of BCPP1 as a novel food, consistent with Section I of this dossier. Exclusive permission is requested on the basis that BCPP1 is produced through a proprietary strain and manufacturing process, and that significant investment has been made in its development.

As BCPP1 is a protein ingredient derived from a new source, it falls within the scope of the Novel Food guidelines. The dossier provides detailed information on its identity, composition, physical and chemical properties, and manufacturing process. Proximate, amino acid, vitamin and mineral analyses are included, together with protein quality evaluations such as Protein Digestibility Corrected Amino Acid Score. Certificates of analysis for multiple production batches establish specifications for identity and purity, and batch data confirm the absence of viable host cells, residual DNA, or other process-related contaminants.

The safety of the production organism has been demonstrated through molecular confirmation of the taxonomic identity of the strain and reference to its safe lineage. Toxicity and allergenicity data are presented for BCPP1 and for related proteins produced using *K. phaffii*. Dietary intake and exposure modelling considers proposed food categories and maximum use levels, with estimated consumption

assessed against Australian and New Zealand food intake data. Nutritional impact assessments show that the inclusion of BCPP1 in the food supply will not lead to dietary imbalance.

Finally, the application provides information on the international regulatory context. Precision-fermented proteins, including dairy proteins, are already approved in other jurisdictions such as the United States and Singapore, supporting the view that BCPP1 can be safely integrated into the food supply of Australia and New Zealand.

E.4 Assurance of data quality

All studies and supporting information have been presented in sufficient detail to allow independent evaluation of methods and results. The evidence demonstrates that BCPP1 is compositionally well-characterised, produced to a consistent specification, and safe for use in foods. Collectively, the data confirm that BCPP1 is as safe and appropriate for consumption as conventional bovine Beta-casein.

F. Assessment Procedure

Eden Brew is anticipating that this application will be considered under the **General Procedure** for Administrative Assessment process by Food Standards Australia New Zealand.

The General Procedure is considered appropriate because:

1. The application does not raise any novel issues of public health and safety that would warrant assessment under the Major Procedure.
2. The technical and safety information provided addresses the requirements of both Guideline 3.5.1 (Foods produced using gene technology) and Guideline 3.5.2 (Novel foods) of the FSANZ Application Handbook (2024); and
3. The scope of the proposed variations is confined to Standard 1.5.2–Food produced using gene technology, with the primary variation sought being to Schedule 26, supported by a consequential specification in Schedule 3–Identity and Purity.

On this basis, Eden Brew respectfully submits that the General Procedure provides an efficient and proportionate pathway for FSANZ to evaluate the safety, identity, and nutritional suitability of BCPP1 as a novel food produced using gene technology.

G. Confidential Commercial Information (CCI)

This application contains certain information that Eden Brew requests be treated as Confidential Commercial Information (CCI) in accordance with section 114 of the *FSANZ Act*. A separate redacted version of the application has been prepared for public release.

The information claimed as CCI is limited to material that, if disclosed, would reveal proprietary details of Eden Brew's strain development, genetic constructs, or manufacturing process. Such information is highlighted in the dossier and clearly identified in the confidential schedule provided to FSANZ.

Consistent with FSANZ's information publication scheme, Eden Brew does not seek to restrict the publication of non-confidential elements of the application, including general descriptions of the product, its purpose, and its proposed uses. Personal information such as signatures, names and contact details of company personnel and consultants is not considered CCI and will be managed by FSANZ in accordance with its privacy policy.

H. Other Confidential Information

No additional confidential material is included in this submission document.

I. Exclusive Capturable Commercial Benefit

Eden Brew is seeking exclusive permission for BCPP1 as a novel food under section 26 of the *FSANZ Act*. This request reflects the significant research, development and investment undertaken to develop the proprietary *Komagataella phaffii* strain (EB-ST-537) and the associated precision fermentation process used to produce BCPP1.

Exclusive permission is sought for the sale of BCPP1 as an ingredient, not for finished food products that contain it. During the exclusivity period, other food manufacturers may use BCPP1 only if sourced directly from Eden Brew and used in accordance with the conditions of approval set by FSANZ.

Eden Brew recognises that FSANZ may grant such exclusivity for a defined period and acknowledges that the exclusivity would apply specifically to BCPP1 produced using Eden Brew's proprietary process.

This request is consistent with Section E.3 above that confirms that the application is being made on an exclusive-use basis.

J. International and Other National Standards

No other submissions for BCPP1 have yet been made to international or national food regulatory authorities. However, precision-fermented dairy proteins are already approved or permitted in several jurisdictions. For example, the United States Food and Drug Administration (FDA) has accepted self-affirmed GRAS notices for whey and casein proteins produced via precision fermentation, and Singapore and Hong Kong have approved products containing animal-free dairy proteins produced using similar technologies.

These international precedents demonstrate regulatory acceptance of precision-fermented proteins as safe and suitable for use in foods. Approval of BCPP1 under Standard 1.5.2 and Schedule 26 of the Code would therefore align Australia and New Zealand with emerging international standards and facilitate future trade opportunities.

K. Statutory Declaration–Australia

[This Statutory Declaration is considered CCI]

L. Checklists Provided With Application

General Requirements

General Requirements (3.1.1)		
Check	**Page No.**	**Mandatory Requirements**
☒		A Form of application ☒ Application in English ☒ Executive Summary (separated from main application electronically) ☒ Relevant sections of Part 3 clearly identified ☒ Pages sequentially numbered ☒ Electronic copy (searchable) ☒ All references provided
☒	3	B Applicant details
☒	3	C Purpose of the application
☒	5	D Justification for the application ☒ Regulatory impact information ☒ Impact on international trade
☒	7	E Information to support the application ☒ Data requirements
☒	8	F Assessment procedure ☒ General ☐ Major ☐ Minor ☐ High level health claim variation
☒	8	G Confidential commercial information ☒ CCI material separated from other application material ☒ Formal request including reasons ☒ Non-confidential summary provided
☒	9	H Other confidential information ☒ Confidential material separated from other application material ☒ Formal request including reasons
☒	9	I Exclusive Capturable Commercial Benefit ☒ Justification provided
☒	9	J International and other national standards ☒ International standards ☒ Other national standards
☒	10	K Statutory Declaration
☒	11-13	M Checklist/s provided with application ☒ 3.1.1 General requirements checklist ☒ All page number references from application included ☒ Any other relevant checklists for Chapters 3.2–3.7

Foods Produced Using Gene Technology

Foods produced using gene technology (3.5.1)		
Check	**Dossier Section**	**Mandatory requirements**
☒	Section A.2.2	A.1 Nature and identity
☒	Section B.2.1	A.2 History of use of host and donor organisms
☒	Section B.2.2 and Section B.2.3	A.3 Nature of genetic modification
☒	Section C.1	B.1 Characterisation and safety assessment
☒	Section C.1.1, C.2 and C.3	B.2 New proteins
☒	Section C.4	B.3 Other (non-protein) new substances
☐	N/A	B.4 Novel herbicide metabolites in GM herbicide-tolerant plants
☒	Section A.5	B.5 Compositional analyses
☒	Section D	C Nutritional impact of GM food
☒	Section C.3.2	D Other information

Novel Foods

Novel foods (3.5.2)		
Check	**Dossier Section**	**Mandatory requirements**
☒	Part 1, Section I; Section A.1	A. Exclusive use
☒	Section A.2.1	B.1 Type of novel food
☒	Section A.3	B.2 Information on potential beneficial outcomes
☒	Section A.4	B.3 Chemical and physical properties
☒	Section A.6	B.4 Impurity profile
☒	Section B.1	B.5 Manufacturing process
☒	Section B.3	B.6 Specification for identity and purity
☒	Section B.4	B.7 Analytical detection method
C.6	Food ingredients derived from a new source	
☒	Section B.2.1	C.6.1 Safety of the source organism, including allergen statement
☒	Section A.5	C.6.2 Composition
☒	Section C.3	C.6.3 Toxicity studies
☒	Part 1, Section J	C.6.4 Overseas safety reports
☒	Section D.1 and Table 17	D.1 List of foods likely to contain the novel food or novel food ingredient
☒	Section D.1 and Table 17	D.2 Proposed levels in foods
☒	Section D.2	D.3 Information on levels of consumption
☒	Section D.3	D.4 Percentage of food group or market
☒	Section D.3 and Section D.5.2	D.5 Where consumption has changed, information on likely consumption
☒	Section A.3 and Section D.4	D.6 Information to show whether the food or ingredient will replace another food
☒	Part 1, Section J	D.7 Use in other countries
☒	Section D.4	E.1 Nutritional impact information
☒	Section C and Section D	E.2 Public health impact
☒	Section D.5.1	F.1 Demonstrated consumer awareness and understanding
☒	Section D.5.2	F.2 Potential behaviour in response to foods
☒	Section D.5.3	F.3 Demonstration of no adverse effects on any population groups

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List of Supplement Reports

1	EB-25-MOL-01-02_Development and Molecular Characterisation of Strain EB-ST-537 [CONFIDENTIAL]
2	EB-25-MOL-02-01_Genetic Stability of the Insertions in Strain EB-ST-537 [CONFIDENTIAL]
3	EB-25-PROT-01-02_Protein Safety Assessment for Strain EB-ST-537 [CONFIDENTIAL]
4	EB-25-PROT-02_01_Allergen and Toxin Evaluation of ORFs in Strain EB-ST-537 [CONFIDENTIAL]
5	EB-25-COMP-01-01_Compositional Assessment of Beta-casein Protein Preparation from Strain EB-ST-537
6	EB-25-MAN-01-01_Manufacture of Precision-Fermented Beta-Casein Powder from Strain EB-ST-537 [CONFIDENTIAL]
7	EB-25-DIET-01_01_Dietary Exposure Assessment of BCPP1 [CONFIDENTIAL]

PART 2: TECHNICAL AND SAFETY ASSESSMENT

This section provides a consolidated technical and safety assessment of the Beta-casein Protein Preparation (BCPP1). The data herein is structured to comprehensively address the mandatory requirements of both Guideline 3.5.1 (Foods produced using gene technology) and Guideline 3.5.2 (Novel foods). Where data requirements overlap, they are presented once to support assessment against both guidelines.

SECTION A: PRODUCT CHARACTERISATION

This section details the identity, composition, purpose, and impurity profile of BCPP1, addressing the technical characterisation requirements for genetically modified foods (Guideline 3.5.1, Sections A.1 and B.5) and novel foods (Guideline 3.5.2, Sections A and B).

A.1 Exclusive Use of Novel Foods

Eden Brew is seeking exclusive permission for the Beta casein protein preparation as a novel food on the basis that it is a highly refined product obtained via proprietary manufacturing processes. There have been significant research and investment by Eden Brew and its partners into the development of the Beta casein protein preparation (BCPP). It is envisioned that exclusivity will be specific to Eden Brew's BCCP when used as a food ingredient, and not to finished food products containing these materials. In practice, this means that during the exclusivity period, a manufacturer may incorporate BCCP into their food products only if they obtain the ingredient from Eden Brew and provided that the use is in accordance with the agreed conditions specified in the approval from FSANZ.

A.2 Nature and Identity of the Food

A.2.1 Type of Novel Food

The subject of this application is a highly purified Beta-casein Protein Preparation (BCPP1), produced via precision fermentation using a genetically modified strain of *Komagataella phaffii* (Designated Strain ID: EB-ST-537). The resulting product is a substance with a protein content of no less than 20% on a dry weight basis and is intended for use as a functional and nutritional protein ingredient in a variety of food products.

The source organism, *K. phaffii*, has a history of safe use in the production of food ingredients, including soy leghemoglobin (A1186) which is approved in Standard 1.5.2. The Beta-casein protein produced is structurally and functionally identical to the A2 Beta-casein protein found in bovine milk. However, as the protein is derived from a new source (a genetically modified microorganism) not traditionally used for producing this protein for human consumption in Australia and New Zealand, it is considered a novel food.

Based on the categories of novel foods identified in Guideline 3.5.2 of the FSANZ Application Handbook, the BCPP1 is most appropriately classified as:

- A food ingredient derived from a new source.

BCPP1 also meets the definition of food produced using gene technology, as it is derived from an organism modified using gene technology (i.e. derived from a GM *K. phaffii* strain). To be permitted for use, express permission for BCPP1 must be given in accordance with Standard 1.5.2 (i.e., BCPP1 must be listed in Schedule 26 and comply with corresponding conditions listed in the Schedule).

This application will provide the necessary technical and safety information to support the approval of BCPP1 under Standard 1.5.2 and its inclusion in Schedule 26 of the Code. Assessment as a novel food would not be

appropriate as BCPP1 is produced through a GM process and the Code does not require a GM food to also be permitted as a novel food.

A.2.2 Food Produced Using Gene Technology

The proprietary yeast production strain, *K. phaffii* EB-ST-537, is a genetically modified derivative of *K. phaffii* Y-7556, developed specifically to enable high-level production and secretion of bovine Beta-casein under industrially relevant, methanol-free fermentation conditions. The genetic modifications were designed to enhance protein expression, improve product stability, and ensure regulatory compliance. Eden Brew has designated the new strain EB-ST-537 to generate a Beta-casein Protein Preparation (BCPP1).

The production strain (EB-ST-537) was constructed using the principles described within the Organisation for Economic Co-operation and Development (OECD) criteria for Good Industrial Large-Scale Practice (GILSP) microorganisms (OECD, 1992; 1993) as well as accepted international best practice criteria for safe production microorganisms (Pariza and Foster, 1983; Pariza and Johnson, 2001). Full details on strain development and molecular characterisation are provided in **Supplemental Report EB-25-MOL-01-02** [CONFIDENTIAL].

To achieve this, several targeted modifications were introduced. The key functional insertion is a single copy of the bovine Beta-casein gene *CSN2* from *Bos taurus*, placed under the control of a synthetic promoter that is activated under methanol free conditions. This allows for regulated, high-yield expression of Beta-casein without the need for methanol as an inducer, a significant advantage for safety, cost, and downstream processing.

To enhance protein folding and secretion, the strain also incorporates a single copy of a helper gene from *Saccharomyces cerevisiae*, along with single copies of two synthetic transcription modulators that facilitate *CSN2* expression.

In addition to these insertions, two endogenous host genes associated with proteolytic degradation, were deleted, reducing unwanted breakdown of the target protein and improving yield and stability.

A summary of the four engineered sites for strain EB-ST-537 is provided (Table 1). Together, these modifications establish *K. phaffii* EB-ST-537 as a stable, efficient, and safe production host tailored for large-scale, methanol-free production of recombinant Beta-casein.

Detailed information on the genetic modifications, engineering methods, and molecular validation of the strain is provided in the **Supplemental Report EB-25-MOL-01-02.**

Table 1. Genes introduced, and deletions made to create strain EB-ST-537

Site	Modification	Donor Organism	Number of Copies
1	Deletion: The entire open reading frame of a host gene was precisely deleted	N/A	N/A
2	Deletion: The entire open reading frame of a host gene was precisely deleted	N/A	N/A
3	Insertion: A synthetic double stranded DNA region encoding a helper gene and a synthetic transcription modulator under the control of synthetic promoters	*S. cerevisiae*	1
4	Insertion: A synthetic double stranded DNA region encoding a synthetic transcription modulator and *CSN2* (Beta-casein) under the control of a synthetic promoter	*B. taurus*	1

A.3 Purpose of Use in Food

The Beta-casein Protein Preparation (BCPP1) is a novel protein ingredient produced via precision fermentation. The preparation is a powder with a total protein content of approximately 22 %, of which the target A2 Beta-casein constitutes about 25 %. The remaining protein consists of co-purified, non-allergenic host cell proteins from the production organism, *K. phaffii*.

The primary purpose of its addition to food is nutritive. It is designed to serve as a high-quality, animal-free source of dietary protein. While BCPP1 is a composite ingredient, its key nutritive value is delivered by the A2 Beta-casein component, which provides essential amino acids necessary for human nutrition. The protein quality of the entire preparation has been demonstrated to be high and comparable to its dairy counterpart. The ingredient's role is substitutional, intended to replace other proteins in a formulation rather than to increase total protein intake in the diet.

In addition to its nutritive role, BCPP1 exhibits valuable technological properties that make it a versatile ingredient. These functions, such as emulsification, heat stability, and contributing to a creamy texture and mouthfeel, are primarily attributed to the Beta-casein fraction within the preparation.

Overall, BCPP1 provides a non-animal protein ingredient that delivers the nutritional and functional benefits of A2 Beta-casein. This allows for the development of innovative products that meet growing consumer demand for animal-free, lactose-free, and sustainable food choices

A.4 Physical and Chemical Properties

The Beta-casein protein preparation BCPP1 is a highly purified, recombinant A2 Beta-casein produced via precision fermentation in *K. phaffii*. It is supplied as a free-flowing, white to off-white powder (<6 % w/w) moisture with a bland taste, high solubility, and excellent dispersibility in aqueous systems. The product is intended for use as a functional and nutritional protein ingredient in a wide range of formulated foods.

A.4.1 Composition and Functional Characteristics

BCPP1 exhibits a consistent macronutrient profile across production batches, with high protein content (mean 22.45 ± 0.3 g/100 g), low fat (1.65 ± 0.2 g/100 g), minimal saturated fat (0.28 ± 0.05 g/100 g), and negligible sugars (<0.2 g/100 g). Moisture and ash levels are low (5.4 ± 0.2 g/100 g and 2.1 ± 0.2 g/100 g, respectively), supporting shelf stability. The carbohydrate fraction (68.3 ± 0.48 g/100 g) consists primarily of non-sugar polysaccharides, presumed to be fermentation-derived mannans, Beta-glucans, and chitin fragments from the host organism’s cell wall. These are non-digestible, physiologically inert, and do not contribute to dietary fibre under FSANZ definitions. Heavy metals, mycotoxins, and microbial contaminants are not detected, consistent with food-grade production standards.

BCPP1 exhibits protein characteristics such as emulsification, foaming, gelation, and water-binding, which are broadly comparable to those observed for bovine Beta-casein. These properties suggest potential utility across a range of food matrices and processing applications.

A.4.2 Uniform Incorporation into Food Matrices

BCPP1 is a soluble, dispersible, and heat-stable protein ingredient designed for uniform integration into a wide variety of food matrices. Its physicochemical properties, specifically high solubility in aqueous systems and resistance to thermal and pH-related denaturation, support its functionality across multiple product categories. The following matrix-specific characteristics are based on pilot formulation trials, ingredient performance testing, and industry-relevant processing conditions.

Dairy analogues and protein beverages

BCPP1 dissolves readily in water and remains fully soluble in dairy analogue bases (e.g., oat, soy, or almond), as well as in high-protein ready-to-drink formulations. It tolerates a pH range of approximately 4.0 to 7.0, disperses uniformly under standard mixing or homogenisation conditions, and does not require pH adjustment or carrier agents. The protein remains stable under pasteurisation (72–85°C) and UHT (135–150 °C) conditions. In refrigerated storage (up to 6 weeks at 4 °C), no precipitation, sedimentation, or visible phase separation has been observed.

Fermented products (e.g., Yoghurt-style products)

In fermented food systems, BCPP1 maintains its solubility and functional stability throughout acidification and cold storage. Formulation trials with pH endpoints between 4.2 and 4.5 confirmed that the ingredient does not precipitate or interfere with fermentation. Product texture and viscosity were retained over 28 days of refrigerated storage, with no evidence of protein destabilisation.

Baked goods and extruded snacks

BCPP1 has demonstrated stability under thermal processing typical of bakery and snack applications. In bread, biscuits, and high-protein snack bars, BCPP1 tolerated baking temperatures up to 100°C without visible browning or degradation. In extruded products, it remained functionally intact during low-moisture extrusion processes (>130 °C barrel temperature), with no negative impact on expansion or textural integrity. Shelf-life stability was observed in ambient storage (6+ months), with no detectable changes in protein solubility or sensory properties.

Dry mixes and shelf-stable powders

In dry applications such as powdered soups, shakes, and meal replacements, BCPP1 is stable under ambient storage conditions. It exhibits low hygroscopicity and does not agglomerate or clump during blending or storage. Accelerated stability studies (25 °C, low water activity) confirmed that the protein retains its structural integrity for at least 12 months, as assessed by SDS-PAGE and nitrogen recovery.

General comments on ingredient incorporation

Across all tested matrices, BCPP1 integrates uniformly without requiring emulsifiers, pre-treatment, or specialised equipment. It is compatible with both cold- and hot-fill processing systems and exhibits no adverse interactions with common food ingredients such as starches, plant proteins, fats, or hydrocolloids.

Where further quantitative data on nutrient retention or claim validation is required (e.g., for nutrition panels or health claims), additional shelf-life and processing stability studies can be undertaken and submitted post-approval as part of labelling or marketing authorisation processes.

A.5 Compositional Analysis of BCPP1

Compositional analysis of four independent commercial 500L batches of BCPP1 were conducted to evaluate the levels of key analytes (Table 2). Specific details of the analysis are provided in **Supplement Report EB-25-COMP-01_01** and summarised in Table 2–Table 11. Results of batch analysis demonstrates that the final BCPP1 complies with the established product specification (see Section B.3). Further, non-viability of GM host cells was confirmed via plating and growth on YPD agar plates (data not shown).

