Final report

Exploring value in pleura removal

Removal of the pleura during dressing and assessment of potential new products

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1.0 Abstract

The parietal pleura, a membrane lining the inner ribcage in cattle, is not currently removed during processing, despite its negative impact on the eating quality of adjoining cuts. This is due to unknown labour requirements, yield impacts, or use-cases for removed pleura. This study explored the ease of pleura removal, reviewed literature and patents on animal pleura, analysed its composition, and identified potential market applications.

Pleurae samples were removed from the 2nd - 8th rib from six hot carcases with relative ease (approximately 10 seconds per sample). The composition of pleura was found to be 43.6 % fat, 40.6 % moisture, and 15.4 % protein. Standard proteomic methods underestimated the collagen content at 0.2 %, but amino acid profiles indicated a high collagen content (>70 % of the protein content).

The study highlighted the lack of literature and analytical methodologies for parietal pleura, necessitating further research and development of specific analytical techniques. Two potential market applications identified for pleura during the project were in advanced wound management (AWM) and pet-care markets. Both are high-growth markets that could value pleura's properties, like high integrity and flexibility, and composition, including collagen and glycosaminoglycans. It is recommended that freedom-to-operate and regulatory requirements are taken into consideration if any products are to be developed from pleura.

This project provides insights into innovative applications for pleura in high growth markets and identifies its potentially valuable components. Removal of pleura could lead to value-addition through new product developments while also benefiting the eating quality of adjoining cuts.

2.0 Executive summary

The parietal pleura is the thin serous thoracic cavity membrane that lines the inside surface of the rib cage and visceral pleura lines the outer surfaces of the lungs. Limited information is available about the composition and properties of the parietal pleura; thus, it remains on the carcase during processing and can impact negatively on adjoining cuts. This study aimed to conduct a literature and patent search on animal pleura, remove and analyse the composition of parietal pleura, and explore market opportunities for its use. This knowledge could inform meat processors about the potential applications of pleura and benefits of pleura removal.

Pleura samples were collected from hot carcases, with an average sample weight of 0.036 kg and a labour requirement of 10 seconds per sample. Samples underwent proximate analysis and quantitative proteomics, revealing high fat (43.6 %) and moderate protein (15.4 %) content. However, current proteomic methodologies highly underestimated the collagen content and highlighted the need for method development specific to the unique pleura matrix. Additional amino acid analysis indicated high collagen content via high hydroxyproline.

A literature review revealed that research on parietal pleura is lacking, with most publications from the 1970s focusing on visceral pleura or the medical field. The literature supported the compositional findings of the current study, and due to a defatting step, collagen and elastin were quantified as the major proteins. The literature search also highlighted that pleura is likely to contain highly valuable health promoting molecules, glycosaminoglycans (GAGs), such as hyaluronic acid, heparin, dermatan sulphate and chondroitin sulphate. A patent search for the term ‘animal pleura’ identified 228 patent families with 36.5 % in medical technology and 26.8 % in pharmaceuticals.

Market analysis identified advanced wound management (AWM) and the pet care sectors as the high growth markets for potential applications. Pleura's composition and unique physical properties make it suitable for AWM products, targeting chronic wound care. Market growth and increased demand will be driven by an ageing population and increased chronic illness. The pet care market also represents a growth opportunity due to increasing pet ownership and humanisation of petfood. Pleura could have value as a source of collagen and GAGs in pet products with joint and mobility claims.

Further research into the development of specific analytical methods for pleura, including defatting steps, is required. The analytical method development should extend to glycosaminoglycan quantification. Prototype products from pleura should be developed to target the AWM and pet care sectors. For any product development from pleura, it is recommended to conduct a cost-benefit analysis, freedom-to-operate search and consider regulatory requirements.

This study highlighted the value-potential of pleura removal. It can be removed with relatively low labour, removal is likely to lead to improved eating quality of adjoining cuts and the properties and composition of pleura could have applications in growth-markets. This could lead to new value-added innovations for the red meat industry.

3.0 Introduction

With 20 % of the carcase accounting for 80 % of the value (MLA, 2024), there are opportunities to value-add in beef processing. Pleura removal and valorisation has the potential to unlock value-addition in beef processing. The pleura is the bi-layer thin serous membrane, whereby one layer (parietal pleura) lines the inside surface of the rib cage and the other layer (visceral pleura) lines the surface of the lungs. In living animals, the purpose of the pleura is lubrication during lung expansion due to respiration. In current processing practices, the parietal pleura remains on the carcase, lining the thoracic cavity. It is not removed as there are several unknowns, notably, the labour required, the impact on yield and benefits of removal or use-cases for pleura. However, chilling regimes increase pleura hardness and adhesion, making it more difficult to remove during boning and resulting in reduced eating quality of the adjoining cuts.

There is lack of existing knowledge on pleura, especially parietal pluera; hence, is was considered necessary to study and summarise existing knowledge on pleura composition and uses, and to conduct full compositional analysis so that an informed value-proposition could be presented. This was performed using CSIRO's library platform to access relevant literature and patents pertaining to animal pleura. Bovine pleurae samples were assessed for proximate analysis (moisture, fat, protein, carbohydrate, ash), amino acid profile and proteomic studies (i.e. protein identification and quantification).

Based on the knowledge gained from literature and analysis, CSIRO worked closely with AMPC to select the two top ideas for pleura applications, including market opportunities for a raw material that currently has no use if removed. This project aims to understand the composition of the pleura and present value propositions for this material if it was to be removed on the kill floor.

4.0 Project objectives

- Conduct a comprehensive review of existing literature, research studies, and patents to gain insights into the composition of pleura and its potential applications.
- Perform detailed compositional analysis and proteomic studies on pleura samples sourced by AMPC.
- Identify two value propositions based on the findings from the compositional analysis and proteomic studies of pleura samples and outline potential applications or market opportunities for utilising pleura or pleura-derived components in meat processing.

5.0 Methodology

5.1 Literature review and patent search

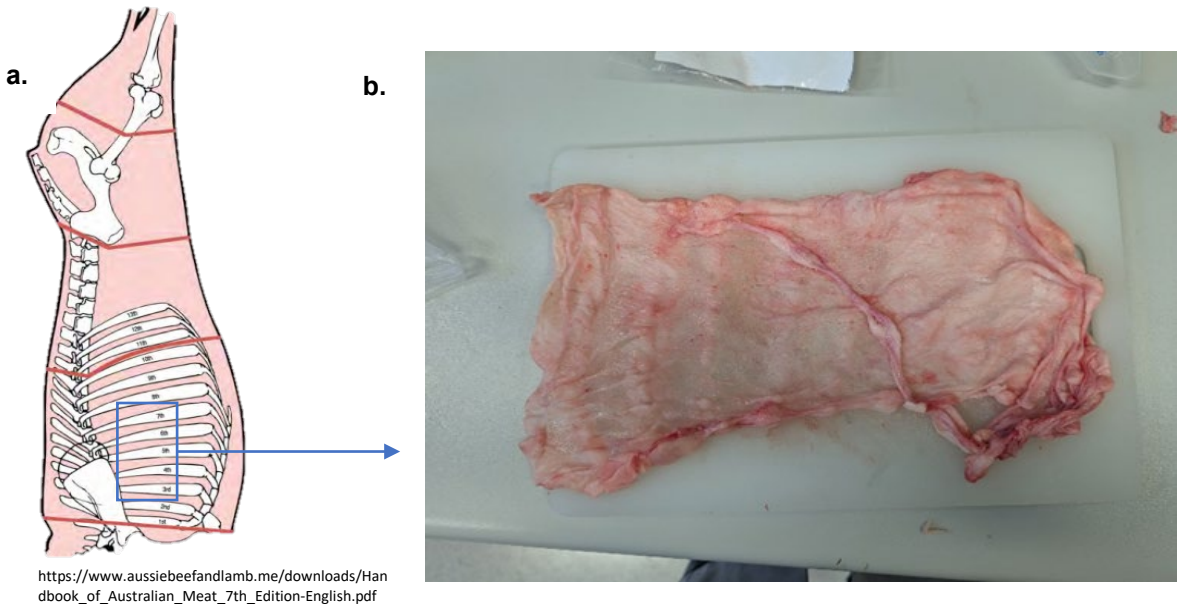
CSIROs library platform was used to conduct a comprehensive desk-based study on pleura composition and current applications of identified proteins. The following search words were used: pleura composition, cattle pleura, bovine pleura.

