final report

Project code: A.BIT.0011

Prepared by: Chris Dahm

Thricor Pty Ltd

Date submitted: December 2010

Date published: May 2011

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Alternative use of hides Literature and patent review

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

1 Abstract

The value of hides and skins to Australia has fallen significantly, from being worth AUS$1,008 million in 2005 to AUS$764 million in 2009[1]. This project conducted a critical review covering the last ten years of novel applications for hides and skins from the red meat industry based on their material composition, other than for leather. The information was reviewed and opportunities assessed within each of seven categories selected, which were application areas such as food and medical. This assessment enabled a ranking of major, medium, and minor opportunities which could be facilitated in three areas of material component focus, these being;

1) The extraction of functional 'collagen fibres' that may be utilised in food, packaging, medical and Industrial applications.
2) The effective hydrolysis of collagen optimally utilising biological, chemical and/or physical means.
3) Characterisation and specification of collagen hydrolysates that accounts for origin, processing and functionality, enabling effective manufacturing utilisation.

Within these areas the opportunities clearly exist to broaden the demand and reduce sensitivity of hide and skin products to the luxury consumer markets.

[1] Ashley, P. (2010). Victorian Food and Fibre Export Performance. C. Jonathan and F. Simon, Dept of Primary Industry.

2 Executive summary

The value of hides and skins to Australia fell significantly, from being worth AUS$1,008 million in 2005 to AUS$764 million in 2009[2] due to a drop in prices by 37% and despite an increase in exports of 20% the value apparently due to of the global financial crisis which caused a drop in demand for luxury consumer goods.[3] The opportunity exists for new applications of hides and skins to broaden the demand and reduce sensitivity to luxury consumer markets.

The objectives of this project included a critical literature and patent review, covering the last ten years, of novel applications for hides and skins from the red meat industry based on their material composition, other than for leather. Additionally, a quantification of, current and recent hide prices, the value derived potentially from these new applications and an appropriately presented report for meat processors to motivate further investigation of value adding options.

Databases were selected based on application relevance and number of peer reviewed journals accessible, additional patent searches were run directly through both the European and United States patent offices. Searches were executed using preliminary keywords such as bovine and hide, which were followed by limiting keywords such as cross-link and collagen and these reviewed with application dependent keywords such as scaffolds and drug delivery. These searches were reversed stepwise as it was noted from article references that relevant material was being filtered due to preliminary keywords.

The information was reviewed and opportunities assessed within each of seven categories selected being; extraction and recovery, food related, research and development, medical, pharmaceutical, packaging and Industrial. A critique was performed on each document, followed by a review within each category group, mapping relationships. This assessment enabled a ranking of major, medium, and minor opportunities based on market volume, value and potential of return to MLA stakeholders. These opportunities can be facilitated in three areas of material component focus, these being as follows with a summary of the opportunities;

[2] Ashley, P. (2010). Victorian Food and Fibre Export Performance. C. Jonathan and F. Simon, Dept of Primary Industry.

[3] Morgan, T. (2010). "Trends in Australian co-product values in 2009." MLA market information, Co- products Brief.

1) The extraction of functional 'collagen fibres' that may be utilised in food, packaging, medical and Industrial applications.

a) Hide stabilisation using new milling process A.MPT.0027 within seconds, so they can be stored or transported. b) Collagen fibres as a matrix for fixing adsorbents and catalysts, having a large surface area and able to be cross-linked.
c) Collagen fibre and powder as 50:50 film components, adding resilience to collagen film.
d) 9M urea extraction process, simple efficient and suitable for tissue engineering. e) Collagen alginate interaction in medical and food products, unique composite structure interaction f) Skin casing change from gut to collagen opportunity for bovine currently going to porcine g) Osmotic drying of meats, exploiting water vapour permeability of collagen film h) Effects of ball milling, may be damaging fibrils, what is the functional impact? i) Membrane recovery of Pt and Pd, using low cost and efficient cross-linked hide. j) Raw material for thermoset and thermoplastic extrusion a large range of options including biodegradable, leather look and feel and leather like paint.

2) The effective hydrolysis of collagen optimally utilising biological, chemical and/or physical means.

a) Optimised chemical and biotechnological hydrolysis of collagen to gelatine and hydrolysates b) Low fat food products from specific gelatines for emulsification, replacing fat in chocolate
c) Specific cleavage of bovine hide collagen using mutant pepsin to increase gelatine yield
d) Thermoplastic extrusion of collagen to thread to make woven articles e) Halal gelatine, a market with growth potential f) Use of soy protein isolate for reduction of water vapour permeability in bovine gelatine film

3) Characterisation and specification of collagen hydrolysates that accounts for origin, processing and functionality, enabling effective manufacturing utilisation.

a) Understanding collagen hydrolysates like 'antifreeze protein'
b) Hydrogels as collagen hydrolysate composites for biodegradable nappies c) Use of collagen hydrolysate for retention in nutritional fortification like iodine & thiamine d) Injected collagen hydrolysates may assist cartilage repair e) Thermal treatment of, dialdehyde starch cross-linked collagen hydrolysate to reduce 'film aging'. f) Biodegradable packaging from polyvinyl blends with collagen hydrolysate.

The potential within these three focused areas supports the premise that opportunities exist for new applications of hides and skins to broaden the demand and reduce sensitivity to luxury consumer markets.

Contents

3 Background

3.1 Introduction

Australian hides and skin prices for 2009 dropped significantly by 37%, despite an increase in exports of 20% the value ostensibly due to of the global financial crisis which caused a drop in demand for luxury consumer goods.[4] Hides and skins constitute over 50% of the total red meat co-products value which totals around 11% value of the slaughtered animal.[5] This significant drop has reduced returns for cattle and sheep prices right across the entire supply chain.

The value of hides and skins to Australia has fallen significantly in the last few years, from being worth a little over AUS$1 billion from 2005 to AUS$764 million in 2009[6]

The MLA's Bioactives program aims at growing the demand and hence the value of red meat through building industry capability to develop red meat derived bioactive products, thereby resulting in an increased share of value added profits for red meat processors and producers. The opportunity exists for new applications of hides and skins to broaden the demand and reduce sensitivity to luxury consumer markets.