Table 2. Summary of screening for analytes and microbes

Proximates (6)		
Fat	Carbohydrates	Ash
Protein	Moisture	
Trace Elements and Heavy Metals (16)		
Lead	Mercury	Cadmium
Arsenic	Calcium	Cobalt
Copper	Iron	Magnesium
Manganese	Molybdenum	Phosphorus
Potassium	Sodium	Sulphur
Zinc		
Vitamins (9)		
Alpha-Carotene	Ascorbic acid	Riboflavin
Beta-carotene	Thiamin	Niacin
Retinol	Pyridoxine	Pantothenic acid
Sugars (5)		
Glucose	Sucrose	
Lactose	Maltose	
Fructose		
Total Amino Acids (22)		
Alanine	Arginine	Aspartic acid
Cysteine	Glutamic acid	Glycine
Histidine	Hydroxyproline	Isoleucine
Leucine	Lysine	Methionine
Phenylalanine	Proline	Serine
Taurine	Threonine	Tryptophan
Tyrosine	Valine	
Pathogenic Microbes (3)		
Escherichia coli	*Listeria spp.*	*Salmonella* spp.
Mycotoxins (12)		
Aflatoxin B1	Aflatoxin B2	Aflatoxin G1
Aflatoxin G2	Deoxynivalenol	Fumonisin-B1
Fumonisin-B2	HT2 toxin	Ochratoxin A
T2 toxin	Zearalenone	Nivalenol

A.5.1 Proximate composition and sugars

The proximate composition of BCPP1 was consistent across four independent production batches (Table 3). Protein content averaged 22.45 g/100 g (± 0.3), confirming BCPP1 as a high-protein product. Carbohydrates were the dominant macronutrient, averaging 68.3 g/100 g (± 0.48), although individual sugars were uniformly <0.2 g/100 g. Fat content was low (1.65 g/100 g ± 0.2), with saturated fat at 0.28 g/100 g (± 0.05). Moisture and ash levels were also stable, averaging 5.4 g/100 g (± 0.2) and 2.1 g/100 g (± 0.2), respectively.

Table 3. Proximate and sugar analysis of BCPP1 from EB-ST-537

Parameter*	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Mean ± SE
Protein	≥20.0	22.7	21.6	22.5	23.0	22.45 ± 0.3
Ash	≤3.0	2.1	2.5	2.2	1.5	2.1 ± 0.2
Moisture	≤6.0	5.6	5.2	4.8	5.8	5.4 ± 0.2
Carbohydrates	≤70.0	67	69	69	68	68.3 ± 0.48
Fat	≤3.0	2.2	1.4	1.2	1.8	1.65 ± 0.2
Saturated Fat	≤0.5	0.4	0.2	0.2	0.3	0.28 ± 0.05
Energy (KJ/100g)	≥1500	1610	1590	1600	1610	1602 ± 4.7
Total Sugars	<1.0	<1	<1	<1	<1	<1
Fructose	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Glucose	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Sucrose	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Maltose	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Lactose	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Mono trans fats	–	<0.1	<0.1	<0.1	<0.1	<0.1
Mono-unsaturated fat	≤1.0	0.8	0.5	0.4	0.6	0.56 ± 0.09
Omega-3 fats	–	<0.1	<0.1	<0.1	<0.1	<0.1
Omega-6 fats	–	0.8	0.6	0.5	0.7	0.65 ± 0.07
Poly trans fat	–	<0.1	<0.1	<0.1	0.1	<0.1
Poly-unsaturated fat	≤1.0	0.9	0.6	0.6	0.8	0.73 ± 0.08
Trans fat	≤0.1	<0.1	<0.1	<0.1	0.1	<0.1

*Unless otherwise stated, units are in g/100g

Energy content averaged 1602 kJ/100 g (± 4.7), reflecting the product's suitability as a concentrated nutritional ingredient. The absence of detectable mono- and disaccharides suggests the carbohydrate content is likely composed of residual, non-digestible polysaccharides. These are presumed to originate from yeast cell walls and post translational modifications to the secreted recombinant protein and are not classified as dietary fibre in the absence of validated analysis.

Based on the production method, the sugars are presumed to be residual yeast-derived structural carbohydrates, such as mannans and Beta-glucans, typical of *Komagataella phaffii*, whose cell wall is well documented to contain these polysaccharide components (Cheng et al., 2024). These compounds are generally resistant to digestion by human enzymes and may behave physiologically like dietary fibre. However, they are not classified as fibre in this context, as no validated dietary fibre analysis has been conducted.

A formal fibre analysis was not undertaken, as BCPP1 undergoes extensive downstream purification, comprising cell separation, protein extraction, ultrafiltration, and polishing, designed to remove host cell biomass and associated polysaccharide residues. These processes significantly reduce the likelihood of functionally relevant fibre-like material being present in the final product.

The remaining carbohydrate content is therefore assumed to consist of trace fermentation-derived polysaccharides or oligosaccharides, possibly including residual medium constituents. These components are considered inert and not intended to contribute physiological fibre effects. Given the origin of the product, its use as a purified protein ingredient, and the absence of supporting functional or compositional data, a fibre claim is not warranted. Should future use cases or labelling considerations require, dietary fibre levels could be quantified using FSANZ-recognised methods such as AOAC 2009.01 or 985.29.

Across all tested batches, BCPP1 demonstrated a consistent nutritional profile: high in protein, low in fat, negligible in sugars, and low in trans-fat. Although total carbohydrates are present in significant amounts, they are neither sugars nor dietary fibre and are likely composed of inert, non-digestible fermentation by-products. This composition supports the suitability of BCPP1 for inclusion in a wide range of high-protein,

low-sugar, and health-focused food formulations, including those aimed at consumers seeking low-glycaemic or animal-free options. In comparison, many dairy analogues exhibit carbohydrate levels ranging from 0.1 to over 10 g/100 mL, often with minimal protein content (up to 3.8 g/100 mL), highlighting BCPP1's nutritional value in protein-forward formulations (Moshtaghian et al., 2024).

In conclusion, the analysis of proximate and sugar profiles demonstrated a consistent composition across the evaluated batches.

A.5.2 Macro and micro-nutrients

The mineral content of BCPP1 was evaluated across four production batches, with results reported in mg/kg unless otherwise specified (Table 4). The data demonstrate a consistent and reproducible mineral profile across batches, with low variability and no anomalous values.

Table 4. Mineral analysis of BCPP1 from EB-ST-537

Parameter*	Batch 1	Batch 2	Batch 3	Batch 4	Mean ± SE
Mineral					
Potassium	2390	4870	3220	3210	3422.5 ± 520.21
Phosphorus	5110	6150	5450	4830	5385.0 ± 284.77
Sulphur	1070	1280	990	1070	1102.5 ± 62.1
Sodium (mg/100g)	463	574	430	435	475.5 ± 33.63
Magnesium	32	45	45	37	39.75 ± 3.2
Calcium	39	41	36	30	36.5 ± 2.4
Zinc	3.2	3	3.4	3.0	3.15 ± 0.1
Iron	23	11	14	13	15.25 ±2.66
Manganese	0.28	0.21	0.24	0.15	0.22 ± 0.03
Molybdenum	2	1.9	1.5	1.9	1.83 ±0.11
Copper	0.29	0.45	0.27	0.47	0.37 ± 0.05

*Unless otherwise stated, units are in mg/Kg

Potassium, sodium and phosphorus were the predominant minerals in BCPP1. Sulphur was also present in notable amounts, with a mean of 1,102.5 mg/kg (± 62.1). Potassium averaged 3,422.5 mg/kg (± 520.2), while phosphorus was present at 5,385.0 mg/kg (± 284.8). Sodium was measured separately in mg/100 g, with values averaging 475.5 mg/100 g (± 33.6), indicating a moderate sodium presence in line with expectations for protein-based preparations. These levels reflect the primary contribution to the product's mineral load and may support nutritional functions such as fluid balance and cellular energy metabolism.

The concentrations of other minerals were comparatively lower but remained consistent across all batches. Magnesium was present at 39.75 mg/kg (± 3.2), and calcium averaged 36.5 mg/kg (± 2.4), both reflecting minor contributions to overall mineral intake. Trace minerals were detected in reliably measurable amounts. Zinc was observed at 3.15 mg/kg (± 0.1), and iron at 15.25 mg/kg (± 2.66), with iron levels particularly noteworthy for their potential nutritional relevance. Manganese was present at 0.22 mg/kg (± 0.03), molybdenum at 1.83 mg/kg (± 0.11), and copper at 0.37 mg/kg (± 0.05).

Among these, iron stands out for its potential contribution to dietary intake, especially in populations where iron status is a concern. Zinc and copper, while present in smaller amounts, contribute to the overall trace mineral profile and are well below any levels of concern in terms of safety or regulatory limits.

In summary, BCPP1 demonstrates a stable and predictable mineral composition across production batches. While not a major source of minerals such as calcium or magnesium, the product does contain nutritionally

relevant levels of potassium, phosphorus, and iron. These results support the safe and consistent use of BCPP1 in food applications where traceability and nutritional profile are key considerations.

A.5.3 Vitamins

The vitamin panel (Table 5) shows that the BCPP1 is essentially free of fat-soluble vitamins and contains only trace quantities of water-soluble B-vitamins. All four batches had α-carotene, β-carotene and retinol below 5 µg/100 g, and ascorbic acid below 1 mg/100 g. Thiamin was <0.02 mg/100 g in every batch, while riboflavin, niacin and pantothenic acid appeared at low, batch-specific levels (riboflavin 0.08–0.14 mg/100 g; niacin <1–3.6 mg/100 g; pantothenic acid 0.05–0.40 mg/100 g). Pyridoxine remained ≤0.04 mg/100 g. These concentrations are two to three orders of magnitude below the adult Recommended Dietary Intakes set by the NHMRC and New Zealand Ministry of Health (e.g., NHMRC 2006: riboflavin 1.3 mg/day; niacin 16 mg/day), confirming that the product is not a significant dietary source of vitamins, and that the genetic modification has not introduced vitamin over-production or imbalance.

Table 5. Vitamin analysis of BCPP1 from EB-ST-537

Parameter*	Batch 1	Batch 2	Batch 3	Batch 4
Alpha-carotene (µg/100g)	<5	<5	<5	<5
Ascorbic acid (Vitamin C)	<1	<1	<1	<1
Beta-carotene (µg /100g)	<5	<5	<5	<5
Thiamin	<0.02	<0.02	<0.02	<0.02
Riboflavin (Vitamin B2)	<0.02	0.14	0.08	0.10
Niacin (Vitamin B3)	<1	3.6	<1	1.5
Retinol (Vitamin A) (µg /100g)	<5	<5	<5	<5
Pyridoxine (Vitamin B6)	0.03	<0.02	0.04	0.05
Pantothenic acid (Vitamin B5)	0.05	0.4	0.07	0.13

*Unless otherwise stated, units are in mg/100g

A.5.4 Amino acids

The amino acid composition was determined for multiple batches to assess consistency. Amino-acid profiling (Table 6) aligns closely with published bovine Beta-casein signatures. Across the three batches, glutamic acid (36–38 g/kg), proline (16–18 g/kg) and aspartic acid (22–25 g/kg) predominate, reflecting the intrinsically flexible, proline-rich casein structure. Essential amino acids represent approximately 44% of total residues; leucine (18–20 g/kg), lysine (11–12 g/kg) and threonine (14–16 g/kg) are the principal contributors, while methionine and tryptophan remain in the expected low range for caseins (approx. 3 g/kg and 1–1.6 g/kg, respectively). Batch-to-batch coefficients of variation are ≤ 16% for every amino acid, demonstrating manufacturing consistency. Hydroxyproline and taurine were below the 50 mg/kg reporting limit, indicating negligible collagen- or bile-derived contamination. Overall, the amino-acid pattern confirms the product's identity as Beta-casein and provides a balanced profile of indispensable amino acids comparable to dairy milk proteins.

For most amino acids analysed, including Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Lysine, Isoleucine, Leucine, Phenylalanine, Methionine, Cysteine, and Tryptophan, analysis indicated no statistically significant differences between the batches (all $P>0.05$). The p-values for these parameters were consistently 0.39.

Overall, the statistical analysis of the amino acid profiles did not reveal any significant differences in the concentrations of the measured amino acids among the evaluated batches. This suggests a consistent amino acid profile across the batches under the conditions tested.

In summary, the amino-acid composition is characteristic of bovine Beta-casein and highly consistent across production batches, supporting the conclusion that the fermentation-derived protein preparation is compositionally concordant to a conventional counterpart.

Table 6. Amino acid analysis of BCPP1 from EB-ST-537

Parameter[1]	Batch 1	Batch 2	Batch 3	Batch 4	Mean ± SE	% of Amino Acid Total
Aspartic acid	2300	2200	2500	2900	2475± 154.8	11.47
Serine	1600	1400	1600	1800	1600 ± 81.6	7.42
Glutamic acid	3700	3600	3800	4400	3875 ± 179.7	17.96
Glycine	660	590	680	730	665 ± 29.0	3.08
Histidine*	240	200	260	280	245 ± 17.1	1.14
Arginine	480	370	450	520	455 ± 31.8	2.11
Threonine*	1500	1400	1600	1700	1550 ± 64.5	7.18
Alanine	1300	1300	1400	1600	1400 ± 70.7	6.49
Proline	1700	1600	1800	1900	1750 ± 64.5	8.11
Tyrosine	510	450	520	550	507.5 ± 21.0	2.35
Valine*	1100	1000	1200	1300	1150 ± 64.5	5.33
Lysine*	1200	1100	1200	1500	1250 ± 86.6	5.79
Isoleucine*	950	910	1100	1100	1015 ± 49.7	4.70
Leucine*	1900	1800	2000	2100	1950 ± 64.5	9.04
Phenylalanine*	1100	1100	1200	1200	1150 ± 28.9	5.33
Methionine*	290	280	300	320	297.5 ± 8.5	1.38
Hydroxyproline	<5	<5	<5	<5	<5	n/a
Taurine	<5	<5	<5	<5	<5	n/a
Cysteine	100	83	62	110	88.8 ± 10.5	0.41
Tryptophan*	140	110	160	150	140 ± 10.8	0.65

1: Unless otherwise stated, units are in mg/100g; *: Essential amino acids

A.5.5 Comparative Nutrient Profile: BCPP1 vs. Conventional Counterpart

An appropriate comparator for BCPP1 is dairy-derived milk protein concentrate (MPC), which maintains the native Beta-casein to whey protein ratio of approximately 80:20. Commercial specifications for MPCs typically include protein levels ranging from 36 % to 90 %, fat content between 0.8 % and 2.5 %, moisture around 4%, ash content between 7.1 % and 8.0 %, and lactose concentrations from 0. 9% up to 52 %, as outlined in the *Australian Dairy Ingredient Reference Manual* (Dairy Australia, 2023).

All macronutrient levels in BCPP1 fall below these typical ranges (Table 7). This is due to a deliberately lower degree of protein enrichment during processing rather than any consequence of the genetic modification. These differences are not considered nutritionally disadvantageous and reflect the intended formulation profile of the product.

Across all measured minerals, BCPP1 consistently exhibits lower concentrations than those typically found in MPC 80. Potassium and phosphorus are the most abundant minerals in BCPP1; however, both fall below the expected levels for MPC 80. Similarly, sulphur is present at reduced levels, aligning with the overall lower mineral density of the product. Sodium was relatively higher than other minerals but falls within the typical range of MCPs.

Of note are the levels of calcium and magnesium. At just 3.65 mg/100 g and 3.98 mg/100 g, respectively, they are well below the conventional ranges observed in dairy-based MPCs. These low concentrations mean that BCPP1 does not function as a meaningful dietary source of these essential minerals. Zinc and copper levels are also reduced and make only minimal contributions toward meeting daily nutritional requirements.

In contrast, iron is present at 1.53 mg/100 g, above the typical concentration reported for MPC 80. Based on the NHMRC Recommended Dietary Intake (RDI), a 100 g serving of BCPP1 would provide approximately 13% of the RDI for adult males (8 mg/day) and around 8.5% for adult females (18 mg/day). Although modest, this contribution may be beneficial in formulations developed to support iron intake, particularly for populations at risk of deficiency.

Manganese is detectable at 0.022 mg/100g, low in absolute terms. Molybdenum and copper are consistently present across batches, though their contributions to dietary intake remain minor. For instance, the mean copper concentration corresponds to about 4% of the adult RDI (1 mg/day).

In summary, the mineral composition of BCPP1 reflects its distinct formulation and processing approach. While it is not a significant source of most minerals, particularly calcium, magnesium, and zinc, it does provide a modest but consistent contribution to iron intake. This may be of nutritional relevance in specific product contexts, such as plant-based applications or dietary supplements designed to address iron insufficiency.

Table 7. Comparative nutrient profile

Mineral	BCPP1*	Typical MPC 80**
Potassium	342.25 ± 52.02	1000–1600
Phosphorus	538.50 ± 28.48	600–900
Sulphur	110.25 ± 6.21	800–1000
Sodium	475.5 ± 33.6	200–500
Magnesium	3.98 ± 0.32	80–120
Calcium	3.65 ± 0.24	500–800
Zinc	0.315 ± 0.01	1–2
Iron	1.53 ± 0.27	<1
Manganese	0.022 ± 0.003	<0.05
Molybdenum	0.183 ± 0.01	not routinely reported
Copper	0.037 ± 0.005	0.05–0.1

*Mean ± SE, mg/100 g; **(mg/100 g)

A.5.6 information on the range of natural variation for each constituent measured to allow for assessment of biological significance should any statistically significant differences be identified

FSANZ requires biological significance to be assessed against natural compositional variability (FSANZ 2024). Published datasets for MPC and whole-milk powders show: protein 36–90 g/100g, fat 0.8–2.5 g/100g, ash 7.1–8 g/100g, lactose 0.9–52 g/100g (Dairy Australia 2023; ADPI, 2023; Foroutan et al., 2019). Mineral variation spans, for example, potassium 300–2600 mg/100g, calcium 30–2000 mg/100g and sodium 40–600 mg/100g (USDA FoodData Central, 2024). The tested batches (potassium 239–487 mg/100g; calcium 3.6–4.1 mg/100 g; sodium 430–574 mg/100g; (Table 4) all lie well below or within these natural ranges. Vitamins were largely below limits of quantification (Table 5) in both the test material and dairy comparators, so differences lack nutritional relevance.

Using the Dairy Australia data set (n = 5 commercial MPCs), the following ranges apply: protein 56–90 g/100g, fat 0.8–2.5 g/100g, ash 7.1–8.0 g/100g, sodium 30–450 mg/100 g, potassium 200–1640 mg/100g and calcium 1250–2200 mg/100g (Dairy Australia 2023). Mineral values for the BCPP1 sit within, or below, these ranges; the relatively higher potassium is expected because casein micelles bind potassium during

pH-controlled recovery and remains well below the upper end of natural Australian MPC values (1640 mg/100g). Calcium levels are 10-fold less than commercial MCPs.

A.5.7 Levels of any other constituents that may potentially be influenced by the genetic modification, as a result, for example, of downstream metabolic effects, compared with the levels in an appropriate comparator as well as the range of natural variation

The strain EB-ST-537 expresses the bovine *CSN2* (Beta-casein) gene and 3 additional genes that support protein secretion; no enzymes that alter core metabolic pathways were introduced. Untargeted screening showed no unexpected secondary metabolites or mycotoxins, and amino-acid profiling was fully consistent with published Beta-casein signatures (e.g., high proline, glutamic acid; low cysteine) (Atamer, 2017). Vitamin and fatty-acid levels were comparable to, or lower than, those found in commercial MPC, indicating that fermentation did not elevate any nutrients of safety concern. Accordingly, no downstream metabolic effects attributable to the genetic modification were observed.

A.5.8 Protein Quality Analysis (PDCAAS)

A protein quality evaluation was conducted on the Beta-casein Protein Preparation to ensure its nutritional adequacy is comparable to its intended substitute, traditional bovine Beta-casein. The assessment used the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which is the internationally accepted method for regulatory protein quality evaluation (FAO/WHO Expert Consultation, 2013).

The analysis was performed on six different BCPP1 preparations using a validated *in vitro* enzymatic assay kit (Megazyme) that shows a high correlation with traditional animal-based digestibility tests. The assay was validated using several controls, including commercial soy protein isolate, a kit-supplied casein control, and purified bovine Beta-casein, which served as the primary reference comparator.

Across the six BCPP1 batches tested, PDCAAS values ranged from 39 % to 52 %, which corresponds to 85 % to 113 % of the score for purified bovine Beta-casein (Table 8). The average PDCAAS for BCPP1 (47.25±2.89) was not significantly different ($F_{[1,6]}$=0.19, p=0.68) to the bovine Beta-casein reference (46.0±0).

Table 8. Protein quality of BCPP1, Soy Protein Isolate and Bovine Beta Casein

Preparation	Amino Acid Score	Digestibility (%)	PDCAAS (%)	PDCAAS Relative to Bovine Beta Casein (%)
BCPP1 Batch B1	0.56	85	48	102.79
BCPP1 Batch B2	0.46	85	39	84.83
BCPP1 Batch B3	0.61	86	52	111.54
BCPP1 Batch B5	0.59	84	50	107.39
BCPP1 Blend B1/B2	0.51	85	43	93.81
BCPP1 Blend B3/B5	0.61	85	52	113.19
Bovine Beta-Casein (Reference)	0.46	100	46	100
Soy Protein Isolate (Control)	1.16	100	100*	-
Casein (Kit Control)	0.95	99	94	-

Note: The PDCAAS score for Soy Protein Isolate was truncated to 100 from a calculated value of 116, in accordance with international guidelines

The *in vitro* PDCAAS determination confirms that BCPP1 produced in *K. phaffii* meets the protein quality standards expected for bovine Beta-casein. This scientifically robust assessment, validated with appropriate controls, supports the conclusion that the product is a suitable and nutritionally equivalent alternative for use in food products.

The slightly lower *in vitro* digestibility value for BCPP1 (84–86 %) compared to the purified bovine Beta-casein control (100 %) in the PDCAAS assay warrants further comment, particularly considering the rapid and complete degradation observed in the pepsin-only model (Section C.1.3). This minor difference is likely attributable to two factors: the differing methodologies of the assays and the presence of post-translational modifications on BCPP1.

The pepsin assay simulates only the initial gastric phase and is primarily a measure of the rate of protein breakdown. In contrast, the PDCAAS assay is a multi-enzyme system designed to measure the final extent of digestion throughout the entire gastrointestinal tract. While experiments showed that the high-mannose *O*-linked glycosylation on BCPP1 did not protect it from rapid initial pepsin digestion, these modifications may subtly reduce the overall extent of hydrolysis in a more complex enzymatic environment, leading to the slightly lower final digestibility score. Nonetheless, this modest difference is not considered nutritionally significant, as the final PDCAAS score for BCPP1 remains equivalent to its bovine counterpart, confirming its suitability as a high-quality protein source.

