Final Report

Final Report: Kokumi flavour ingredient from beef lung- development of pilot scale processing

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Abstract

The potential of beef lung and liver as kokumi seasoning ingredients was first evaluated at bench-scale in a previous project (No: 2024-1087). Building on those findings, this project (No: 2025-1063) advanced to pilot-scale production, cost estimation and the nutritional assessment of beef lung (Lung–G) for human consumption and potential export to Australia. Lung-G is designed to enrich food flavour by adding depth and savoury complexity, while also supporting sustainable use of underutilised carcass parts.

The project consisted of three milestones:

Milestone 1 established a food-grade pilot-scale production process and collected mass balance data (ingredient inputs and waste outputs). From 54 kg (wet basis) of beef lung, the process yielded 7.35 kg (dry basis) of Lung-G. Product's kokumi taste potential was confirmed by the calcium-sensing receptor (CaSR) assay.

Milestone 2 completed a preliminary cost estimate for the commercial production of Lung-G, accounting for raw material, yields, and equipment costs. Two drying scenarios were modelled: 1) fully in–house freeze-drying processing, and 2) outsourcing freeze-drying and milling. Following this analysis, a third option, roller dryer without ultra-high temperature (UHT) treatment was evaluated and shown to significantly reduce the costs from approximately AUD 100/kg to AUD 60/kg.

Milestone 3 determined the nutrient profile of Lung-G, including amino acids, vitamins, minerals, and bioactive peptides. The results highlight opportunities to enhance its nutritional value, reduce sodium content to strengthen its positioning as an iron- and B12-rich supplement, and achieve cost efficiencies through streamlined processing.

Overall, this study confirmed beef lung's potential as a kokumi ingredient, offering the meat industry a value addition pathway to utilise animal offal.

1.0 Executive summary

AgResearch, in partnership with the Australian Meat Processor Corporation (AMPC), successfully scaled up the production of Lung-G, a kokumi-rich extract derived from beef lung to pilot scale. Building on the lab-scale findings that demonstrated its flavour-enhancing potential, 54 kg of beef lung (indicate fresh weight or dry solids basis here) was processed to yield 7.3 kg of freeze-dried powder. The commercial feasibility was modelled through a benefit cost analysis based on this pilot-scale process. The project aims to convert underutilised bovine offal into a high-value, natural flavour enhancer, meeting consumer demand for natural ingredients while supporting sustainability and creating added value for red meat processors.

This initiative addresses two industry challenges:

- Underutilisation of edible co-products like beef lung.
- Market signals for natural, functional flavour ingredients to replace synthetic additives.

Red meat processors, product developers, and AMPC levy payers stand to benefit through increased revenue, greater resource efficiency, and delivering an innovative product to market. This project establishes the foundation for process optimisation and industry adoption, opening new commercial opportunities for transforming low-value co-products into high-value ingredients.

Objectives and Methodologies

Objective 1: Scale-Up and Kokumi Flavour Integrity

A food safety risk assessment ensured compliance with Australian export standards. Pilot-scale processing achieved 68.1% dry matter recovery, with key parameters (enzyme activity, pH, temperature, drying) optimised. Kokumi flavour was verified using in vitro taste receptor assays.

Objective 2: Manufacturing Cost Estimation

Three cost models were developed: in-house processing (AUD $100/kg, $2.03M/year), outsourced freeze drying ($96/kg, $1.95M/year), and drum drying ($61/kg, $1.23M/year). Analysis identified freeze drying and enzyme use as key cost drivers, with batch size and utility rates needing refinement.

Objective 3: Chemical Analysis of Nutrient Profile

- Lung-G is rich in B-complex vitamins, particularly B12 (11.4 µg/100 g), minerals such as iron (24 mg/100 g), with 62.7% of total amino acids as free amino acids, and bioactive peptides. At a 1% inclusion rate in a 250 g serving, it meets 11.8% of the recommended daily intake for B12 and 7.5% for iron. High sodium content (5,100 mg/ 100g Lung-G) from enzymatic treatments caused it to fail the FSANZ Food Standard 1.2.7 Nutrient Profiling Scoring Criterion. The elevated sodium levels stem from the use of sodium hydroxide to optimise glutaminase enzyme activity at pH 10.0. To reduce sodium content, an alternative alkali, such as potassium hydroxide, could be considered.

Key Results and Findings

- Pilot production retained kokumi activity and passed microbiological safety tests, qualifying as food-grade.
- Cost estimates showed drum drying as the most cost-effective option.
- High sodium levels limited health claim eligibility under FSANZ standards.
- The product contains high levels of B-complex vitamins, particularly vitamin B12 (11.4 µg/100g), and iron (24 mg/100g), which could contribute to 12% of the Recommended Dietary Intake (RDI) for B12 and 7.5% of the RDI for iron per 250 g serving of food with 1% Lung-G inclusion.

Benefits to Industry

- Enables scalable upcycling of beef lung into a premium ingredient.
- Creates new revenue streams through improved offal utilisation.
- Supports innovation in natural flavour development, aligning with market trends.

Future Research and Recommendations

- Optimise enzyme use and explore membrane filtration to reduce costs.
- Investigate sodium reduction to meet FSANZ standards.

- Investigate various final product formats, such as liquid, concentrate, or powder, prepared using different drying methods, including drum drying.
- Validate site-specific costs (utilities, labour) via industry collaboration.
- In-market consumer sensory test of the kokumi enhancing ability of Lung-G in food applications.
- Conduct market research to determine the selling price and complete the next level of techno-economic analysis which would use the selling price to estimate internal rate of return and payback time.

This project positions Lung-G as a sustainable, high-value ingredient, with potential for global market adoption. Although a pilot-scale manufacturing cost estimate was completed, full economic viability was not assessed and would require a detailed comprehensive techno-economic analysis (TEA), with market analysis. Nevertheless, Lung-G demonstrates preliminary product value chain potential: it is desirable (natural, flavour-enhancing, nutrient-rich), feasible (pilot-scale production retained functional peptides), and potentially viable (low inclusion rate and minimal per-serving cost), supporting its commercial appeal in premium markets. However, sensory testing in actual food systems is still required to validate these dosages and confirm that the kokumi effects translate to real consumer perception.

2.0 Introduction

The red meat industry continues to face challenges related to the underutilisation of edible co-products (Lynch et al., 2018) and of growing consumer demand for clean-label, natural ingredients (Balasubramaniam et al., 2023). One such underutilised co-product is beef lung, which is often rendered or discarded despite its nutritional potential (Jayawardena, 2020). At the same time, the food industry is seeking alternatives to synthetic flavour enhancers that align with consumer expectations of natural product. This project addresses both issues by exploring the commercial potential of Lung-G, a kokumi-rich extract derived from beef lung, as a natural flavour enhancer.

Kokumi taste compounds are known to enhance distinct sensory attributes of mouthfulness, continuity, and complexity in foods (Kuroda and Miyamura, 2015). We previously through laboratory-scale research with AMPC identified that enzymatic hydrolysis of beef lung can generate kokumi-active peptides. The current project builds on these findings by scaling up Lung-G production to a pilot scale, verifying its kokumi activity using a taste receptor assay, and generating a manufacturing cost estimate to inform future commercialisation efforts and to identify potential nutrient benefits.

The primary audience for this research includes red meat processors, product developers, and co-product value chain stakeholders such as AMPC. The outcomes aim to support these groups by identifying new revenue opportunities, improving co-product utilisation, and contributing to the development of innovative, clean-label food ingredients.

3.0 Project objectives

- Scale up food-grade Lung-G production in a pilot plant and test its kokumi flavour integrity through a receptor assay.
- Estimate the manufacturing cost of the Lung-G pilot plant production process.
- Determine the nutrient profile of Lung-G and identify its health claims potentials.

4.0 Methodology

Objective 1: Scaling Up Food-Grade Lung-G Production and Flavour Validation

Based on our risk assessment process, we modified our enzymatic process as reported in Milestone 1 report to make it safe for human food consumption (Figure 1) and to fit within the operating constraints of the pilot plant.

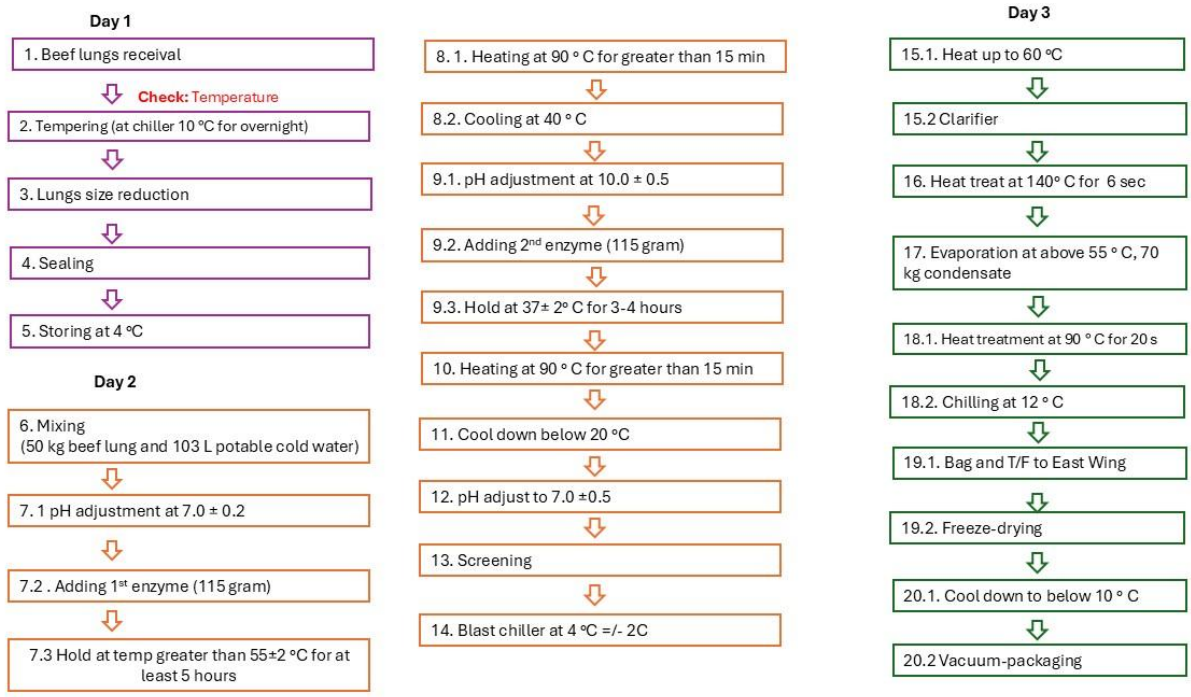

Figure 1. *Schematic methodology for the pilot plant scale-up of Lung-G kokumi extract production.*

Processing of beef lung hydrolysate for kokumi-rich ingredient development

Frozen beef lung from Taylor Preston Ltd NZ, a NZ MPI-registered meat processing company was tempered at 4°C for two days. It was chopped and 54 kg combined with 104 L of water in an Optimix paddle mixer vat. A two-stage enzymatic hydrolysis followed. In Stage 1, the pH was adjusted to 7.0 ± 0.2 for protease–A, Amano 2SD (115 g), incubated at 55 ± 2°C for 5 hours. The enzyme was inactivated by heating to 90°C for 15 minutes, then cooled to 40°C. Stage 2 aimed to enhance γ-glutamyl compound formation using glutaminase SD-C100S (115 g) at pH 10.0 ± 0.5, incubated at 37°C for 4 hours. A CCP heating step at 90°C for 15 minutes followed, and the hydrolysate was cooled to 20°C and screened to remove tissue residue.