This project seeks to complete a literature and patent review of novel uses of hides and skins other than for leather, identifying opportunities to reduce the reliance of the industry on the clothing and industrial leather markets.

Potential Industry Benefits

Adding value to red meat co-products, which together make up the largest portion of the slaughtered animal, being approximately 62% of the weight, is an opportunity which needs attention. Hides and skins have carried the largest value part of this segment and as such when significantly impacted, as has happened with the global financial crisis, have come under significant focus.

Hide material composition has been relatively well investigated as being the natural source component of leather and gelatine. However new potential applications of its components are being found which fall into the area of bioactives as a result of altering component functionality through processes like cross-linking, digestion, polymerisation and spinning.

These new potential applications need to be identified and evaluated as opportunities to encourage further investigation of value adding, reducing the reliance of the industry on the clothing and industrial leather markets.

4 Project objectives

This project will conduct a critical review of novel applications for hides and skins from the red meat industry based on their material composition, other than for leather.

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[4] Morgan, T. (2010). "Trends in Australian co-product values in 2009." MLA market information, Co- products Brief.

[5] Anonymous (2010). "Co_products." from http://www.redmeatinnovation.com.au/innovation-areas/value- adding/co-products.

[6] Ashley, P. (2010). Victorian Food and Fibre Export Performance. C. Jonathan and F. Simon, Dept of Primary Industry.

The objectives of this project include

1. A critical literature and patent review evaluating these new applications
2. A quantification of the value
    a. derived from hides, both current and recent
    b. derived potentially from these new applications
3. A consideration of structural, nutritional and food processing applications including the potential for altering functionality of hide products and components through various biochemical, chemical and physical processes including cross-linking, digestion, polymerisation and spinning
4. An appropriately presented report for meat processors to motivate further investigation of value adding options.

Additional modifications to the project objectives that have been agreed upon include limiting the search period to the last ten years and ensuring novel applications.

5 Methodology

5.1 Database selection

Database access was carried out through both University libraries and through the internet directly where appropriate. University library databases were grouped under subjective 'area headings' (e.g. Agriculture, Applied Science...Medicine … Science etc). Because ‘material composition’ was used as the evaluation basis, ‘area headings’ were selected which were most likely to cover this in relation to the major application areas.

From within each selected ‘area heading’ the Databases, numbering from 6 to 20 per area, were reviewed for number of peer reviewed journals and application relevance. These were reduced to the best 3 or 4 Databases in total to ensure that some diversity was maintained. The Databases chosen were Science Direct, Scopus, Proquest and Business source premier

5.2 Searching Strategy

An initial Boolean search of selected keywords (using OR type expression) was carried out in each selected database under an advanced search and refined with added keyword limitation (utilising AND/AND NOT type expression depending on the database) until total search numbers fell into the 60 to 150 range. The preliminary keywords used were: bovine, ovine, goat, hide, skin and dermis. These were then limited through material composition terms like collagen, gelatine, GAG, fib(), cross-linking.

This strategy was then modified a number of times to explore variations in terminology which tended to be application dependent like ‘scaffolds’ and ‘drug delivery’.

It was later found through analysis of the list of references for most articles, that considerable information was not being caught due to the use of the preliminary keywords which were filtering a large number of potentially relevant articles.

For each database these abstracts were assessed for relevance. Full text documents were then sourced and a bibliography constructed - using Endnote. As an example: A large body of work on collagen type I does not reference any relationship to the preliminary key words. So when collagen was used as a keyword limitation, most of these were already excluded. The search strategy was then modified to start with each of the material composition terms and filter with the preliminary terms where necessary. While this process extended the time involved in reviewing what appeared to be relevant articles, it significantly improved information sourcing.

While patent searching was included in the above strategy, additional patent searches were run in esp@cnet (European patents office) and The US Patent office which have additional patent researching tools.

A search for economic data was also completed. Some of this was accessed through MLA with respect to hide's current and recent value. However, for selected new applications a business/economic database *Business source premier* was searched along with industry associations in the relevant application areas. The information sourced was selected based on its appropriateness for the new applications, ensuring a global perspective. In some application areas validity of information was difficult to verify and in such situations either printed corroboration was sought or a 'parent' market segment used that would include the application.