A.6 Impurity Profile

BCPP1 is produced using a genetically modified strain of *K. phaffii* and undergoes a multi-stage purification process designed to remove host-derived materials and process-related impurities. Analytical testing was conducted across multiple production batches to confirm that residual levels of potential contaminants are either not detected or are present at concentrations well below internationally recognised safety thresholds.

A.6.1 Residual Host Cell Proteins and Relative Contribution

Quantitative assessment of residual host cell proteins (rHCPs) was performed using a *K. phaffii* LC-MS (see Section C.1

While the total protein content of BCPP1 is approximately 22 %, and Beta-casein accounts for roughly 25 % of that fraction, the remaining protein content consists of low-abundance, co-purified *K. phaffii* proteins. These have been characterised by LC-MS/MS (see Section C.1), and the 20 most abundant host-derived proteins identified are neither allergenic nor toxic (see Table 13). Their presence at low levels, combined with the absence of allergenicity and toxicity, supports the safety and compositional integrity of BCPP1.

Given the QPS status of *K. phaffii*, the absence of known allergens or toxins, and the low residual abundance of host proteins, the impurity profile is consistent with international safety expectations for food-grade recombinant protein preparations.

A.6.2 Residual Host Cell DNA

Quantitative PCR (qPCR) assays targeting *K. phaffii*-specific genomic sequences were used to evaluate residual DNA content in BCPP1. All tested batches contained less than 10 ng/g of host DNA, which is well below internationally recognised thresholds for residual host DNA in food ingredients produced using microbial fermentation (see **Supplement Report EB-25-PROT-01-02** [CONFIDENTIAL]). These results align with established methodologies and acceptance criteria used in safety assessments of enzyme and protein products derived from genetically modified microorganisms (Pariza et al., 2015; EFSA BIOHAZ Panel, 2024).

A.6.3 Process-Related Residues

All reagents and process aids used during fermentation and purification are either food-grade or have GRAS-equivalent status. The final product is free from antibiotics, selection markers, or metabolic inducers. Common purification agents, such as phosphate buffers and sodium chloride, are removed during diafiltration and polishing. Testing using LC-MS and ICP-MS methods confirmed that residual levels of these compounds were either not detectable or present at trace concentrations (<1 ppm), well below applicable food safety limits (e.g., ICH Q3D for elemental impurities).

A.6.4 By-Products and Degradation Products

Stability studies conducted under accelerated and real-time storage conditions have shown no evidence of protein hydrolysis, aggregation, or formation of truncated or oxidised forms of Beta-casein. Analytical methods (LC-MS/MS) confirmed that the protein retains its structural integrity under expected shelf-life conditions. No measurable degradation products were detected in the final product.

A.6.5 Microbiological, Mycotoxin and Heavy Metal Contaminants

Comprehensive safety testing of BCPP1 was conducted across four independent production batches to confirm compliance with relevant microbial, chemical, and toxin-related safety standards for food-grade protein ingredients.

Microbiological Contaminants

Microbial testing followed methods consistent with Schedule 27 of the Australia New Zealand Food Standards Code (Table 9). No pathogens were detected in any batch, including:

- *Salmonella spp.*: Not detected in 25 g
- *Listeria spp.*: Not detected in 25 g
- *Escherichia coli* (thermotolerant coliforms): Not detected in 1 g

Analytical testing confirmed the absence of Escherichia coli, Salmonella spp., Listeria spp., and mycotoxins, consistent with food-grade production standards. These results confirm that the fermentation and downstream processing steps, including pasteurisation-equivalent thermal treatment and sterile filtration, effectively control microbial risks.

Mycotoxins

Mycotoxin screening was performed using ELISA and/or HPLC-based assays with detection limits aligned to international guidance (e.g., <2 µg/kg for aflatoxins; Table 9). Target analytes included aflatoxin B1, ochratoxin A, fumonisins, and zearalenone. All batches tested below detection limits for these mycotoxins. As *K. phaffii* is a non-mycotoxin-producing yeast and fermentation is conducted under controlled, sterile conditions, the risk of mycotoxin presence is considered negligible.

Heavy Metals

Food safety testing for heavy metals and trace elements was performed (Table 10). Testing included a standard panel of heavy metals (i.e., arsenic, lead, cadmium and mercury) that may have been introduced during the fermentation runs and bioprocessing steps. The additional elements tested for were selected as they were added to the fermentation media as supplements to assist the fermentation of EB-ST-537. Their

levels in the resulting products were therefore monitored to detect if they accumulated during the production processes.

Heavy-metal residues were <0.01 mg/kg in all batches (Table 10), well below the Codex maximum levels for milk powders (e.g., lead 0.02 mg/kg; Codex 2023) and well below the limits in Schedule 19 of the Australia New Zealand Food Standards Code (e.g., lead in infant-formula products 0.01 mg/kg). No other toxicants of concern were identified.

Table 9. Microbiological and mycotoxin analysis of BCPP1 from EB-ST-537

Parameter*	Specification	Batch 1	Batch 2	Batch 3	Batch 4
Microbiology					
E. coli (MPN/g)	<1	<1	<1	<1	<1
Salmonella spp. / 25g	ND	ND	ND	ND	ND
Listeria spp. / 25g	ND	ND	ND	ND	ND
Mycotoxins					
Nivalenol		<100	<100	<100	<100
Ochratoxin A		<1	<1	<1	<1
T2		<25	<25	<25	<25
Zearalenone		<20	<20	<20	<20
Aflatoxin B1		<1	<1	<1	<1
Aflatoxin B2		<1	<1	<1	<1
Aflatoxin G1		<1	<1	<1	<1
Aflatoxin G2		<1	<1	<1	<1
Total Aflatoxin (B1, B2, G1, G2)		<4	<4	<4	<4
Deoxynivalenol		<100	<100	<100	<100
Fumonisin B1		<20	<20	<20	<20
Fumonisin B2		<20	<20	<20	<20
HT2		<20	<20	<20	<20

ND= not detected; *Unless otherwise stated, units are in µg/Kg

Table 10. Heavy metal impurities

Heavy Metals*	Specification	Batch 1	Batch 2	Batch 3	Batch 4
Lead	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	<0.05	<0.05	<0.05	<0.05	<0.05
Cadmium	<0.01	<0.01	<0.01	<0.01	<0.01
Cobalt	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury	<0.01	<0.01	<0.01	<0.01	<0.01

*Unless otherwise stated, units are in mg/Kg

Impurity Profile Summary

Together, these findings support the microbiological and chemical safety of BCPP1 for use in food, consistent with international best practices for recombinant protein ingredients produced via microbial fermentation.

A.6.6 Analysis of Antinutrients

Compositional and impurity analyses of BCPP1 confirm that the preparation does not contain any known anti-nutritional factors. Screening across multiple independent production batches demonstrated the absence of compounds typically associated with anti-nutrient activity, such as protease inhibitors, lectins,

phytates, or oxalates. The carbohydrate fraction was shown to consist primarily of inert, fermentation-derived polysaccharides, which are non-toxic and physiologically inert under FSANZ definitions. No unexpected secondary metabolites, mycotoxins, or other constituents of concern were detected, and amino-acid, vitamin, and mineral analyses did not reveal any anomalous patterns attributable to anti-nutritional substances. Collectively, these findings support the conclusion that BCPP1 is free from anti-nutrients and suitable for its intended role as a nutritive protein ingredient.

SECTION B: MANUFACTURING AND SPECIFICATIONS

This section describes the manufacturing process, provides a full molecular characterisation of the genetically modified production organism, and establishes product specifications and analytical methods for BCPP1, addressing the requirements of Guideline 3.5.1 (Sections A.2 and A.3) and Guideline 3.5.2 (Sections B.5, B.6, B.7, and C.6.1).

B.1 Manufacturing Process

The Beta-casein protein preparation is manufactured under a Hazard Analysis and Critical Control Points (HACCP)-based Food Safety Program, consistent with Standard 3.2.1 of the Australia New Zealand Food Standards Code. All production activities are performed in compliance with current Good Manufacturing Practice (GMP) and food-grade ingredient requirements. Detailed information on the manufacturing process, validation parameters, and quality control checkpoints is provided in the **Supplemental Report EB-25-MAN-01_01** [CONFIDENTIAL].

A schematic representation of the manufacturing process is outlined in Figure 1 and is summarised as follows:

1. A vial of genetically modified *Komagataella phaffii* from a qualified working cell bank is used to initiate the inoculation and seeding stages.
2. The seed culture 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 a soluble form.
3. The fermentation medium containing the Beta-casein protein is harvested and separated from the yeast biomass by centrifugation.
4. The liquid is subjected to micro-filtration the pH is adjusted and the solution heat treated ready for downstream processing.
5. The resulting filtrate is concentrated by ultrafiltration and diafiltration to exchange the fermentation medium for water.
6. The pH is adjusted, and the final solution is dried to produce a white to off-white powder containing Beta-casein protein.

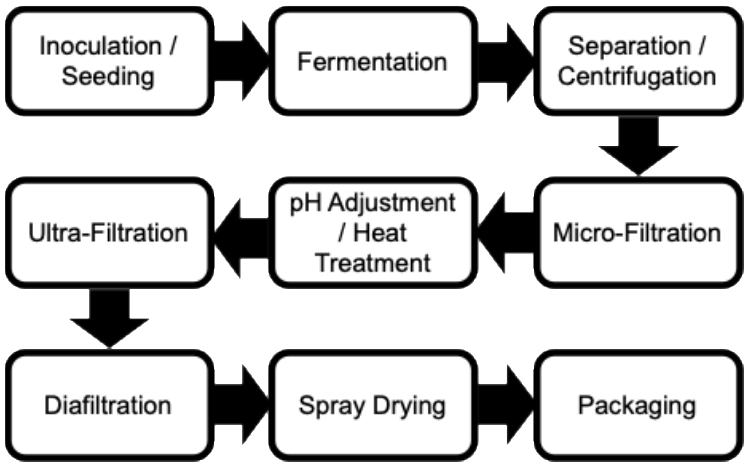


Figure 1. Schematic of the manufacturing process for the Eden Brew BCPP1

B.2 Characterisation of the Genetically Modified Production Organism

B.2.1 History of use of the host and donor organisms

All donor organisms from which genetic elements were sourced in the construction of strain EB-ST-537 have a documented history of safe use or environmental exposure, as detailed in this section. None of the inserted genetic elements are associated with known pathogenic or toxigenic properties. There are no reports of pathogenicity or toxicity linked to the donor organisms, or the specific sequences introduced into the production strain.

This section provides information on the donor organisms from which the introduced genetic elements are derived. It addresses any known concerns related to pathogenicity, toxicity, or allergenicity that may be relevant to food safety. Additionally, it outlines the history of use of these organisms in the food supply or other known routes of human exposure, including incidental or environmental contact, to support an assessment of potential risks associated with their genetic material. Full details on the donor organisms is provided in **Supplemental Report EB-25-MOL-01-02** [CONFIDENTIAL].

EB-ST-537 Host History of safe use in food

The host organism is *Komagataella phaffii*:

KINGDOM	Fungi
PHYLUM	Ascoymycota
CLASS	Hemiascomycetes
ORDER:	Saccharomycetales
FAMILY:	Endomycetaceae
GENUS:	*Komagatella* (formerly *Pichia*)
SPECIES:	*phaffii*
COMMON NAME:	Pichia

The methylotrophic yeast *Pichia pastoris*, reclassified as *Komagataella phaffii* (Kurtzman 2005), is widely used for heterologous protein production. Despite the taxonomic change, the organism continues to be commonly referred to as *P. pastoris* in scientific literature and regulatory submissions. It was first introduced in the 1970s as a livestock feed additive due to its ability to grow to high cell densities on methanol as its sole carbon and energy source (Ahmad et al., 2014). *K. phaffii* has been extensively used in the production of recombinant proteins because it provides the advantages of post-translational modification in a eukaryotic single-cell system. Protein production in *K. phaffii* is typically driven by the AOX1 promoter, which occurs in response to methanol induction due to its strong and regulatable characteristics (Cregg et al., 2000).

While there is limited evidence that *K. phaffii* has been consumed by humans, this organism does have a long history of safe use to produce pharmaceuticals and industrial chemicals, including several enzyme processing aids approved by European Food Safety Authority (EFSA), U.S Food and Drug Administration (FDA) and Food Standards Australia New Zealand (FSANZ) (Spohner et al., 2015). *K. phaffii* has been designated as GRAS (Generally Recognized As Safe) by the FDA and has been approved for the production of a recombinant proteins such as phospholipase C enzyme (GRN No 204), soy leghaemoglobin as used by Impossible Foods Inc. (GRN No. 737), egg-white protein produced by *K. phaffii* ATCC GSD-1235 (GRN No. 1104), and β-lactoglobulin produced by *K. phaffii* strain yRMK-66 (GRN No. 1056). FSANZ recently approved the use of *P. pastoris* (*K. phaffii*) to produce soy leghaemoglobin (branded LegH Prep) to be used as a meat analogue additive (FSANZ 2020).

The safety of live *K. phaffii* has been evaluated in several animal models that support that this strain is not toxic or pathogenic. Studies conducted in chicks support that *K. phaffii* is safe for use in feed as a probiotic and can increase feed efficiency compared to untreated control feeds (Gil de los Santos et al., 2012; Gil de los Santos et al., 2018).

Strain EB-ST-537 is derived from a species that has a Qualified Presumption of Safety (QPS) status. The QPS list is a tool used by the European Food Safety Authority (EFSA). It provides a list of biological agents, like bacteria, fungi, and viruses, that are presumed to be safe for human, animal, or plant health when used in food or feed (EFSA BIOHAZ Panel, 2024). This presumption is based on scientific evidence and is only given to those agents with a clear and established history of safe use.

The main purpose of the QPS list is to streamline the safety assessment process for microorganisms used in the food and feed industry. When a microorganism is on the QPS list, applicants looking to use it in human food or animal feed do not need to provide detailed safety information in their authorisation applications to EFSA. Instead, they can refer to its QPS status, significantly reducing the complexity and cost of applications.

To be included on the QPS list, a microorganism must meet specific criteria related to its identification, safety history, and absence of pathogenic properties. The list is regularly updated to include new organisms or to remove those that no longer meet the safety criteria. This ensures that the list remains current with the latest scientific knowledge and safety standards.

The host strain used for strain EB-ST-537 is *Komagataella phaffii* (*Pichia pastoris*) that is included in the QPS list with the following qualification (EFSA BIOHAZ Panel, 2024)

> "QPS applies for 'production purposes only' (the qualification 'for production purpose only' implies the absence of viable cells of the production organism in the final product and can also be applied for food and feed products based on microbial biomass)."

Strain EB-ST-537 can be traced back to the progenitor *Pichia* strain NRRL Y-7556, also known as NCYC 2543 and CBS 2612, a wild-type strain (Phaff and Knapp, 1956; Figure 2). NRRL Y-7556 is the parental source for several commonly used recombinant protein production strains, including YB-378, YB-4289, and Y-17741. The genetics of these strains is discussed in Kurtzman (2005).

The host strain is a protease deletion strain, derived from NRRL Y-7556. Protease deficient strains have been shown to be effective in reducing the degradation of some heterologous proteins. This is especially noticeable in fermenter cultures of secreted recombinant proteins, because the combination of high cell density and lysis of a small percentage of cells results in a relatively high concentration of these vacuolar proteases.

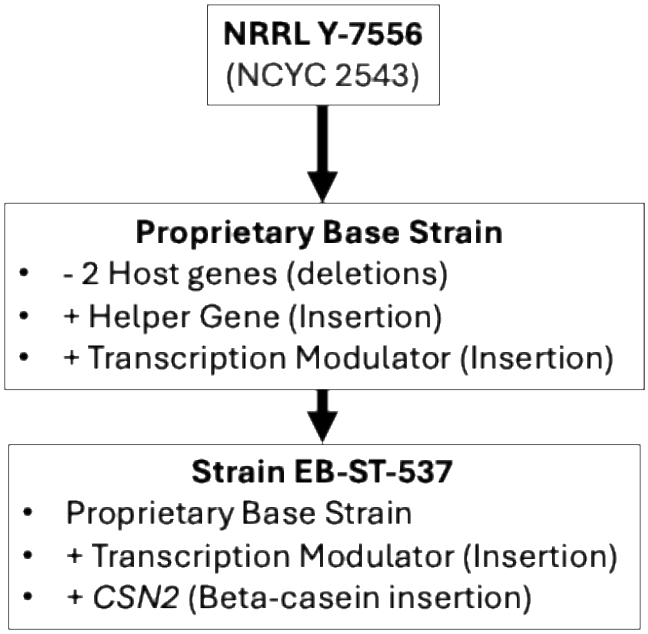


Figure 2. Strain lineage and genomic modification overview for EB-ST-537
Diagrammatic representation of the engineering pathway used to construct EB-ST-537 from the parental *Komagataella phaffii* Y-7556 strain. Four genomic loci were targeted: Site 1 and Site 2 involved precise deletions of host proteolytic genes; Site 3 included insertion of a helper gene and synthetic transcription modulator; and Site 4 included insertion of the bovine *CSN2* gene and synthetic transcription modulator. Temporary antibiotic selection markers were excised via Cre-lox recombination. All modifications were confirmed through next-generation sequencing.

Safe Strain Lineage of *Komagataella phaffii* EB-ST-537

The safety of the production strain, EB-ST-537, is firmly established through a weight-of-evidence assessment of its safe lineage. This assessment is founded on the established safety of the host species, the non-pathogenic nature of the progenitor strain, and the well-characterised, targeted genetic modifications that do not introduce any safety concerns.

The host organism, *Komagataella phaffii,* has an extensive history of safe use in the production of food ingredients and has been granted Qualified Presumption of Safety (QPS) status by EFSA. FSANZ has also previously approved ingredients from *K. phaffii*. The specific lineage of EB-ST-537 traces back to the wild-type progenitor strain NRRL Y-7556, a well-characterised, non-pathogenic, and non-toxigenic organism that has served as the parental source for numerous industrial strains for decades.

The genetic modifications made to create EB-ST-537 were precise and targeted. They involved the deletion of two endogenous protease genes and the insertion of genetic material sourced exclusively from organisms with a long history of safe use in food (*Bos taurus* and *Saccharomyces cerevisiae*) or synthetic sequences confirmed to have no homology to known toxins or allergens. Comprehensive molecular characterisation has confirmed these modifications are stable, integrated only at the intended sites, and that no open reading frames with similarity to known hazards were created.

This lineage from a safe, non-pathogenic progenitor, combined with well-characterised and targeted genetic modifications, provides strong evidence for the safety of the production strain. A comprehensive analysis and the primary data supporting this safe lineage assessment are provided in **Supplemental Report EB-25-MOL-01-02** [CONFIDENTIAL]**.**

Helper gene and transcription modulators (Enhanced secretion)

A helper gene from *Saccharomyces cerevisiae*, along with two synthetic transcription modulator genes, were inserted into the host to enhance Beta-casein protein secretion. *S. cerevisiae* is widely recognised as safe for the general population, supported by its millennia-long use in food and beverages and its designation as GRAS by the U.S. FDA (Cosmetic Ingredient Review, 2021). However, it is also an established opportunistic pathogen (Raghaven et al., 2019). Infections caused by *S. cerevisiae*, ranging from superficial to systemic (e.g., fungemia), primarily affect individuals with severe immunocompromise, critical illness, or indwelling medical devices (Yan et al., 2024). Additional risk factors include the use of broad-spectrum antibiotics and administration of *S. cerevisiae*-based probiotics, particularly in hospital settings (Raghaven et al., 2019). Genomic analyses have linked pathogenic strains to those originating from food sources and probiotic supplements (Morard et al., 2023).

S. cerevisiae is not considered intrinsically toxic under typical exposure conditions and is widely used as a model organism for evaluating chemical toxicity (Välimaa et al., 2008). However, allergenicity is a recognised concern for sensitised individuals. Exposure, primarily via inhalation or ingestion, can cause respiratory symptoms (e.g., baker's asthma), skin reactions, and, in rare cases, systemic anaphylaxis. Specific proteins, including enolase (Sac c enolase), have been identified as major allergens (Belchi-Hernandez et al., 1996).

The helper protein is confined to intracellular functions and does not contribute directly to pathogenicity, toxicity, or allergenicity. It is not secreted and is therefore absent from the final BCPP1 product. It is not a known virulence factor and is not recognised as an allergen.

Human exposure to *S. cerevisiae* has occurred for thousands of years through its use in bread making, brewing, and winemaking (Sicard and Legras, 2011). Over time, strains have been domesticated and optimised for these specific applications. In addition to traditional uses, *S. cerevisiae* is consumed today in the form of nutritional yeast, probiotic supplements, and as a production platform in various biotechnological applications (Czerucka et al., 2007; Nevoigt, 2008).

Incidental exposure is also widespread due to its natural presence in the environment (e.g., on fruits, trees, soil) and in human-associated habitats such as vineyards and wineries (Stefanini et al., 2012; Morard et al., 2023). Recent research has identified *S. cerevisiae* as a component of the human gut mycobiome, where it may persist and interact metabolically with the host via mucin utilisation (Caetano et al., 2023). Although generally considered a dietary transient, emerging evidence suggests strain-specific capacities for gut colonisation. These may contribute to beneficial effects (e.g., probiotic activity) or adverse outcomes in susceptible individuals (e.g., exacerbation of inflammatory bowel conditions; Mercurio et al., 2021).

The transcription modulators are required to activate the promoters, driving expression of the *helper* and *CSN2* (Beta-casein) genes. Neither is recognised as an allergen or toxin.