The screened hydrolysate was stored overnight at 4°C, clarified at 60°C (Westfalia clarifier), and sterilised at 140°C for 6 seconds (Appendix 1). Concentration via a scraped-surface evaporator (Tetra Pak) at 55°C reduced volume before freeze drying. A secondary heat treatment (90°C for 20 seconds) was applied before freeze drying, yielding 7.35 kg of powder. During processing, two types of waste were generated: tissue waste and liquid waste. Tissue waste (25.0 kg wet weight; 3.6 kg dry matter) refers to the solid residue remaining after enzymatic hydrolysis processing of the lungs. Liquid waste (26.0 kg wet weight; 1.8 kg dry matter) refers to the aqueous by-products released during processing, including residual water, soluble

compounds, and any other liquids separated from the tissue. The combined dry matter from tissue and liquid waste amounted to 5.4 kg.

A 500 g sample underwent comprehensive safety and quality testing, including pathogen testing, aerobic plate counts, water activity, and moisture. Kokumi activity was assessed via in-vitro taste receptor assays at three stages: post-evaporation (PE), freeze-dried (FD), and oven-dried (OD, from PE at 55°C to constant weight). The total peptide contents of 1% solutions of the three extracts in water were determined, and dilutions containing 1 mg/mL of peptides were then prepared. Serial dilutions in water were performed from 1 mg/mL down to 0.00039 mg/mL and tested for receptor activation in cell-based fluorescence assay following our published method (Kim, 2024). Potency (strength) and efficacy (maximum response and highest dose) were calculated from relative fluorescence units (RFU) to evaluate kokumi compound stability and effectiveness across drying methods.

Data were analysed in GraphPad Prism software using a non-linear regression (four-parameter model) for dose–response fitting, and two-way ANOVA followed by Tukey's post hoc test for multiple comparisons.

Objective 2: Estimating Manufacturing Costs for Lung-G Production

The manufacturing cost estimate was based on data from the pilot-scale trial (Milestone 1), including process yields and product stream masses. These informed the development of a process flow diagram (PFD) and mass balance to:

Determine process yield and material flows

- Quantify raw materials and outputs
- Identify and size main plant items (MPIs)
- Estimate utility requirements

Additional input (e.g., utility costs, production rate) was provided by David Carew (AMPC).

The manufacturing cost was calculated using the method described by Coulson et al. (1983) **Error! R eference source not found.**. It was recognised early in the costing process that freeze drying would be a significant cost (either as a capital investment or as outsourcing to a contract freeze dryer). For this reason, three scenarios were developed:

- **Scenario 1:** Freeze drying - all processing in-house
- **Scenario 2:** Freeze drying and milling outsourced
- **Scenario 3**: Roller dryer and no UHT

Scenario's 1 and 2 are based on the pilot-scale process used in Milestone 1. Scenario 3 is a lower cost option that is based on the following assumptions:

A roller dryer can be used instead of a freeze dryer. This type of dryer is lower cost, but the product is exposed to a much higher temperature than when freeze drying. Our preliminary results indicate this should not be detrimental to the Lung-G.

The UHT is not needed. The UHT was added as a microbial control step for running the pilot-scale process in our food grade facility. Scenario 3 assumes this is not needed on a commercial scale – this assumption needs verification.

Table 1. Summary of production costs.

Variable costs		
1	Raw materials	From mass balance
2	Miscellaneous consumables	10% of item (5)
3	Utilities	From mass balance
4	Shipping and packaging A	Usually, negligible
	Sub-total A	
Fixed costs		
5	Maintenance	5-10% of fixed capital
6	Operating labour	From manning estimates
7	Supervision	20% of item (6)
8	Plant overheads	50-100% of item (6)
9	Capital charges	10-20% of fixed capital
10	Insurance	1-2% of fixed capital
11	Rates	1-2% of fixed capital
12	Royalties	1-5% of fixed capital
	Sub-total B	
	Direct production cost (A + B)	
Other costs		
13	Sales expense	20-30% of
14	General overheads	direct production
15	Research and development	cost
	Sub-total C	
	Annual production cost (A + B + C)	
	Production cost $/kg = Annual production cost / Annual production rate	

[A] For this costing, the process ends when the bulk extract has been milled after drying.

Key Inputs and Assumptions

- **Raw Materials:** Quantities measured during the trial; prices sourced from suppliers and AMPC. Currency conversion used NZD 1 = AUD 0.93.
- **Utilities:** Included steam, electricity, and cooling water. Steam and electricity costs from AMPC; water costs estimated from literature. Heating/cooling demands were calculated using thermodynamic equations.
- **Electricity:** Estimated from MPI power ratings multiplied by runtime and energy cost.
- **Freeze Drying:** Costs obtained from an Australian contract provider (Forager Foods).
- **Labour:** Based on pilot-scale experience.
- **Equipment Costing:** MPIs sized for a batch of 600 kg lung (yielding 81 kg powder). Vendor quotes were obtained and converted to AUD as needed.
- **Fixed Capital Estimation:** Lang factors of 2.0 (brownfield) and 3.2 (greenfield) were applied to the total equipment cost, assuming modular, turnkey equipment.

- **Production Rate:** 600 kg/day for 250 days/year = 20,250 kg powder annually, based on a 13.5% yield.

Objective 3: Analysis of Nutritional (amino acid composition, vitamin, and mineral) and Functional (bioactive peptide identification) Properties of Lung–G

Chemical analysis of the nutrient profile included free and total amino acid compositions, vitamin A, E and B-complex, minerals, and bioactive peptides. These analyses were performed to characterise the nutritional and functional components of Lung-G. In this study, vitamin and mineral analyses were conducted at the Massey Nutrition Laboratory, while amino acid analysis and bioactive peptide analysis were performed at AgResearch Ltd. Detailed analytical methods are described below.

Vitamin B1, B2, B3, and B6 were measured following a method of AOAC 2015.14. Vitamin B5 was measured following a method of AOAC 2012.16. Vitamin B7 was measured following a method of AOAC 2016.02. Vitamin B9 was measured following a method of AOAC 2013.13. Vitamin B12 was measured following a method of AOAC 2014.02. Minerals were measured by using Inductively-Coupled Plasma (ICP) mass-spectroscopy. Vitamin A and E were measured by using high-performance liquid chromatography with diode array detection method.

Regarding total amino acid composition analysis, three different analytical methods are required to measure the full range of protein-derived amino acids, due to chemical sensitivity and instability of some residues. The methods are:

- Acid stable amino acid by acid hydrolysis AOAC 994.12 (modified)
- Sulphur amino acids by performic oxidation AOAC 994.12
- Tryptophan by alkaline hydrolysis AOAC988.15

Free amino acid analysis was conducted using a modified Pickering Method (MA 383). Samples were prepared by lithium buffer SSA extraction with an internal standard to ensure accurate quantification. Amino acids were separated via Shimadzu 20Ai ion exchange chromatography, utilising a lithium buffer system for optimal resolution. Post-column derivatisation was performed with the Pickering Pinnacle PCX system, followed by UV/Vis detection at 440/570 nm to identify and quantify the amino acids.

Peptides and proteins were identified by liquid chromatography-trapped ion mobility spectrometry-tandem mass spectrometry (LC-TIMS-MS/MS) analysis. Subsequently, bioactive peptides analysis was performed by matching the peptides identified in Lung-G against a database containing 53,360 known bioactive peptides that was constructed from 16 online databases and directly from the scientific literature.

5.0 Results

5.1 Milestone 1: Successful Scale-Up and Kokumi Flavour Integrity Testing

The first milestone of this project involved scaling up the production of Lung-G, a kokumi-active extract derived from beef lung, validating its flavour functionality, and confirming its food safety and regulatory compliance. This section summarises the key outcomes of the pilot-scale process, including production yields, CaSR assay for kokumi activity, and microbiological testing for food safety.

5.1.1 Scale-Up Production Yield and Mass Balance

Table 2. *Summary of the scaled-up process with ingredients and final product details.*

Category	Wet weight (kg)	Dry matter (kg)	Protein (kg)	Protein % (%DM)	Collagen (kg)	Collagen % (%DM)
INPUTS						
Raw beef lung	54	10.8	9.2	85.6 %	2.5	22.8%
Protease enzyme		0.1				
Glutaminase enzyme		0.1				
NaOH (DM)		0.7				
Lactic acid (DM)		0.5				
Sum of inputs		12.2				
OUTPUTS						
Final product (Lung-G)	7.3	6.8	4.3	64.3 %	1.0	15.4%
Side streams						
Tissue	25	3.6	2.8	78.1 %		
Liquid	26	1.8				
Sum of outputs		12.2				

Note - DM, dry matter

Table 2 provides a summary of the mass balance. From 10.8 kg of raw beef lung dry matter, 7.3 kg of freeze-dried Lung-G powder was produced, achieving a 68.1% yield. The dry matter of Lung-G was 6.8 kg, calculated based on the freeze-dried powder's moisture content of 7.6%. The total input materials, summing to 12.2 kg, correspond exactly with the 12.2 kg of total output products after processing.

The Lung-G powder contains 64.3 % protein (dry matter basis), representing a 46.7% recovery of proteins in the raw beef lung. On a wet basis, the protein content of Lung-G is 54.9 %, equivalent to a total amino acid content of 538.6 mg/g, as detailed in Table 9.

Two side streams were generated by this process, an insoluble tissue residue and a liquid stream. The tissue residue stream accounts for about ~30% of the raw beef lung material, with a considerably high protein content (78.1%). The process generated a liquid waste stream with 1.8% solids.

The raw beef lung material contained 22.8% collagen (2.5 kg), with 1.0 kg recovered in Lung-G, equating to 15.4% collagen in the powder. The remaining 1.5 kg of collagen is likely retained as insoluble proteins in the tissue residue stream. This collagen-rich side stream could potentially be used for applications such as cosmetics or wound products, pending further analysis of collagen quality.