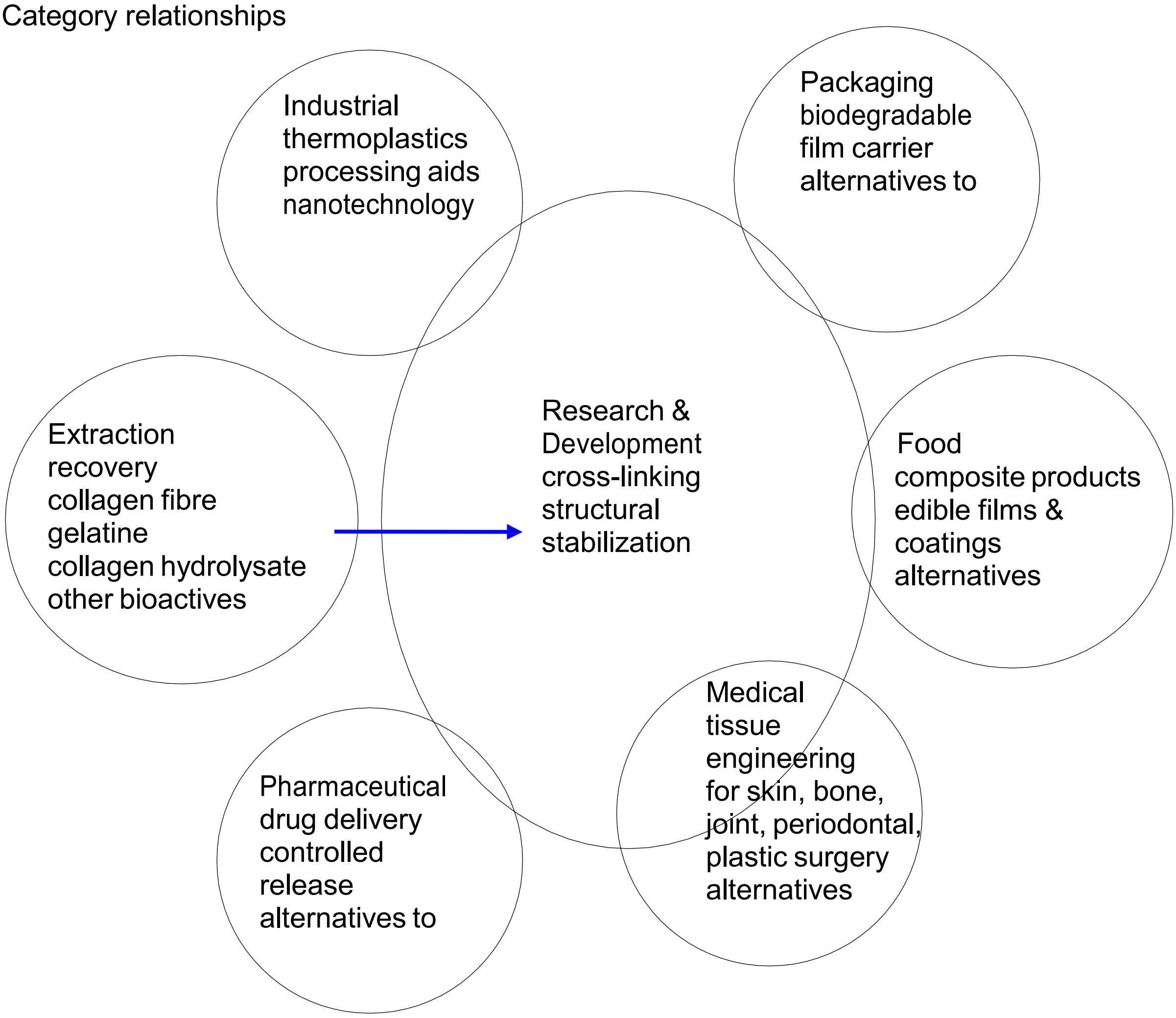
5.3 Categories

As shown in Figure 1 there is significant commonality with respect to the 'material composition' of hides and skins (hides), which drive the investigations, within each application area. These include aspects such as cross-linking, biocompatibility, denaturation, hydrolysis and structure.

However, these are recognized as drivers for investigations and not 'market defining' so these have been allocated across the following Categories:

- Extraction - those applications concerned with providing a primary product
- Food related – those applications which must meet food regulations
- Industrial – applicable to all fields but not specific to one
- Medical surgery – those directly related to surgical use
- Packaging – all packaging other than edible
- Pharmaceutical – any chemical used for medical diagnosis, treatment, cure, or prevention of disease that is non surgical.
- Research & Development – modifications through stabilization or structural

Figure 1 Category relationships



5.4 Critical Analysis

Full text documents have been reviewed to critically understand aspects of context. A critique was completed for each document assessing credibility and value. This was followed by a review within each category group, mapping relationships.
Any apparent gaps in the literature were identified and the search design was reviewed (order and/or new keywords) and carried out again.

This process has resulted in a significantly longer time to complete the review than planned, as each of the categories are very large and yet the articles are highly specialized.

6 Results and discussion

The following sections are a broad review of the body of work that has been completed over the last 10 years concerning hide components other than leather and include the consequences of the bovine spongiform encephalopathy occurrences. Where possible market opportunities are identified and potentials assessed, in all cases, it is recommended that those areas of interest chosen to be explored further, also be subjected to a more rigorous value assessment.

Opportunities that have been highlighted have been ranked taking into account market volume, value and potential of return to MLA stakeholders. These rankings appear as shade weighting of the frames as follows; Major Medium Minor

6.1 Extraction and recovery

6.1.1 Extraction

Collagen makes up 20 to 30% of the slaughtered animal and 70 to 90% of this is collagen type 1.[7] Most bovine hides, usually the corium from leather processing, are processed to produce either collagen dispersions, used mainly for sausage skins, or gelatine processing.[8] Traditionally gelatine has been made by extracting collagen from hides under alkaline conditions[9] generating type B gelatine. For food this has changed more to sourcing from porcine both in Europe (80%)[10] and America (70%)[11] which is extracted under acid conditions making gelatine type A.

Application of chemical and biotechnological processes to maximise value output from extraction of collagen using tendons has been shown[12] (opportunity to explore with application to hides). This process begins with dilute acetic acid yielding 5% (300 kDa), followed by the extraction of gelatine with water (acetic acid concentration 0.08 – 0.15 mol/l and temperature 70 – 75 °C), to yield gelatine of 350 – 410° Bloom and finally enzymatic hydrolysis into collagen hydrolysate 500 to 1000 Da.[13] Through this approach, yields for each stream are maximised and targeted for a premium product in each phase.

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[7] Zeugolis, D. I., S. T. Khew, et al. (2008). "Electro-spinning of pure collagen nano-fibres - Just an expensive way to make gelatin?" Biomaterials 29(15): 2293-2305.

[8] de Wolf, F. A., Ed. (2003). Progress in Biotechnology, Industrial Proteins in Perspective Chapter 5. Collagen and gelatin.

[9] Galea, C. A., B. P. Dalrymple, et al. (2000). "Modification of the substrate specificity of porcine pepsin for the enzymatic production of bovine hide gelatin." Protein Science 9(10): 1947-1959.

[10] Anonymous (2010). "Raw materials." Gelatin.org. from http:/www.gelatine.org/en/about- gelatine/manufacturing/raw-materials.html.

[11] Anonymous (2010). "Raw materials & Production." GMIA. from http:/www.gelatin- gmia.com/html/rawmaterials.html.

[12] Mokrejs, P., F. Langmaier, et al. (2009). "Extraction of collagen and gelatine from meat industry by- products for food and non food uses." Waste Management and Research 27(1): 31-37.