For the patent search, a brief check of the freely available information source, Google Patents, was conducted to locate any obvious patent disclosures in this area and to assist with the development of the search strategy. Formal patent database searching using the Orbit FAMPAT Database was then conducted. The full patent search methodologies and considerations are detailed further in Appendix 1.

5.2 Sample preparation & analysis

Parietal pleura samples were collected from a local abattoir in southeast Queensland by an AMPC staff member. Rectangular portions of the pleura were excised from the 2nd rib to the 8th rib along the dorsal edge of the ribs and aligned with the ventral edge along the brisket scribe line (Figure 1). Each sample was weighed and the average time to remove the pleura sample was recorded. Samples from six animals were collected and transported to CSIRO's laboratories on the same day.

Portions of each of the six pleurae, representing approximately a quarter of each sample, were removed and reduced into small pieces using a knife. The samples were mixed to create a representative, composite sample and immediately frozen at – 80 °C. The sample was then shipped frozen to a National Association of Testing Authorities (NATA) accredited commercial laboratory for proximate analysis and amino acid profiling. Three pleurae samples were then randomly chosen and a representative sample from each was prepared, frozen at – 80 °C and sent to a second NATA accredited laboratory for quantitative proteomics analysis.



https://www.aussiebeefandlamb.me/downloads/Handbook_of_Australian_Meat_7th_Edition-English.pdf

Figure 1 a) A pictorial depiction of the parietal pleura sampling area and b) a representative image of a collected sample.

5.3 Identification of two value chain propositions

At the completion of Milestone 1, the findings from the literature review and patent search were presented to AMPC, highlighting various applications of the pleura and the value proposition for each of the application. Several potential market segments for pleura application were considered including nutraceuticals, food and beverage, cosmetics, feed and regenerative medicine. It was concluded from the discussions that two ideas should be investigated further and the value propositions refined by researching the market growth potential. They were:

1. Advanced wound management
2. Pet-care market

To understand the relevant industries, market intelligence was gathered from enterprise-approved databases produced by leading global growth consulting companies. These include Frost & Sullivan and BCC Research. Using keywords that match the scientific and technical needs of the specific markets, various reports were gathered from the databases to evaluate the following key variables: Market size in terms of revenue (in USD), compound annual growth rates (CAGRs) over defined periods of time and the drivers and restraints of individual markets. In some cases, it was crucial to understand the business strategies of key industry players to further comprehend proposed value chains.

6.0 Results

6.1 Literature review

The pleura is an anatomic part of the body of the cattle that covers the lung and interior of the thorax. There are two types of the pleurae known as visceral and parietal for each lung. While the focus of this project was on parietal pleura, there is no literature on the chemical composition of parietal pleura. Therefore, some of the information presented is on visceral pleura. The visceral pleura covers the lung, whereas the parietal pleura covers the thoracic cavity. The fluid filled space between the visceral and parietal pleura is called pleural cavity (Aung et al., 2019). The connective tissue of the visceral pleura is connected to the connective tissue of the pulmonary lobular septae (Jennings & Premanandan, 2017). The parietal pleura is subdivided into locational sections known as the costal pleura, diaphragmatic and mediastinal pleura (Miroshnikova et al., 2018). The visceral pleura consists of a single layer of squamous mesothelial cell with microvilli, resting on a thin basement membrane, which is strongly attached to dense connective tissue consisting of elastin and collagen fibres (Peake & Pinkerton, 2015). The dense tissue is underlined by loose connective tissue, extending into the space between the alveoli. The visceral pleural connective tissue is typically thicker in large animal domestic species than in small animal domestic species (Jennings & Premanandan, 2017). The visceral pleura is lined on the outside with squamous mesothelial cells which express proteins typical for mesothelial and epithelial cells (Jennings & Premanandan, 2017). The mesothelial cells secrete a serous liquid which lubricates the pleural surfaces and permit the lungs to move over the thoracic wall reducing the friction while breathing (Aung et al., 2019; Jennings & Premanandan, 2017).

On a microstructure level, parietal pleura has been characterised in sheep to compose of a single layer of mesothelial cells and a layer of loose, irregular connective tissue with thickness of 23 micrometers (Albertine et al., 1984). Figure 2 shows immunofluorescent staining of the connective tissue (collagen and elastin layers) of bovine visceral pleura (Lu et al., 2022).

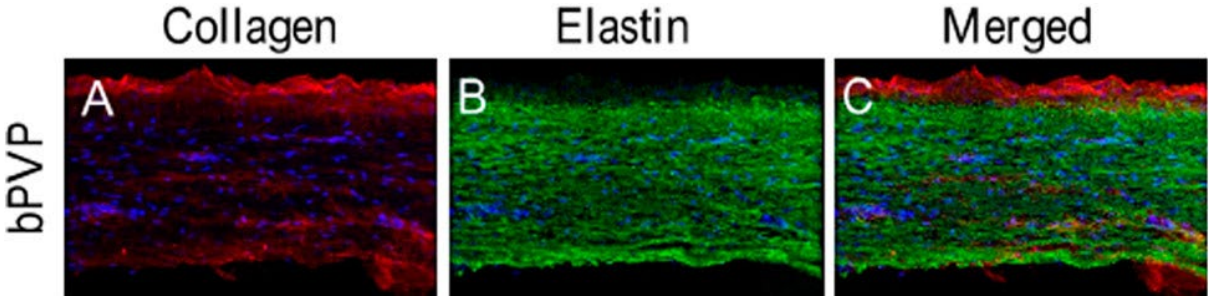


Figure 2 Bovine visceral pleura connective tissue visualized with immunofluorescent staining and fluorescent microscopy (Lu et al., 2022)

Furthermore, there is very little literature on the chemical composition of pleura and almost no literature on the chemical composition of parietal pleura. The existing literature reports that visceral pleura contains about 16 % nitrogen, between 22 and 66 % collagen and between 25 and 36 % elastin on a dry weight basis (Table 1).

Table 1 Composition of bovine visceral pleura based on the published literature

Proteins and carbohydrates in bovine visceral pleura	John and Thomas (1972)	Wusteman (1972)	Francis and Thomas (1975)	Paz et al. (1976)	Lu et al. (2022)
Unit	% (g/100 g)	mg/g dry weight of pleura	g/100 g (salt extracted defatted)	% dry weight	µg/mg dry weight
Collagen		393 (39.3 %)	68		216.7 (21.6 %)
Elastin	27.8	310 (31 %)	28	25	252.1 (soluble), 361.4 (soluble) (25.2 % and 36.1 %)
GAG		6.67 (0.667 %)	4		
Hydroxyproline			9.76		
Carbohydrate			0.94		
Nitrogen			16.6		

On the other hand, there are few articles that characterise the composition of glycosaminoglycans (GAGs) in bovine visceral pleura, with low presence of highly valuable compounds such as hyaluronic acid, heparin, heparan sulphate, dermatan sulphate and chondroitin sulphate (Table 2).

Table 2 Glycosaminoglycans (GAGs) composition of bovine visceral pleura

Glycosaminoglycans (GAGs) in visceral pleura	Wusteman (1972)	Seethanathan et al. (1975)
	per g dry weight of pleura	mg uronic acid/g dry defatted tissue
Total	6.67 (µmol glucuronic acid)	1.19
Hyaluronic acid	0.77 (µmol glucuronic acid)	0.04
Heparin	0.88 (µmol glucosamine)	0.25
Heparan sulphate	0.89 (µmol glucosamine)	0.28
Dermatan sulphate	2.52 (µmol galactosamine)	0.21
Chondroitin sulphate 1/4	0.53/0.41 (µmol galactosamine)	0.31

There are several applications of compounds of pleura reported in literature (Table 3), including cosmetics, biomaterials and food and feed applications. Compounds like collagen tend to be extracted using denaturation and enzymatic procedures (Figure 3). Processed pleura applications include a variety of biomaterials for medical purposes (Table 3). Collagen has numerous applications across cosmetics, biomaterials and food and feed (Figure 4). In addition, smaller compounds, such as bioactive peptides and GAGs, have also numerous applications for medical and food purposes (Table 3). However, many focus on visceral pleura, highlighting the opportunity to further explore parietal pleura physical properties, composition and applications.