5.1.2 Flavour Integrity: Kokumi Taste Receptor Assay

The kokumi activity of Lung-G was evaluated using a CaSR bioassay (Kim, 2024, Kim et al., 2022). Three different processing formats were tested:

- **PE**: Post-evaporation liquid concentrate
- **OD**: Oven-dried powder
- **FD**: Freeze-dried powder

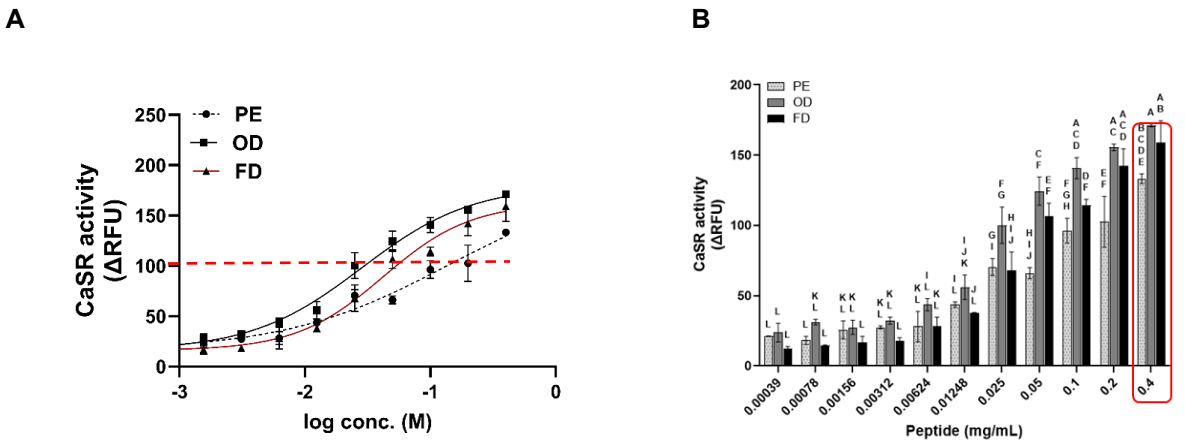

Figure 2. *Results of in vitro taste receptor assay for three beef lung kokumi extract samples: post-evaporation (PE), oven-dried (OD), and freeze-dried (FD).* ***Panel A:*** *Dose–response curves illustrating the half-maximal response (EC_{50}) for each sample.* ***Panel B:*** *Efficacy, shown in red box as the maximum response at the highest concentration (0.4mg/mL) of Lung-G tested. Different letters above bars represent significant differences between different concentrations, p<0.05.* Red dotted line represents human sensory threshold for kokumi flavour intensity.

Table 3. *Potency values as the half-maximal protein concentration (EC_{50} in mg/mL) and efficacy values of the maximum response at the highest concentration as relative fluorescent units (RFU) for three samples of beef lung kokumi extract.*

Sample	Potency as EC50 (mg/mL)	Efficacy as Max Response (RFU)
OD	0.028	160

FD	0.042	170
PE	0.081	133

All samples showed a clear ability to activate the kokumi taste receptor (Figure 2A, B; Table 3), confirming that kokumi flavour-enhancing components were retained during scale-up. Potency comparisons showed that the oven-dried sample reached the target response at the lowest concentration (lowest EC50) suggesting greater efficiency despite not having the highest overall response at highest concentration (efficacy) (Table 3). In terms of efficacy, both the freeze-dried (FD) and oven-dried (OD) samples achieved similarly high maximum responses, which were greater than the post-evaporation sample (PE) (Table 3). This suggests that functional kokumi peptides were largely preserved during drying whereas lower efficacy of PE may partly reflect uncertainty in solids determination rather than an inherent loss of activity.

The oven-dried (OD) sample exhibited the highest potency, followed by freeze-dried (FD), and then post-evaporation (PE). These results suggest that while drying method may influence potency and efficacy, kokumi peptide integrity is largely retained across processing types.

5.1.3 Food Safety and Regulatory Compliance

A critical aspect of this milestone was verifying food safety and ensuring compliance with New Zealand Ministry for Primary Industries (MPI) standards. A full risk assessment was conducted, identifying **three critical control points (CCPs):**

- **UHT treatment** ≥140 °C for ≥6 seconds
- **Heat treatments** at ≥90 °C for ≥15 minutes
- **Water activity reduction** to below 0.6

Final product testing confirmed compliance with microbiological safety parameters as shown in Table 4.

Table 4. *Microbial safety parameters, water activity, and moisture content of final beef lung hydrolysate powder as analysed by an accredited laboratory.*

Parameter	Result	Standard Met
Listeria spp.	Not detected	✓
Salmonella spp.	Not detected	✓
E. coli	<1 cfu/g	✓
Staph. aureus	<10 cfu/g	✓
B. cereus	<10 cfu/g	✓
C. perfringens	<10 cfu/g	✓
Aerobic Plate Count	<100 cfu/g	✓
Moisture Content	5.7%	✓
Water Activity (aw)	0.247	✓

These results confirm the microbial safety of Lung-G (Table 4) and validate the process as suitable for producing a food-grade ingredient for human consumption.

5.2 Milestone 2: Manufacturing Cost Estimation for Pilot Plant Production

Three processing scenarios were assessed based on a streamlined production process (Figure 1, Appendix 2):

- **Scenario 1** – Freeze drying - all processing in-house
- **Scenario 2** – Freeze drying and milling outsourced
- **Scenario 3** – Roller dryer and no UHT

The estimated production cost was AUD $100/kg of product for the in-house freeze-drying scenario, $96/kg for the outsourced freeze-drying scenario, and $61/kg for the roller drying scenario, as shown in Table 5. For the assumed production rate this gives annual production costs of $2.03 million (freeze drying in-house), $1.95 million (freeze drying outsourced) and $1.23 million (roller drying), with the outsourced option proving slightly more economical overall than freeze drying in-house, and using a roller dryer (and no UHT) being the lowest cost option.

The fixed capital investment required was lowest for the roller dryer scenario, followed by the outsourced freeze-drying scenario. As expected, there is a significant cost difference between a brownfield (within an existing facility) setup and a greenfield (new site) setup (Table 6).

Table 5. *Summary of estimated annual production costs.*

Scenario	Cost per kg (AUD)	Annual Cost (AUD)
Freeze drying - all processing in-house	$100	$2,033,000
Freeze drying and milling outsourced	$96	$1,952,000
Roller dryer and no UHT	$61	$1,234,000

Table 6. *Kokumi process total fixed capital cost for a production rate of 20,250 kg per year.*

Scenario	Greenfield (AUD)	Brownfield (AUD)
Freeze drying - all processing in-house	$6,940,000	$4,340,000
Freeze drying and milling outsourced	$2,590,000	$1,620,000
Roller dryer and no UHT	$2,870,000	$1,790,000

Table 7 provides a detailed breakdown of both variable and fixed costs, showing that raw materials, enzymes, and contract freeze drying (in the outsourced model) are the most significant contributors to total costs.

To visually demonstrate where costs are concentrated, Figure 2, Figure 3 and Figure 4 illustrate the breakdown of variable manufacturing costs for each scenario. These figures help highlight key areas like enzyme use and freeze drying where cost reduction strategies could be focused. The most expensive equipment items (if purchased in-house) were the freeze dryer, UHT unit, and evaporator, as detailed in Table 8. Opportunities to reduce capital costs include exploring alternatives to freeze drying, such as roller drying (Scenario 3) or producing a concentrated liquid instead of a powder. Similarly, membrane filtration could potentially replace the costly evaporator step. A preliminary sensitivity analysis found that if contract freeze drying costs increase by 50%, the outsourced scenario becomes more expensive than the in-house

model. This reinforces the need for ongoing vendor validation and process optimisation. The cost assumptions and basis for this analysis are provided in Appendix 2, ensuring transparency and supporting future refinements of the production model.

Table 7. *Breakdown of production cost for a plant producing 20,250 kg of product per year.*

Cost Category	Scenario 1 Freeze drying In-House (AUD)	Scenario 2 Freeze drying Outsourced (AUD)	Scenario 3 Roller dryer and no UHT (AUD)
Variable Costs			
Raw materials	$405,000	$405,000	$405,000
Consumables	$21,700	$8,100	$5,500
Utilities	$18,400	$7,900	$12,000
Contract freeze drying	$0	$476,000	$0
Sum of variable costs	$445,100	$897,000	$422,500
Fixed Costs			
Maintenance	$217,000	$81,000	$55,000
Operating labour	$210,000	$210,000	$210,000
Supervision	$42,000	$42,000	$42,000
Overheads	$105,000	$105,000	$105,000
Capital charges	$434,000	$162,000	$109,000
Insurance	$43,400	$16,200	$11,000
Rates	$86,800	$32,400	$21,900
Royalties	$43,400	$16,200	$11,000
Sum of fixed costs	$1,181,600	$664,800	$564,900
Other Costs			
General overheads	$406,700	$390,500	$246,800
Total Annual Cost	$2,033,000	$1,952,000	$1,234,000
Cost per kg of product	$100	$96	$61

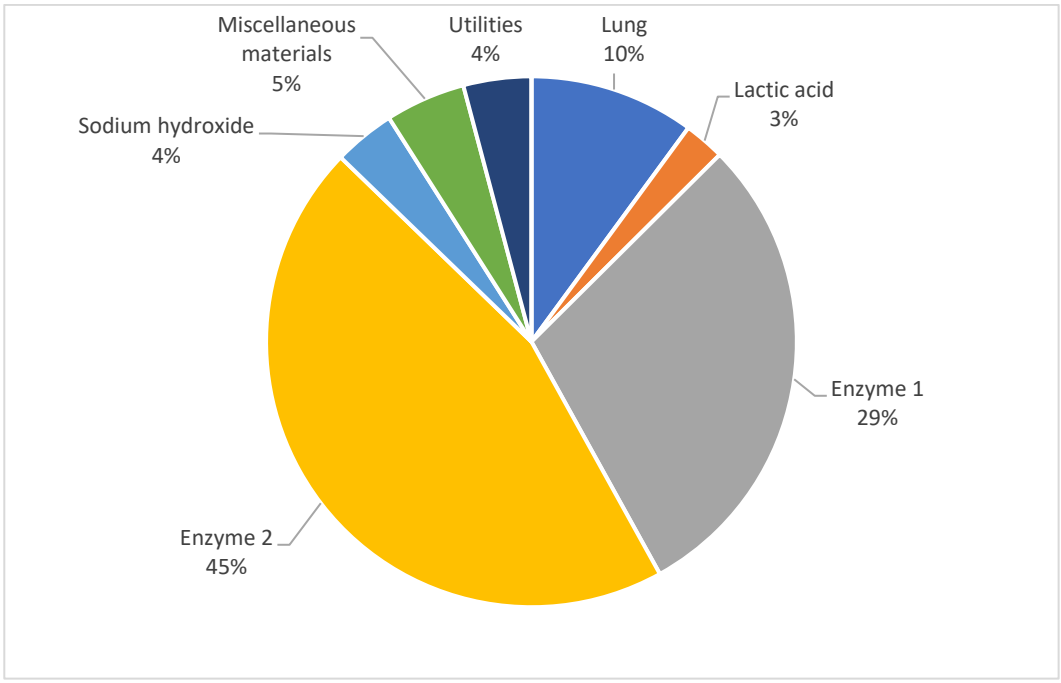

Figure 2. *Breakdown of variable costs for Scenario 1: In-house using freeze drying.*