Beta-casein from *Bos taurus*

Beta-casein from *Bos taurus* is not known to possess inherent pathogenicity or toxicity (El-Agamy, 2007). However, it is a well-recognised allergen and a major contributor to cow's milk allergy (Restani et al., 2002). Cow's milk allergy is an immune-mediated adverse reaction to several milk proteins, with Beta-casein being one of the primary allergens, alongside Alpha-S1 casein, Alpha-S2 casein, and Kappa-casein, as well as whey proteins like Beta-lactoglobulin and Alpha-lactalbumin (Restani et al., 2002; Host, 2002). Clinical manifestations can range from mild symptoms (e.g., hives, digestive issues) to severe reactions such as anaphylaxis (Host, 2002). The allergenicity is related to the specific protein structure and the individual's immune system response (Wal, 2004; Jensen et al 2022).

As a source of Beta-casein, *Bos taurus* has a long and well-documented history of use in the human food supply. Cattle have been domesticated for millennia, and milk and milk-derived products have been staple components of the human diet across many cultures.

It is important to clarify that human exposure is to the protein product (Beta-casein), not to the organism *Bos taurus* itself. The organism is not a "normal contaminant" in the context of food; rather, it is the source animal from which the milk, and subsequently Beta-casein, is obtained. Therefore, regulatory considerations should focus on the safety and history of consumption of the purified protein rather than the host organism.

Description of non-coding sequences

A range of non-coding genetic elements were used in the development of strain EB-ST-537 to enable regulated and efficient expression of recombinant genes. These include promoters, regulatory elements, and polyadenylation signals. None of the sequences are derived from organisms known to be toxic or allergenic to humans. Full details of each element are provided in the **Supplemental Report EB-25-MOL-01-02** [CONFIDENTIAL].

The non-coding sequences include regulatory promoters responsive to external signals, synthetic promoter elements built from non-host DNA, and transcriptional control sequences sourced from non-toxic microbial strains. Transcription termination elements are also included to support transcript stability and expression efficiency.

These components work together to create a controlled and functional genetic system, supporting precise gene expression in EB-ST-537 without involving host organisms of safety concern.

B.2.2 Nature of the Genetic Modification

The production strain is a proprietary strain developed by Ginkgo Bioworks and provided to Eden Brew under the designation EB-ST-537. It was derived from the parental *Komagataella phaffii* strain Y-7556 (also known as YB-4920 or CBS 2612), which was originally isolated from a Black Oak tree in California. This wild-type strain has a well-established history of use in the biotechnology industry to produce food-grade proteins.

Strain EB-ST-537 (original Ginkgo Bioworks ID t2329144) was constructed through a series of targeted genetic modifications of the Y-7556 parental line. The modifications were achieved using standard molecular biology techniques. Four distinct genomic loci were engineered, each validated by whole genome sequencing (WGS) using both long-read (Oxford Nanopore) and short-read (Illumina) technologies.

The genetic modifications introduced into the strain include:

- Deletion of a host protease gene (Site 1)
- Deletion of a host protease gene (Site 2)
- Insertion of a helper gene from *S. cerevisiae* along with a synthetic transcription modulator (Site 3)
- Insertion of the *CSN2* bovine Beta-casein A2 variant, under the control of a synthetic promoter along with a synthetic transcription modulator (Site 4).

During strain construction, antibiotic resistance markers were temporarily used for selection purposes and subsequently removed via Cre-lox recombination. The excision of these markers was confirmed through phenotypic analysis, PCR, and WGS.

Strain EB-ST-537 is a clonal, genetically stable production strain. The version submitted for regulatory evaluation is the final engineered form of the strain, with no further breeding, recombination, or selective propagation beyond standard clonal expansion. All production and validation data presented in this dossier are derived directly from this confirmed and characterised lineage.

A description of the method used to transform the host organism

The proprietary strain EB-ST-537 was developed from *Komagataella phaffii* Y-7556 using multiple genome engineering strategies. Antibiotic selection markers were employed during transformation and subsequently excised using *Cre*-mediated recombination, leaving behind lox72 sequences. Specific details are provided in **Supplemental Report EB-25-MOL-01-02** [CONFIDENTIAL].

A description of the construct and the transformation vectors used

Synthetic DNA constructs were designed for each genomic modification. They included:

- A cassette containing the Helper gene from *Saccharomyces cerevisiae*, driven by a synthetic promoter, alongside a synthetic transcription modulator to enhance expression
- A cassette containing the bovine *CSN2* (Beta-casein) gene driven by a synthetic promoter, alongside a synthetic transcription modulator to enhance secretion of Beta-casein.

The *CSN2* gene expressed by EB-ST-537 is identical in amino acid sequence to the natural Beta-casein protein produced by *Bos taurus* (Figure 3). Specifically, it corresponds to the A2 variant of Beta-casein (referred to as Beta-Casein A2-5P), which consists of 209 amino acid residues. The reference sequence is available in ExPASy under the entry name CASB_BOVIN and accession number P02666. A2 Beta-casein is one of two naturally occurring genetic variants, the other being A1 that is the predominant form found in milk produced in Australia. Due to its prevalence and consumer preference, the A2 variant was selected for expression in this production strain. Beta-casein, as a family, makes up approximately 35 % of the total casein content in bovine milk.

This 209-amino acid protein has a molecular mass of 23.6 kDa for the primary structure prior to post-translational phosphorylation, and 24.0 kDa following phosphorylation of 5 Ser residues, that is, Ser15, Ser17, Ser18, Ser19, and Ser35; of these, the first four form a centre of phosphorylation (De Kruif and Holt 2003).

In bovine species, Beta-casein is characterised by genetic polymorphism. The two most common protein variants are beta-casein A2 (i.e., P02666) and A1, differing from A2 for one amino acid substitution (Pro67 to His67; Figure 4). There are also several other genetic variants of bovine Beta-casein (Han et al., 2000; Caroli et al., 2016).

The protein expression cassette utilised by Eden Brew consists of the 209 amino acid bovine Beta-casein protein A2 variant (Figure 4).

MKVLILACLVALALARELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPI**H**NSL
PQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQ
PLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV*

Figure 3. A1 Beta-casein (*Bos taurus*), NCBI Accession AAA30431.
Amino acids 1-15 (highlighted in red) represent a signal peptide; the natural variant amino acid 67 (highlighted in bold blue)

```
AAA30431   13  ALARELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPL
               |*|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||*||||||||||
Eden Brew 402  AEARELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPL

AAA30431   93  TQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMF
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Eden Brew 482  TQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMF

AAA30431   173 PPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV*
               ||||||||||||||||||||||||||||||||||||||||||||||||||||
Eden Brew 562  PPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV*
```

Figure 4. Beta-casein (*Bos taurus*), NCBI Accession AAA30431 aligned with the Eden Brew Beta-casein sequence.
Amino acids highlighted in red represent part of the Beta-casein signal peptide; the A1/A2 variant amino acid is highlighted in bold blue.

B.2.3 A full molecular characterisation of the genetic modification in the new organism

Full molecular characterisation was performed using next-generation sequencing (Oxford Nanopore for long reads and Illumina for high-accuracy short reads). Results confirmed:

- Precise, targeted integration of all synthetic elements at their intended loci
- Deletion of host genes without unintended alterations
- Absence of off-target integrations, chromosomal rearrangements, or unexpected mutations
- Complete removal of all antibiotic resistance markers, leaving only the lox72 sequence at deletion sites
- Genetic stability of engineered regions was confirmed under standard culture conditions using DNA sequencing.

Further information is provided in the **Supplement Report EB-24-MOL-01_01** [CONFIDENTIAL]**.**

Structure and organisation of the Insertion in *K. phaffii*

The structure and organisation of the insertions in *K. phaffii* strain EB-ST-537 are summarised in Table 1, which details the modifications, and inserted elements. Each genetic modification involved either the insertion of synthetic cassettes (including bovine *CSN2*, the Helper gene, and two synthetic transcription modulators) or precise deletions of endogenous proteolytic genes. Sequencing analysis confirmed that each engineered element was integrated as a single copy at its intended site, with no additional insertions or rearrangements detected. The copy number of inserted genes was validated to be one copy each, consistent with the design specifications.

Details of an analysis of the insert and junction regions for the occurrence of any open reading frames (ORFs)

The sequence of each insertion and the flanking regions were subjected to an ORF analysis (see **Supplement Report EB-25-PROT-02_01** [CONFIDENTIAL]). None of the peptides that might be hypothetically produced from these ORFs were identified as homologs of known toxins or allergens (see Section C.2).

B2.4 Evidence of the stability of the genetic changes

The genetic stability of strain EB-ST-537 was demonstrated by quantifying the absolute copy number of its inserted genes using droplet digital PCR (ddPCR). The strain underwent a 96-hour fermentation process, during which it completed approximately 10–11 generations, with genetic assessments performed at the beginning (t_o) and end (t_{96}) of fermentation to detect potential variations in copy number. Details of the assessment of strain stability is provided in **Supplemental Report EB-25-MOL-02-01** [CONFIDENTIAL].

ddPCR analysis confirmed that the copy number ratios for all four inserted genes remained stable throughout fermentation, with only minor fluctuations observed. Statistical analysis showed no significant variation associated with time ($F_{[1,19]}$=0.52, p=0.479), no significant differences between loci ($F_{[3,19]}$=2.63, p=0.076), and no significant interaction between time and loci ($F_{[3,19]}$=1.98, p=0.163). These results indicate that any observed fluctuations were within the expected range of experimental variation and do not suggest meaningful genomic instability.

The findings confirm that strain EB-ST-537 maintained stable gene copy numbers throughout fermentation, despite undergoing extensive replication over 10–11 generations, supporting its suitability for consistent Beta-casein production.

B2.5 Summary Statement

Strain EB-ST-537, a proprietary production strain developed by Ginkgo Bioworks and provided to Eden Brew, was derived from *Komagataella phaffii* Y-7556 through precise genome engineering techniques. The genetic modifications involved the targeted insertion of synthetic cassettes encoding bovine *CSN2* (Beta-casein), a helper gene from *Saccharomyces cerevisiae*, and synthetic transcription modulators, alongside the deletion of two endogenous proteolytic genes.

All modifications were confirmed by next-generation sequencing to be site-specific, structurally intact, and free of off-target integrations or residual antibiotic resistance markers. The final engineered strain exhibits a stable genomic architecture and robust expression of the target protein under methanol-free fermentation conditions.

B.3 Product Specifications and Certificates of Analysis

The BCPP1 Beta-casein protein preparation is a white to off-white powder intended for use as a nutritive protein source in formulated foods. Its specifications have been developed based on manufacturing process capability, compositional consistency, and international benchmarks for food-grade recombinant proteins.

As there is currently no monograph or specification for recombinant Beta-casein listed under **Schedule 3–Identity and Purity Standards** of the Australia New Zealand Food Standards Code, a new specification is proposed. The specification is aligned with those applied to microbial food enzymes, dairy protein ingredients, and fermentation-derived nutritive substances.

Section A.5 above provides a comprehensive assessment of the composition of BCPP1. Certificates of analysis (CoA) for independent production batches are provided in **Supplement Report EB-25-COMP-01_01**. The CoAs provide evidence that BCPP1 consistently meets its specifications for purity, composition and the absence of contaminants.

The proposed specification for BCPP1 for inclusion in Schedule 3 is as follows:

(a) Appearance: White to off-white powder
(b) Composition:
- (i) Total Protein (%)– no less than 20; and
- (ii) Carbohydrates (%)– no more than 70; and
- (iii) Fat (%)– no more than 3; and
- (iv) Ash (%)–no more than 3; and
- (v) Moisture (%)–no more than 6
- (vi) Total Sugars (%)–no more than 1

(c) Purity:
- (i) Beta-casein protein (%)–more than 20

(d) Metals
- (i) Lead (mg/Kg)–no more than 0.05
- (ii) Arsenic (mg/Kg)–no more than 0.05
- (iii) Mercury (mg/Kg)–no more than 0.05
- (iv) Cadmium (mg/Kg)–no more than 0.05

(e) Microbiological
- (i) *Escherichia coli*–negative to test
- (ii) *Salmonella spp*.–negative to test
- (iii) *Listeria spp*.–negative to test.

This specification, and the detailed specification in Table 11, ensures the identity, compositional integrity, and safety of the product and reflects both the intended use (as a protein source) and the safety principles outlined in Schedule 3. Justification for parameters is based on analytical results, product function, and production system controls.

Table 11. Product specification for BCPP1

Source			
Genetically modified strain of *K. phaffii* (EB-ST-537)			
Parameter	**Units**	**Specification**	**Method[a]**
Proximates			
Protein	g/100g	≥20	VL299
Carbohydrates	g/100g	≤70	VL412
Sugars	g/100g	<1	VL295
Ash	g/100g	≤3	VL286
Moisture	g/100g	≤6	VL298
Fat	g/100g	≤3	VL302
Energy	KJ/100g	≥1500	VL412
Sugars			
Fructose	g/100g	<0.2	VL295
Glucose	g/100g	<0.2	VL295
Sucrose	g/100g	<0.2	VL295
Maltose	g/100g	<0.2	VL295
Lactose	g/100g	<0.2	VL295
Fats			
Saturated	g/100g	≤0.5	VL289
Mono-unsaturated	g/100g	≤1	VL289
Poly-unsaturated	g/100g	≤1	VL289
Trans fat	g/100g	≤0.1	VL289
Heavy metals			
Lead	mg/Kg	<0.01	NT2_46
Arsenic	mg/Kg	<0.05	NT2_46
Mercury	mg/Kg	<0.01	NT2_46
Cadmium	mg/Kg	<0.01	NT2_46
Cobalt	mg/Kg	<0.01	NT2_46
Microbiology			
E. coli	MPN/g	<1	VM1.09C
Salmonella spp. /25g		ND	VM1.20B
Listeria spp. /25g		ND	VM1.16C

a. National Measurement Institute Method. NOTE: NMI holds ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories accreditation through the National Association of Testing Authorities (NATA)

B.4 Analytical Detection Method

The identity and concentration of recombinant Beta-casein in the BCPP1 ingredient can routinely be confirmed using validated analytical techniques including SDS-PAGE, reversed-phase high-performance liquid chromatography (RP-HPLC), and liquid chromatography–tandem mass spectrometry (LC-MS/MS). These methods allow for batch-specific verification of protein purity, integrity, and Beta-casein content during production.

For detection and quantification of Beta-casein in finished food products, immunoassay and mass spectrometry-based methods are suitable and available. Specifically:

- ELISA-based methods targeting bovine Beta-casein are commercially available and have been widely used for quantitative analysis in dairy matrices. These kits typically detect both A1 and A2 variants and can be applied to food matrices such as beverages, baked goods, and yoghurt analogues. Sample preparation steps (e.g., extraction buffers, heating) are tailored to the matrix.

- LC-MS/MS offers high specificity and can be used to detect signature tryptic peptides unique to the A2 variant of Beta-casein. This approach allows for precise quantification in complex food systems, particularly where immunoreactivity is diminished or where differentiation from native milk proteins is required.

These methods are considered suitable for regulatory compliance testing to verify the presence and concentration of Beta-casein in final products containing BCPP1. Method validation data, including limits of detection and quantification, can be provided upon request or developed for specific food matrices prior to commercial launch.

SECTION C: SAFETY AND RISK ASSESSMENT

This section presents the characterisation and safety assessment of the Beta-casein protein preparation produced using gene technology. It includes compositional analysis, structural identity confirmation, and an evaluation of the physicochemical properties of the expressed protein. The safety assessment considers the potential for toxicity, allergenicity, and bioactivity, including a review of the digestibility and metabolic fate of the protein and its degradation products. Where relevant, comparisons are made to conventional dairy-derived Beta-casein to establish compositional and functional equivalence. The data presented support the conclusion that BCPP1 is safe for human consumption and consistent with the objectives of food safety regulation under the Australia New Zealand Food Standards Code.

C.1 Characterisation and Safety Assessment of New Substances

The primary protein produced in strain EB-ST-537 is Beta-casein, a milk-derived protein produced using precision fermentation in the yeast *K. phaffii*. The expressed Beta-casein is structurally and functionally equivalent to its naturally occurring counterpart in bovine milk, and it is intended for use in food products as a protein ingredient.

Strain EB-ST-537 also expresses a Helper gene and two synthetic transcription modulators. The production strain does not persist in the final product, and purification steps ensure removal of most host-derived material including the Helper protein and synthetic transcription modulators. Beta-casein has a well-established history of safe consumption in the human diet. Bioinformatic and LC-MS analysis of the expressed protein and surrounding open reading frames confirmed no significant homology to known toxins or allergens. No post-translational modifications were observed that would alter the safety profile compared to the naturally occurring protein.

C.1.1 a full description of the biochemical function and phenotypic effects of all new substances (e.g. a protein or an untranslated RNA) that are expressed in the new GM organism, including their levels and site of accumulation, particularly in edible portions

The commercially available *K. phaffii* yeast strain Y-7556 was modified to contain a *Bos taurus* Beta-casein expression cassette to generate strain EB-ST-537. The strain was developed to produce a Beta-casein protein preparation.

Four new proteins are expressed in strain EB-ST-537. In addition, the preparation also contains some *K. phaffii* host protein.

Further information is provided in the following Supplement Reports:

- EB-25-PROT-01-02_Protein Safety Assessment for Strain EB-ST-537 [CONFIDENTIAL].
- EB-25-PROT-02-01_Allergen and Toxin Evaluation of ORFs in Strain EB-ST-537 [CONFIDENTIAL]
- EB-25-MOL-02-01_01 Genetic Stability of the Insertions in Strain EB-ST-537 [CONFIDENTIAL]

Beta-Casein is one of the principal proteins found in bovine milk, accounting for approximately 27 to 36 % of the total milk protein content. It plays essential roles in both the nutritional value of milk and its functional properties during processing.

Structurally, Beta-casein is a single-chain polypeptide composed of 209 amino acids with an approximate molecular mass of 24 kDa (Duarte-Vázquez et al., 2017). The gene encoding this protein, *CSN2*, is located on chromosome 6 of *Bos taurus* and is known to be highly polymorphic, with over 15 naturally occurring variants identified (Sebastiani et al., 2020). Among these, the A1 and A2 variants are the most prevalent in

dairy cattle populations. These two forms differ by a single amino acid substitution at position 67, histidine in A1 and proline in A2 (Cieślińska et al., 2022), a change that has functional implications during digestion.

Functionally, Beta-casein is involved in several critical processes. It facilitates the transport of calcium and phosphate, contributing to mineral balance in milk. It also plays a key role in curd formation, which is central to cheese production, and helps stabilise casein micelles, thereby influencing the physicochemical characteristics and processing performance of milk.

Beyond these physical functions, Beta-casein also exhibits bioactive properties. The A1 variant, when subjected to enzymatic digestion, can release Beta-casomorphin-7 (BCM-7), a peptide with opioid-like activity (He et al., 2017). This peptide has been associated with a range of physiological responses, including effects on gastrointestinal function and inflammation. By contrast, the A2 variant, which is used in the Beta-casein protein preparation expressed in strain EB-ST-537, does not produce BCM-7 under typical digestive conditions and is increasingly recognised for its potential benefits in improving digestive comfort.

From a nutritional perspective, Beta-casein is classified as a complete protein, supplying all nine essential amino acids required by humans. Its high biological value makes it a significant contributor to the overall protein quality and density of milk and dairy products (Haug et al., 2007). The slow digestion rate of Beta-casein also supports sustained amino acid release, promoting prolonged muscle protein synthesis and satiety (Boirie et al., 1997).

Technologically, Beta-casein variants influence the behaviour of milk during processing. The A1 and A2 genetic variants, distinguished by a single amino acid substitution at position 67, can impact curd formation, cheese yield, and fermentation efficiency (Caroli et al., 2009; Mishra et al., 2009). Studies have shown that milk containing the A2 variant tends to exhibit improved rennet-induced coagulation properties and may produce firmer curds, which are favourable for cheese-making applications (Faggion et al., 2025). These properties make the selection of Beta-casein variant relevant for both industrial functionality and product quality.

Finally, while research continues into the health implications of Beta-casein variants, there is growing interest in the potential differential effects of A1 and A2 on digestion and tolerance, particularly among individuals reporting milk-related discomfort (Hohmann et al., 2020).

Characterisation of N-terminal sequences of Beta-casein from EB-ST-537

The N-terminal identity of recombinant Beta-casein produced by strain EB-ST-537 was thoroughly investigated using two orthogonal analytical methods: high-sensitivity liquid chromatography-tandem mass spectrometry (LC-MS/MS) with iTRAQ labelling and definitive sequencing by Edman degradation (see **Supplement Report EB-25-PROT-01-02** [CONFIDENTIAL]).

The initial analysis by LC-MS/MS, a technique capable of detecting low-abundance peptide species, identified two N-terminal forms in the BCPP1 samples. The major form detected was an extended N-terminus with a four-amino-acid EAEA prefix (EAEARELEEL...). A second, minor form was also identified, corresponding to a truncated sequence lacking the N-terminal arginine residue (ELEELN...).

To confirm these findings and definitively identify the primary N-terminal sequence, all three batches were subsequently analysed using Edman degradation, the gold-standard method for N-terminal sequencing. This analysis unambiguously identified a single N-terminal sequence across all samples: EAEARE.... The truncated variant observed by LC-MS/MS was not detected by this method, confirming its status as a minor species present at a very low relative abundance.

The combined results from these complementary techniques provide a comprehensive characterisation of the BCPP1 N-terminus. The product consists of a single, predominant protein species with the N-terminus EAEARELEELNVPGEIVES..., which arises from a consistent and predictable alternative cleavage of the

secretion signal peptide. The data confirms that the BCPP1 product is well-characterised and demonstrates a high degree of homogeneity.