Table 3 Applications of pleura and pleura derived ingredients in the published literature

Application		Reference
Biomaterials		(Lu et al., 2022)
Vanous patch for reconstruction of veins		(Lu et al., 2020)
Heart valve (cusps)		(Chen et al., 2022; Lu et al., 2023)
Artery graft (made from porcine visceral pleura)		(Lu et al., 2019)
Bio scaffold for solving air leaks as a consequence of surgery, physical trauma and pneumothoraces		(Vikranth et al., 2024)
Collagen		
Food applications	Food additive /Ingredient (collagen, gelatin of hydrolysates, peptides)	(Tang et al., 2022)
	Food packaging and preservation material (collagen or gelatin)	
	Functional foods (skin health, cardiovascular disease, muscle health)	
Wound healing	Guiding function, chemotactic properties, nucleation, reutilization	(Chvapil et al., 1973)
	Review	
Biopolymer for prosthetic material		(Chvapil et al., 1973)
Biomaterial		(Chvapil et al., 1973; Ferraro et al., 2016)
Cosmetics		(Avila Rodríguez et al., 2018)
Elastin		
Biomaterial		(Del Prado Audelo et al., 2020)
Biomaterial for tissue engineering		(Daamen et al., 2007) (Ferraro et al., 2016)
Bioactive peptides		
Antioxidant, ACE inhibitor, antiphotaging, DPP IV inhibitor, cryoprotective, antimicrobial, inhibition of lipid oxidation		(Ferraro et al., 2016)

GAGs	
Sugar drugs for parasitic and viral infections	(Yamada & Sugahara, 2008)
Regenerative medicine (Growth factors for tissue regeneration)	(Yamada & Sugahara, 2008)
Anti- tumour drugs	(Yamada & Sugahara, 2008)

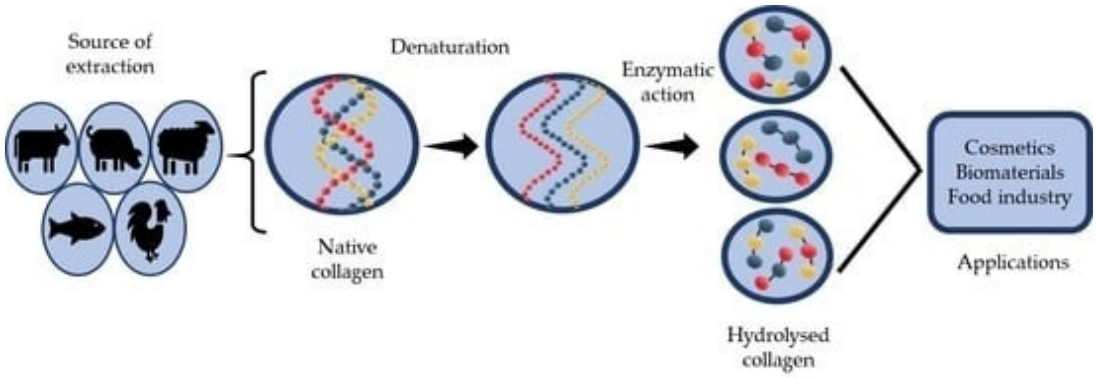


Figure 3 Usual process flow for treatment and applications of collagen (León-López et al., 2019)

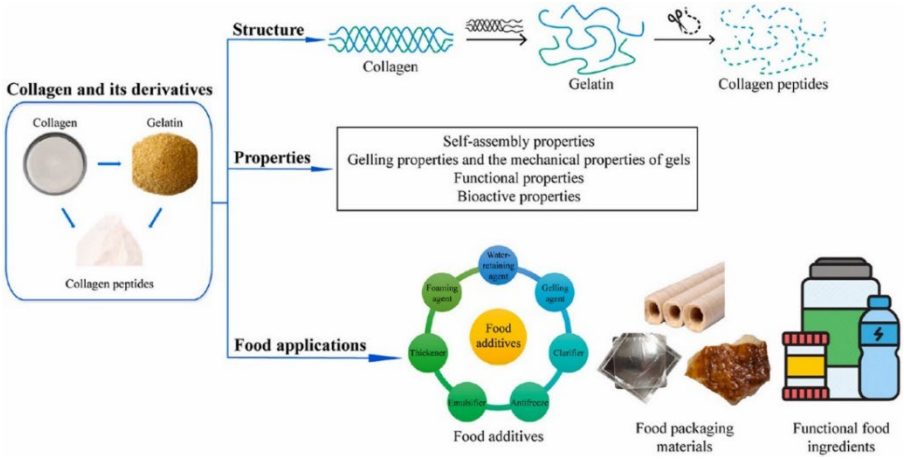


Figure 4 Collagen applications in the food industry (Tang et al., 2022)

6.2 Patent search

A patent search was conducted for the term ‘animal pleura’ with details of the search aspects and number of patent families shown in Table 4, including other search aspects and number of patent families. In total, the search led to 228 identified patents families.

Table 4 Patent search aspects and number of patent families identified

Search Aspect	No. of Patent Families
Landscape area Animal pleura including, but not limited to, bovine pleura	228
Use in selected specific applications:	
▪ Biomaterials / biopolymers in general including specifically use in packaging materials / sheets	77
▪ Use in medical applications including specifically medical bioscaffolds, prosthesis or stents, or wound healing use including use in medical dressings	112
▪ Use in cosmetics	8
▪ Use in foods as specifically an additive or functional ingredient, or as a pet food / animal fodder	51
Application of selected specific individual components of the pleura:	
▪ Collagen or Gelatin	111
▪ Elastin	22
▪ Polysaccharides and Mucopolysaccharides including Glycosaminoglycans such as Glucosamine, Hyaluronic acid, Chondroitin, Heparan sulphate, Chondroitin 4-sulphate, Chondroitin 6-sulphate, Dermatan sulphate and Heparin	80
Removal of pleura from a carcass during meat processing, for use in applications	15

Medical technology (36.5 %) and pharmaceuticals (26.8 %) were the top applications (Figure 5). In terms of countries, the US is the leading country with 92 patent families, followed by China with 89. Australia is ranked 5th globally with 8 patent families.

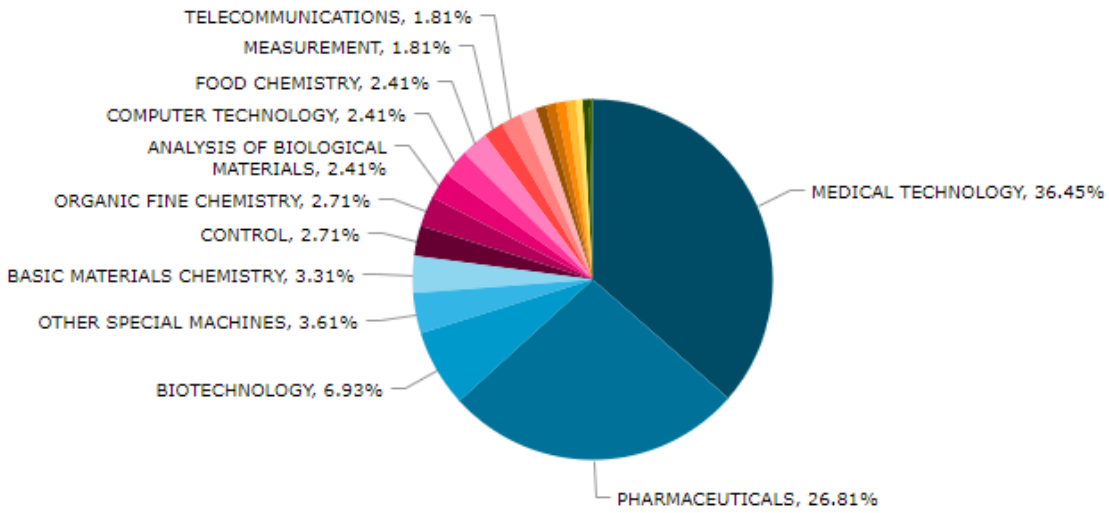


Figure 5. Patent classifications to show a summary of where the patent families sit in terms of technology domains

Of the 228 patents,17 % owned by the top 10 players (Figure 6). Polares Medical, headquartered in the United States (US) and Switzerland, hold the most patent families (n=5) and all their patent families are live (Figure 6). Polares Medical specialise in treating mitral valve regurgitation, a heart condition, and they have a patented device in which part-of may be composed of pleura. Therefore, while there were many patent families identified in the medical sector, not all are related to wound dressings. On further investigation, many were aligned to veterinary surgery of animal pleura rather than pleura applications.