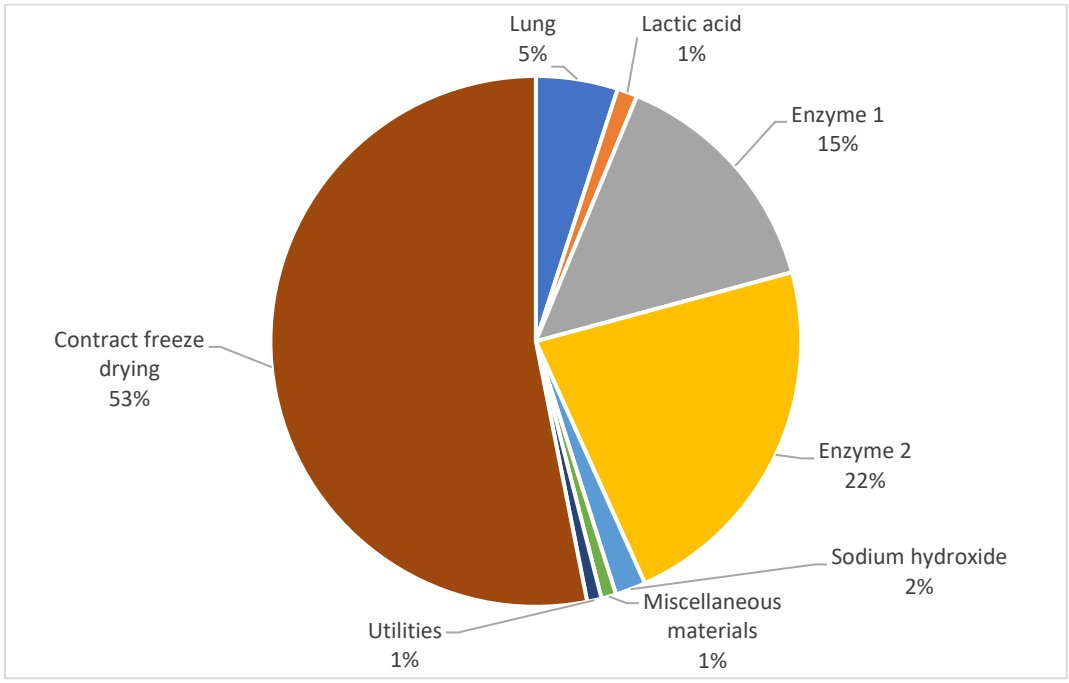

Figure 3. *Breakdown of variable costs for Scenario 2: Outsourced freeze drying and milling.*

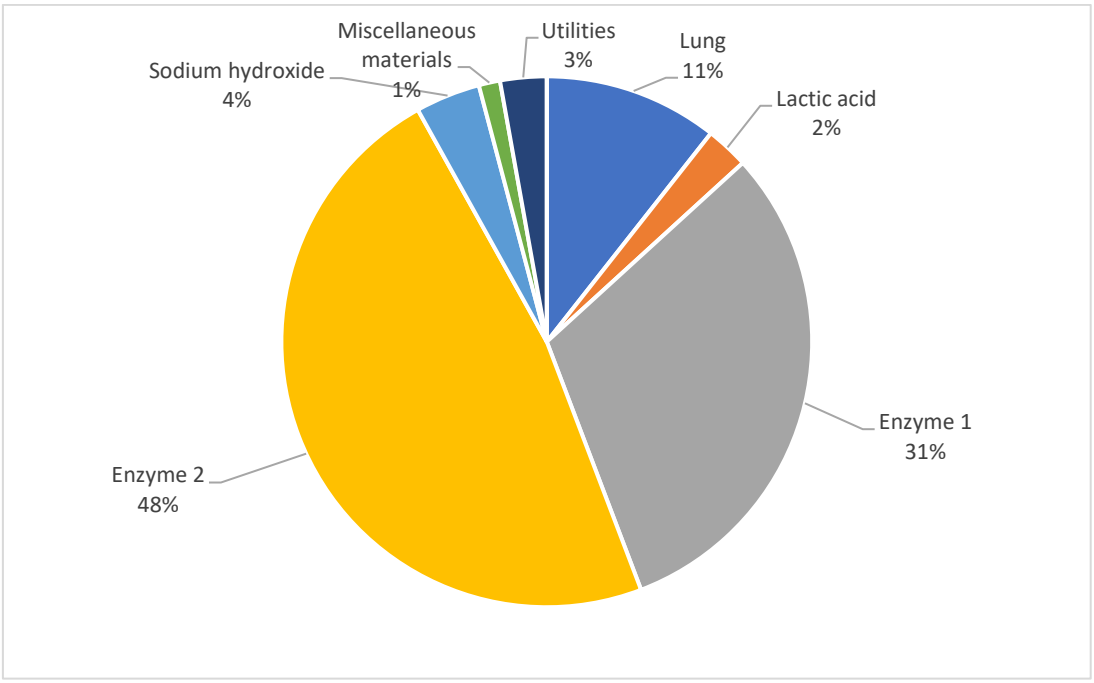

***Figure 4**. Breakdown of variable costs for Scenario 3: In-house using a roller dryer.*

***Table 8.** Main plant items purchase cost (AUD).*

MPI	Description	Scenario 1	Scenario 2	Scenario 3
D1	Dicer	$28,900	$28,900	$28,900
T1	Tank 1: Hydrolysis	$18,500	$18,500	$18,500
S1	Screen	$9,000	$9,000	$9,000
T2	Tank 2: Filtrate	$13,500	$13,500	$13,500
C1	Clarifier	$109,000	$109,000	$109,000
T3	Tank 3: Centrate	$13,500	$13,500	$13,500
UHT1	Ultra Heat Treatment plant	$349,000	$349,000	$349,000
T4	Tank 4: Evaporator inlet	$13,500	$13,500	$13,500
E1	Evaporator	$251,000	$251,000	$251,000
T5	Tank 5: Evaporator outlet	$4,500	$4,500	$4,500
FD1	Freeze dryer	$1,345,000		
RD1	Roller dryer			$71,000
M1	Mill	$14,700		$14,700
	Total equipment purchase cost	$2,170,000	$810,000	$896,000

5.3 Milestone 3: Nutritional and Functional Benefits of Kokumi Lung-G

5.3.1. Total amino acids

Table 9. *Total amino acid concentration (mg/g of freeze-dried powder) of kokumi-rich Lung hydrolysate (Lung-G) freeze-dried powder.*

Classification	Amino acid	Concentration (mg/g)	RDI (adult-70 Kg-mg/day)	% RDI for a 250 g serving of food with 1% Lung-G incorporation
Essential amino acids (EAA)	Leucine	42.4	2,730	3.9
	Lysine	39.1	2,100	4.7
	Valine	29.3	1,820	4.0
	Threonine	23.0	1,050	5.5
	Phenylalanine	22.0	1,750	3.1
	Isoleucine	15.2	1,400	2.7
	Histidine	13.1	700	4.7
	Methionine	5.6	1,050	1.3
	Tryptophan	5.6	280	5.0
	Total EAA	195.3		
Non-essential amino acids (NEAA)	Glutamic Acid	70.3		
	Glycine	53.5		
	Aspartic Acid	48.5		
	Alanine	39.9		
	Proline	34.8		
	Arginine	31.3		
	Serine	24.4		
	Hydroxyproline	18.9		
	Tyrosine	14.4		
	Cysteine	3.9		
	Hydroxylysine	3.4		
	Total NEAA	343.3		
Total EAA+NEAA (= TAA)		538.6		
EAA/TAA		36.2%		
EAA/NEAA		56.8%		

Table 9 provides a summary of 20 amino acids identified and grouped into essential amino acids (9 amino acids), and non-essential amino acids (11 amino acids). Amino acids serve roles beyond being building blocks of proteins, performing multiple regulatory functions in cells, including cell signalling, appetite regulation, blood flow modulation, growth and development, antioxidant defence, energy provision, and supporting immunity and overall health (Wu, 2013). Essential amino acids, comprising nine amino acids that the human body cannot synthesize and must obtain through the diet, are critical for these functions. In

contrast, non-essential amino acids can be produced by the body and do not need to be consumed. Since the non-essential amino acids are synthesised by the body, so no recommended intake is provided.

Essential amino acids (EAA) can be provided 1.3-5.5 % RDI when included at 1% in a 250 g serving. Threonine and tryptophan contribute the highest % RDI at 5.5% and 5.0%, respectively. Threonine supports protein synthesis and serves as an energy substrate for mitochondrial ATP production (Matsumoto et al., 2025). Tryptophane is a key precursor for the neurotransmitters serotonin and tryptamine (Heine et al., 1995).

Among non-essential amino acids (NEAA), glutamic acid and aspartic acid dominate this category, suggesting Lung-G is rich in these amino acids. Glycine, proline, and hydroxyproline were dominant in the total amino acid (TAA) profile, consistent with their abundance in collagen, a major structural protein in lung tissue. Hydroxyproline is a key component of collagen, playing a critical role in stabilising its structure. These NEAA including arginine, glutamic acid, glycine and proline play important roles in health such as protein synthesis, cell signalling, blood flow and regulating gene expression (Wu, 2013). According to the WHO/FAO recommendations for the ideal amino acid composition of dietary proteins (Hisaki et al., 2024), the EAA/TAA ratio is 40%, and the EAA/NEAA ratio is ≥60%. Additionally, when added at 1% to food and for 250 g serving, the total essential amino acids in Lung-G provide only a modest contribution, generally below 5% of the recommended daily intake for an average 70 kg adult (Table 9). The total amino acid profile of Lung-G is slightly below the ideal composition, as it contains a higher proportion of non-essential amino acids relative to essential ones. It can support tissue repair, immune function, and energy metabolism, particularly for those with limited protein intake. Combining it with EAA-rich foods or supplements can optimise its benefits.