Identity and function of the Helper protein

[COMMERCIALLY SENSITIVE SECTION REMOVED]

Identity and function of the Transcription Modulator proteins

[COMMERCIALLY SENSITIVE SECTION REMOVED]

Beta-casein protein content from EB-ST-537 fermentation

The Beta-casein protein content and overall protein composition of BCPP1s from EB-ST-537 were evaluated through quantitative mass spectrometry and compositional analysis (see **Supplemental Report EB-25-PROT-01-02** [CONFIDENTIAL] and **Supplemental Report EB-25-COMP-01-01**). A summary of the findings across three independent test materials is provided in Table 12. Beta-casein comprised approximately 5–6 % of the total weight in each sample, with Beta-casein purity, expressed as a proportion of total protein, ranging from 22-28 %. Total protein content across the three batches was consistent, measuring at 22.7 %, 21.6 %, and 22.5 % respectively. These results demonstrate consistent Beta-casein production and overall protein yield in BCPP1s from EB-ST-537.

Table 12. Beta-casein protein levels from EB-ST-537 fermentation

	Test Material		
	Batch 1	**Batch 2**	**Batch 3**
Composition			
Beta-casein (w/w)	5-6%	5-6%	5-6%
Beta-casein purity per total protein (w/w)	22-26%	23-28%	22-27%
Protein (w/w)	22.8%	21.6%	22.5%

Host proteins in Beta-casein protein preparations from EB-ST-537

Profiling Liquid Chromatography-Mass Spectrometry (LC-MS) combined with routine database searching was employed to identify the most frequently occurring host cell proteins in three independent protein preparations (see **Supplement Report EB-25-PROT-01-02** [CONFIDENTIAL]). The samples were digested with trypsin and subsequently analysed using single shot information dependant acquisition mass spectrometry to identify the top twenty host proteins. Using the Codex Alimentarius framework for protein quantification (Codex, 2010), the identified proteins were ranked by their peptide-spectrum match (PSM) counts to estimate their relative abundance.

The top twenty proteins identified by mass spectrometry along with their UniProt database accession numbers and corresponding protein lengths are provided in Table 13. The top-ranked proteins were all *Komagataella* origin, except for the recombinant bovine Beta-casein.

C.1.2 Information about prior history of human consumption of the new substances, if any, or their similarity to substances previously consumed in food.

The primary new substance, bovine Beta-casein, has an extensive and well-established history of safe human consumption as a major nutritional protein in cow's milk and dairy products, which have been part of the human diet for millennia. Comprehensive characterisation of the Beta-casein protein preparation confirms that the expressed A2 Beta-casein is structurally and functionally equivalent to its conventional counterpart. The final preparation also contains a mixture of low-abundance, co-purified host cell proteins derived from the production organism, *Komagataella phaffii*. The host organism has a documented history of safe use for producing food ingredients and enzymes that have been approved by global regulatory bodies, including FSANZ, the U.S. FDA, and EFSA, thereby establishing a precedent for the safety of its residual components in purified food-grade preparations. Other new proteins expressed intracellularly in the production strain (i.e., the helper protein and transcription modulators) are not secreted and have been shown to be absent from the final purified product. Thus, the new substances present in BCPP1 are either identical to proteins with a long history of safe dietary exposure or are residual proteins from a production organism with a history of safe use in food manufacturing.

C.1.3 information on whether any new protein has undergone any unexpected post-translational modification in the new host

Post-translational glycosylation, which involves the attachment of carbohydrate groups to proteins, is a suggested key structural characteristic distinguishing allergenic proteins from non-allergenic ones (Altman 2007).

Most secreted and cell membrane proteins in mammals undergo glycosylation. Many of these glycoproteins are also prevalent in milk, where they play essential roles in modulating biological functions and significantly enhance the nutritional quality of milk.

Bovine milk contains two types of protein glycosylation: N-linked glycosylation, where the glycan chain is covalently linked via an N-acetyl glucosamine molecule to the amide side chain of an asparagine residue, and O-linked glycosylation, where the glycan chain is covalently attached to the hydroxyl oxygen of a serine or threonine residue (Vance et al., 1997). However, Beta-casein lacks naturally occurring glycosylation sites (Dingess et al., 2021). A study on the potential O-linked glycosylation of human milk Beta-casein found no evidence of glycosylation on bovine Beta-casein (Molinari et al., 2012).

Table 13. Top ranked proteins detected in BCPP1s

Protein Name[1]	Accession	Protein Length	Relative Frequency[2]	Proportion of BCPP1 Protein (%)[3]
Beta-casein	P02666	224		26
Endoplasmic reticulum chaperone BiP	Q56D08	678	99.33±6.17	7
BA75_04779T0	A0A1B2JGS7	645	76.33±2.03	3
BA75_00236T0	A0A1B2J5W9	656	37.33±1.2	3
Glyceraldehyde-3-phosphate dehydrogenase	Q92263	333	36.0±0	6
Protein disulfide-isomerase	Q9C1Z8	517	36.0±1.53	4
Glyceraldehyde-3-phosphate dehydrogenase	A0A1B2JAL9	333	35.67±1.2	6
Acetyl-coenzyme A synthetase	A0A1B2JDV8	668	35.0±0	3
Elongation factor 2	Q874B9	842	34.33±0.88	3
Phosphoglycerate kinase	Q7ZA46	416	30.0±1.53	4
5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase	Q6GYJ7	768	29.0±1.0	2
BA75_00945T0	A0A1B2J6S7	706	28.33±1.2	2
Transketolase	C0LQF6	684	27.0±1.0	2
Transaldolase	A0A1B2JB79	324	26.33±0.88	5
5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase	A0A1B2JIW1	768	26.67±0.88	2
Elongation factor 1-alpha	A0A1B2JFK5	459	25.33±1.45	3
Phosphotransferase	A0A1B2JFF8	496	24.33±1.2	3
Phosphoglycerate kinase	A0A1B2J5L5	416	24.33±0.88	3
BA75_03811T0	A0A1B2JG62	613	22.67±1.67	2
6-phosphogluconate dehydrogenase, decarboxylating	A0A1B2J6Y7	492	22.0±0.58	3
Triosephosphate isomerase	A0A1B2JFA7	248	21.0±0.58	5

1: Except for Beta-casein, all other proteins matched *K. pastoris* when compared to the UniProt database; **2:** Numbers in column indicate the mean frequency (± standard error) of peptide-spectrum matches with 95% confidence or higher; **3:** Proportion of the total protein fraction (%)

Micro-organisms producing recombinant proteins are known to produce glycosylated protein forms with variations in the glycan structures (SECRETERS-European Union's Horizon 2020 Programme 2022). In yeast, both N- and O-glycosylation pathways exist, but glycan maturation in the Golgi apparatus typically results in a higher mannose content than in higher eukaryotes.

Beta-casein variants expressed in *K. phaffii* undergo post-translational glycosylation, accounting for up to 30 % of the total expressed protein (Choi and Jiménez-Flores, 2001). This glycosylation is of the high-mannose type and is pH-dependent, occurring in buffered minimal media. Unlike hyper-glycosylation observed in

other yeast systems, most bovine Beta-casein expressed in *K. phaffii* is not hyper-glycosylated (Choi and Jiménez-Flores, 2001).

Analysis of the Beta-casein from strain EB-ST-537, assessed using mass spectrometry, supports a finding that that only a limited number of glycan structures are detectable on the recombinant protein. The evidence suggests that only O-linked glycosylation is present on the recombinant Beta-casein protein (**Supplement Report EB-25-PROT-01-02** [CONFIDENTIAL]).

Identification of glycosylation in EB-ST-537 Beta-casein products

The potential for glycosylation in the EB-ST-537 Beta-casein protein preparation was evaluated using LC-MS analysis. Protein production using non-native hosts such as *K. phaffii* can result in post-translational modifications, including glycosylation. As such, three independent batches of the recombinant Beta-casein product were assessed for evidence of glycan attachment.

N-linked glycosylation was investigated by identifying glycopeptides containing the canonical N-linked sequon (Asn-X-Ser/Thr, where X is any amino acid except proline). Peptide spectrum matches were filtered to retain only those with ≥85 % confidence. In cases where N-linked glycosylation was detected in the absence of this consensus sequence, results were considered artefactual and excluded. Based on this criterion, no credible N-linked glycosylation sites were identified in the Beta-casein protein across any of the tested batches.

In contrast, O-linked glycosylation was consistently observed at five distinct serine (S) or threonine (T) residues (Table 14). These modifications were detected by LC-MS peptide mapping with supporting spectral data containing diagnostic oxonium ions and were present across all three production batches. The glycan structures identified at these positions were simple mannose-based units typical of glycosylation patterns in *K. phaffii*-derived proteins. Some degree of structural variability was observed at amino acid positions 35 and 41, which exhibited both Man(5) and Man(6) glycans. No variation was noted at the remaining sites.

Table 14. O-linked glycan structures identified on recombinant Beta-casein products

Position	Amino acid	Glycan structure/s
15	S	dMan(1)Man(1)
35	S	Man(5)
		Man(6)
41	T	Man(5)
		Man(6)
57	S	Man(5)
78	T	Man(1)

The glycosylation profile observed in these recombinant Beta-casein preparations is consistent with known host cell glycosylation behaviour in *K. phaffii*. The O-linked glycans are structurally simple and unlikely to affect the nutritional or safety profile of the protein, particularly given the minimal degree of glycosylation detected and the absence of complex or immunogenic glycan structures.

The EB-ST-537 Beta-casein protein preparation, assessed using mass spectrometry, supports a finding that only a limited number of glycan structures are detectable on the recombinant protein. The evidence suggests that only O-linked glycosylation is present on the recombinant Beta-casein protein. These findings support the compositional integrity of the product and are consistent with expectations for recombinant proteins expressed in *K. phaffii*.

Information on the stability of the protein to proteolysis in appropriate gastrointestinal model systems

Beta-casein is widely published as an easily digested and absorbed natural food component of bovine milk (Guzmán et al., 2019). To confirm the digestibility of Beta-casein protein, potential cleavage sites were investigated using the amino acid sequences of each of the proteins and the PeptideCutter tool in the ExPASy Proteomics Site (Gasteiger et al., 2005).

Beta-casein has multiple cleavage sites for pepsin (31 sites at pH 1.3 and 36 sites at pH >2), trypsin (15 sites), chymotrypsin (14 high-specificity sites, 25 low-specificity sites), Proteinase K (72 sites) and endopeptidases (63 sites). On this basis, Beta-casein is considered likely to be as susceptible to digestion as most dietary proteins.

Whilst the additional proteins are not present in BCPP1, they were also analysed for potential cleavage sites. The helper protein has multiple cleavage sites for pepsin (55 sites at pH 1.3 and 61 sites at pH >2), trypsin (27 sites), chymotrypsin (15 high-specificity sites, 48 low-specificity sites), Proteinase K (107 sites) and endopeptidases (57 sites). Transcription modulator 1 has multiple cleavage sites for pepsin (55 sites at pH 1.3 and 61 sites at pH >2), trypsin (27 sites), chymotrypsin (15 high-specificity sites, 48 low-specificity sites), Proteinase K (107 sites) and endopeptidases (57 sites). Transcription modulator 2 has multiple cleavage sites for pepsin (162 sites at pH 1.3 and 172 sites at pH >2), trypsin (38 sites), chymotrypsin (31 high-specificity sites, 138 low-specificity sites), Proteinase K (274 sites) and endopeptidases (142 sites).

On this basis, if BCCP1s contained any of these proteins, they are likely to be as susceptible to digestion as most dietary proteins.

A pepsin digestibility study was also undertaken on BCPP1 samples (see below).

Pepsin Digestibility of Recombinant Beta-Casein

The digestibility of the recombinant Beta-casein protein preparation BCPP1 produced via precision fermentation in strain EB-ST-537 was assessed under simulated gastric conditions using a standardised pepsin digestion assay. BCPP1 samples from three independent 500 L fermentation batches (EDB-500-011525, EDB-500-020425, and EDB-500-022425) were compared with bovine Beta-casein as a reference control. Both untreated and α-mannosidase-treated BCPP1 samples were analysed to evaluate the impact of glycosylation on proteolytic susceptibility.

Coomassie-stained SDS-PAGE gels showed that BCPP1 and bovine Beta-casein protein is rapidly degraded upon exposure to pepsin. In all samples, the major intact protein band at ~28 kDa diminished substantially within 5 minutes of digestion. By 60 minutes, full-length Beta-casein was no longer detectable, and only low molecular weight peptide fragments remained visible Figure 5. The degradation profile of BCPP1 closely mirrored that of bovine Beta-casein, with no observable differences in the timing or extent of proteolysis between them.

α-Mannosidase-treated BCPP1 samples displayed a similar degradation pattern to untreated counterparts. Although de-glycosylation led to a shift in molecular weight consistent with the removal of glycan moieties, it did not alter the rate or extent of pepsin-mediated digestion (Figure 5).

Immunoblotting with an anti-Beta-casein antibody confirmed the rapid loss of immunoreactive signal for both BCPP1 and bovine Beta-casein (Figure 5 and Figure 6). In all samples, the Beta-casein signal was markedly reduced by 10 minutes and absent by 60 minutes. No Beta-casein was detectable at the 120- or 240-minute time points (Figure 5). Control samples lacking pepsin retained strong Beta-casein signals throughout the 240-minute incubation period, confirming that degradation was pepsin-dependent.

Western blot analysis of mannosidase-treated samples showed a shift in the Beta-casein band to a lower molecular weight, consistent with de-glycosylation. However, the digestion profile over time was

indistinguishable from that of untreated samples, indicating that glycosylation did not provide protection against pepsin cleavage under the conditions tested (Figure 6).

In summary, BCPP1 exhibited rapid and extensive degradation by pepsin, consistent with the known digestibility of bovine Beta-casein. De-glycosylation with α-mannosidase did not affect the protein's susceptibility to enzymatic hydrolysis. These results demonstrate that BCPP1 produced in *K. phaffi* is readily digested under simulated gastric conditions and behaves comparably to naturally derived bovine Beta-casein.

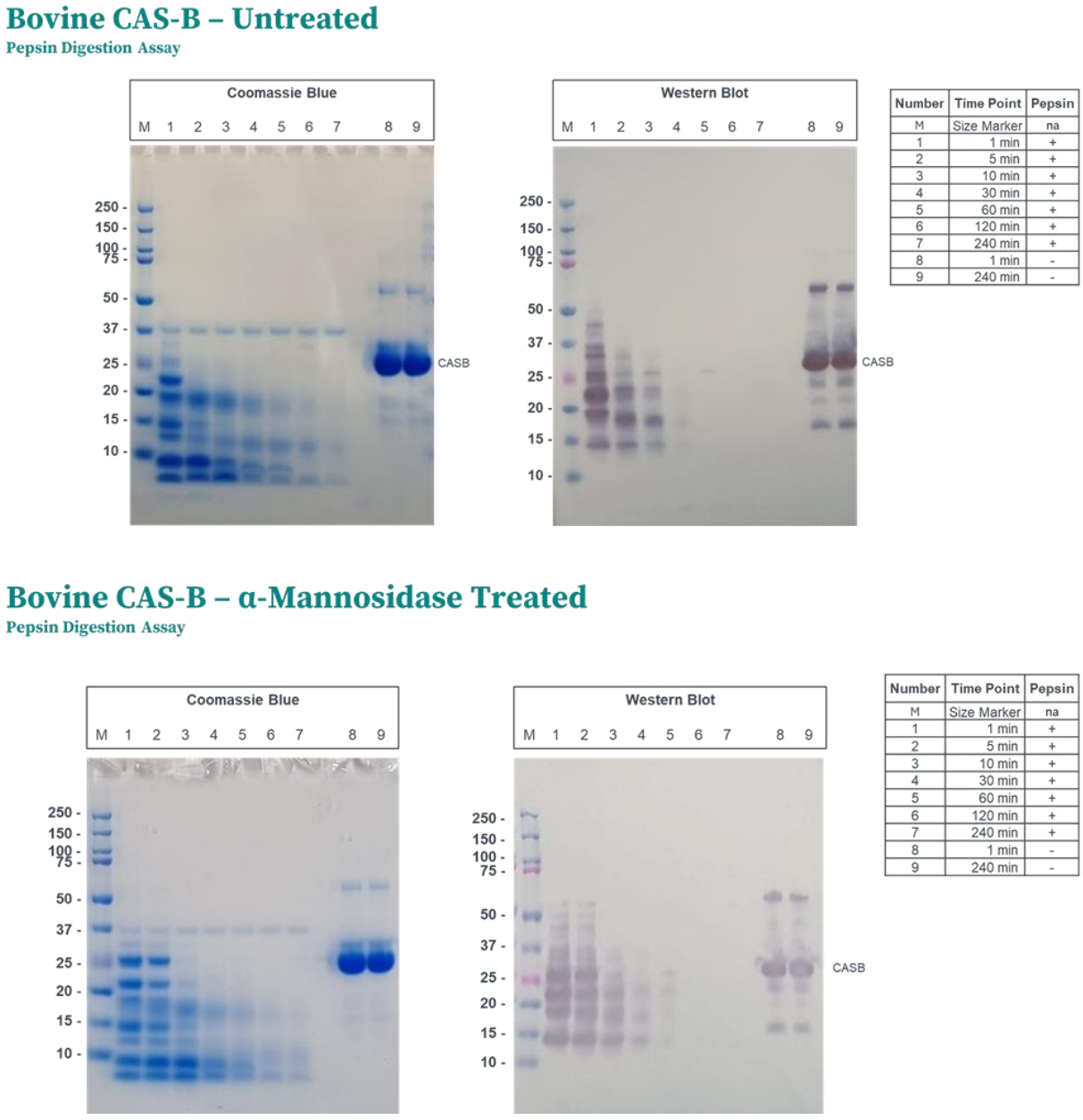
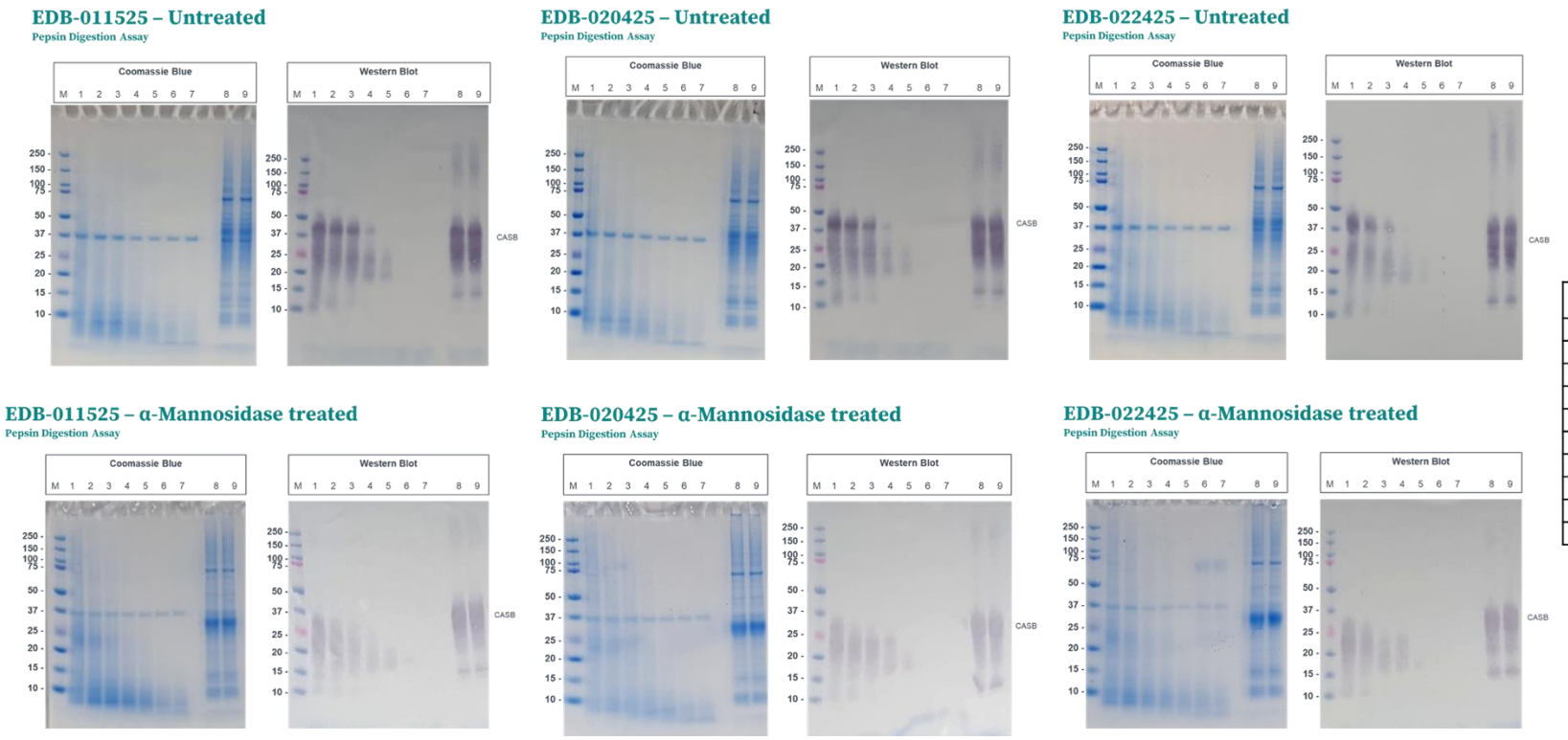


Figure 5. Pepsin digestibility of bovine Beta-casein
Lane M: Protein size markers (KDa); Lanes 1-7: Bovine Beta-casein pepsin digestibility over time (1-240 min); Lanes 8 & 9: Bovine Beta-casein non-pepsin treated (1 and 240 min respectively). Upper panels: Coomassie Blue and Western Blot of Bovine Beta-casein; Lower panels: Coomassie Blue and Western Blot of α-mannosidase-treated Bovine Beta-casein.



Number	Time Point	Pepsin
M	Size Marker	na
1	1 min	+
2	5 min	+
3	10 min	+
4	30 min	+
5	60 min	+
6	120 min	+
7	240 min	+
8	1 min	-
9	240 min	-

Figure 6. Pepsin digestibility of BCPP1

Lane M: Protein size markers (KDa); Lanes 1-7: BCPP1 pepsin digestibility over time (1-240 min); Lanes 8 & 9: BCPP1 non-pepsin treated (1 and 240 min respectively). Upper panels: Coomassie Blue and Western Blot of three independent BCPP1 batches; Lower panels: Coomassie Blue and Western Blot of three independent BCPP1 batches treated with α-mannosidase.