[13] *Ibid*.

The challenge with this approach for mature bovine hide is the low yields achieved with acid hydrolysis of 20-30 % of solubilised collagen.[14] Enzyme hydrolysis has been shown to be effective at improving the extraction yield of collagen from bovine hide in use with acid hydrolysis, but yield conversions to gelatine were low due to excessive degradation of the collagen.[15] Through site directed mutagenesis of porcine pepsin it has been proposed that mutant pepsins can be generated to enable specific cleavage of bovine hide collagen enabling solubilisation under much milder conditions, and hence minimise the denaturation and proteolytic cleavage that results from extraction under alkaline conditions.[16] Initial results with selective peptide sequences have proven successful, similar to Cathepsin D cleavage occurring at just one site Leu-Ser and at a rate 23 times that of the wild type pepsin.[17]

This work was done in Australia and could be beneficial. There was no evidence found that any further work was undertaken and this has been confirmed through contact with one of the authors. With the progress that has been made in the last 10 years it is definitely worth reviewing this as it may provide a mechanism to produce high grade gelatine with a higher yield from mature bovine hide. Bovine hide accounts for only 28%[18] of the total gelatine production where porcine accounts for 42%, and this is primarily as a result of the food market which requires more high bloom gelatine.

A new extraction procedure recovering collagen (from tendons) for tissue engineering utilises a two step purification starting by extraction with 9M Urea at 25 °C for 20 hours and centrifuged at 4000 g for 30 minutes and is then followed by Superose 12 chromatography, the column is equilibrated with 8 M urea at p H 7.5 and 100mM NaCl.[19] The purified collagen is re-natured to form triple helices by dialysis against water at 4 °C for 2 days. Recovery was shown to be 95% of collagen type I. Of additional interest is that previously acetic acid extracted collagen type I from tendons could not be solubilised in 9M urea. The collagen extract was assessed and found suitable for biotechnological applications and tissue engineering. This is a simple, high yielding process but may require further work on the dialysis stage for scale-up. It is recognised that this was developed on tendons and may be less effective on hides.

An alternative to the biochemical 'clip and snip' is the physical 'smash and bash' where limed cattle hides have been split and the corium dried to 15 to 20% w/w moisture and adjusted to pH 8 to 9. These were ground to flakes and subjected to 9 – 10 MJ/kg of work through a Haake Rheomix 90 to form soluble hydrolysates that were still able to form stiff gels when cooled. The product contained a wide, evenly spread range of molecular weight sizes with none of the peaks

[14] Galea, C. A., B. P. Dalrymple, et al. (2000). "Modification of the substrate specificity of porcine pepsin for the enzymatic production of bovine hide gelatin." Protein Science 9(10): 1947-1959.

[15] Chomarat, N., L. Robert, et al. (1994). "Comparative efficiency of pepsin and proctase for the preparation of bovine skin gelatin." Enzyme and Microbial Technology 16(9): 756-760.

[16] Galea, C. A., B. P. Dalrymple, et al. (2000). "Modification of the substrate specificity of porcine pepsin for the enzymatic production of bovine hide gelatin." Protein Science 9(10): 1947-1959.

[17] *Ibid*.

[18] Jarvis, L. (2002). "Gelatin Market Stable as BSE Moves From the Spotlight." Chemical market reporter 261(6): 8.

[19] Xiong, X., R. Ghosh, et al. (2009). "A new procedure for rapid, high yield purification of Type I collagen for tissue engineering." Process Biochemistry 44(11): 1200-1212.

indicating α-chains and β components that both collagen and gelatine do, although gelatine is more spread.[20]

This is a relatively quick procedure with a high yield that appears simple and may be suitably applied to standard twin screw extrusion. While all collagen was thought to be made soluble, the gels were turbid and had gel strengths around half that of the first extract gelatine from cattle hides. Further work looking at application of the gels would better define the value of this product as it may interact well in composite systems as found in food products where opaqueness often can be a benefit, especially in diet products like low fat cheese.

Ultrasonic irradiation has also been used to assist pepsin extraction of collagen type I from tendons, this showed an increase in extraction of 124 %, believed to be as a result of opening up the fibre structure to improve the enzymes access.[21]

An MLA project recently completed on a novel milling process, A.MPT.0027, may be applicable with some of the above extraction options. This process was shown to be able to stabilise fresh wet hides with two passes, each less than 1 second, to a water activity of 0.2. This should provide an option to store and transport raw materials not being processed for leather. While the collagen fibres are torn apart in the milling operation, they remain essentially as fibres with diameters less than 10 µm. This should enable both rapid solubilisation via 9M urea or efficient enzyme activity utilising mutant pepsin.

6.1.2 Recovery

Though this review does not include leather, it is pertinent to consider opportunities which are applicable to existing leather processing that would assist in either alternative applications to leather, and hence facilitate existing asset utilisation, or may be applied as an adjunct.

Aqueous two phase systems (ATPS) are an alternative separation process for recovering biomolecules from multifarious mixtures.[22] An ATPS occurs when a number of hydrophilic solutes show incompatibility in aqueous solutions above critical concentrations.[23]

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[20] Meyer, M., R. Mühlbach, et al. (2005). "Solubilisation of cattle hide collagen by thermo-mechanical treatment." Polymer Degradation and Stability 87(1): 137-142.

[21] Li, D., C. Mu, et al. (2009). "Ultrasonic irradiation in the enzymatic extraction of collagen." Ultrasonics Sonochemistry 16(5): 605-609.

[22] Rito-Palomares, M., C. Dale, et al. (2000). "Generic application of an aqueous two-phase process for protein recovery from animal blood." Process Biochemistry 35(7): 665-673.
, Dallora, N. L. P., J. G. D. Klemz, et al. (2007). "Partitioning of model proteins in aqueous two- phase systems containing polyethylene glycol and ammonium carbamate." Biochemical Engineering Journal 34(1): 92-97.
, Saravanan, S., J. R. Rao, et al. (2007). "Partition of tannery wastewater proteins in aqueous two- phase poly (ethylene glycol)-magnesium sulfate systems: Effects of molecular weights and pH." Chemical Engineering Science 62(4): 969-978.
, Saravanan, S., J. R. Rao, et al. (2008). "Aqueous two-phase poly(ethylene glycol)-poly(acrylic acid) system for protein partitioning: Influence of molecular weight, pH and temperature." Process Biochemistry 43(9): 905-911.