5.3.2. Free amino acids

Free amino acids (FAAs) are primarily obtained through enzymatic hydrolysis of tissue proteins, such as with Protease A digestion. This process facilitates faster amino acid absorption and enhances plasma amino acid availability during digestion (Weijzen et al., 2022). Lung-G exhibits a relatively high free-to-total amino acid ratio of 44.5%, indicating that nearly half of its amino acids are present in a readily bioavailable form (Table 10). 62.7% of essential amino acids, equivalent to 122.5 mg/g as free amino acids, and 37.3% as bound amino acids, were found, indicating a high level of bio-accessible amino acids in Lung-G. The FAA profile is dominated by leucine (29.0 mg/g; 68% of total leucine), glutamic acid (28.4 mg/g; 40% of total), and lysine (23.4 mg/g; 60% of total), which are central to protein metabolism, taste modulation, and nitrogen balance.

From a dietary perspective, when incorporated at 1% in a 250 g serving of food, Lung-G provides modest but meaningful contributions to daily amino acid intake. For example:

- Tryptophan accounts for 5% of its RDI and as precursor of serotonin and melatonin important in supporting mood, cognition and sleep (Richard et al., 2009).
- Leucine contributes ~4.3% of its adult RDI per serving, with over two-thirds in free form, supporting efficient stimulation of muscle protein synthesis.
- Lysine reaches ~4.0% of RDI per serving, with ~60% free, suggesting potential benefits for collagen crosslinking, immunity, and tissue repair.
- Valine at ~4% and Threonine at ~5.5% RDI has potential in supporting muscle growth, energy production and fat metabolism and immune function respectively.
- Glutamic acid, while non-essential present in good amount (70mg/g) contributes additional umami/kokumi flavour activity alongside its metabolic role.

The overall EFAA/TFAA ratio (51.1%) and EFAA/NEFAA ratio (104.4%) in the free fraction surpass WHO/FAO reference requirements (EFAA/TFAA ≥40%; EFAA/NEFAA ≥60%), highlighting Lung-G as a particularly EAA-rich hydrolysate. This balance, combined with the high FAA availability, positions Lung-G as a promising functional food ingredient capable of supporting protein metabolism and metabolic health, while also contributing flavour-enhancing properties through glutamic acid and related kokumi-active FAAs.

***Table 10**. Free amino acid concentrations (mg/g of freeze-dried powder) of kokumi-rich Lung hydrolysate (Lung-G) freeze-dried powder.*

Classification	Compound	Concentration (mg/g)	% of Total AA
Essential free amino acids (EFAA)	Leucine	29.0	68.5
	Lysine	23.4	59.8
	Valine	19.7	67
	Phenylalanine	13.7	51.7
	Threonine	11.9	62
	Isoleucine	9.3	61
	Histidine	6.3	48
	Methionine	5.6	100
	Tryptophan	3.6	64
	Total EFAA	**122.5**	
Non-essential free amino acids (NEFAA)	Glutamic Acid	28.4	40
	Alanine	19.5	49
	Arginine	13.9	44
	Serine	12.7	52
	Glycine	11.2	21
	Tyrosine	8.8	61
	Aspartic Acid	7.2	15
	Proline	6.1	
	Taurine	3.8	
	Asparagine	2.0	
	L-Ornithine	1.1	
	D,L-O-Phosphoserine	0.8	
	Ammonia	*0.8*	
	Ethanolamine	*0.7*	
	O-Phosphoethanolamine	0.3	
	Cysteine	-	
	Hydroxproline	-	
	Hydroxylysine	-	
	Total NEFAA	**117.3**	
Total EFAA+NEFAA (TFAA)		**239.8**	
EFAA/TFAA		51.1%	
EFAA/NEFAA		104.4%	

5.3.3. *Mineral profile*

Table 11. *Mineral concentrations of kokumi-rich Lung hydrolysate (Lung-G) freeze-dried powder.*

Mineral	Unit	Concentration	RDI/AI (adult- 70 Kg- mg/day or ug/day)	% RDI for a 250 g serving of food with 1% Lung-G incorporation
Calcium	mg/100 g	33	1000 mg	0.08
Magnesium	mg/100 g	47	420 mg	0.28
Potassium	mg/100 g	1100	3800 mg (AI)	0.72
Sodium	mg/100 g	5100	2000 mg (AI)	6.4
Phosphorus	mg/100 g	840	1000 mg	2.10
Iron	mg/100 g	24	8 mg	7.50
Copper	mg/100 g	0.5	1.7 mg	0.73
Iodine	µg/100 g	37	150 ug	0.63
Manganese	µg/100 g	0.069	5.5 mg (AI)	0.00
Selenium	µg/100 g	0.02	70 ug	0.00
Zinc	µg/100 g	5.5	14 mg	0.00
Chloride	µg/100 g	1730	2300 mg	0.00

Note: Recommended Daily Intake (RDI) for an adult weighing over 70 kg (Nutrient Reference Values for Australia and New Zealand, 2017). Adequate Intake (AI) values are used, as they are common for minerals like sodium, potassium, and chloride.

Table 11 shows the mineral contents in Lung-G freeze-dried powder and their contributions to the Recommended Daily Intake (RDI) or Adequate Intake (AI) when included at 1% in a 250 g serving. Its high iron content is particularly valuable for oxygen transport and preventing deficiencies, Zinc, magnesium, copper, iodine, selenium, and calcium play roles in immune support, enzymatic reactions, and thyroid regulation, but when included at 1% in a 250 g serving, their contribution is minimal, close to 0%. Sodium is the most prevalent nutrient. According to the Australian and New Zealand government recommends a daily sodium intake of 2,000 mg, which is roughly equivalent to 5g of salt). A 250 g serving of food with 1% Lung-G provides 128 mg sodium, or 6.4% of the RDI, which is low. While Lung-G powder is high in sodium (5,100 mg/100 g), using it at 1% as a flavour ingredient keeps the food's sodium content minimal. The sodium content arises from the use of sodium hydroxide in the enzymatic hydrolysis process and could potentially be reduced by substituting or partially replacing it with other alkali alternatives, such as potassium hydroxide.

5.3.4. Vitamin profile

Table 12. *Vitamins concentrations of kokumi-rich Lung hydrolysate (Lung-G) freeze-dried powder.*

Compound	Unit	Concentration	RDI/AI (adult-70 Kg-mg/day or µg/day)	% RDI for a 250 g serving of food with 1% Lung-G incorporation
Vitamin A	µg/100 g	<40	900 ug	0.00
Vitamin E	mg/100 g	<0.16	10 mg	0.00
B1	mg/100 g	0.04	1.2 mg	0.08
B2	mg/100 g	0.98	1.3 mg	1.88
B3	mg/100 g	10.1	16 mg	1.58
B5	mg/100 g	1.66	6 mg (AI)	0.70
B6	mg/100 g	0.08	1.3 mg	0.15
B7	µg/100 g	3.61	30 µg (AI)	0.30
B9	µg/100 g	4.83	400 µg	0.03
B12	µg/100 g	11.4	2.4 µg	11.88
Choline	mg/100 g	295	550 mg (AI)	1.35

Table 12 provides the vitamin contents in Lung-G and their contributions to RDI/AI when included at 1% in a 250 g serving. Lung-G is a particularly good source of vitamin B12 containing 11.4 µg per 100 g. At 1% inclusion in a 250 g serving, it could contribute to 11.88% of RDI of Vitamin B12. Vitamin B12 is essential for red blood cell formation and neurological health.

5.3.5. Bioactive peptides

Table 13. *The 10 most abundant proteins identified in Lung-G. Data shown are the number of MS/MS spectra (a measure of abundance), the percentage of the protein sequence covered by observed peptides, and the number of peptides.*

Rank	Gene	Protein	No of Spectra	Coverage (%)	No of Peptides
1	COL1A1	collagen alpha-1(I) chain	2211	61.3	360
2	HBA	hemoglobin subunit alpha	1941	84.4	170
3	COL1A2	collagen alpha-2(I) chain	1828	61.3	310
4	HBB	hemoglobin subunit beta	1617	86.9	152
5	COL3A1	collagen alpha-1(III) chain	1267	76.1	184
6	ACTG1	actin alpha 2, smooth muscle	807	56.0	125
7	ELN	elastin	632	53.2	136
8	ACTC1	actin alpha cardiac muscle 1	550	50.9	90
9	ACTA1	actin, alpha 1, skeletal muscle	485	48.8	81
10	ALB	albumin	384	26.5	51

Table 14. Bioactive peptides identified in Lung-G.

Bioactive Peptide	Source Protein	Activity
AERVGAGAPVY	histone H2	antibacterial, antifungal, antimicrobial
AERVGAGAPVYL	histone H2	antibacterial, antifungal, antimicrobial
AEYGAEAL	haemoglobin	hemopoietic
ASHLPSDFTPAVHASL	haemoglobin	peptide potentiator
EVGGEALGRL	haemoglobin	hemopoietic
GAEALER	haemoglobin	hemopoietic
GKVKVDEVGGEALGRL	haemoglobin	hemopoietic
GPAGPIGPVG	collagen alpha-1(I) chain	antioxidant
IDGRPGPIGPA	collagen alpha-2(I) chain	antioxidant
LVVYPW	haemoglobin	immunomodulator
MLTAEEKAAVT	haemoglobin	hemopoietic
VGGHAAEYGAEA	haemoglobin	hemopoietic
VGGHAAEYGAEAL	haemoglobin	hemopoietic
VLSAADKGNVKAA	haemoglobin	hemopoietic
VVYPW	haemoglobin	antihypertensive, ACE inhibitor, DPP-3 inhibitor, neuropeptide
VVYPWTQ	haemoglobin	ACE inhibitor, DPP-3 inhibitor, neuropeptide, opioid, toxin
VVYPWTQR	haemoglobin	toxin

A large number of peptides (3,764 in total) were identified in the Lung-G sample. The 10 most abundant proteins (as judged from the number of MS/MS spectra recorded for each) where these peptides originated are shown in Table 13. Collagen type I and haemoglobin were the two most abundant proteins and the sources of the most peptides following digestion with protease A. Collagen type III was also prominent. A full list of all the collagens identified is given in Appendix 3.

Several of the identified peptides matched sequences in the database of known bioactive peptides (Table 14), mostly derived from haemoglobin. This was not unexpected, as the database of haemoglobin-derived bioactive peptides is far more diverse and extensively characterized compared with that of collagen-derived peptides. Consequently, relatively few matches were observed for collagen peptides. The predominant activities of the exact sequence matches included hemopoietic, antihypertensive/ACE inhibitory, and antimicrobial functions. Further research is needed to determine whether these peptides occur in Lung-G at sufficiently high concentrations to exert meaningful functional effects when consumed.