Heat stability

The stability of milk proteins is well-established in the scientific literature (Huppert 2016). Heat treatment, including pasteurisation, is a widely used method to extend shelf life and maintain the functionality of milk and dairy products (Daniloski et al., 2022). As Beta-casein is a key component of dairy products, it is important that recombinant Beta-casein maintains its stability when exposed to heat. To assess this stability, BCPP1 samples were subjected to heating at 80°C for 10 minutes and compared with commercially obtained Beta-casein isolated from bovine milk (Figure 7). This heating protocol exceeds the conditions typically required for pasteurisation of common dairy products (ANZDAC 2007), providing a more rigorous test of protein stability. The extended heating time and high temperature used in this experiment were chosen to evaluate the protein's stability under more extreme conditions than those typically encountered in standard dairy processing. This approach allows for a comprehensive assessment of the heat stability of the BCPP1 compared to its native counterpart.

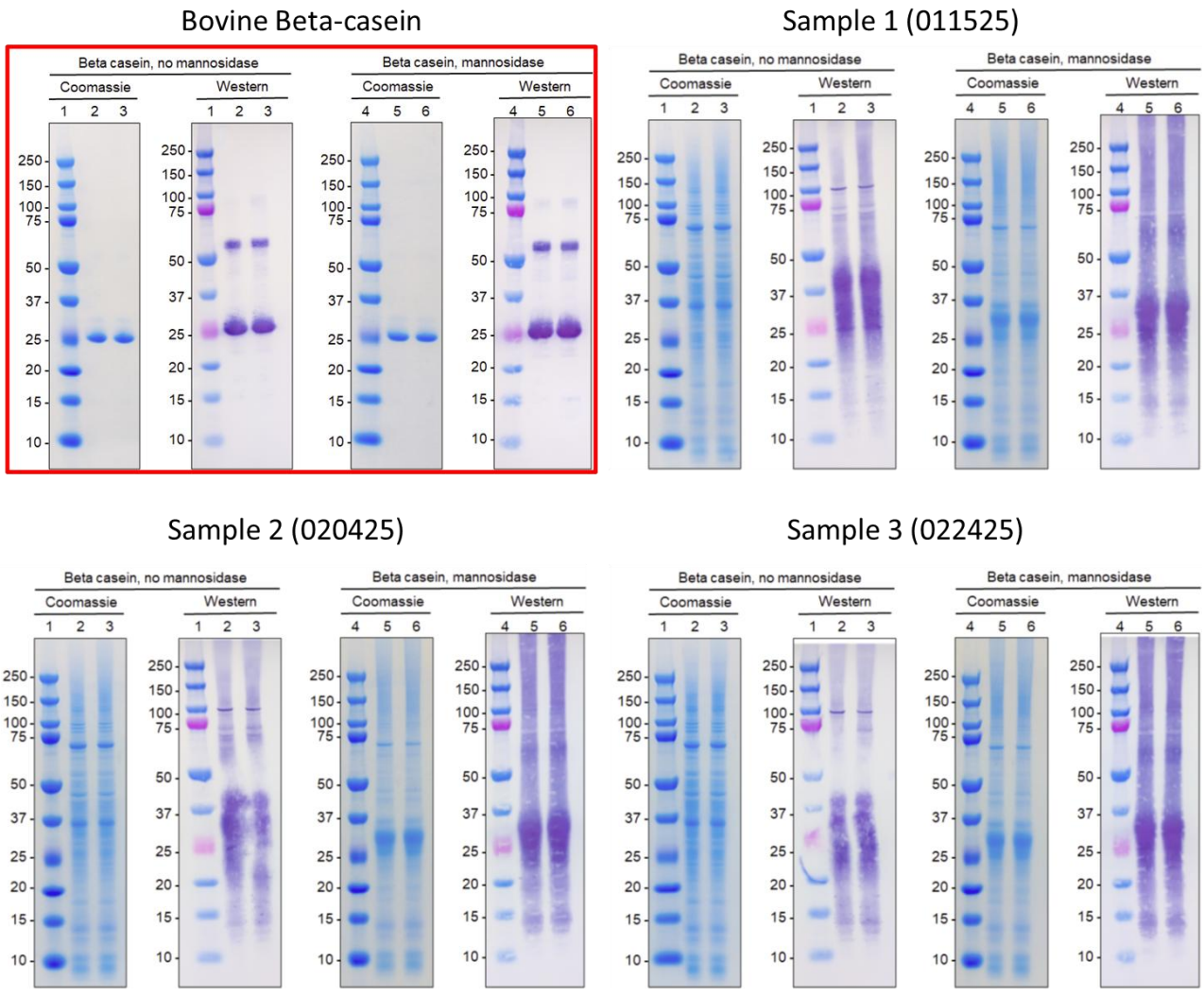


Figure 7. SDS-PAGE and Western Blot showing negligible impact of heat treatment on the Beta-casein preparations.

Lane 1 & 4: Protein size markers (KDa); Lane 2: Beta-casein untreated, 2 µg, 4°C; Lane 3: Beta-casein untreated, 2 µg, 80°C 10 minutes; Lane 5: Beta-casein treated with mannosidase, 2 µg 4°C; Lane 6: Beta-casein treated with mannosidase, 2 µg, 80°C 10 minutes.

As mentioned above, Beta-casein samples produced in EB-ST-537 treated with a mannosidase releases a ~28 kDa band comprising Beta-casein. This band is not visible in samples prior to mannosidase treatment. Heat stability studies were performed with and without mannosidase treatment.

Comparing the Beta-casein band patterns of the non-heat-treated samples of lanes 2, 5 with their heat-treated counterparts in lanes 3, 6 suggests that heat treatment has negligible impact on the Beta-casein samples tested (Figure 7).

C.2 Potential Allergenicity of New Proteins

Allergenicity assessment for BCPP1 was undertaken following the FSANZ weight-of-evidence framework (Guideline 3.5.1.B.2). This framework integrates information on the source of each protein, amino acid sequence homology to known allergens, protein stability characteristics, and relevant immunological considerations.

C.2.1 Protein Sources and Expression

BCPP1 contains bovine Beta-casein expressed in *K. phaffii* together with residual host cell proteins. Other auxiliary proteins (helper protein and transcription modulators) are confined intracellularly and are not secreted; they are below the level of detection in the final product following validated downstream processing.

Beta casein: *Bos taurus* (A2 variant, 209 amino acids)

Host Cell Proteins: Endogenous to *K. phaffii*

Helper Protein: *S. cerevisiae* (intracellular, undetected)

Transcription Modulator 1 and 2: Synthetic derived from *S. cerevisiae* and *E. coli* (intracellular, undetectable)

C.2.1 Host Cell Protein–Related Allergenicity

BCPP1 contains approximately 22 % total protein, of which 75 % is derived from host cell proteins (HCPs) originating from the *K. phaffii* production strain. Proteomic profiling shows that no single protein predominates. Among the top 20 identified proteins, the maximum contribution of any one protein is 7 % of the total protein fraction, with the majority present between 3-5 % (Table 13). This indicates a heterogeneous mixture of HCPs without dominance of a single protein species at high levels.

This profile contrasts with the findings of Ye et al. (2024), where immunological responses in mice were attributed to impurities in partially purified porcine myoglobin preparations (60 % and 88 % purity). In that study, the impurity fraction was undefined, chemically uncharacterised, and present at relatively high levels. Importantly, the absence of analytical verification meant it was not possible to determine whether the observed immune responses were driven by host cell proteins, other process-derived constituents, or artefacts of the experimental system. As such, the Ye et al. study demonstrates a correlation between low-purity protein fractions and immune signals but does not establish causality.

By contrast, BCPP1 is manufactured under controlled fermentation and downstream processing conditions, with validated impurity monitoring and full proteomic characterisation. The defined and broadly distributed HCP profile, combined with the relatively low abundance of individual proteins, reduces the likelihood that any single HCP could act as a dominant sensitising antigen. This approach aligns with international regulatory precedents, including FSANZ's assessment of soy leghemoglobin (A1186) and EFSA evaluations of other

microbial proteins, where residual HCPs were analytically profiled and not considered to present allergenicity or toxicity concerns when appropriately managed.

C.2.2 Allergenicity of Milk Proteins

A recent review published by the World Allergy Organization provides a comprehensive overview of milk protein allergens, their immunogenicity and allergenicity in the context of industrial processing, and milk-related immune mechanisms triggering IgE-mediated immediate type hypersensitivity reactions, mixed reactions and non-IgE mediated hypersensitivities (Jensen et al., 2022).

More than 20 proteins in cattle milk are known to cause allergic reactions; of these, the casein fractions (especially αs2-, αs1-, and κ-caseins as well as, to some extent, Beta-casein), lactoferrin, serum albumin, and Beta-lactoglobulin are considered the most common cattle milk allergens (Docena et al., 1996; Lam et al., 2008; El-Agamy 2007; Natale et al., 2004).

A recent study reported a current prevalence of immunoglobulin E–mediated cow's milk allergy of 1.3% in a population-based sample of 1-year-old Australian infants (Soriano et al., 2023). Early eczema, parent country of birth, and parent history of food allergy increased the risk of milk allergy. Most children outgrow cow's milk allergy by the age of 3 to 5 years, but it can remain a lifelong allergy.

Several studies have examined the degree of allergenicity of non-cattle milk protein (Restani et al., 2002; Infante et al., 2003; Martín et al., 2004). Despite the increasing interest with respect to the suitability of non-cattle milk as a hypoallergenic option to cattle milk (Hinz et al., 2012), the overall, the scientific evidence indicates that there is little basis for promoting non-cattle milk or milk proteins as an alternative to cattle milk for people suffering from cattle (or cow) milk allergy.

In summary, Beta-casein is a multifunctional milk protein that plays critical roles in the nutritional, physiological, and technological aspects of cow's milk. Its genetic variants, particularly A1 and A2, have become a focus of research due to their potential differential effects on human health and milk processing. The Beta-casein expressed in BCPP1 is identical in amino acid sequence to the naturally occurring A2 bovine variant and therefore shares the same allergenic profile. It has an extensive history of safe dietary exposure in conventional milk.

C.2.3 Potential Allergenicity of the Production Strain EB-ST-537

Bioinformatic evaluation of open reading frames (ORFs) using established databases and search algorithms (Goodman et al., 2008; Ladics et al., 2007; Terrat and Ducancel, 2013) at the four engineered loci and their flanking regions (**Supplemental Report EB-25-PROT-02_01**) found no significant similarity to known allergens or toxins, apart from the introduced Beta-casein ORF (Table 15). Thus, no novel allergenic concerns arise from the genetic modifications in the host organism.

C.2.4 Potential Allergenicity of BCPP1

The Helper protein and Transcription Modulators function exclusively within the yeast cell, are not secreted into the extracellular environment, and remain below the level of detection in the final product. Accordingly, BCPP1 contains only bovine Beta-casein and residual host cell proteins.

Comprehensive bioinformatic analysis demonstrated no relevant similarity between the peptides derived from BCPP1 (other than Beta-casein) and known allergens. The Beta-casein protein expressed in BCPP1 shares 100% amino acid sequence identity with the naturally occurring bovine Beta-casein (A2 variant). As such, while Beta-casein is recognised as a known allergen in cow's milk, it has a well-established history of

dietary exposure and safe use. The risk profile is therefore consistent with existing dietary exposures to bovine milk proteins.

The most abundant HCPs present in BCPP1 are listed in Table 13. Each was assessed against the AllergenOnline database (version 23, current as of January 2025, comprising 2334 entries across 969 allergen groups). In line with Codex and FAO/WHO recommendations, homology searches were conducted using both an 80-mer amino acid sliding window and an 8-mer exact match approach. No HCPs exhibited ≥35% identity over an 80-amino acid segment, nor did they share identical 8-amino acid motifs with any known allergenic epitope. These findings demonstrate that the residual proteins in BCPP1 do not present sequence-based evidence of allergenicity.

To assess whether the N-terminal heterogeneities identified in BCPP1 could generate novel allergenic or toxic peptide motifs, a comprehensive *in silico* analysis was conducted. This assessment included all N-terminal variants detected by high-sensitivity LC-MS/MS, ensuring a conservative safety evaluation. The sequences were subjected to simulated human digestion with pepsin, trypsin, and chymotrypsin. The resulting peptide fragments were screened against the AllergenOnline and UniProtKB toxin databases. No digestion fragments from any N-terminal variant showed significant homology to known allergens or toxins, nor were any predicted to be toxic. This confirms that the minor N-terminal variations do not introduce any new or unexpected safety risks.

Conclusion

The weight of evidence demonstrates that BCPP1 contains only Beta-casein, a known milk allergen with an extensive history of safe dietary use, and a mixture of well-characterised, low-abundance host cell proteins that show no homology to known allergens. Proteins unique to the production system (Helper and Transcription Modulators) are absent from the final preparation. Importantly, unlike the undefined impurity fractions described by Ye et al. (2024), the residual proteins in BCPP1 are fully characterised and controlled, ensuring a reproducible safety profile. Taken together, these findings support the conclusion that BCPP1 introduces no new or unexpected allergenicity risks beyond those already associated with conventional bovine milk proteins, and that the presence of residual host cell proteins does not raise additional allergenicity concerns under intended conditions of use.

Table 15. Allergen similarity of ORF9 in Site 4

Database	AllergenOnline Database version 23; January 30, 2025
Input Query	>ORF9 MQFSTVASIAAVAAVASAAAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDAAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEARELE ELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVK EAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQA FLLYQEPVLGPVRGPFPIIV
Length	299
Number of hits	The best scores are: opt bits E(2291) %_id %_sim alen gi|942073448|gid|374|PREDICTED: beta-casein isoform (259) 1428 291.8 2.6e-080 0.995 0.995 212 gi|459292|gid|374|beta-casein A3 [Bos taurus] (224) 1419 290.1 7.3e-080 0.991 0.995 212 gi|162805|gid|374|beta-casein (224) 1416 289.5 1.1e-079 0.991 0.991 212 gi|162797|gid|374|beta-casein precursor (224) 1397 285.7 1.5e-078 0.976 0.986 212 gi|508732623|gid|150|omega-gliadin, partial [Triticu (366) 239 55.7 4.4e-009 0.292 0.524 185 gi|208605344|gid|150|D-type LMW glutenin subunit [Tr (359) 209 49.7 2.7e-007 0.274 0.520 179 gi|170702|gid|152|gamma gliadin precursor (302) 170 42.1 4.3e-005 0.296 0.497 159 gi|21783|gid|154|LMW glutenin-like protein product [(356) 169 41.8 6.4e-005 0.242 0.516 161
Number of 80-mers	220
Number of sequences with hits	8
Number of 8-mers	292
Number of sequences with hits	1. GI:162797 Hits:178-- Def:Beta-casein precursor 2. GI:162805 Hits:195-- Def:Beta-casein 3. GI:459292 Hits:195-- Def:Beta-casein A3 [*Bos taurus*] 4. GI:942073448 Hits:203-- Def:PREDICTED: Beta-casein isoform X2 [*Bos mutus*]

C.3 Potential Toxicity of New Proteins

This section evaluates the potential toxicity of BCPP1 in alignment with the FSANZ Application Handbook requirements for Guideline 3.5.1 (Foods produced using gene technology) and Guideline 3.5.2 (Novel foods). Both frameworks require a systematic, weight-of-evidence approach that considers the source of the protein, its toxicokinetics and metabolism, sequence homology with known protein toxins, and results from relevant toxicological studies. The assessment presented here integrates:

(i) bioinformatic analyses of BCPP1 and residual host cell proteins

(ii) literature evidence from acute, sub-chronic, genotoxicity, and reproductive/developmental studies of comparable *K. phaffii*-derived proteins, and

(iii) consideration of the long history of safe dietary exposure to bovine Beta-casein. By addressing the criteria of both 3.5.1 and 3.5.2, this section provides a comprehensive evaluation of whether BCPP1 introduces any novel or unexpected toxicity concerns under its intended use.

C.3.1 Toxicokinetics and Metabolism

Beta-casein (A2 variant) and residual host cell proteins from *K. phaffii* follow the expected digestive fate of dietary proteins. Beta-casein is hydrolysed into peptides and amino acids during gastrointestinal digestion, with no evidence that novel metabolites of toxicological concern are generated. Residual HCPs represent a heterogeneous mixture of yeast proteins and are similarly degraded by gastric and intestinal proteases into common amino acids and small peptides. Collectively, these characteristics demonstrate that the metabolic fate of proteins in BCPP1 does not deviate from that of proteins with a long history of safe dietary use.

C.3.2 Potential Toxicity of K. phaffii

The foundation of the safety case for any microbial production system is the intrinsic safety of the host organism itself. As established in Section B.2.1.1 (Safe Strain Lineage), the production strain EB-ST-537 is derived from a well-characterised, non-pathogenic, and non-toxigenic progenitor. The safety of *K. phaffii* as a production platform is further supported by its Qualified Presumption of Safety (QPS) status with EFSA and an extensive record of safe use in the production of food ingredients and pharmaceuticals globally. Furthermore, a long-standing and well-documented body of toxicological evidence affirms its non-pathogenic and non-toxigenic nature.

Sub-chronic Oral Toxicity Studies

Several 90-day oral toxicity studies in Sprague-Dawley rats have been conducted on various protein preparations produced in *K. phaffii* (Table 16). These studies consistently establish a high No Observed Adverse Effect Level (NOAEL), which is the highest dose at which no statistically or biologically significant adverse effects are found. In nearly all cases, the NOAEL is established at the highest dose tested, indicating a very low order of toxicity.

The consistent outcome across these diverse proteins is a lack of any treatment-related mortality, clinical signs of toxicity, or adverse changes in body weight, food consumption, clinical pathology, or histopathology. This strong and consistent dataset supports the conclusion that purified protein preparations from *K. phaffii* do not cause systemic toxicity.

Table 16. Summary of Key Sub-chronic Oral Toxicity Studies on *K. phaffii*-Derived Protein Preparations

Test Substance / Recombinant Protein	Study Duration (days)	Species	Route of Exposure	NOAEL (mg/kg bw/day)	Key Finding / Comment	Reference
Soy Leghemoglobin Prep.	90	Sprague-Dawley rat	Dietary	Male: 4798.3 Female: 5761.5	No adverse effects observed; NOAEL was the highest dose tested.	Reyes et al., (2023)
Brazzein	90	Rat	Oral	2000	No adverse systemic effects; NOAEL was the highest dose tested.	Meetro et al., 2025
Recombinant Humanized Type III Collagen	90	Sprague-Dawley rat	Oral gavage	4500	No adverse findings observed; NOAEL was the highest dose tested.	Dai et al., (2025)
Phospholipase C (TOS)	90	Rat	Oral	≥ 1672	No adverse effects observed; NOAEL was the highest dose tested.	EFSA (2019)
Fungal Immunomodulatory Protein (GMI)	91	Sprague-Dawley rat	Oral gavage	100	No mortality or moribundity; NOAEL was the highest dose tested.	Fu and Hseu (2022)
Soy Leghemoglobin Prep.	28	Sprague-Dawley rat	Dietary	750 (as LegH)	No adverse effects attributable to consumption.	Fraser et al., (2018)
Recombinant Human Lactoferrin (rhLF)	14 & 28	Sprague-Dawley rat	Oral gavage	≥ 2000	Well tolerated; no toxicologically significant changes observed.	Peterson et al., (2024)

Shorter-term studies, typically 14 or 28 days in duration, are also used, often as dose-range-finding studies or to investigate specific endpoints. A 28-day dietary study on soy leghemoglobin established a NOAEL of 750 mg/kg/day (as leghemoglobin) and found no adverse effects (Fraser et al., 2018). Similarly, 14- and 28-day studies on recombinant human lactoferrin demonstrated that the product was well tolerated at doses up to 2,000 mg/kg/day with no toxicologically significant changes observed (Peterson et al., 2024). These shorter studies corroborate the findings of the 90-day studies and reinforce the overall conclusion of low systemic toxicity.

Genotoxicity and Mutagenicity Assessments

Genotoxicity testing is a critical safety endpoint designed to determine if a substance has the potential to damage DNA, which could lead to mutations or cancer. A suite of *in vitro* tests is available to examine this potential hazard. Protein preparations from *K. phaffii* have been subjected to these tests, with consistently negative results.

The bacterial reverse mutation assay (Ames test) is a widely used method for detecting a substance's potential to cause gene mutations. The test uses several strains of bacteria (e.g., *Salmonella typhimurium*, *Escherichia coli*) with pre-existing mutations that render them unable to synthesise a specific amino acid. A positive result occurs if the test substance causes a reverse mutation, allowing the bacteria to grow on a medium lacking that amino acid. Studies on soy leghemoglobin (Fraser et al., 2018; Reyes et al., 2022), brazzein (Meetro et al., 2025), and recombinant collagen (Dai et al., 2025) have all been conducted using the standard panel of bacterial strains, both with and without an external metabolic activation system (S9 mix) to mimic mammalian metabolism. In all reported cases, the results were negative, indicating that these preparations are not mutagenic.

To assess the potential for a substance to cause larger-scale damage to chromosomes (clastogenicity), *in vitro* assays using mammalian cells are employed. The most common are the chromosome aberration assay or the micronucleus assay, often conducted using human peripheral blood lymphocytes. These tests detect breaks, rearrangements, or loss of chromosomes following exposure to the test substance. As with the Ames test, preparations of soy leghemoglobin (Fraser et al., 2018; Reyes et al., 2022) and brazzein (Meetro et al., 2025) have been evaluated in these assays. The results have been uniformly negative, providing no evidence of clastogenic potential. The consistent negative findings across the full battery of genotoxicity tests provide strong evidence that food ingredients produced from *K. phaffii* do not pose a genotoxic risk.