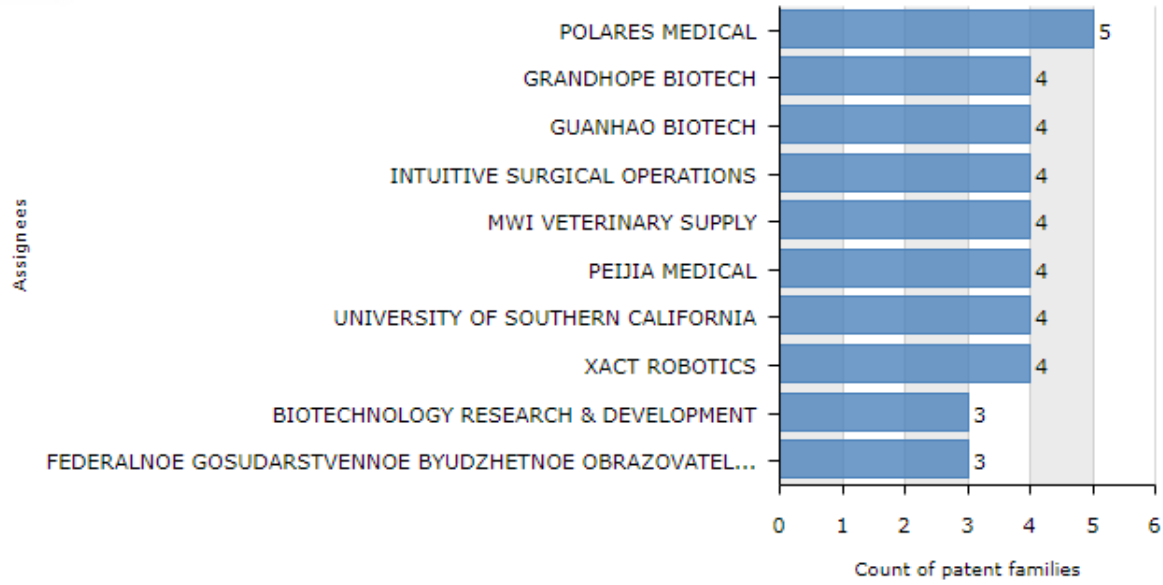


Figure 66 Summary of top 10 assignees of various patent families for animal pleura

Of the 228 patents, several key patents were further identified within this group, with relevance to advanced wound management (Appendix 1). For example, a patent (CN102085386) for the preparation of medical porcine or bovine pleura biological dressing, filed in China by Hebei Iron and Steel Co Ltd Handan Branch, describes their process of

wound dressing formation from pleura as simple and low cost. The patent also states that the process leads to a product that is relatively stable, flexible, transparent for wound observation, elastic and with key medical benefits of reduced rejection, pain and scarring. Steps are outlined for processing the pleura which include fat removal, disinfection, preservation and storage. While this highlights the suitability of pleura for this application, it also highlights the need to ensure novelty of pleura applications for dressings.

A total of 51 patents were identified for pleura use in foods, specifically an additive or functional ingredient, or as a pet food / animal fodder, however none mentioned using pleura directly as a petfood ingredient or a pet treat. On closer inspection, within those patent families that appeared in the category for pleura removal during processing, the majority were also aligned to the medical field. Therefore, once a specific invention with use of pleura is determined, a more specific freedom-to-operate search is recommended.

6.3 Pleura collection and analysis

6.3.1 Sample collection

The labour requirement for pleura removal was 10 seconds of skilled slaughterman time per carcase. It was removed with ease from the hot carcase before chilling. The weight of the removed pleurae was 0.036 ± 0.165 kg (n=6). It was removed from the section adhering to the chuck rib, rib-prepared and short rib. The side weights were 201.6 ± 17.0 kg and the removed pleurae represented 0.018 ± 0.01 % of side weight.

6.3.2 Proximate Analysis

To help understand the potential uses for the parietal pleura, particularly with minimal processing, it is important to determine its chemical composition. As mentioned in the previous section, literature describing the composition of bovine pleura, particularly the parietal pleura, is scarce. The proximate and amino acid results are reported on a percentage basis in Table 5. Results are reported on a ‘fresh weight’ basis and a ‘dried and defatted’ basis was estimated from the fresh weight data to allow easier comparison to existing literature.

Table 55. Proximate and amino acid results, ± the measurement of uncertainty for the parietal bovine pleura composite sample. Results are reported on a fresh weight and dried and defatted equivalent’ basis as a percentage of the total sample weight.

Parameter	Fresh weight %	Dried & defatted equivalent %
Moisture	40.6 ± 0.8	-
Protein (N x 6.25)	15.4 ± 0.6	97.5 ± 3.8
Total Fat	43.6 ± 1.2	-
Ash	0.5 ± 0.1	3.2 ± 0.6
Carbohydrate (by difference)	< 1	-
Aspartic Acid	1.10 ± 0.11	7.0 ± 0.7
Serine	0.69 ± 0.07	4.4 ± 0.44

Glutamic Acid	2.00 ± 0.2	13.0 ± 1.3
Glycine	3.50 ± 0.35	22.0 ± 2.2
Histidine	0.12 ± 0.01	0.8 ± 0.08
Arginine	1.20 ± 0.12	7.6 ± 0.8
Threonine	0.40 ± 0.04	2.5 ± 0.3
Alanine	1.60 ± 0.16	10.0 ± 1.0
Proline	1.80 ± 0.18	11.0 ± 1.1
Tyrosine	0.23 ± 0.02	1.5 ± 0.15
Valine	0.47 ± 0.05	3.0 ± 0.25
Lysine	0.78 ± 0.08	4.9 ± 0.49
Isoleucine	0.29 ± 0.03	1.8 ± 0.18
Leucine	0.74 ± 0.07	4.7 ± 0.47
Phenylalanine	0.39 ± 0.04	2.5 ± 0.25
Methionine	0.17 ± 0.02	1.1 ± 0.11
Hydroxyproline	1.60 ± 0.16	10.0 ± 1.0
Taurine	0.01 ± 0.0007	0.04 ± 0.004
Cysteine	0.10 ± 0.01	0.6 ± 0.06
Tryptophan	0.03 ± 0.003	0.2 ± 0.02

Overall, the parietal bovine pleura can be considered high in fat, low in moisture and of moderate protein content when comparing it to other bovine tissues, such as muscle. In this study, the fresh pleura moisture content was 40.6 %, fat 43.6 % and protein 15.4 %. The ash content, representing the mineral content of the pleura, was 0.5 %.

The amino acid profile is shown in Table 5. The parietal pleura is rich in glycine, glutamic acid, proline, alanine, and hydroxyproline at fresh weight values of 3.5, 2.0, 1.8, 1.6 & 1.6 % respectively, and is low in tryptophan at 0.03 %. Collagen is primarily made up of glycine, proline and hydroxyproline (Gauza-Włodarczyk et al., 2017), with the latter being a typical marker to calculate total collagen content in meat products (Stoilov et al., 2018). As per Table 1, Francis & Thomas (1075) found salt extracted, defatted and dried visceral pleura to have a hydroxyproline content of 9.76 %, which corresponded to a collagen content of 68 % in that study. That result for hydroxyproline is very similar to our findings of 10.0 % in dried and defatted pleura. Based on the amino acid profile of the pleura, it is expected that collagen would form the dominant part of the proteome of the pleura, making up a significant proportion of the total protein. It is estimated that collagen would make up 74 % of the dried and defatted pleura, however this number may be somewhat overestimated due to presence of elastin in the pleura which contains around 1 % hydroxyproline by weight (Stoilov et al., 2018).

6.3.3 Quantitative proteomics

To our knowledge there is no published research on the full proteome of bovine parietal pleura. In total, 2541 individual proteins were identified, and the abundance of each protein was normalised to a percentage of the total protein abundance and then reported as both a percentage of the pleura fresh weight, and then calculated on a dried and defatted pleura weight basis. Haemoglobin, alpha and beta subunits, were detected in the proteomic data but removed from the analysis as they were likely detected due to small amounts residual blood contamination on the received samples.