A very large number of cryptides (short bioactive sequences) were encoded in the sequences of the peptides identified in Lung-G. This analysis suggests that, following oral consumption, large quantities of short antihypertensive/ACE inhibitory and of dipeptidyl peptidase IV (DPP–4) inhibitory peptides will be released, imparting benefits for cardiovascular health and improved glycaemic regulation.

6.0 Discussion

The successful completion of Milestone 1 established the technical feasibility of producing Lung-G, a freeze-dried kokumi powder derived from beef lung at pilot scale. The scale-up process, involving a 54 kg batch of raw material, resulted in 7.3 kg of freeze-dried Lung-G powder with a dry matter yield of 68.1%. The kokumi-active peptides responsible for taste enhancement were retained across all processing formats post-evaporation, oven-dried, and freeze-dried with consistent dose-dependent activation of the kokumi receptor. EC_{50} values between 0.028 and 0.081 mg show how little Lung-G is needed to activate the kokumi receptor and create a flavour-enhancing effect. A lower EC_{50} means higher potency, so these results confirm that Lung-G remains highly effective after processing. This supports its use as a natural flavour booster that meets clean-label food ingredient trends. However, sensory testing in actual food systems is still required to validate these dosages and confirm that the kokumi effects translate to real consumer perception.

The process also passed critical food safety benchmarks, supported by microbiological and physicochemical testing, and was aligned with MPI food-grade standards through hazard identification and control point validation. These factors collectively support Lung-G's readiness for food industry applications. However, the pilot run highlighted technical challenges, particularly the need for strict control over enzymatic activity, temperature, and pH to ensure consistent product quality. Insights gained from these challenges will guide future commercial scale-up. Importantly, alternative drying approaches like oven drying also retained functional activity, suggesting potential for significant cost savings in future production. This finding opens avenues for reducing reliance on high-cost technologies such as freeze drying and aligns with observations from the cost analysis in Milestone 2.

Milestone 2 built on this technical foundation by assessing the manufacturing cost of scaling the Lung-G production process. Rather than conducting a full techno-economic analysis, this step focused specifically on estimating the production costs to provide an early indication of commercial viability. Three cost scenarios were evaluated: one involving complete in-house processing with freeze drying, another with outsourcing the freeze drying and milling, and the third was in-house but using a roller dryer instead of a freeze dryer. The estimated cost per kg of Lung-G was AUD $100 for in-house processing, $96 for the outsourced model, and $61 when using a roller dryer (based on an assumed annual output of 20,250 kg per year). The outsourcing scenario also required significantly lower capital investment, making it potentially attractive for small processors or startups with limited resources.

This cost analysis identified drying as the most expensive operation. Importantly, alternative drying methods were shown in Milestone 1 to maintain the functional properties of Lung-G, indicating that process modifications can reduce costs without compromising kokumi activity. These findings highlight opportunities for process optimisation, improving cost-efficiency, and supporting the development of a commercially viable production model.

Milestone 3 revealed nutrient profile of Lung-G including amino acid composition, minerals, vitamins and bioactive peptides. While Lung-G has interesting and distinct composition, there is very little opportunity to capitalise on it as a nutritional supplement. The inherently high content of sodium, which is a consequence of the enzymatic treatments, means that it fails the FSANZ Food Standard 1.2.7 Nutrient Profiling Scoring Criterion. Lung-G is food safe and saleable, but label claims CANNOT be made about its nutrition content ("A good source of B12") nor any general association to health benefit ("Necessary for normal cell division"). While process modifications might reduce sodium content, the current composition does not support positioning Lung-G as a supplement. This restriction does not affect its value as a flavouring or tastant in food applications. It is noted that this relates only to presenting Lung-G as a supplement and does not impinge on its utility and value as a flavouring or tastant when added to foods.

Reframing the Cost Estimate and Highlighting Product Value

While the indicative manufacturing cost of ~AUD $61–100/kg may appear high at first glance, it is essential to contextualise this figure appropriately. Firstly, this is not a techno-economic analysis (TEA), but a preliminary engineering estimate with ±30% accuracy, based on generic assumptions. A full TEA is recommended to integrate detailed inputs such as labour rates, utility costs, market-based pricing scenarios, and site-specific capital costs, enabling better-informed investment and adoption decisions.

Secondly, efficacy testing, which measures the maximum effect a sample can achieve, indicates that Lung–G produces maximum kokumi enhancement at the highest concentration tested in receptor assays (0.4 mg peptide of Lung-G /mL of water), generating responses over 150 RFU, approximately 100 times higher than its potency (0.04 mg/mL). Here, potency refers to the concentration needed to reach 50% of the maximum response (EC_{50}). The Lung-G powder contains approximately 570 mg of peptides/amino acids per gram. This corresponds to roughly 700 mg per 100 g of food (~0.7% w/w powder) in practical formulations. Even at this level, the actual ingredient cost per serving remains low. However, the current production cost is still estimated at around AUD 60/kg, indicating the need for further research to reduce costs. Lung-G demonstrates significant potential for flavour enhancement at low concentrations, owing to its natural origin and robust kokumi properties. However, its potential bioactivity properties and health benefits from vitamins and minerals are limited by its high sodium content, which restricts consumption levels and makes achieving these functional health benefits challenging. This limitation, however, can be addressed through reformulation strategies (e.g. replacing sodium salts with potassium salts), thereby reducing sodium content while preserving flavour functionality.

These factors suggest that even at current cost estimates, Lung-G remains a viable and attractive option for processors, especially in premium ingredient markets. Moreover, further optimisation of enzyme usage, concentration methods, and drying technology could substantially reduce costs reinforcing the importance of ongoing process development and R&D.

From an industry perspective, these outcomes are promising. The ability to transform a low-value co–product (beef lung) into a functional, kokumi-active food ingredient offers a pathway for value addition, sustainability, and innovation within the meat processing sector. Lung-G could help processors diversify their product portfolios, upcycle waste streams, and meet growing consumer demand for savoury, flavour-rich ingredients.

Three key comparative insights provide context for Lung-G's potential in the market

1. Functional and cost comparison with commercial protein ingredients:

A direct comparison with OXO beef stock powder (**Figure 55A**), a commercially available flavour enhancer, shows that Lung-G has approximately three to four times higher protein concentration (60% vs. 17.4%). To deliver an equivalent 0.5 g of protein in a 100 mL stock, Lung-G requires just ~1 g, compared to ~2.87 g of OXO. Moreover, Lung-G demonstrated strong kokumi receptor activation in terms of higher fluorescent units (>100 RFU) in receptor assay suggesting enrichment of potent kokumi tastants. To enable a fair value comparison between products with differing compositions, cost is normalised on a per-gram-of-protein basis. However, a direct comparison of OXO powder Lung-G requires confirmation through taste receptor activation. At the pilot scale, Lung-G's production cost is approximately AUD 0.16/g protein, compared with OXO's retail price of around AUD 0.50/g protein. While a direct comparison is constrained by the absence of data on profit margins and additional processing costs (e.g., formulation, packaging, transport), preliminary estimates suggest that even with an assumed 20–30% margin, Lung–G would maintain strong price competitiveness.

	Per 100g	Per 100mL of prepared stock
Energy	1117KJ/265 Kcal	35 KJ/8kcal
Fat	4.9 g	<0.5 g
(Of which saturated)	2.4 g	<0.1g
Carbohydrate	0g	0g
(Of which sugars)	2.2g	<0.5g
Fibre	4.2 g	<0.5 g
Protein	**17.4g**	**0.5g**
Salt	**30.70 g**	**0.95g**

Figure 55A. *Example of commercial flavour product (OXO beef stock) and their nutritional information.*

We also compared Lung-G with savoury yeast extract (**Figure 5B**) as yeast extracts are commonly used as flavouring ingredients and have been reported in the literature to contain kokumi-active peptides (Chang et al., 2022) and, in some cases, monosodium glutamate. However, it is not established whether the particular yeast extract product (Our mate) here contains these components. Lung-G has higher amount of protein per 100g (59 g vs 39 g in Our Mate yeast extract spread). Since protein is a key contributor to taste, we normalised the retail price to the cost per gram of protein, resulting in AUD 0.10 per g of protein for the yeast extract. In contrast, Lung-G's production cost is AUD 0.16 per g of protein, making the commercial yeast extract protein less expensive, though the minimum effective dose as a flavour enhancer may differ. Lung-G is formulated to be rich in kokumi flavour peptides, unlike yeast extract, positioning it as a premium, high-value flavour ingredient with potential for a niche market. The sodium content of yeast extract is 3.9%, compared with 5.1% in Lung-G. This higher sodium content in Lung-G could be addressed through reformulation with alternative ingredients, enabling its development into a final product with improved nutritional balance.

Nutritional Information Table		
Nutrient	**Per Serving**	**Per 100g / 100ml**
Energy	43.00kJ	1070.00kJ
Protein	1.60g	39.00g
Fat Total	<0.50g	<0.50g
Saturated	<0.10g	<0.10g
Carbohydrate	1.00g	24.00g
Sugars	<1.00g	1.10g
Sodium	156.00mg	3900.00mg

Figure 5B. Savoury Yeast extract spread with nutritional information

2. Relevance in processed meat food applications:

Lung-G also presents a compelling case as a premium flavour enhancer in processed meats, such as frankfurters (Figure 6), where protein additives like soy protein isolate (SPI) are commonly used for texture and bulk. At the suggested concentration, Lung-G is unlikely to affect bulk or texture but will markedly enhance flavour. In contrast, while vegetable protein extracts can improve functionality, they may also introduce off-flavours or be perceived negatively by consumers. Incorporating meat-derived hydrolysates such as Lung-G could therefore be more positively received, though this warrants confirmation through consumer research. Although SPI is relatively inexpensive (~AUD 0.06/g, Bulksupplements.com), Lung-G delivers distinct sensory advantages by enhancing umami and meaty notes. At functional use levels (0.1–0.5%), the cost difference is minimal, and Lung-G may offer the additional benefit of reducing reliance on MSG or yeast extracts, which can be viewed less favourably by some consumers. Furthermore, its potential to contribute to texture and yield at higher inclusion rates could further increase its value in meat product reformulation strategies.