Reproductive and Developmental Toxicity

Full reproductive and developmental toxicity studies are not always required for food ingredients unless suggested by the substance's structure or results from sub-chronic studies. However, there is some data available for *K. phaffii*-derived products. During a 28-day study on soy leghemoglobin, a potential effect on the oestrous cycle distribution in female rats was suggested, which prompted a follow-up study specifically designed to evaluate female reproductive health. This subsequent study found that female reproductive parameters were comparable between treated and control rats, alleviating the initial concern (Fraser et al., 2018).

In a 90-day study on soy leghemoglobin at target dietary levels of 30,000, 60,000, and 90,000 ppm, there were no adverse effects on terminal body weights, organ weights, or clinical parameters such as hematology, coagulation, clinical chemistry, thyroid hormones, and urinalysis parameters during either the main study or recovery phases (Reyes et al., 2025). Further, there were no significant alterations were found in the oestrous cycle distribution between the control and high dose groups during the main phase or recovery phase of the 90-day study (Reyes et al., 2025).

A developmental toxicity study was also conducted for recombinant humanised Type III collagen, which found no evidence of developmental toxicity in rats at doses up to 4.5 g/kg bw/day (Dai et al., 2025).

Collectively, these studies, while limited, suggest the lack of reproductive or developmental hazards associated with the consumption of *K. phaffii* host cell protein.

C.3.3 Toxicity of Beta-Casein

Beta-casein is a major milk protein constituting approximately 30-37 % of total casein content in bovine milk. It exists predominantly in two genetic variants: A1 and A2, which differ by a single amino acid at position 67 (histidine in A1 vs. proline in A2). This structural variation affects enzymatic cleavage during digestion, notably leading to the differential release of the opioid peptide Beta-casomorphin-7 (BCM-7) from A1 but not A2 Beta-casein (Chitra, 2021). While Beta-casein is broadly recognised as a high-quality nutritional protein, concerns have emerged about the potential adverse physiological effects of A1 Beta-casein and its metabolites, particularly BCM-7. This section reviews relevant toxicological data from both animal and human studies.

Evidence from Animal Studies

Multiple rodent studies have explored physiological responses to A1 versus A2 Beta-casein. In rats, A1 Beta-casein consumption has been associated with slower gastrointestinal transit times, a response attenuated by the opioid antagonist naloxone, implicating BCM-7 in the mechanism (Pal et al., 2015). Additional findings include elevated markers of gut inflammation (myeloperoxidase and IL-4), increased jejunal dipeptidyl peptidase-4 (DPP-4) activity, enhanced mucus secretion, and shifts in gut microbiota composition (Bolat et al., 2024).

In autoimmune-prone non-obese diabetic (NOD) mice, early-life exposure to A1 Beta-casein increased the incidence of type 1 diabetes in later generations (F3 and F4), accompanied by subclinical insulitis, impaired glucose regulation, and reductions in regulatory T cell populations, suggesting possible epigenetic effects (Chia et al., 2018).

In rabbit models, A1 Beta-casein consumption was linked to increased atherosclerotic risk markers including total cholesterol, LDL, triglycerides, and vascular lesions (Tailford et al., 2003; Fernández-Rico et al., 2022). However, similar effects have not been consistently demonstrated in humans.

Beyond BCM-7, other Beta-casein-derived peptides exhibit potential bioactivity. For example, a peptide fragment spanning residues 54-59 has shown immunostimulatory effects *in vitro*, enhancing mycobacterial clearance in human monocytic cells. Separately, a cationic peptide (INKKI) from Beta-casein hydrolysates demonstrated cytotoxicity against melanoma cells and reduced tumour progression in mouse models.

Evidence from Human Studies

Several double-blind, randomised controlled trials in adults and children have found that A1 Beta-casein may provoke gastrointestinal symptoms, particularly in individuals with self-reported milk intolerance or irritable bowel syndrome. In a crossover trial with 41 adults, A1 milk was associated with increased abdominal pain, bloating, and looser stools compared to A2 milk (Ho et al., 2014). Similar findings were reported in lactose-intolerant individuals and children, even when lactose levels were consistent across test products (Sun et al., 2015; Choi et al., 2024).

BCM-7 has been shown to stimulate histamine release *in vitro*. In a clinical study, intradermal administration of BCM-7 induced erythema and blistering in healthy individuals, consistent with a pseudoallergic response (Kurek et al., 1992). In children, A2 milk consumption was associated with modulation of immune markers,

including increased levels of IL-4, immunoglobulins (IgG, IgE, IgG1), and glutathione (Choi et al., 2024). Beta-casein has also been implicated in cow's milk protein allergy, though such reactions are not specific to the A1 variant (Host 2002).

BCM-7 may influence redox balance and neurological function through interference with glutathione synthesis. A randomised crossover trial reported lower circulating glutathione levels following A1 milk consumption, potentially via inhibition of cysteine uptake by the EAAT3 transporter (Deth et al., 2016). In infants, elevated blood BCM-7 concentrations after cow's milk formula feeding were associated with delayed psychomotor development (Wasilewska et al., 2011). While suggestive, these findings require further validation.

Ecological studies have proposed associations between A1 Beta-casein intake and non-communicable diseases such as type 1 diabetes (T1D), ischaemic heart disease, autism, and schizophrenia (Laugesen and Elliott, 2003). However, these correlations are inherently limited by confounding variables and cannot establish causality. More robust clinical trials have failed to consistently replicate such associations. For example, no meaningful changes in cardiovascular risk markers were observed in healthy adults following A1 or A2 milk consumption, although one study reported minor changes in HDL cholesterol, glucose, diastolic blood pressure, and heart rate (Tailford et al., 2003).

Safety assessment reports prepared by international agencies or other national government agencies, if available

The potential health risks associated with Beta-casomorphin-7 (BCM-7), a bioactive peptide released during digestion of A1 Beta-casein, have been the subject of multiple reviews by international and national regulatory agencies.

In 2009, the European Food Safety Authority (EFSA) conducted a comprehensive scientific evaluation of BCM-7 and related casomorphins. EFSA concluded that the available evidence did not support a causal relationship between the oral intake of BCM-7 and the development of non-communicable diseases such as cardiovascular disease (CVD), type 1 diabetes (T1D), autism spectrum disorders, or sudden infant death syndrome (SIDS) (EFSA, 2009). This conclusion was based on several key factors: the methodological limitations of the epidemiological studies cited, the absence of adverse effects following oral administration in experimental models (in contrast to effects seen with direct injection), and the physiological observation that BCM-7 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) and is poorly absorbed in healthy adults.

Subsequent evaluations by other agencies have generally aligned with EFSA's position. The New Zealand Food Safety Authority (NZFSA) reviewed similar data and did not find sufficient grounds to impose regulatory restrictions or warnings on A1 Beta-casein-containing milk (NZSFA 2004). Likewise, the United States Food and Drug Administration (US FDA) has not raised specific safety concerns regarding A1 Beta-casein or BCM-7; milk containing A1 Beta-casein continues to be permitted for general sale without labelling or warnings specific to BCM-7 content (FDA 2025). These regulatory positions imply that current scientific evidence does not justify classification of BCM-7 as a public health risk under normal dietary exposure conditions.

From a toxicological perspective, Beta-casein itself is widely recognised as a safe, high-quality dietary protein. The safety discussion has primarily focused on BCM-7 due to its opioid-like activity observed *in vitro* and in certain animal models. However, the physiological relevance of these effects following oral exposure remains uncertain. BCM-7 undergoes significant enzymatic degradation in the human gastrointestinal tract, particularly by DPP-4, which limits systemic bioavailability (EFSA, 2009).

No No-Observed-Adverse-Effect Level (NOAEL) or Acceptable Daily Intake (ADI) for BCM-7 has been established through standard toxicological protocols. According to the Globally Harmonized System (GHS) classification framework, BCM-7 falls within Category 5 for acute oral and dermal toxicity, which indicates it may be harmful if swallowed or in contact with skin, but only at relatively high doses (GHS 2021). This

classification supports the position that BCM-7 poses low acute toxicity risk under typical consumption levels.

Overall, existing safety assessments by leading regulatory bodies do not support restrictions on A1 Beta-casein or its metabolite BCM-7 for the general population. However, comparative studies suggest that milk containing only A2 Beta-casein may offer improved gastrointestinal tolerance in individuals with functional digestive sensitivity, which may guide product differentiation and consumer preference rather than regulatory action.

Safety Conclusions and Regulatory Perspective

The European Food Safety Authority reviewed the evidence in 2009 and concluded that a causal relationship between A1 Beta-casein (or BCM-7) and non-communicable diseases was not supported by sufficient evidence, particularly in humans. While some studies suggest that BCM-7 may influence gut function or immune responses, the data are inconsistent and not yet adequate for risk assessment purposes (EFSA, 2009).

Overall, Beta-casein, regardless of variant, is a widely consumed dietary protein with a long history of safe use. While there is evidence of differential effects between A1 and A2 variants, particularly on gastrointestinal symptoms in sensitive individuals, there is no robust evidence of toxicity or adverse effects in the general population. Further investigation is warranted, particularly to clarify the biological relevance of BCM-7 in infant and adult populations.

C.3.4 Potential Toxicity of BCPP1

Bioinformatic Analysis

To evaluate whether BCPP1 or its residual host cell proteins share identity with known protein toxins, bioinformatic analyses were conducted using BLAST (blastp) against the UniProtKB toxin dataset (7,839 reviewed and 94,680 unreviewed sequences as of 2 December 2024). No significant homologies were identified. This outcome indicates that BCPP1 does not share sequence features with recognised protein toxins, consistent with international guidance for protein safety assessments.

To assess whether the N-terminal heterogeneities identified in BCPP1 could generate novel allergenic or toxic peptide motifs, a comprehensive *in silico* analysis was conducted. This assessment included all N-terminal variants detected by high-sensitivity LC-MS/MS, ensuring a conservative safety evaluation. The sequences were subjected to simulated human digestion with pepsin, trypsin, and chymotrypsin. The resulting peptide fragments were screened against the AllergenOnline and UniProtKB toxin databases. No digestion fragments from any N-terminal variant showed significant homology to known allergens or toxins, nor were any predicted to be toxic. This confirms that the minor N-terminal variations do not introduce any new or unexpected safety risks.

Acute and Sub-chronic Toxicity Studies

Toxicity assessments of food proteins produced in *K. phaffii* (soy leghemoglobin, brazzein, recombinant collagen, phospholipase C, lactoferrin) demonstrate no adverse systemic effects in rodents even at high dietary inclusion levels (see above). Sub-chronic 90-day studies reported no changes in body/organ weights, clinical chemistry, or haematological parameters, establishing high NOAELs.

Collectively, these data demonstrate that purified proteins from *K. phaffii* are well tolerated and do not cause systemic toxicity.

Genotoxicity and Mutagenicity

Ames assays, micronucleus tests, and mammalian chromosome aberration studies conducted on comparable *K. phaffii*-derived proteins consistently returned negative results, providing strong evidence that food ingredients produced from this expression system do not pose genotoxic risk.

Reproductive and Developmental Toxicity

Reproductive and developmental endpoints have been examined for *K. phaffii*-derived soy leghemoglobin and recombinant humanised collagen. These studies, including a 90-day dietary study in rats and developmental toxicity studies in rodents, found no adverse effects on oestrous cyclicity, fertility, or developmental outcomes at high doses (up to 4.5 g/kg bw/day). No reproductive or developmental hazards are expected for BCPP1 given its structural identity to bovine Beta-casein and the absence of concerning findings in related proteins.

Special Considerations

Some studies have suggested that digestion of A1 Beta-casein releases BCM-7, which has been investigated for potential effects on immune and neurological function. However, BCPP1 encodes only the A2 variant of Beta-casein, which does not yield BCM-7 upon digestion. Consistent with EFSA's 2009 conclusion, no causal link has been established between Beta-casein intake and non-communicable diseases. Therefore, no additional toxicity concerns are raised by BCPP1.

Safety Conclusions

The evidence presented in this section demonstrates that BCPP1 does not raise novel or unexpected toxicity concerns. Bioinformatic analyses show no similarity to known protein toxins, toxicokinetic and metabolic considerations align with established dietary proteins, and data from comparable *K. phaffii*-derived proteins confirm the absence of systemic, genotoxic, reproductive, or developmental hazards. Furthermore, BCPP1 contains only the A2 variant of bovine Beta-casein, which avoids BCM-7 release and has a long history of safe dietary use. Taken together, these findings meet the requirements of both Guideline 3.5.1 and Guideline 3.5.2, supporting the conclusion that BCPP1 is safe for its intended use as a nutritive protein ingredient.

C.4 Other (non-protein) new substances

If other (non-protein) substances are produced as a result of the introduced DNA, information must be provided on the following:

2B.3(a) the identity and biological function of the substance

2B.3(b) whether the substance has previously been safely consumed in food

2B.3(c) potential dietary exposure to the substance

No other non-protein substances are produced through precision fermentation.

C.5 Novel herbicide metabolites in GM herbicide tolerant plants

Not applicable to this application

SECTION D: DIETARY INTAKE AND IMPACT

This section provides a comprehensive assessment of the dietary exposure, nutritional impact, and potential consumer behavioural impact associated with the introduction of the Beta-casein Protein Preparation into the Australian and New Zealand food supply. The assessment has been prepared in accordance with the requirements of the FSANZ Application Handbook, primarily Guidelines 3.5.1 (Section C) and 3.5.2 (Sections D, E, F).

D.1 Proposed Foods, Use Levels

BCPP1 is intended for use as a functional and nutritional protein ingredient in a range of food products, serving as a direct substitute for conventional dairy- and plant-derived proteins. The proposed food categories, specific product examples, and corresponding maximum use levels are detailed in Table 17.

These levels have been determined based on the minimum amount required to achieve a specific technological function (e.g., texture, emulsification, structural stability) in each product type, while ensuring organoleptic properties are not compromised. The rationale for the different use levels across various food matrices is also provided in the table. To aid in the nutritional assessment, the table includes a calculation of the final protein contributed to the food by BCPP1 at its maximum use level, based on the preparation's minimum 22% protein content.

Furthermore, to assist with a refined dietary exposure assessment, Section D.1.1 provides a list of food categories where BCPP1 would be technologically or functionally unsuitable for use.

D.1.1 Technologically Unsuitable Food Categories

To aid the dietary exposure assessment, BCPP1 would not be added to the following food categories for technological or functional reasons:

- Whole, unprocessed foods such as fresh fruits and vegetables, unprocessed meat, poultry, and fish, and whole eggs
- Clear beverages such as soft drinks, cordials, and flavoured mineral waters, where the opacity provided by casein would be undesirable
- Certain high-acid beverages (pH < 4.0): Where casein may precipitate and cause instability without specific formulation adjustments (e.g., addition of stabilisers)
- Standardised foods with strict compositional requirements: Such as butter, where the addition of a protein ingredient is not permitted under Standard 2.5.5 of the Code
- Foods that typically contain no protein: Such as sugar confectionery, herbs, spices, and pure fats and oils, where its addition serves no functional purpose.

D.1.2 Rationale for Proposed Maximum Use Levels

The proposed maximum use levels detailed in Table 17 are based on achieving a specific technological function and are not intended to position BCPP1 as the sole source of protein in most food categories.

Table 17. Proposed Food Categories and Maximum Use Levels for BCPP1

Food Category	Example Product Type	Applicable Regulatory Standard(s)	Proposed Maximum Use Level (% w/w)	Rationale for Use Level and Technological Function	Final Protein Contribution from BCPP1 (% w/w)*
Dairy Analogues	Milk Alternatives (e.g., oat, soy, almond)	Standard 2.6.2 (Non-alcoholic beverages and brewed soft drinks).	3.00 %	To provide a source of high-quality, animal-free protein. The use level is optimised to also contribute valuable creaminess, opacity, and mouthfeel characteristic of dairy milk.	~0.66 %
	Yoghurt-style Products	General food permissions. Not covered by Standard 2.5.3 (Fermented milk products).	5.00 %	To serve as a nutritive protein source. A higher level is required for the Beta-casein component to contribute to gel structure, texture, and water-holding capacity.	~1.10 %
	Cheese Analogues	General food permissions. Not covered by Standard 2.5.4 (Cheese).	5.00 %	To provide nutritive protein in an animal-free format. The use level allows the Beta-casein component to aid emulsification, melt characteristics, and flavour.	~1.10 %
Protein Beverages	Ready-to-drink (RTD) Protein Shakes	Standard 2.6.2 (Non-alcoholic beverages and brewed soft drinks).	10.00 %	To serve as a primary source of high-quality protein. The preparation's high solubility and stability are key functional properties for this application	~2.20 %
	Powdered Beverage Mixes	Standard 2.9.3 (Formulated supplementary foods).	15.00 %	To act as a concentrated source of nutritive protein in a dry mix format intended for reconstitution.	~3.30 %
Nutritional Products	Formulated Meal Replacements	Standard 2.9.3 (Formulated meal replacements and formulated supplementary foods).	15.00 %	To serve as a primary, high-quality protein source to meet specific compositional requirements for formulated foods.	~3.30 %
Bakery Products	Protein-enriched Breads, Biscuits, Snack Bars	Standard 2.1.1 (Cereal and Cereal Products).	8.00 %	For nutritional fortification with a high-quality protein. The use level is suitable to also contribute to dough texture, binding, and moisture retention.	~1.76 %
Mixed Foods	Soups, Sauces, Dressings	General food permissions, e.g., Standard 2.10.4 (Miscellaneous standards for other foods).	3.00 %	To provide nutritive protein. Lower levels are used where the Beta-casein component's properties of emulsification and creaminess are also desired.	~0.66 %

*Calculated based on the BCPP1 preparation containing 22% protein by weight as per Section A.5.1 of this dossier. These levels are comparable to, or supplement existing protein levels found in conventional versions of these products as reported in AUSNUT 2023 and the Australian Food Composition Database-release 2 (FSANZ 2023).

An analysis was conducted to determine the use level required for BCPP1 to provide the entire protein content for several benchmark products, based on typical protein levels in the AUSNUT database. This analysis revealed that achieving total protein replacement would require technologically and sensorially unfeasible inclusion rates. For example, to match the typical protein content of dairy milk (~3.4 %), a milk alternative would require a BCPP1 inclusion level of approximately 15.0 %. Such a high concentration would severely and negatively impact the product's texture, taste, and overall palatability, making it commercially non-viable.

Therefore, the proposed maximum use levels reflect a balance between delivering functional and nutritional benefits while maintaining the high quality and consumer acceptability of the final food product. This confirms BCPP1's intended role as a high-value functional ingredient rather than a bulk protein replacer in many applications.

D.2 Dietary Exposure Assessment

A dietary exposure assessment (DEA) was conducted to estimate the potential intake of BCPP1 for consumers in Australia and New Zealand (also see Supplement Report DIET_01_01). To provide a comprehensive risk characterisation, a two-model approach was used:

1. A highly conservative Maximum Potential Dietary Exposure model to establish a theoretical upper bound of safety.
2. A Refined Estimated Dietary Intake model to reflect a more realistic, likely intake scenario based on proposed product formulation and market projections.

D.2.1 Model 1: Maximum Potential Dietary Exposure

This worst-case model was designed to produce a conservative overestimation of intake to ensure safety is assessed under the most exaggerated, yet plausible, conditions. The model is based on the key assumption that BCPP1 replaces 100 % of all theoretically "replaceable" dietary protein. This replaceable fraction was determined to be 48 % for Australia and 64.5 % for New Zealand, based on an analysis of primary protein sources (e.g., whole meats, fish) that cannot be technologically substituted.

The model uses mean protein intake and body weight data from the latest available national nutrition surveys. Because the BCPP1 ingredient is 22 % protein by weight, the mass of the preparation required to supply the protein equivalent is 4.5-fold higher.

It is important to note that this model does not estimate exposure for high consumers (e.g., the 90th or 95th percentile), as this requires individual dietary records. The data is therefore provided to establish a conservative baseline mean exposure, with the understanding that FSANZ will conduct its own refined modelling to confirm high-consumer exposure levels.

Analysis of Mean Exposure in Australia and New Zealand

The analysis of the mean dietary exposure from the conservative "worst-case" model reveals a clear and expected trend related to age and body weight.

As shown in Table 18, the estimated mean exposure to the BCPP1 ingredient, when expressed on a body weight basis (g/kg bw/day), is highest in the youngest age groups and progressively decreases with age. This is a typical finding in dietary exposure assessments, as young children consume significantly more food relative to their low body weight to support rapid growth and development. The population group with the highest estimated mean exposure is males aged 2-3 years, at 8.73 g/kg bw/day.

When compared to the NOAEL of 4,798 mg/kg bw/day (see Table 16), even this mean exposure level for the most sensitive group presents a low Margin of Exposure (MOE). The mean protein intake for this group is calculated to be 1.9 g/kg bw/day (8.7 g/kg bw/day × 0.22 protein), resulting in an MOE of approximately 2.5.

This low MOE for an average consumer demonstrates the highly conservative nature of this theoretical model. It confirms that the exaggerated assumptions of the worst-case scenario are not suitable for a final risk conclusion and highlights the necessity of the Refined Estimated Dietary Intake (Model 2) to determine a more realistic exposure estimate and a meaningful margin of safety.

Similar trends are observed in the New Zealand population, with younger adult males exhibiting the highest exposures on a body weight basis. The group with the highest estimated mean exposure is males aged 19-30 years, at 4.91 g/kg bw/day.

This equates to a mean protein intake of 1,080 mg/kg bw/day (4,910 mg/kg bw/day × 0.22 protein). When compared to the NOAEL of 4,798 mg/kg bw/day (Reyes et al., 2024), this results in an MOE of approximately 4.4. Notably, high intakes were also observed for Māori and Pacific male populations, reflecting different dietary patterns.

The low MOEs calculated for mean consumers in the highest exposure groups in both countries underscore the highly conservative and theoretical nature of this model. This analysis confirms that the exaggerated assumptions are not suitable for a final risk conclusion but serve to establish a theoretical upper boundary, demonstrating the necessity of the Refined Estimated Dietary Intake (Model 2) to determine a realistic safety margin.