[23] Rito-Palomares, M. (2004). "Practical application of aqueous two-phase partition to process development for the recovery of biological products." Journal of

Chromatography B 807(1): 3-11.

Two solutes which have shown functionality with tannery waste water are polyethylene glycol (PEG) and polyacrylic acid (PAA).[24] An examination of the impacts of the molecular weight of PEG, tie line length (tll), pH, temperature and sodium chloride (NaCl) concentration on yield for recovery of glycosaminoglycans (GAGs) in tannery waste water has been investigated and partition coefficients along with yields calculated.[25]

The yields for this work were better in PEG with a molecular weight of 4000 (the lowest reviewed), tll (highest), pH of 8.0 (highest), temperature of 20 °C (lowest), NaCl of 0M (lowest) and were 91.5% and 83.7% for standards of dermatan sulphate (DS) and chondroitin sulphate (CS) respectively. For GAGs extracted from tannery waste water a yield of 69.7% was achieved at the tll 54.7 (% w/w) (highest).

This process would appear to have significant potential for efficient recovery because it involves very few steps including mixing, centrifugation, settling and recovery. Further exploration would be required to optimise the process, especially as the best yields were all at the limits investigated. Also most work in this separation process appears to have occurred over the last 5 years, hence a few fundamental aspects may yet need elucidation.

The content of GAGs in bovine hides is 0.2 – 0.3 % based on raw weight.[26] Therefore, if a tannery is processing 2000 kg/day of hides[27] a maximum theoretical yield of around 2.8kg/day of GAGs may be expected. Market values vary widely depending on grade, volume and application, however as an indication: food grade chondroitin sulphate, minimum purchased 100kg, varies between US$59 to US$82 per kg.[28] Hence the number of hides being processed would need to be very high and, as such, the recovery of solutes PAA and PEG would also need to be very efficient. The process also seems to have sensitivity to salts which are generally quite high in tannery waste water.

An alternative process for preparation of hides claims the use of dimethyl ether (DMS) as a reactor solvent to degrease more than 98% of fat and dehydrate more than 90% of water from animal skins. It is proposed that it takes less than 1 hour to process, having an extractor contact time of up to 30 minutes. It is claimed that the solvent is easy to recover using 1 litre of extractor solvent per kg of skin and that the system is better run under pressure, 4 bar and 25 °C in the reactor. The inventors claim that the process is not impacted by either salts or acid and alkalai detergents.[29]

This process may benefit high volume processing where the investment to install the process correctly would provide the returns through efficiency very quickly. Being a solvent with a low vaporisation temperature of -23.6 °C and flash point of – 41 °C[30] an automated process would be

[24] Saravanan, S., J. R. Rao, et al. (2006). "Recovery of value-added globular proteins from tannery wastewaters using PEG-salt aqueous two-phase systems." Journal of Chemical Technology and Biotechnology 81(11): 1814-1819.

[25] Raghava Rao, J. and B. U. Nair "Novel approach towards recovery of glycosaminoglycans from tannery wastewater." Bioresource Technology In Press, Corrected Proof.

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[27] Anonymous (2002). "TRADE WASTE MANAGEMENT PLANS." from http://www.vicwater.org.au/uploads/Downloads/TWMPG%20&%20Template04.pdf.

[28] Anonymous (2010). from http://au.alibaba.com/products/Chondroitin-sulphate.html.

[29] Berkhout, H. J. and J. R. Garcia Del Rio (2003). Process for treating animal skins. European patent office. W. I. P. Organisation. WO 2005/059184 A3.

[30] Windholz, M., Ed. (1976). The Merck Index, Merck & Co Inc.

required, especially as the process would need to be run under inert or extinguisher gases such as nitrogen or carbon dioxide. What the process may provide, but is not claimed, is the concentrated recovery of the by-products, oils/fats and some water soluble components in a form which may be easily exploited.

A new hair saving unhairing method containing an enzymatic preparation (NUE 0.6 MPX), sodium hydroxide, sodium sulphide and disodium hydrophosphate (2%) uses an enzymatic dehairing mechanism though removal of sulphated proteoglycans (decorin)[31] which the authors claim results in a high quality pelt and reduces the pollution caused be sodium sulphide.[32] While the process is identified as being 10 – 15% more expensive than conventional liming, the reduction in waste as pollution load is likely to redeem some of this. The modelled optimum time for this process is 5.2 to 7 hours and use of enzyme 0.6 – 0.7 % of the hide mass.

In summary the refinement in extraction recovery of components as collagen, gelatine or collagen hydrolysates will assist all sectors and benefit stakeholders directly.

6.2 Research & Development

As indicated in the classification section at the front of this document, research and development is a significant aspect in all applications of hide products. This results in overlaps so that investigation outcomes are frequently being applied across categories. Broadly, most of the investigations in this area can be divided as being stabilisation or structurally motivated. The body of work in this area is significant and has many different threads, which are not constrained by the categories chosen for this review. A proportional sample is provided for both areas, where possible an assessment on where this work may have application is made.

6.2.1 Stabilisation

A study was undertaken to stabilise collagen with a plant polyphenol from Acacia Mollissima in the presence of an acrylic polymer.[33] Three approaches were explored, firstly collagen with 1% acrylic for 12 hours (CA12), secondly collagen with 5% polyphenol for 12 hours (CP12) and thirdly collagen with 1% acrylic polymer for 1 hour followed by 5% polyphenol for 12 hours (CA1P12). It was found that both CP12 and CA1P12 increased the hydrothermal stability, with CA1P12 increasing it by 25 °C. Investigations point to the acrylic polymer opening up the collagen structure and enabling the polyphenol better penetration.