Table 66. The top 50 most abundant proteins found in the pleura by quantitative proteomics. The abundance of each protein was normalised to the total protein abundance and presented as a percentage of the fresh weight and dried and defatted protein content of the parietal pleura.

Protein	Fresh weight pleura (%)	Dried and defatted pleura equivalent (%)
Albumin	1.22	7.75
Actin, gamma-enteric smooth muscle	0.76	4.79
Histone H2A.Z	0.66	4.15
Decorin	0.49	3.09
Histone H4	0.48	3.04
Histone H2B type 1-K	0.45	2.82
Actin, cytoplasmic 1	0.40	2.56
Serotransferrin	0.36	2.27
Mimecan	0.31	1.97
Lumican	0.28	1.77
Histone H3.3	0.27	1.71
Vimentin	0.27	1.70
Serpin A3-7	0.20	1.30
Annexin A2	0.20	1.26
Histone H1.3	0.17	1.11
Apolipoprotein A-I	0.17	1.05
Fatty acid-binding protein, adipocyte	0.15	0.92
Prolargin	0.14	0.87
Hemopexin	0.14	0.86
Keratin, type II cytoskeletal 8	0.13	0.80

Annexin A5	0.12	0.76
Protein S100-A4	0.11	0.67
Histone H1.1	0.10	0.64
Collagen alpha-1(I) chain	0.10	0.63
Keratin, type I cytoskeletal 19	0.09	0.58
14-3-3 protein epsilon	0.09	0.57
Peptidyl-prolyl cis-trans isomerase B	0.09	0.57
Phosphatidylethanolamine-binding protein 1	0.09	0.54
Elongation factor 1-alpha 1	0.08	0.50
Pancreatic trypsin inhibitor	0.08	0.50
Galectin-1	0.08	0.49
Heat shock protein beta-1	0.08	0.49
L-lactate dehydrogenase B chain	0.07	0.47
Transthyretin	0.07	0.47
Endoplasmic reticulum chaperone BiP	0.07	0.46
Glyceraldehyde-3-phosphate dehydrogenase	0.07	0.44
Alpha-2-HS-glycoprotein	0.07	0.42
Tubulin alpha-1B chain	0.06	0.40
Serpin A3-8	0.06	0.38
Peptidyl-prolyl cis-trans isomerase A	0.05	0.33
Annexin A1	0.05	0.33
Protein S100-A10	0.05	0.32
Profilin-1	0.05	0.31
Asporin	0.05	0.30
Biglycan	0.05	0.30
14-3-3 protein beta/alpha	0.05	0.29
Transgelin-2	0.04	0.28

Complement C3	0.04	0.28
Collagen alpha-2(I) chain	0.04	0.28
Histone H2A type 2-C	0.04	0.27

The top 50 most abundant proteins found using quantitative proteomics are shown in Table 6. Unexpectedly, albumin was the most abundant protein found at 1.22 % fresh weight which may be elevated due to the small amount of blood contamination on the pleura samples. Of the expected proteins to be dominant in the pleura proteome only collagen alpha-1(I) and collagen alpha-2(I) chain were present in the top 50 most abundant proteins at 0.1 & 0.04 % fresh weight basis, respectively. Based on the small amount of published literature of collagen and elastin content in visceral bovine pleura, this result is surprising. Upon further investigation however, some reasons for the underrepresentation of these proteins in the proteomic data set start to become apparent.

The pleura is rich in extracellular matrix (ECM) proteins, which form the complex structural network in multicellular organisms. This complexity presents challenges for sample preparation methodologies, making it difficult to extract and quantify these proteins using modern proteomics workflows. In a review by Naba (2023), the challenges in analysing tissues with high levels of ECM proteins are presented.

Table 77. Quantitative proteomic results for collagen types and elastin found in the parietal pleura. The abundance of each protein was normalised to the total protein abundance and presented as a percentage of the fresh weight and dried and defatted protein content of the parietal pleura.

Protein	% of protein content	% of dried and defatted pleura equivalent
Collagen alpha-1(I) chain	0.10	0.63
Collagen alpha-2(I) chain	0.04	0.28
Collagen alpha-1(III) chain	0.03	0.20
Elastin	0.01	0.03
Collagen alpha-1(IV) chain	0.01	0.03
Collagen alpha-3(IV) chain (Fragment)	0.002	0.012
Collagen alpha-4(IV) chain (Fragment)	0.002	0.012
Collagen alpha-2(IV) chain (Fragment)	0.001	0.005
Collagen alpha-1(XII) chain (Fragment)	0.0003	0.002
Collagen alpha-1(XI) chain (Fragment)	0.0001	0.001

Firstly, tissues that are rich in ECM proteins undergo comprehensive post-translational modifications (PTMs), which include glycosylation, and lysine and proline hydroxylation reactions, which occur to provide stability (Rappu et al., 2019). These PTMs present two challenges for accurate proteomics assessment, being accurate protein database identification and protein solubility. Firstly, any peptides generated during sample processing that have undergone PTM will not be identified in database searches used to identify individual proteins unless the PTMs are accounted

for. Secondly, ECM proteins are naturally insoluble due to their large size and function as biological scaffolds, making protein extraction difficult, and leading to an underrepresentation of these proteins (Naba, 2023). As these proteins are in high proportions in tissues such as pleura, protein extraction is challenging and modifications to proteomics extraction methodologies are required. In this study, a re-analysis of the protein data was undertaken to account for the expected PTMs, resulting in the identification of the expected ECM proteins, although in very low concentrations. Proteins expected to be present in significant proportions in the pleura, such as elastin and collagen types, were detected as shown in Table 7, but they made up only 0.2 % of the pleura fresh protein weight. This indicates that the methodology used to extract these key ECM proteins was insufficient for the pleura matrix and further method development will be needed to accurately describe the parietal pleura proteome.

6.4 Value propositions

Bovine pleura is a unique material composed primarily of moisture, fat and connective proteins such as elastin and collagen. The global collagen market is expected to reach $6.2 billion USD in revenue by 2027, growing at a CAGR of 5.9 % from 2022 to 2027 (BCC Publishing, 2022). Interestingly, the highest growth is expected in Asia-Pacific with a CAGR of 10.4 % from 2022 to 2030, followed by North America at 9.7 %. Currently, the main sources of animal collagen are skin, tendons, bones, cartilage from cattle (37 %), pigs (26.9 %), fish (30.9 %) and poultry (4.3 %) (BCC Publishing, 2022). Bovine sources dominate due to easier accessibility of raw materials and lower costs compared to other sources. Extracted collagens type I and type II, gelatin and collagen peptides find applications in various markets; nutritional supplements (42.4 %), food and beverage (25.5 %), pharmaceuticals (16.1 %), cosmetics (9.2 %) and others (6.8 %). With an increasingly ageing global population and more consumer awareness around the health benefits of collagen, the number of clinical trials for collagen-based products and treatments has more than doubled, from 105 in 2010 to 225 in 2022 (BCC Publishing, 2022; Dhiman, 2022). However, it must be noted that the majority of clinical trials involve collagen type II, predominantly from poultry (i.e. chicken sternum cartilage). Collagen type II is most valuable for its health benefits on bones & joints but has higher production costs due to lower abundance and specialised extraction methods.

While the components of pleura, such as collagen, could be extracted for various applications, it may be more economical to leverage the intrinsic characteristics (thin, translucent, resilient, flexible) of pleura for unique applications within growth application markets. Various growing markets for potential pleura application were identified and discussed with AMPC staff. The two market segments identified for more detailed investigation are discussed further.

6.4.1 Advanced wound management

According to the World Health Organisation (WHO), there will be 2.1 billion people over the age of 60 years by 2050 (WHO, 2024). This will represent 16.7 % of the global population being over 65 years by 2050, up from 9 % in 2019 (BCC Publishing, 2023). The largest increases were seen in developed countries, for example, Japan (Figure 7). However, there is an increasing ageing population in every country in the world, and by 2050, the number of people over 80 years of age is predicted to triple (WHO, 2024). With increased aging, along with increasing obesity, there will be a rise in chronic illnesses like diabetes. Collectively, this will lead to more people experiencing chronic wounds, i.e., a wound that fails to progress through the normal stages of healing. Chronic wounds include diabetic foot ulcers, pressure ulcers and venous leg ulcers. The increased prevalence of ulcers will be closely correlated to the increase in type 2 diabetes, as a result of obesity. More than 365 million people worldwide who are obese have type 2 diabetes. Associated diabetic foot ulcers result in over 50,000 amputations annually in the US. This wound sector will need innovations that can accelerate healing times and reduce the risk of infection.