D.2.2 Model 2: Refined Dietary Intake Assessment

To provide a more realistic estimate of likely dietary intake than the theoretical substitution model (Model 1), refined deterministic assessments were conducted for both Australia and New Zealand. Due to differences in the structure of the publicly available national food consumption datasets, the specific methodologies for calculating intake were adapted for each country. Both approaches are scientifically sound and provide a conservative, health-protective estimate of mean dietary exposure.

Australia: Direct Ingredient-Based Assessment

For the Australian population, the dietary exposure assessment was conducted using a direct, ingredient-based methodology. The available data from the Australian Bureau of Statistics (2023-24) provides mean per capita consumption in grams per day for specific food categories. This allowed for a direct calculation of the BCPP1 ingredient intake for each food category. The methodology for this model is as follows:

1. **Food Consumption:** Mean daily per capita consumption data for relevant food groups was sourced from the Australian Bureau of Statistics (2025a).
2. **Use Levels and Market Share:** The proposed maximum use levels (from Table 17) and estimated market penetration rates (from 1-5 %) were applied to the consumption data for each food category.
3. **Exposure Calculation:** The final exposure (mg/kg bw/day) was calculated for two key demographic groups: young children (2-4 years) and the general population (15+ years), using mean body weights for each group sourced from the Australian Bureau of Statistics (2022).

Analysis shows that the primary dietary contributors to BCPP1 exposure are Bakery Products and Mixed Foods (Table 20). As expected, the final exposure on a body weight basis is highest for young children. For the 2–4-year-old age group, the estimated mean exposure ranges from 6.66 to 33.3 mg/kg bw/day, depending on the market penetration assumptions for different food categories.

Table 18. Protein consumption and estimated BCPP1 consumption in Australia

Demographic	Protein intake (g/d)[a]	Average Body Weight (Kg)[b]	Max Potential Protein from BCPP1 (g/day)[c]	Max Potential BCPP1 Ingredient intake (g/day) [d]	Final Exposure (g/kg bw/day)
Persons (years)					
2-4	54.6	16.4	26.208	119.13	7.26
5-11	71.7	25.5	34.416	156.44	6.13
12-17	96.4	65.4	46.272	210.33	3.22
18-29	96	73.6	46.08	209.45	2.85
30-49	95.6	80.45	45.888	208.58	2.59
50-64	88.8	82.4	42.624	193.75	2.35
65-74	82	80.2	39.36	178.91	2.23
75 and over	77.2	75	37.056	168.44	2.25
Males (years)					
2-4	57.1	16.6	27.408	124.58	7.50
5-11	77.2	25.7	37.056	168.44	6.55
12-17	113.5	70.1	54.48	247.64	3.53
18-29	111.7	80	53.616	243.71	3.05
30-49	108.7	87.9	52.176	237.16	2.70
50-64	100.6	89.5	48.288	219.49	2.45
65-74	90.7	87.4	43.536	197.89	2.26
75 and over	85.6	82.2	41.088	186.76	2.27
Females (years)					
2-4	52	16.2	24.96	113.45	7.00
5-11	65.8	25.3	31.584	143.56	5.67
12-17	78.5	60.3	37.68	171.27	2.84
18-29	79.5	66.7	38.16	173.45	2.60
30-49	83.2	73.05	39.936	181.53	2.48
50-64	77.5	75.6	37.2	169.09	2.24
65-74	73.9	73.6	35.472	161.24	2.19
75 and over	69.9	68.7	33.552	152.51	2.22

a: Protein intake was from Australian Bureau of Statistics. (2025b); **b**: Body weight data from Australian Bureau of Statistics (2022) was converted/interpolated using weighted averages to the same age categories of the Australian Bureau of Statistics. (2023) protein intake age ranges; **c**: an estimate of maximum potential protein for BCPP1 calculated based on 48 % of total protein intake; **d**: the maximum potential BCPP1 ingredient intake is the maximum potential protein for BCPP1 divided by the protein content of the BCPP1 (22 %).

Table 19. Protein consumption and estimated BCPP1 consumption in New Zealand

Demographic	Protein intake (g/day)[a]	Average Body Weight (Kg)[b]	Max Potential Protein from BCPP1 (g/day)[c]	Max Potential BCPP1 Ingredient intake (g/day)[d]	Final Exposure (g/kg bw/day)
Total Population	88	82	57.2	260	3.17
Males (years)					
15-18	108	81.5	70.2	319.09	3.92
19-30	113	84.4	73.45	333.86	3.96
31-50	113	89.6	73.45	333.86	3.73
51-70	92	87.8	59.8	271.82	3.10
71+	79	84.8	51.35	233.41	2.75
Total*	104	87	67.6	307.27	3.53
Females (years)					
15-18	69	71.9	44.85	203.86	2.84
19-30	73	75.2	47.45	215.68	2.87
31-50	79	78.3	51.35	233.41	2.98
51-70	71	74.1	46.15	209.77	2.83
71+	62	70.7	40.3	183.18	2.59
Total*	73	76.1	47.45	215.68	2.83
Māori					
Males (Years)					
15-18	102		66.3	301.36	
19-30	127		82.55	375.23	
31-50	118		76.7	348.64	
51+	95		61.75	280.68	
Total*	114	95.8	74.1	336.82	3.52
Female (years)					
15-18	67		43.55	197.95	
19-30	81		52.65	239.32	
31-50	76		49.4	224.55	
50+	68		44.2	200.91	
Total*	76	84.8	49.4	224.55	2.65
Pacific					
Males (years)					
15-18	117		76.05	345.68	
19-30	114		74.1	336.82	
31-50	167		108.55	493.41	
50+	86		55.9	254.09	
Total*	109	103.2	70.85	322.05	3.12
Female (years)					
15-18	66		42.9	195	
19-30	81		52.65	239.32	
31-50	87		56.55	257.05	
50+	73		47.45	215.68	
Total*	81	97.2	52.65	239.32	2.46

a: Protein intake was from University of Otago and Ministry of Health. (2011) **b:** Data from Ministry of Health. 2024; **c:** an estimate of maximum potential protein for BCPP1 calculated based on 65 % of total protein intake; **d:** the maximum potential BCPP1 ingredient intake is the maximum potential protein for BCPP1 divided by the protein content of the BCPP1 (22 %).

New Zealand: Protein-Based Extrapolation Assessment

For the New Zealand population, the available national survey data reports food consumption in terms of protein contribution (%) from major food groups, rather than per capita consumption in grams per day. Therefore, a protein-based extrapolation was required to estimate the likely intake of the BCPP1 ingredient.

This scientifically sound, multi-step approach was conducted as follows:

1. **Estimate Protein Intake per Food Group:** The total mean protein intake for New Zealanders was multiplied by the percentage contribution from each relevant food group to determine the grams of protein consumed from that group per day. For example: Total Replaceable Protein (64.5 g/day) × Milk Protein Contribution (8.8 %) = 5.68 g of protein from milk per day.
2. **Estimate Food Consumption (g/day):** The estimated protein intake for each food group was then used to back-calculate an estimated total consumption of the food itself, using a benchmark protein concentration for that food category (e.g., assuming dairy milk contains approx. 3.4% protein). For example: 5.68 g protein / 0.034 (protein content) = 167 g of milk consumed per day.
3. **Calculate BCPP1 Ingredient Intake**: This estimated food consumption amount (g/day) was then used in the same formula as the Australian assessment to determine the final intake of the BCPP1 ingredient. For example: 167 g/day × 5 % (Use Level) × 5 % (Market Share) = 0.417 g/day of BCPP1 ingredient.
4. **Exposure Calculation:** The final exposure (mg/kg bw/day) was calculated for key demographic groups using mean body weights for each group sourced from the Ministry of Health (2024).

While this method for New Zealand involves an additional modelling step, it represents the most rigorous and realistic approach possible given the available data. It provides a significantly more refined and scientifically robust estimate than the theoretical 'worst-case' substitution model (Model 1).

The major contributors to exposure in New Zealand are non-alcoholic beverages, bread, and milk (Table 21). The highest potential exposure was identified for New Zealand females, with an estimated mean intake ranging from 1.98 to 9.88 mg/kg bw/day. The contribution from "non-alcoholic beverages" is a particularly conservative estimate, as this food group consists predominantly of waters and juices with negligible protein, where BCPP1 would be added for novel functional purposes.

Risk Characterisation for Australia and New Zealand

The risk is characterised by comparing the highest estimated exposure from this refined model to the NOAEL of 4,798 mg/kg bw/day (see Table 16). A Margin of Exposure (MOE) of 100 or greater is generally considered protective of public health.

In Australia, the highest potential exposure identified across all populations and scenarios is for children aged 2-4 years, at 33.3 mg/kg bw/day. The resulting MOE of approximately 144 is well above the safety benchmark of 100. This provides a high degree of confidence that the proposed uses of BCPP1 in Australia are safe for even the most sensitive Australian population group.

In New Zealand, The highest potential exposure is for females, at 49.4 mg/kg bw/day. An MOE of approximately 97 is effectively at the safety benchmark of 100. This result is generated from a deliberately conservative model that incorporates multiple health-protective assumptions, including the use of the absolute maximum proposed use level in every calculation.

Overall, the refined dietary exposure models for both countries demonstrate a sufficient margin of safety. The MOE for the highest-exposed Australian group exceeds the safety benchmark, while the MOE for the highest-exposed New Zealand group is effectively at the benchmark. Given that these results are derived from conservative models, they provide a robust basis for concluding that the proposed uses of BCPP1 are safe and pose no toxicological concerns.

Table 20. Estimated Dietary Intake-Australia

Food Category	Example Product Type(s)	Relevant ABS Food Group(s) (2023–24)[1]	Daily Consumption (g/person/day)	Proposed Maximum Use Level (% w/w)	Calculated BCPP1 Intake per day (mg/day)[2]	Final Exposure (mg/kg BW/day)[3]	
						2-4 yr olds	**15+ yr olds**
Dairy Analogues – Milk Alternatives	Oat, soy, almond, coconut milks	Dairy and meat substitutes: Dairy milk substitutes (unflavoured + flavoured)	17.7	3.0	5.31–26.55	0.32–1.62	0.07–0.34
Dairy Analogues – Yoghurt-style Products	Soy or coconut yoghurt	Dairy and meat substitutes: Soy-based yoghurts	0.4	5.0	0.2–1.0	0.01–0.06	0.00–0.01
Dairy Analogues – Cheese Analogues	Plant-based cheese	Dairy and meat substitutes: Cheese substitute	0	5.0	0	0	0
Protein Beverages – RTD Shakes	Ready-to-drink protein shakes	Non-alcoholic beverages: Electrolyte, energy and fortified drinks	24.6	10.0	24.6–123	1.50–7.5	0.31–1.55
Protein Beverages – Powdered Mixes	Protein powders for reconstitution	Special dietary foods: Formula dietary foods	1.5	15.0	2.25–11.25	0.14–0.69	0.03–0.14
Nutritional Products – Meal Replacements	Formulated shakes or bars	Special dietary foods: Formula dietary foods	1.5	15.0	2.25–11.25	0.14–0.69	0.03–0.14
Bakery Products	Protein-enriched breads, biscuits, snack bars	Cereals and cereal products: Regular breads (53.0) + biscuits (21.8) + cereal/nut/fruit/seed bars (4.8)	79.6	8.0	63.68-318.4	3.88–19.41	0.80–4.02
Mixed Foods – Soups, Sauces, Dressings	Cream-based soups, pasta sauces, salad dressings, dips	Soup (4.0) + Savoury sauces and condiments (32.4)	36.4	3.0	10.92-54.6	0.67–3.33	0.14–0.69
					TOTAL ESTIMATED MEAN INTAKE	**6.66–33.30**	**1.38–6.89**
Milk, yoghurt, cheese and/or alternatives	All milk, yoghurt and cheese including alternatives	Total milk, yoghurt, cheese and/or alternatives (discretionary and non-discretionary)	264	5.0	132–660.0	8.05–40.24	1.67–8.33
Total Milk	Regular and Low Fat Alternative Milk	Milk Alternative Total	7.1	5.0	3.55–17.75	0.22–1.08	0.04–0.22

1: Apparent Consumption of Selected Foodstuffs, Australia, 2023–24 (Australian Bureau of Statistics, 2025a); **2&3**: Based on 1-5 % Market Share; **3:** Body weight data from Australian Bureau of Statistics (2022)

Table 21. Estimated Dietary Intake-New Zealand

Food Category[1]	Frequency (% of total population)[1]	Estimated Protein Intake (g/day)[2]	Estimated Food Consumption (g/day)[3]	BCPP1 Ingredient Intake (mg/day)[4]	Final Exposure (mg/kg BW/day)[5]					
					Males	Females	Māori Males	Māori Females	Pacific Males	Pacific Females
Milk	8.0	5.68	166.94	83.47–417.35	0.96–4.80	1.10–5.48	0.87–4.36	0.98–4.92	0.81–4.04	0.86–4.29
Dairy Products	2.0	1.29	28.67	8.60–43.00	0.10–0.49	0.11–0.57	0.09–0.45	0.10–0.51	0.08–0.42	0.09–0.44
Non-Alcoholic Beverages	2.5	1.61	537.50	537.50–2,687.50	6.18–30.89	7.06–35.32	5.61–28.05	6.34–31.69	5.21–26.04	5.53–27.65
Cheese	3.1	2.00	8.00	4.00–20.00	0.05–0.23	0.05–0.26	0.04–0.21	0.05–0.24	0.04–0.19	0.04–0.21
Dietary Supplements	0.4	0.26	0.43	0.65–3.23	0.01–0.04	0.01–0.04	0.01–0.03	0.01–0.04	0.01–0.03	0.01–0.03
Snack Bars	0.4	0.26	3.23	2.58–12.90	0.03–0.15	0.03–0.17	0.03–0.13	0.03–0.15	0.03–0.13	0.03–0.13
Bread	11.1	7.16	81.36	65.09–325.43	0.75–3.74	0.86–4.28	0.68–3.40	0.77–3.84	0.63–3.15	0.67–3.35
Biscuits	1.1	0.71	11.83	9.46–47.30	0.11–0.54	0.12–0.62	0.10–0.49	0.11–0.56	0.09–0.46	0.10–0.49
Cakes and Muffins	1.8	1.16	23.22	18.58–92.88	0.21–1.07	0.24–1.22	0.19–0.97	0.22–1.10	0.18–0.90	0.19–0.96
Soups and Stocks	1.1	0.71	47.30	14.19–70.95	0.16–0.82	0.19–0.93	0.15–0.74	0.17–0.84	0.14–0.69	0.15–0.73
Savoury Sauces	0.8	0.52	25.80	7.74–38.70	0.09–0.44	0.10-0.51	0.08–0.40	0.09–0.46	0.08–0.38	0.08–0.40
ESTIMATED MEAN INTAKE				**751.85–3,759.23**	**8.64–43.21**	**9.88–49.40**	**7.85–39.24**	**8.87–44.33**	**7.29–36.43**	**7.74–38.68**

1: Food groups and frequency used from the 2008/09 New Zealand Adult Nutrition Survey; **2:** The total mean protein intake for New Zealanders was multiplied by the percentage contribution from each relevant food group to determine the grams of protein consumed from that group per day; **3:** Estimated protein intake for each food group was then used to back-calculate an estimated total consumption of the food itself, using a benchmark protein concentration for that food category (e.g., assuming dairy milk contains approx. 3.4 % protein); **4:** This estimated BCPP1 ingredient intake based on proposed maximum use level and a 1-5 % market share; 5: Final exposure calculated using body weight data from Ministry of Health (2024).

D.3 Market Share and Consumption Trends

While the DEA uses a 100 % substitution model for a worst-case assessment, a more realistic scenario considers likely market penetration. Products positioned as "animal-free" or "plant-based" currently occupy a small market share (e.g., dairy and meat substitutes contribute ≤ 0.7 % of total dietary protein in Australia).

Eden Brew anticipates BCPP1 will be used in niche, value-added products rather than across mainstream commodity foods. Based on optimistic growth projections, it is assumed that BCPP1-containing products will achieve a maximum market penetration of between 1 % and 5 % depending on the food category over five years post-launch.

D.4 Information related to potential impact on consumer understanding and behaviour

The introduction of BCPP1 as a protein ingredient is not expected to cause nutritional imbalance. Its primary role is substitutional, and it provides a high-quality, complete protein source nutritionally equivalent to its bovine-derived counterpart. However, two key differences are noted:

1. **Mineral Content:** As detailed in Section A.5.5, BCPP1 has a lower mineral content, particularly calcium and magnesium, compared to dairy-derived Milk Protein Concentrate (MPC). Foods formulated with BCPP1 as a replacement for MPC would not be equivalent sources of these minerals unless fortified. This is not considered a risk, as consumers do not rely on a single ingredient for mineral intake, and final products will be required to declare their nutrient content on the nutrition information panel.
2. **Lactose Absence**: BCPP1 is free from lactose. This provides a nutritional benefit for individuals with lactose intolerance, allowing them to consume products with the functional and nutritional properties of casein without adverse gastrointestinal effects.

The use of BCPP1 is not expected to negatively impact the bioavailability of other nutrients. Its composition is free from anti-nutrients (e.g., phytates, lectins) that could interfere with mineral absorption.

D.5 Consumer Understanding and Behaviour

This assessment addresses the potential impact of BCPP1 on consumer understanding and dietary patterns.

D.5.1 Consumer Awareness and Understanding

Consumer awareness of Beta-casein as a milk protein is well-established, particularly within markets such as Australia and New Zealand where dairy plays a central role in dietary habits. The distinction between A1 and A2 Beta-casein variants is especially prominent due to widespread marketing and consumer education by the A2 Milk Company and related brands.

One survey indicated that one in four consumers can differentiate between A1 and A2 milk types, associating A2 Beta-casein with improved digestive comfort or reduced symptoms of milk intolerance (Roper, 2015). This alignment with a pre-existing consumer need for a gentler dairy option has been a cornerstone of the A2 milk category's growth and its ability to command a premium price.

While precision fermentation remains a relatively new concept to the average consumer, awareness is growing rapidly. A 2023 report from the Good Food Institute (GFI) indicates increasing consumer familiarity and acceptance of “animal-free dairy” produced using microbial fermentation. Education campaigns, sustainability messaging, and transparency in labelling have contributed to favourable perceptions, especially among younger demographics and environmentally motivated consumers (GFI, 2025).

Because the Beta-casein protein preparation proposed for use in foods is structurally identical to dairy-derived A2 Beta-casein, and because its functional role is consistent with consumer expectations for milk proteins (e.g., nutritional value, high-quality protein), the risk of confusion or misinterpretation is low. Appropriate product labelling and ingredient declarations will further support informed consumer choice, particularly when transparency is maintained regarding the source (e.g., "fermentation-derived" or "non-animal").

D.5.2 Potential Consumer Behaviour

The introduction of precision fermentation-derived Beta-casein is expected to complement, rather than displace, current consumer behaviours. Products containing this nutritive substance are typically positioned as animal-free, allergen-reduced, or sustainable alternatives to traditional dairy. As such, the primary behavioural shift is expected to occur among:

- Lactose-intolerant individuals, who may be drawn to formulations made without lactose or with reduced allergenic potential
- Environmentally conscious consumers, seeking lower-impact protein sources
- Ethical consumers, including vegetarians or flexitarians, who avoid animal-derived ingredients but still seek the nutritional functionality of dairy proteins.

No evidence suggests that the availability of such products would lead to inappropriate dietary substitutions or displace more nutrient-dense foods. On the contrary, the proposed Beta-casein preparation offers a complete amino acid profile, comparable digestibility, and is free from other common dairy allergens (e.g., whey proteins such as Beta-lactoglobulin), potentially broadening nutritional access for sensitive populations (Keppler et al., 2021).

Because the ingredient is expected to be used primarily in formulated dairy analogues (e.g., milk alternatives, protein beverages, yoghurts), consumer expectations around usage, serving size, and nutritional contribution are already well established. These products fall into familiar categories, and their format and labelling are unlikely to disrupt normal eating patterns.

Market research in Australia and globally (e.g. BCG 2021; GFI 2025) suggests that consumers are increasingly accepting of precision-fermented ingredients, particularly where their function, safety, and source are clearly explained. The positioning of BCPP1 as an animal-free protein with the nutritional benefits of dairy aligns with these emerging preferences. Similar products on market, such as animal-free whey protein or casein analogues, have demonstrated consumer uptake when accompanied by clear labelling and communication.

D.5.3 Impact on Population Groups

The Beta-casein protein preparation is not associated with any specific health risks to vulnerable population groups, including children, pregnant women, older adults, or culturally defined subgroups.

- For infants and young children, the product is not intended for use in infant formula or follow-on formula unless specifically evaluated under separate regulatory pathways. Its use in general foods or beverages aligns with standard dietary exposure to casein proteins, which are routinely consumed from dairy
- Among culturally defined populations (e.g., those following vegetarian, Halal, or Kosher diets), the fermentation-derived nature of the ingredient offers potential advantages. It enables inclusion of dairy-like functionality without requiring animal husbandry, which aligns with certain ethical, environmental, or religious preferences. Provided production strain use and purification methods

meet relevant religious standards, the ingredient may be acceptable under Halal or Kosher certification

- Allergenicity risk is low. As a single protein fraction, the Beta-casein preparation excludes major allergenic whey proteins such as Beta-lactoglobulin, which are more often implicated in cow's milk protein allergy. However, as a milk-identical protein, the ingredient may still elicit reactions in individuals with casein-specific IgE sensitisation, and this would be appropriately managed through allergen labelling in accordance with Standard 1.2.3 (Information Requirements–Warning Statements and Declarations).

Consumption of this Beta-casein protein preparation is not expected to adversely impact any specific population groups. Rather, it provides an alternative protein source with comparable nutritional benefits, expanded accessibility, and minimal potential for unintended behavioural or health impacts when used as intended.

PART 3: REFERENCES CITED

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