An exploration of the thermal, dielectric, and piezoelectric properties of galactomannan collagen cross-linked films was conducted to find a new material to be used with electronic devices, such as surface acoustic wave devices for pressure sensors in biological applications.[34] The investigation utilised 0 to 70%w/w proportions of soluble collagen additions to the galactomannan solutions to prepare a number of films. These films were then cross-linked with glutaraldehyde with variable concentrations from 0.001 to 1.5%w/w. While the galactomannan was retained in

[31] Sivasubramanian, S., B. M. Manohar, et al. (2008). "Mechanism of enzymatic dehairing of skins using a bacterial alkaline protease." Chemosphere 70(6): 1025-1034.

[32] Valeika, V., K. Beleška, et al. (2009). "An approach to cleaner production: from hair burning to hair saving using a lime-free unhairing system." Journal of Cleaner Production 17(2): 214-221.

[33] Madhan, B., C. Muralidharan, et al. (2002). "Study on the stabilisation of collagen with vegetable tannins in the presence of acrylic polymer." Biomaterials 23(14): 2841-2847.

[34] Figueiró, S. D., J. C. Góes, et al. (2004). "On the physico-chemical and dielectric properties of glutaraldehyde crosslinked galactomannan-collagen films." Carbohydrate Polymers 56(3): 313-320.

the film, it remained a heterogeneous system. Increasing amounts of galactomannan were found to increase the swelling, which was inconsistent with the glutaraldehyde increments.[35] This appears to be a gel in a network in which the glutaraldehyde may be unable to access the collagen linking sites due to the gum. This body of work was focussed on the dielectric measurements, which were achieved for galactomannan-collagen film (60%) yielding the piezoelectric strain d_{14} of o.o81 pC/N.

6.2.2 Structure

Investigation into the effect of the extracellular matrix microstructure and composition on the mechanical behaviour of the dermal layers has shown that depletion of the interfibrillar proteins, proteoglycans and GAGs decreased the dynamic moduli.[36] The upper layer was impacted more by the enzymatic action showing a remarkable decrease in both storage (G') and loss (G") moduli inferring that the structural assemblage of elastin and collagen networks and their interactions dictate the dynamical mechanical response of the dermis.[37]

A high resolution scanning electron microscopic investigation of the interactions of the GAGs and small leucine-rich proteoglycans (SLRPs) *invitro*[38] showed that they all bound to the collagen fibrils in a specific way and influenced its size and shape except for chondroitin 4-sulphate. Without their protein cores they still performed in the same manner. Decorin was the only participant to organise the fibrils into large fibrillar groups of natural appearing tissue.[39] This is a significant finding for understanding the determinants of self assembly, which should lead to advances in scaffold technology and general tissue engineering.

An investigation into the nano-structural fibrillar networks of collagen using scanning electron and atomic force microscopy, at varying concentrations between 1mg/ml and 7 mg/ml with and without alginate at varying ratios of 0, 20, 50 and 70 % was carried out in vitro.[40] It was revealed that with increasing alginate the collagen fibrillar structure became looser with thicker twisted interlaced fibres made up of denser aggregates of normally banded smaller fibrils. The authors proposed that the electrostatic interaction between collagen and alginate played a role in this observed aggregation.[41] This may have applications for pore size control with scaffolds for wound healing or texture control in food products.

Through using electric currents to generate a pH gradient, collagen type 1 molecules have been aligned within a plane using the isoelectric focusing process.[42] The cross-linked fibres produced

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[35] Ibid.

[36] Ventre, M., F. Mollica, et al. (2009). "The effect of composition and microstructure on the viscoelastic properties of dermis." Journal of Biomechanics 42(4): 430-435.

[37] *Ibid*.

[38] Raspanti, M., M. Viola, et al. (2008). "Glycosaminoglycans show a specific periodic interaction with type I collagen fibrils." Journal of Structural Biology 164(1): 134-139.

[39] *Ibid*.

[40] Sang, L., X. Wang, et al. (2010). "Assembly of collagen fibrillar networks in the presence of alginate." Carbohydrate Polymers 82(4): 1264-1270.

[41] *Ibid*.

[42] Cheng, X., U. A. Gurkan, et al. (2008). "An electrochemical fabrication process for the assembly of anisotropically oriented collagen bundles." Biomaterials 29(22): 3278-3288.

were evaluated as being 30 times stronger than the randomly oriented cross-linked fibres and half that of natural tendon. Fibroblasts were found to be able to migrate and populate the bundles at a rate of 0.5 mm/day. This is a relatively simple but slow process, however, the simplicity would enable scaling, which would give it a significant advantage for mass scaffold production.

The physical aging of glassy gelatine films was investigated through both enthalpy using Differential Scanning Calorimetry (DSC) and mechanical relaxation with Dynamic Mechanical Thermal Analysis (DMTA). It was shown that DSC can be effectively used as an indicator of mechanical changes with aging. Also identified was that the rate and extent of aging increases with water content.[43] Recently a phase diagram was constructed using DSC for water:glycerol content in films used to make capsules to explore the states of the film after equilibrium was reached.[44] It was proposed that the phase diagram is of gelatine film based on the ratio of hydroxyl groups (water or glycerol) per residue instead of water: glycerol. This is very useful for managing glassy gelatine films (capsules) for storage.

Manufacture of collagen threads through wet spinning collagen dispersion at 26 °C (needed to be precipitated by organic solvents due to low dry matter) and melt spinning of thermoplastic collagen was achieved.[45] A range of cross-linking agents was assessed for cytotoxic effect, both non-cross-linked and 0.1% gluteraldehye showing no measured effect against mouse fibroblast.[46] The spinerette is an old system and appears to have worked satisfactorily for porcine collagen, but not for adult bovine. The thermoplastic threads ran best at a glycerol content of 25 to 35%. The process may have benefited more from a few more additives to better prepare the melt. This process looks positive for a range of options including thread.