Ingredients
Pork (74%), water, salt, acidity regulators (262, 326), spices, pea protein, mineral salts (450, 451), dextrose, vegetable protein extract, antioxidant (316), preservative (250), edible protein casing, natural wood smoke

Nutrient	Per serving	Per 100g / 100 mL
Energy	612 kJ	914 kJ
Protein	8.3 g	12.4 g
Carbohydrate	6.4 g	9.6 g
Sugars	0.8 g	1.2 g
Sodium	557 mg	832 mg

Figure 6. *Example of commercial product (Verkerks, Frankfurters), and their ingredient list and nutritional information.*

3. Detailed Comparison and Health Implications

When compared to other commercial health supplements derived from bovine by-products, based solely on the information provided on product labels, Beef Lung-G demonstrates competitive protein and iron content (see Table 15). However, its levels of vitamin A, B-Complex, minerals excluding iron are relatively lower. As previously noted, the sodium content in Beef Lung-G remains notably high.

It is worth noting that the Beef Liver & Spleen product lists a protein content of 71 mg per 100 g, which seems unusually low equivalent to just 0.07% protein by weight (w/w) compared to the protein content expressed in gram quantities in other products. This product is processed into a tasteless and odourless freeze-dried form, suggesting that a significant portion of the protein may have been pre-extracted during processing, with other nutrients concentrated separately. As protein is a primary contributor to flavour, its removal likely results in the neutral sensory profile observed. This processing method indicates the potential for dual revenue streams: one from the isolated protein fraction and another from the nutrient-concentrated residue.

Furthermore, analysis has identified functional peptides in Beef Lung-G. As other products have not been examined for functional peptides, a direct comparison cannot be made. However, the presence of these peptides in Beef Lung-G indicates potential additional nutritional benefits. These benefits, however, can only be realised if the product is consumed in larger quantities, with the important caveat that its sodium content must first be substantially reduced.

Table 15. *Nutritional profile comparison of beef-derived commercial supplements and Lung-G (unit: per 100 g).*

Per 100g	Mitchell's Beef Liver & Spleen	Beef Liver and Heart Blend | Awaken Wellness	HEART + LUNG HOMEGROWN-Primal	Beef Lung-G (AMPC)
Co-products used	Beef Liver and Spleen	Beef Liver and Heart	Beef Heart and Lung	Beef Lung
Product image				
Protein	71 mg	72 g	65 g	59 g
Vitamin A	24,555 µg	1,504 µg		< 40 µg
Vitamin B12	132 µg	185.2 µg	High potency	11 µg
Vitamin B2	5.56 mg			0.98 mg
Vitamin B3	35 mg			10.1 mg
Iron (Heme)	400.56 mg	22 mg	25 mg	24 mg[1]
Selenium	0.05 µg	0.05 mg	0.1 mg	0.02 mg
Copper		3.4 mg		0.5 mg
Zinc	9.44 mg	10 mg		5.5 mg
Sodium	1,207 mg	150 mg		5,100 mg

[1.] The iron content measured in Lung-G includes both heme and non-heme iron.

Key Priorities for Future Development and Adoption

- **Conduct sensory evaluation in real food systems**

Conduct sensory evaluation in real food systems, including comparison with existing market alternatives, to validate consumer perception of Lung-G's flavour enhancement and determine optimal market positioning. This will complement existing receptor assay data and confirm at practical dosage levels.

- **Reduce sodium level in Lung-G**

Investigate alkali alternatives to replace sodium hydroxide, reducing sodium content in the final product.

- **Explore alternative drying methods**

Investigate options such as spray drying, drum drying, or eliminating drying altogether via concentrated liquid formats to lower energy use and production costs.

- **Refine evaporation and filtration steps**

Enhance upstream concentration processes to reduce drying load, increase throughput, and improve overall processing efficiency.

- **Conduct a comprehensive techno-economic analysis**

Integrate detailed cost inputs (capital, labour, utilities), market-based pricing, and scalability metrics in line with AMPC expectations to support commercial investment decisions.

- **Leveraging Flavour Substances and Supplement Production through a New Strategy for Dual Revenue Generation**

Through this study, the potential of Beef Lung protein as a kokumi substance has been confirmed. However, despite the co-extraction of many nutritionally valuable substances, their value may be diminished or their use as nutritional supplements may be limited due to processing methods for enhancing kokumi substances (e.g., high-temperature treatment, prolonged enzymatic hydrolysis, pH changes, etc.). This suggests that if Beef Lung protein were first chemically extracted, followed by enzymatic hydrolysis, and the remaining non-protein components were reprocessed into health supplements, it could create two revenue streams.

In summary, this project demonstrated both the technical scalability and preliminary commercial feasibility of Lung-G production. With targeted optimisation and further validation, Lung-G holds strong potential to be adopted as a novel, cost-effective flavour enhancer that supports sustainable meat processing and commercial innovation.

7.0 Conclusions

This project successfully demonstrated the technical feasibility and preliminary commercial potential of producing Lung-G, a kokumi-enhancing powder derived from beef lung. The pilot-scale production confirmed that Lung-G can be manufactured at scale while retaining its kokumi functionality. Taste receptor assays showed consistent dose-dependent activation across all processing formats post-evaporation, oven-dried, and freeze-dried validating that the active peptides remain effective after processing. This confirms Lung-G's potential as a natural, clean-label flavour enhancer suitable for human consumption. Key challenges in transitioning from lab-scale to pilot-scale—such as maintaining enzyme activity and flavour integrity were effectively addressed through process optimisation, control of pH and temperature, and compliance with regulatory requirements (e.g. MPI standards). These learnings have not only secured a high-quality product but also established a strong foundation for future scale-ups. The pilot run also provided essential insights into production logistics, highlighting critical areas such as enzymatic control and drying methods. Notably, the demonstration that alternative drying methods (e.g. oven drying) preserved functionality opens opportunities for reducing production costs, which will be key in future commercial planning.

Building on this, Milestone 2 assessed the manufacturing costs associated with scaling up Lung-G production. Three cost scenarios were evaluated, complete in-house production using freeze drying, outsourcing freeze drying, and in-house production using a roller dryer, yielding estimated costs of AUD $100/kg, $96/kg and $61/kg respectively. These preliminary figures derived using standard industry methods with an expected accuracy of ±30%, offer early guidance for investment planning and technology adoption. Importantly, this costing exercise revealed high-cost drivers (enzymes and freeze-drying) and identified opportunities for optimisation. It also underscored the sensitivity of outsourcing to market fluctuations, particularly in freeze-drying costs. These economic insights are valuable for future commercial strategy but are not sufficient alone to determine Lung-G's market viability. A full TEA will be necessary to integrate cost data, capital requirements, market assumptions, and AMPC's commercialisation expectations. This will enable more robust decisions about investment, pricing, and business models.

Chemical analysis from Milestone 3 established Lung-G powder nutrient profile, with 59 g protein/100 g, a balanced amino acid profile (538.6 mg/g total, 44.5% free amino acids), essential minerals (e.g., 24 mg iron, 1,100 mg potassium), and vitamins (e.g., 2,880 µg Vitamin A, 11.4 µg B12). The identification of bioactive peptides, predominantly from collagen and haemoglobin, suggests additional functional benefits like antihypertensive and glycaemic regulatory properties, though high sodium

(5,100 mg/100 g) limits supplement claims under FSANZ standards. Comparative analysis with other beef organ supplements (e.g., Mitchell's Beef Liver & Spleen with higher iron but lower protein usability) underscores Lung-G's specialised niche for nutrients and peptides, warranting further peptide research.

For industry stakeholders, these results suggest enhanced profitability through upcycling. Lung-G presents a cost-effective source of protein, making it attractive for processed meats (e.g., as a replacement for MSG or soy isolates). Adoption could reduce waste and create additional revenue via dual streams 1) kokumi flavour from protein hydrolysis, and 2) supplements from non-protein residues and meet consumer demand for natural enhancers.

Future research should prioritise sensory trials in foods, process refinements (e.g., spray drying, sodium reduction), a full techno-economic analysis, and peptide bioactivity studies to unlock health applications. Overall, Lung-G represents a sustainable innovation for the meat sector, transforming co-products into high-value assets with broad market potential.

8.0 Recommendations

Following the successful pilot-scale production and initial cost estimation, this project has laid a strong foundation for the commercialisation of Lung-G. To support industry adoption and guide future research, development and extension (RD&E) activities, the following recommendations are made:

1. **Support Industry Adoption Through Application of Project Learnings**

To enable immediate uptake of project findings and stimulate commercial interest, the following RD&E and extension activities are recommended:

- **Sensory Evaluation and Market Positioning**

Conduct comprehensive sensory testing across a range of food applications to confirm Lung-G's flavour-enhancing effect and guide product positioning and messaging.

- **Industry Communication**

Present findings at upcoming industry showcases (e.g., the scheduled at Innovation workshop, AMPC, Australia) to build interest and attract partners.

- **Commercial Validation**

Collaborate with processors to test Lung-G in real-world conditions, validating performance, cost, and format flexibility.

Run formulation trials across various applications (e.g., soups, meat products, plant-based foods) with food manufacturers.

- **Immediate Industry Use**

Encourage food manufacturers to explore Lung-G in food formulations. Its proven peptide stability across processing methods allows flexibility in product development (e.g., broths, gravies, plant-based dishes).

2. **Strengthen Economic Case Through Cost and Market Analysis**

To guide industry investment decisions and future development, RD&E efforts should include:

- **Planning Benchmark**

Use the estimated manufacturing cost (AUD $61–100/kg; ±30% accuracy) as a guide for early-stage planning and feasibility screening.

- **Market Research**

Conduct targeted studies to define realistic selling price ranges, assess customer demand across sectors (e.g., meat, ready meals, snacks), and benchmark against competitive flavour enhancers.

- **Techno-Economic Analysis (TEA)**

Develop a comprehensive TEA to build on initial cost models. This should include production costs, capital requirements, market data, and pricing forecasts to meet AMPC's expectations and inform commercial go/no-go decisions.

- **Process Optimisation Opportunities**

Reassess drying methods—e.g., spray, oven, or drum drying as cost-saving alternatives to freeze drying, particularly since functionality was retained in other formats.

Optimise enzyme dosage to reduce one of the largest input costs without compromising kokumi activity.

- **Industry Partnerships**

Work with processors to validate site-specific cost variables (e.g., utilities, labour, capital installation), reducing dependence on estimation methods like Lang factors.

3. Advance Product Value Through Bioactivity and Nutritional RD&E

Future RD&E activities should explore Lung-G's bioactive and nutritional potential to enhance market value:

- **Bioactive Compound Characterisation**

Use mass spectrometry to identify and quantify γ-glutamyl peptides and collagen-derived peptides, followed by bioassays to assess antioxidant or health-promoting activity.

4. Improve Process Efficiency and Sustainability Through Further RD&E

Several areas offer potential for improving Lung-G's processing sustainability and operational efficiency:

- **Process Research**

Through initial protein extraction processing, enzymatically treat the extract to produce kokumi flavour compounds, while separately concentrating the remaining non-protein extract for testing as a functional health food, aiming to generate dual revenue streams.