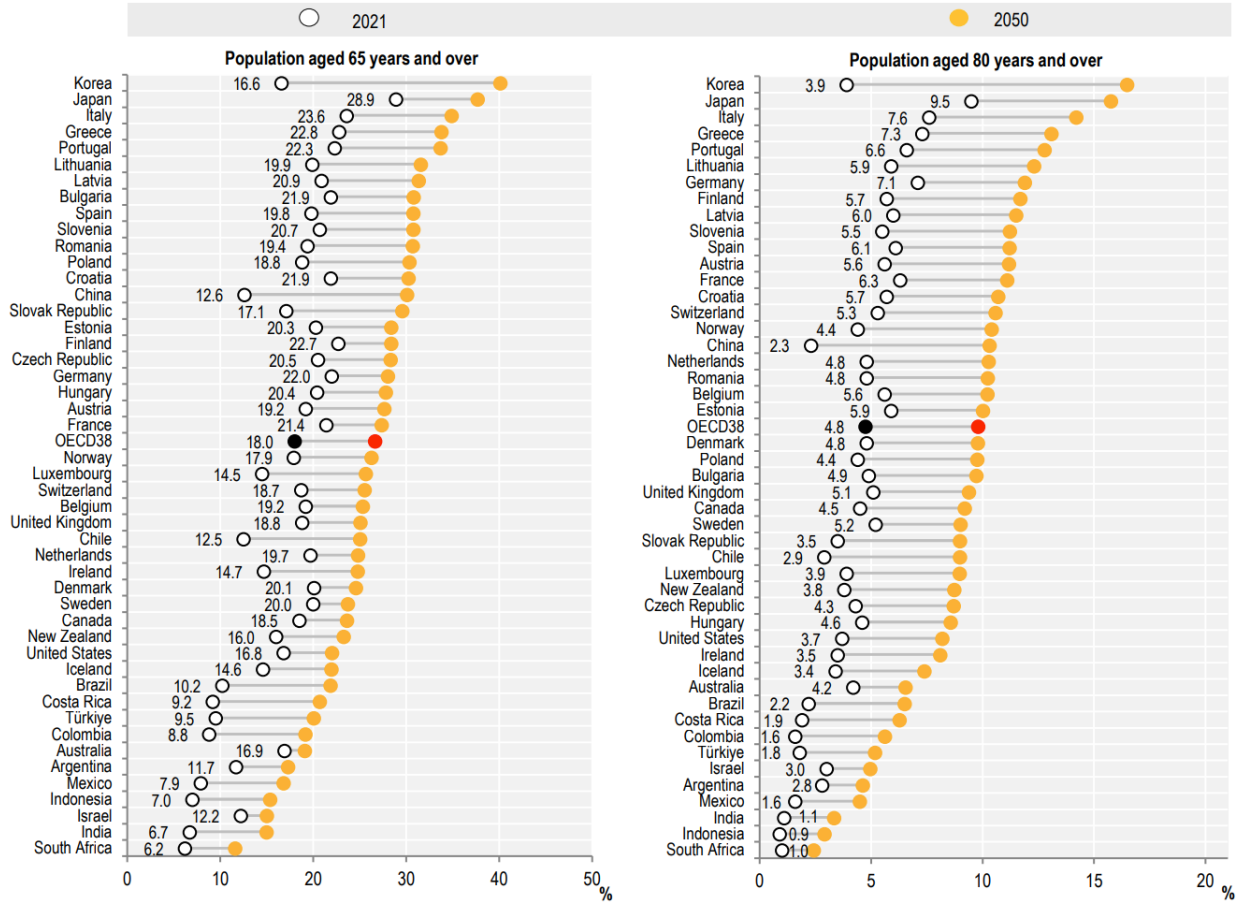


Figure 77 Share of the population aged 65 and over and 80 and over, 2021 and 2050. Sources: OECD Health Statistics 2023, OECD Historical Population Data and Projections (1950-2060) database (OECD, 2023).

The global advanced wound management (AWM) sector was valued at $10.7 billion in 2022 and is projected to increase at a CAGR of 6.2 % to 2027. Within this sector, the chronic wound management products account for 50.2 %, with an expected annual growth of 12 % (BCC Publishing, 2023). A pleura-derived wound dressing would be categorised as a wound biologic/bioactive and this category of AWM products has a CAGR of 12.2 % from 2023-2028 (Fig. 8). The main growth markets for AWM in Asia-Pacific are India (10.1 %), China (9.6 %) and Japan (8.2 %) due to population growth and ageing populations, and these represent untapped markets (BCC Publishing, 2023).

In 2021, there were 16 clinical trials for wound biologics specifically for the treatment of diabetic foot ulcers (BCC Publishing, 2023). Ulcers are treated with wound dressings, and bovine collagen dressings are commercially available. However, it is also possible to treat ulcers with xenografts, more commonly used for burns, and the properties of pleura are more aligned with some specialist products in this market segment. The most specialist xenografts are Porcine Small Intestinal Submucosa (SIS) Xenografts, marketed as promoting healing and tissue regeneration due to the presence of collagen, elastin, glycoproteins (fibronetin), glycosaminoglycans (heparin, hyaluronic acid) and proteoglycans (heparan sulphate, proteoglycan) (Cook BioTech, 2024). Some of these components are found in pleura as mentioned previously. A study in 2017 found that while SIS xenograft application could cost $3019.89 USD, it could reduce overall costs per patient for subsequent healthcare by $105 USD per year and have 32 % increased probability of healing and a 42 % increase in the number of ulcer-free months (Guest et al., 2017).

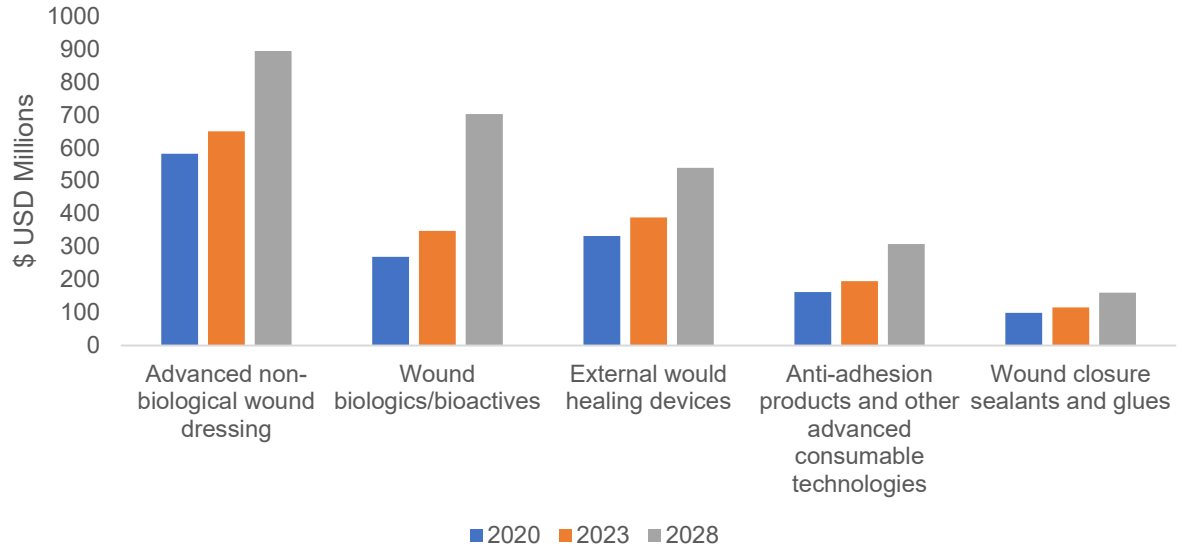


Figure 88 Asia-Pacific advanced wound-management products, by type, through 2028 (BCC Publishing, 2023)

In terms of developing a product with pleura in this sector, there are two key opportunities. Firstly, as wound management is complex and often requires a range of products, healthcare purchasers are consolidating to streamline procurement. This has led to established medical companies buying smaller companies so that they can offer a more diverse product portfolio, while also acquiring intellectual property. Secondly, there are many untapped markets outside of the US for AWM products, notably, Japan, China and India. However, there are also market restraints to consider. For example, any new development is subject to stringent regulatory environments. Products would require approval from the Food and Drug Administration (FDA) in the US or the Therapeutic Goods Administration (TGA) in Australia, or other relevant markets. Therefore, considerations for time to market should be included in any business plan.