Gelatine and collagen hydrolysate both can be used to form hydrogels[47]. Hydrogels are macromolecules characterised by their hydrophilicity and insolubility in water. They swell to an equilibrium volume maintaining their shape. Gelatine hybrid hydrogels have been assessed for in vitro and in vivo biodegradation and controlled release of gentamicin as they can take around 15 hours to reach 0.63 of their equilibrium swelling. Collagen hydrolysate hybrid hydrogels have the capacity to perform as nappies, sanitary pads and horticultural uses which puts them in the class of super absorbents[48].

It is not likely that these hydrogels will be able to compete with the synthetic super absorbents until oil prices start to increase consistently. However, they present as viable alternatives that may find niche markets initially being more biodegradable.

The opportunity assessment of this research and development body of work would be speculative at best.

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[43] Badii, F. (2006). "Enthalpy and mechanical relaxation of glassy gelatin films." Food Hydrocolloids 20(6): 879-884.

[44] Coppola, M., M. Djabourov, et al. (2008). "Phase Diagram of Gelatin Plasticized by Water and Glycerol." Macromolecular Symposia 273(1): 56-65.

[45] Meyer, M. (2010). "Collagen fibres by thermoplastic and wet spinning." Materials science & engineering. C, Biomimetic materials, sensors and systems 30(8): 1266-1271.

[46] Ibid.

[47] Zohuriaan-Mehr, M., A. Pourjavadi, et al. (2009). "Protein- and homo poly(amino acid)-based hydrogels with super-swelling properties." Polymers for advanced technologies 20(8): 655-671.

[48] *Ibid*.

6.3 Food related

Food is a commodity that is likely to stay in demand and, because the red meat industry is intimately involved in the food industry, it would follow that opportunities in this area are likely to be aligned.

6.3.1 Edible films and coatings

Edible films and coatings can guard food products by maintaining flavour and aroma, providing structural integrity, moisture migration barrier properties, and vapour permeability by reducing exposure to oxygen. The following are new developments in this field.

Collagen is still the primary raw material being used to produce edible films for the meat and poultry industry,[49] the largest of which is sausage skins. Devro, one of the world's leading casings manufacturers, with 2009 revenue of over AU$350 million, identifies that "the conversion of food casings from using gut as a raw material to being produced from collagen is a major driver towards future sales growth."[50] However, Devro is focusing on porcine collagen, with new co-extrusion technology forming the film around the product as it is extruded.[51] Opportunities, like market changes away from gut, could be worth investment.

A composite biodegradable gelatine chitosan film with essential oils, especially clove, inhibited all 6 fish spoilage microorganisms tested against, chitosan slowed the breakdown, hence may have extended the activity release. The level of essential oils used was 0.17 ml/g of gelatine or gelatine/chitosan (G or GC) – this seems high as the film is prepared using 8g (G or GC)/100ml of water plus plasticizers at 0.15g/g (G or GC). It may have been used to substitute other modifiers.

An evaluation of galactomannan, collagen and glycerol coating blends on mangoes and apples, clearly showed how wettability can be used to optimise coating performance. This work highlights the importance of understanding both the coating process and the properties of what is being coated. The gas balance inside the fruit was reasonably maintained due to reduced CO_2 production and O_2 consumption.[52]

A determination of the water vapour permeability and the mechanical and thermal properties of bovine and porcine edible gelatine films have been completed for sorbitol of levels 15 – 65 g/100g gelatine. With increasing sorbitol the puncture force decreased and the puncture deformation and water vapour permeability increased.[53] While the authors identify that the origin

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[49] Ustunol, Z. (2009). Edible Films and Coatings for Meat and Poultry. Edible Films and Coatings for Food Applications. K. C. Huber and M. E. Embuscado, Springer New York: 245-268.

[50] Anonymous (2009). Annual Report & Accounts 2009, Devro plc.

[51] Ustunol, Z. (2009). Edible Films and Coatings for Meat and Poultry. Edible Films and Coatings for Food Applications. K. C. Huber and M. E. Embuscado, Springer New York: 245-268.

[52] Lima, A. M., M. A. Cerqueira, et al. (2010). "New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits - Influence on fruits gas transfer rate." Journal of Food Engineering 97(1): 101-109.

[53] Sobral, P. J. A., F. C. Menegalli, et al. (2001). "Mechanical, water vapor barrier and thermal

properties of gelatin based edible films." Food Hydrocolloids 15(4-6): 423-432.

of gelatine was important, only over 25g/100g of gelatine, this is more than half of the investigation. Careful note should be taken of these results as the identified phase transition impacts bovine hide results.

A new invention has been patented describing a process where fresh minced meat is pumped between two films sealed at the edges, which are permeable to moisture. The inventors identify either cellulose or animal fibres (collagen?) as suitable films.[54] This stuffed tube is rolled flat and passed through a brine bath where the meat effectively dehydrates, presumably by osmosis, and forms a reformed meat product. The films are separated and the product is sliced.

Given the water vapour permeability of collagen/gelatine films, potential use as osmotic membranes may be exploited for controlling moisture levels in a wide range of products. The principle of pervaporation of collagen membranes[55] may possibly be applied to enhance this process.

Currently a portion of reformed meat is processed using a collagen flat film fitted over formed product and clipped within tubular netting and set, following which the netting is removed and a desired dimpled surface remains.[56] Development of a collagen tube film and a suitable device now enables forming and fitting of film and net in a single process reducing over use of film.[57]

A laser multi perforated collagen film has been developed for absorption of steam or water during processing of cooked formed meats to ensure product appearance, eliminating poor visual appearances[58]. It appears the moisture is held in the film, a unique solution. It may be applied as a method of controlled release of antioxidants or natural preservatives through exploiting leaching.