Explore membrane filtration as a more energy-efficient concentration method than evaporation.

Test shelf-life and product stability across different drying methods and formats.

Finalise the most suitable product format (e.g., powder, liquid concentrate, paste) based on market feedback and process compatibility.

- **Sustainability and Circular Economy**

Assess options for by-product valorisation (e.g., animal feed applications).

Quantify the environmental and cost advantages of alternative drying and concentration technologies.

9.0 Project outputs

Milestone 1 Report: Pilot-Scale Production of Lung-G

- **Submitted:** 02nd May 2025
- **Description:** This technical report documented the successful scale-up of Lung-G production from laboratory to pilot scale. It confirmed kokumi activity retention through enzymatic hydrolysis and different drying methods (freeze drying, oven drying, post-evaporation). Key outputs included peptide retention validation using taste receptor assays, process optimisation insights and strategies.
- **Output Type:** Technical report on AMPC template
- **Target Audience:** AMPC, food processors, ingredient manufacturers, product development teams

Milestone 2 Report: Preliminary Manufacturing Cost Analysis

- **Submitted:** 14th May 2025
- **Description:** This report provided an economic assessment of Lung-G production at pilot scale, including estimates of fixed capital costs, manufacturing costs and key cost drivers such as enzyme use and freeze drying. It also outlined improvement opportunities through alternative drying, process optimisation, and commercial partnerships.
- **Output Type:** Technical report
- **Target Audience:** AMPC, commercialisation teams, food industry investors, R&D strategists

Milestone 3 Report: Chemical Analysis of Nutrient Profile

- **Submitted:** 8th Aug 2025
- **Description:** This report provided a detailed chemical analysis results of nutrient profile including amino acid composition, vitamin, minerals, and bioactive peptides. It outlined comprehensive nutrient profile of Lung-G, and potential health implementation.
- **Output Type:** Technical report
- **Target Audience:** AMPC, commercialisation teams, food industry investors, R&D strategists

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11.0 Appendices

11.1 Appendix 1: Milestone 1. Images of enzymatic hydrolysis process

Supplementary Figure 1A-1. *Photographs of the beef lung mixing step 6.*

Supplementary Figure 1A-2. *Photographs of the two enzymatic hydrolysis steps 7 to 9.*

Supplementary Figure 1A-3. *Photographs of the clarification step 15 to remove suspended solids from liquid.*

Supplementary Figure 1A-4.

12.2 Appendix 2: Milestone 2. Manufacturing cost inputs.

Table A2-1. *Raw material cost.*

Raw material	Unit	Unit price (AUD)	Amount needed per kg of product	Cost per kg of product (AUD)	Cost per year (AUD)	Percent of total raw materials cost
Enzyme1	Kg	$412.00	0.016	$6.45	$131,000	32%
Enzyme2	Kg	$634.00	0.016	$9.92	$201,000	50%
Latic acid (80%)	L	$7.69	0.071	$0.54	$11,000	3%
Lung	kg	$0.30	7.390	$2.22	$45,000	11%
Sodium hydroxide	Kg	$8.90	0.093	$0.82	$16,700	4%
Water	L	$0.002	14.010	$0.03	$570	0%
Total raw material cost				**$20.00**	**$405,000**	

Table A2-2a. *Utilities cost – Scenario 1 freeze drying and milling in-house.*

Description	Utility	Cost per kg of product	Cost per year	Percent of total utility cost
Power for equipment	Electricity	$0.74	$15,000	82%
All process liquor heating	Steam	$0.06	$1,200	7%
Concentrating process liquor by evaporation	Steam	$0.10	$2,000	11%
All process liquor cooling	Cooling water	$0.01	$200	1%
Total utility cost for Scenario 1		**$0.91**	**$18,400**	

Table A2-2b. *Utilities cost – Scenario 2 freeze drying and milling outsourced.*

Description	Utility	Cost per kg of product	Cost per year	Percent of total utility cost
Power for equipment	Electricity	$0.22	$4,500	57%
All process liquor heating	Steam	$0.06	$1,200	15%
Concentrating process liquor by evaporation	Steam	$0.10	$2,000	25%
All process liquor cooling	Cooling water	$0.01	$200	3%
Total utility cost for Scenario 2		**$0.39**	**$7,900**	

Table A2-3. *Operating labour.*

Description	Number required	Salary (AUD)	Cost per year (AUD)
Operating labour	3	$70,000	$210,000

Table A1-4. Outsourced freeze drying and milling cost.

Description	Price per batch (AUD)	Batches per year	Cost per year (AUD)
Freeze drying	$1,500	250	$375,000
Milling	$400	250	$101,250
Total			**$476,250**

Table A2-4. MPI size basis and cost source.

MPI	Description	Size basis and details	Purchase cost (AUD)	Supplier
D1	Dicer	600 kg per batch processed in 0.5 hrs	$28,900	Barnco (Australia)
T1	Tank 1: Hydrolysis	Working volume 2,000 L Scrape surface agitator Heating and cooling jackets	$18,500	HD Process
S1	Screen	1,800 L per batch processed in 0.5 hrs	$9,000	Openshaw Plant Machinery Limited
T2	Tank 2: Filtrate	Working volume 2,000 L No agitator, no heating, no cooling	$13,500	HD Process
C1	Clarifier	1,600 L per batch processed in 0.5 hrs	$109,000	HD Process
T3	Tank 3: Centrate	Working volume 2,000 L No agitator, no heating, no cooling	$13,500	HD Process
UHT1	Ultra Heat Treatment plant	1,300 L per batch processed in 0.5 hrs	$349,000	GEA and Thermaflo
T4	Tank 4: Evaporator inlet	Working volume 2,000 L No agitator, no heating, no cooling	$13,500	HD Process
E1	Evaporator	1,000 L to evaporate per batch processed in 4 h	$251,000	Thermaflo
T5	Tank 5: Evaporator outlet	Working volume 500 L No agitator, no heating, no cooling	$4,500	HD Process
FD1	Freeze dryer	300 kg liquor per batch	$1,345,000	Cuddon Freeze Dry
M1	Mill	80 kg product per batch processed in 1 h	$14,700	Jiangyin Yinda Machinery Co
RD1	Roller dryer	50 kg/hour evaporation rate	$71,200	Jiangsu Shengman Drying Equipment Engineering Co.Ltd, Changzhou City.

12.3. Appendix 3: Milestone 3. Collagens identified in the Lung-G

Table A3-1. *Collagens identified in Lung-G. Data shown are rank of the collagen in the 187 identified proteins, the number of MS/MS spectra (a measure of abundance), the percentage of the protein sequence covered by observed peptides, and the number of peptides.*

Rank	Gene	Protein	No of MS/MS Spectra	Coverage (%)	No of Peptides
1	COL1A1	collagen alpha-1(I) chain	2211	61.3	360
3	COL1A2	collagen alpha-2(I) chain	1828	61.3	310
5	COL3A1	collagen alpha-1(III) chain	1267	76.1	184
12	COL2A1	collagen alpha-1(II) chain	220	32.8	62
14	COL6A1	collagen alpha-1(VI) chain	190	26.2	43
15	COL6A3	collagen alpha-3(VI) chain	160	11.6	52
16	COL6A2	collagen alpha-2(VI) chain	157	18.7	39
36	COL5A2	collagen alpha-2(V) chain	56	7.7	17
43	COL4A2	collagen alpha-2(IV) chain	36	7.1	13
52	COL8A1	collagen alpha-1 (VIII) chain	29	4.2	4
65	COL5A3	collagen alpha-3(V) chain	19	1.7	5
69	COL5A1	collagen alpha-1(V) chain	18	3.0	4
70	COL7A1	collagen alpha-1(VII) chain	18	0.9	4
89	COL11A1	collagen alpha-1(XI) chain	11	3.6	6
90	COL6A5	collagen alpha-5(VI) chain	11	0.6	2
96	COL4A4	collagen alpha-4(IV) chain	9	1.9	5
102	COL22A1	collagen alpha-1(XII) chain	8	1.6	3
106	COL13A1	collagen alpha-1(XIII) chain	7	4.8	3
111	COL24A1	collagen alpha-1(XXIV) chain	6	1.6	3
120	COL17A1	collagen alpha-1(XVII) chain	5	2.7	3
121	COL8A2	collagen alpha-2(VIII) chain	5	3.4	3
142	COL4A3	collagen alpha-3(IV) chain	3	3.6	2
143	COL9A1	collagen alpha-1(IX) chain	3	2.8	2
155	COL18A1	collagen alpha-1(XVIII) chain	2	1.4	2
156	COL4A5	collagen alpha-5(IV) chain	2	2.1	2
154	COL10A1	collagen alpha-1(X) chain	2	1.6	1
171	COL25A1	collagen alpha-1(XXV) chain	1	1.4	1
172	COL28A1	collagen alpha-1(XVIII) chain	1	1.8	1

Table 3-1. Mass balance for a 600 kg batch of lung producing 81 kg product.

Step		Enzyme hydrolysis							Purfication1 - Screen		Purfication2 - Clarifier		Concentration		Drying	
Stream name		Lung	Water	2M NaOH	Enzyme1	Enzyme2	Lactic acid	Meat slurry	Tissue	Filtrate	Solids	Centrate	Water	Concentrate	Water	Dry extract
Stream type		Input	Input	Input	Input	Input	Input	Process	Waste	Process	Waste	Process	Waste	Process	Waste	Output
Total mass	kg	600	1138	84	1.3	1.3	6.2	1831	271	1560	282	1278	1008	271	190	81
Total solids	kg	120	0.0	6.7	1.3	1.3	5.0	134	39	95	19	76	0	76	0	76
Protein	kg	103	0.0	0.0	0.8	0.1	0.0	104	30	74	12	61	0	61	0	61
Non-protein solids	kg	17	0.0	6.7	0.4	1.2	5.0	30	8.5	22	6.8	15	0	15	0	15
Moisture	kg	480	1138	77	0.0	0.0	1.2	1697	232	1465	263	1202	1008	195	190	5
Total solids	%	20.0	0.0	8.0	100.0	100.0	80.0	7.3	14.4	6.1	6.8	6.0	0.0	28.1	0.0	93.7
Protein	%	17.2	0.0	0.0	65.0	9.0	0.0	5.7	11.3	4.7	4.4	4.8	0.0	22.7	0.0	75.5
Non-protein solids	%	2.8	0.0	8.0	35.0	91.0	80.0	1.6	3.2	1.4	2.4	1.2	0.0	5.4	0.0	18.1
Moisture	%	80.0	100.0	92.0	0.0	0.0	20.0	92.7	85.6	93.9	93.2	94.0	100.0	71.9	100.0	6.3