6.4.3 Pet care market

The pet care market was valued at $1,883.5M USD in 2023 and has a revenue CAGR of 5.7 % through to 2030 (Fig. 9) (Frost&Sullivan, 2024). The main market drivers for growth include increasing pet ownership. For example, in Australia the number of pets (29 million) has outnumbered the human population (26 million) (RSPCA, 2024). Another market driver is increased pet-owner affluency, as 82 % of Australia pet-owners are from households with income over $100,000 AUD. Asia-Pacific is the main growth market globally with a CAGR of 8.4 % from 2023-2030, where China is the primary driver, but Japan is also a key market, where the pet population has outnumbered children under 15 years of age. A final key driver of market growth is the humanisation of pet-food, which has resulted in pet owners seeking products with health claims.

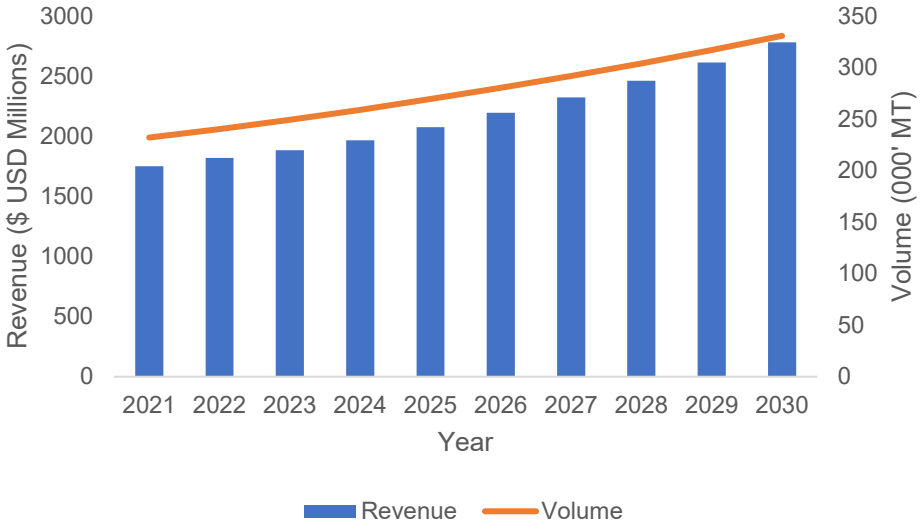


Figure 99 Pet-food ingredients revenue and volume forecast, global, 2021-2030 (Frost & Sullivan, 2024)

There is an opportunity to innovate in this sector by combining ingredients that draw on the collagen and elastin content, as well as GAGs content to create pet foods with mobility claims for older pets like dogs (Beynen, 2016). GAGs are functional mucopolysaccharides associated with health benefits. Collagen and glucosamine and chondroitin (G&C) are projected to have revenue growth of 8.9 % and 5.7 % CAGR, respectively, through to 2030 (Figure 10). They are desirable petfood ingredients for their association with hip and joint health. The price point for these products in market ranges between $15-29 per kg.

Alternatively, purified GAGs achieve a better price point, with chondroitin sulphate for example being priced at $65-150/kg. However, the challenge of adding purified ingredients, such as GAGs from pleura, is that they are in low concentrations in pleura, therefore use/applications needs to be found for the rest of the material once the GAGs are extracted. Also, pleura as a source for GAGs would need to compete against other sources like cartilage.

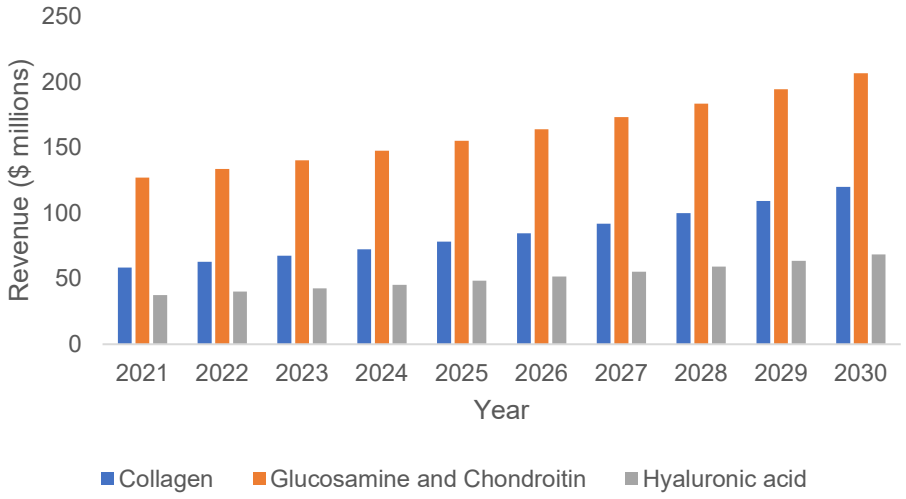


Figure 1010 Collagen, glucosamine and chondroitin and hyaluronic acid revenue forecast, global, 2021-2030 (Frost & Sullivan, 2024)

Hip and joint health claims on products account for 14 % of petfood revenue (Frost & Sullivan, 2024). This is driven by the fact that about 25 % of dogs and up to 90 % of cats will get arthritis. G & C function in synergy for joint health. Glucosamine stimulates glycosaminoglycan production, which holds moisture, acting as an anti-inflammatory allowing cartilage synthesis, while chondroitin inhibits white blood cell enzymes that damage cartilage. Both are also thought to have beneficial effects on the renal and circulatory systems (Frost & Sullivan, 2024).

Despite the demand for G & C in petfood, the Food and Drug Administration (FDA) in the US does not permit the addition of extracted G & C ingredients to pet products. This is a regulatory challenge for market growth for extracted ingredients; however, it could present as an opportunity for pleura. Petfood manufacturers who desire to make health claims in the US add natural sources such as poultry meal or hydrolysed crustaceans. Similarly, pleura could be manufactured into an ingredient within petfood formulations, adding to the ability for hip and joint claims. In Australia, G & C can be added to pet products as ingredients; however, they need to follow the regulatory requirements of Australian Pesticides and Veterinary Medicines Authority (APVMA).

7.0 Discussion

This study presents findings on the literature and patents pertaining to animal pleura and associated applications. The literature search highlighted the lack of research on parietal pleura, so findings on visceral pleura, while also limited, were presented. The majority of compositional data on pleura was from several decades ago, with the exception of Lu et al (2022). There are varied reports on the quantities of collagen and elastin in pleura, ranging from 20-70 % in dried and defatted pleura. Further research is required to confirm the composition of pleura, and this should be performed using newly developed methods specific to the unique pleura matrix. Assuming that pleura collagen content ranges from 20-70 %, it might be more economical to leverage the unique intrinsic physical characteristics of pleura such as high integrity and flexibility. While 228 patents were identified for animal pleura, many were for the veterinary field, e.g., referencing to live animal surgery, and not applicable to this study. However, several patents of interest were identified pertaining to wound dressing applications, highlighting the requirement for freedom-to-operate searches pertaining to an invention.

Regarding removal and analysis, the parietal pleura was removed from the section adhering to the chuck rib, rib-prepared and short rib, therefore yield impacts should be considered. Given the proposed value-propositions for pleura applications and the likely improved eating-quality improvement for these cuts, any yield impact could be off-set but this requires a full cost-benefit analysis during any prototype development. As there is no known published proximate analysis of bovine parietal pleura, some comparisons are drawn to lung. Selmane et al. (2008) assayed the proximate composition of bovine lung tissue and found that it was much higher in moisture, much lower in fat and had a slightly lower protein content when compared to pleura. In that study, bovine lung was found to contain 78.5 % moisture, 13.8 % protein and 2.9 % fat. The high fat content of the pleura may be a challenge for any minimal processing use cases of the pleura and at a minimum, a defatting step may need to be included as an initial step, with options to capture the removed fat as a value-added side stream. Drying and defatting the pleura results in a product that has a very high in protein content at 97.5 %, which may be useful for several applications outlined in this report such as wound dressings or pet ingredients/treats. The proteomic data was much lower than anticipated and the reasons are thought to be a) post-translational modifications within standard proteomic methods which are not suitable to pleura, and b) the large protein size within pleura resulting in poor extraction. This highlights the need for proteomic methodology development specific to pleura.

Leveraging the unique properties of pleura, two value propositions based on its integrity and composition were presented and the associated markets were discussed. The AWM and pet-care markets are growing at 6.2 and 5.7 % CAGR, respectively, and they offer key opportunities for pleura. However, two key considerations for any product development are freedom-to-operate and regulatory hurdles.

8.0 Conclusions

- Parietal pleura can be removed from the hot carcase with relative ease requiring as little as 10 seconds per animal
- The parietal pleura is high in fat (40 %) and contains moderate protein (15 %)
- Drying and defatting pleura could yield a product with very high in protein content (97.5 %)
- Pleura is high in glutamic acid, glycine, alanine, proline and hydroxyproline, indicating collagen content
- Proteomic and glycosaminoglycans analytical techniques for bovine tissue are not directly applicable to pleura
- There is a lack of literature on bovine parietal pleura, with most publications occurring in the 1970s
- The advanced wound management and petfood sectors represent growth markets where pleura could find an innovative application
- There are already several patents in the medical field referring to animal pleura

9.0 Recommendations

- Analytical methods should be developed specifically for pleura as the current methods do not accurately quantify the pleura proteome
- A defatting step should be included in pleura proteome analysis
- The GAGs content of pleura should be assessed
- Research should be undertaken for prototype product development using pleura for the AWM and pet care markets, as these have been identified as the high growth areas
- Any innovation involving pleura should include a thorough cost-benefit analysis and a freedom-to-operate search
- For all potential applications, regulatory requirements should be considered

10.0 Project outputs

- Milestone report and PowerPoint presentation delivered 21st October 2024 online to AMPC by CSIRO project team.
- Final report

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12.0 Appendix 1 – Patent Search

Patent search strategy

The search strategy used combinations of the following key words to locate relevant documents: pleura, pleurae, pleuras, nonhuman+, non_human+, “non+ human+”, animal+, livestock+, live_stock+, “live+ stock+”, bovid+, bovin+, ovine+, ruminant+, cattle+, cow?, bison+, buffalo+, antelope+, sheep+, goat+, ewe?, ram?, lamb+, bull?, heifer?, calf, calves, oxen, alpaca+, llama+, camel+, deer?, biomaterial+, bio_material+, “bio+ material+”, biopolym+, bio_polym+, “bio+ polym+”, packag+, sheet+, material+, biodegrad+, bio_grad+, “bio+ grad+”, bioscaffold+, bio_scaffold+, “bio+ scaffold+”, prosthes+, strent+, “wound+ heal+”, dressing+, bandag+, cosmetic+, toiletr+, food?, fodder+, collagen, gelatin+, elastin+, polysaccharide+, mucopolysaccharide+, glycosaminoglycan+, glucosamin+, hyaluronicacid+, hyaluronic_acid+, “hyaluronic+ acid+”, “heparan+ sulphate+”, “heparan+ sulfate+”, chondroitin+, “dermatan+ sulphate+”, “dermatan+ sulfate+”, heparin+, removal+, extract+, cut+ and separat+ (where + indicates a word truncation, ? indicates a single character substitution, “ “ indicates a combination of words within a certain proximity, and _ represents a space or single character between two words)

These key words were searched in all cases in the title, abstract and claims fields, and extended to the full description as required. The search also used the following International Patent Classifications (IPCs) and / or Cooperative Patent Classifications (CPCs): A22+, A23+, A23J-003/06, A23J-003/342, A23V-2250/51+, A23V-2250/5422, A23V-2250/5432, A61C-013+, A61D-009+, A61F-002+, A61F-013+, A61K-006+, A61K-008+, A61K-008/65, A61K-008/73+, A61K-2800+, A61L+, A61L-027+, A61L-027/24 and A61Q+ (where + indicates a class truncation). Finally, a check to identify any relevant patents where CSIRO is the applicant was performed as part of the search strategy. Results are presented as patent families rather than individual patents.

Patent search limitations

There are several limitations relating to patent searching that make it impossible to conduct an exhaustive patent search and hence guarantee that all relevant patents have been located. The following are three examples of these limitations:

- A patent search strategy included the use of keywords and/or patent classifications. It is not possible to conceive and use every possible keyword variation in the search strategy. It is also not possible to guarantee that all relevant classifications will be discovered and searched or that patents will be correctly classified.

- The search strategy was implemented in an appropriate database. Each database has respective limitations as to its patent coverage and the level of information available for patent records. There is no single database that has complete coverage of all patent data. The same key word search in one particular database will not necessarily provide the same results in another database.

- Irrespective of the database used, patent information is not published in databases when a patent is first filed (i.e., at the priority date). The first publication of information for a patent may not occur for at least 18 months in most cases. Therefore, it is likely that any searching results for more recent years do not yet include all patent applications.

Table 1A. Examples of key patent families identified with reference to medical applications

Publication numbers	Publication dates	Earliest priority date	Title	Latest standardized assignees - inventors removed
CA1234653 WO85/01651 EP0160025 EP0160025 EP0160025 DE3480693 ATE48532 US4681588 AU559373 AU3553284 JPS61-500302	1988-04-05 1985-04-25 1989-12-13 1987-01-20 1985-11-06 1990-01-18 1989-12-15 1987-07-21 1987-03-05 1985-05-07 1986-02-27	1983-10-20	(EP0160025) Biomaterial	BIO NOVA NEO TECHNICS OF VICTORIA WILKRACHT
US20230285634 US20160067031	2023-09-14 2016-03-10	2014-09-08	(US20230285634) Methods and uses of mediastinal pleura tissue for various stent and other medical applications	CVDEVICES
US11000285 US20180235634 WO2015/095380 GB2536174 GB2536174 GB201611887 US10314686 US20160331511	2021-05-11 2018-08-23 2015-06-25 2020-12-16 2016-09-07 2016-08-24 2019-06-11 2016-11-17	2013-12-17	(US11000285) Luminal grafts and methods of making and using the same	3DT HOLDINGS
CN118304484	2024-07-09	2024-04-11	(CN118304484) Guided tissue regeneration membrane and application thereof	HANGZHOU HUAMAI MEDICAL TECHNOLOGY
CN1522767	2004-08-25	2003-02-21	(CN1522767) Preparation method and use of biological membrane and material adapted to the membrane for medical use	CAO XU
WO2015/142509 EP3119446 EP3119446 ES2742701 PL3119446 JP6502376 JP2017512563 AU2015231836 AU2015231836 CN106132450 BR112016020367 US9801910 US20150258142	2015-09-24 2019-06-12 2017-01-25 2020-02-17 2019-10-31 2019-04-17 2017-05-25 2018-03-29 2016-09-15 2016-11-16 2016-11-01 2017-10-31 2015-09-17	2014-03-17	(EP3119446) Decellularized pleural matrix	ETHICON

CN102085386 CN102085386	2013-06-05 2011-06-08	2011-01-30	(CN102085386) Preparation method of medical porcine or bovine pleura biological dressing	HANDAN BRANCH HEBEI IRON & STEEL
US20210161970 WO2013/120082 US10940167 US20150064140 EP2811939 EP2811939 EP2811939 EP2811939 EP3281608 EP3281608	2021-06-03 2013-08-15 2021-03-09 2015-03-05 2017-11-15 2017-09-20 2015-10-07 2014-12-17 2020-09-16 2018-02-14	2012-02-10	(EP3281608) Medical product comprising a frame and visceral pleura	CVDEVICES
CN117771442	2024-03-29	2023-12-28	(CN117771442) Multilayer soft tissue repair patch and preparation method thereof	SHENZHEN LANDO BIOLOGICAL MATERIALS
CN106390201	2017-02-15	2016-09-21	(CN106390201) Absorbable medical biological membrane and preparation method thereof	TIANJIN OUERKE MEDICINE TECHNOLOGY