6.3.2 Collagen and Gelatine

Global gelatine production in 2002 was 266,150 tonne[59], valued at US$1.2 billion which grew to 320,000[60] to 340,000[61] tonne in 2008. Currently the global value is estimated at US$1.65 billion[62]

[54] Suirami. Jean and Lhoutellier. Pascal (2007). Method for continuos production of minced meat dried and restored to thin slabs. European Patent Office. France. EP 1406501 (A1)

[55] Maser, F., C. Ströher-Glowienka, et al. (1991). "Collagen film as a new pervaporation membrane." Journal of Membrane Science 61: 269-278.
, Suzuki, F., H. Kimura, et al. (2000). "Formation having a tanning gradient structure of collagen membrane by the pervaporation technique." Journal of Membrane Science 165(2): 169-175.

[56] BRUNO, W. (1990). Process for the production of meat products. European Patent Office. Germany. US 4910034 (A).

[57] Mysker, T. (2005). Apparatus and method to net food products in shirred tubular casing*Ibid*. U.S. Pat. and Trademark Office. USA.

[58] Maser, F. and O. Tuerk (2004). Multi-perforated collagen film. U. S. P. T. Office. United States of America. US 2004/0161500 A1.

[59] Jarvis, L. (2002). "Gelatin Market Stable as BSE Moves From the Spotlight." Chemical market reporter 261(6): 8.

[60] Lavers, B. (2009). "Collagen cash cow." Food Manufacture(February): 37-38.

[61] Roldan (2009). "The rise of pharmaceutical gelatin plant - uncertainty of the market." from http://news.frbiz.com/the_rise_of_pharmaceutical-227675.html.

Composite food modifiers

There has been a need for specific information, rather than empirical, when using collagen or gelatine in formulated food products, which are complex systems that are often composite gels[63] having two or more gelling agents. These gels are utilised primarily for their structural and physical properties, the main groups being proteins and polysaccharides.

Sugars are a large part of the confectionery market and have been shown to destabilise polysaccharide gel networks when used at levels of 40-60%, but increase gelatine networks at these levels[64] and the thermodynamic stability increases for gelatine networks and continues to do so above these levels.[65] Both starches and sugars also help to stabilise the gelatine gel arrangement, sucrose and glucose syrup mixtures can create a continuous fluid phase with gelatine. These co-solutes can increase the strength of the gel up to a point where the gel starts to weaken because of a lack of available water.[66]

Recently, collagen fibres as an emulsifier in oil water emulsions were assessed against pH, protein concentration and homogenisation pressure.[67] The collagen emulsions for pH values of 4.5, 5.5, and 7.5 and the lowest homogenisation pressure all showed that they were stabilised by a microscopic network and not by an electrostatically stabilised emulsion. The pH 3.5 emulsions were stabilised electrostatically at concentration of 0.5-3.0% w/w and at high homogenisation pressure up to 100MPa was maintained. It was concluded that collagen fibre would make a good emulsifier in acid food systems.[68] It is possible that evaluation of the isoelectric point of the collagen sourced could have assisted this investigation.

An investigation of the viscoelastic properties of collagen fibre at concentrations of 4, 6, 8 and 10% showed a large increase in the storage modulus (G') from 4-6 % and a decrease from 8-10% that did not recover after heating/cooling.[69] The latter result was believed to be caused by the heating cycle strengthening the network. Addition of xanthan gum at 0.1 % showed an increase in gel strength especially following heating-cooling; at 0.3% xanthan the gel was weaker and this was believed to be an indication of thermodynamic incompatibility. Mixtures of collagen fibres and maltodextrin at 4 and 12% showed more fluid structures than collagen fibres alone

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[62] Anonymous (2010, 12 Oct). "The Gelita group is world leader." from http://www.gelita.com/DGF- english/index.html.

[63] Burey, P., B. R. Bhandari, et al. (2009). "Confectionery Gels: A Review on Formulation, Rheological and Structural Aspects." International Journal of Food Properties 12(1): 176-210.

[64] Kasapis. S, Mitchell. J, et al. (2004). "Rubber-to-glass transitions in high sugar biopolymer mixtures." Trends in Food Science & Technology 15(6): 293-304.

[65] Kasapis, S., I. M. Al-Marhoobi, et al. (2003). "Gelatin vs polysaccharide in mixture with sugar." Biomacromolecules 4(5): 1142-1149.

[66] Burey, P., B. R. Bhandari, et al. (2009). "Confectionery Gels: A Review on Formulation, Rheological and Structural Aspects." International Journal of Food Properties 12(1): 176-210.

[67] Santana, R. C., F. A. Perrechil, et al. "Emulsifying properties of collagen fibers: Effect of pH, protein concentration and homogenization pressure." Food Hydrocolloids In Press, Corrected Proof.

[68] *Ibid*.

[69] Nicoleti, J. and V. Telis (2009). "Viscoelastic and Thermal Properties of Collagen–Xanthan Gum and Collagen–Maltodextrin Suspensions During Heating and Cooling." Food Biophysics 4(3): 135-146.

across the range of temperatures and concentrations indicating an incompatibility.[70] It is important when investigating systems, especially component systems, to be familiar with the physicochemical, mechanical and thermodynamic aspects of the individual components to ensure that an understanding of these is gained first.

The DSC results from the above work are very useful, as they show the damage that can be done from days of ball milling ground dried hide. This may assist with the extraction decision making, with respect to the aforementioned biochemical 'clip and snip' or the physical 'smash and bash.'

A linear viscoelastic assessment of bovine gelatine stabilised gel-like emulsions with low lipid content was carried out with different molecular weight sourced gelatines of 60, 80 and 120 kD.[71] Emulsion stability was found to be associated with the molecular weight and not to droplet size. Analysis of the dynamic oscillatory results led to the assessment that both the viscosity and the elastic characteristics increased as the molecular weight increased. Changes in material characteristics inferred a relationship between molecular weight and the amount of cross-linking. A fundamental investigation for how low fat products with temperature sensitive raw materials should be developed. A significant challenge for the food industry is product structure and consumer perception when fat/oil is removed from a product (see 'low fat product', under 'Some new products and opportunities')

An investigation into the effect of collagen preparations on iodine and thiamine retention found that both collagen fibre and collagen hydrolysate, when impregnated with potassium iodide, increased the stability of iodine and thiamine through processing and storage.[72] It is proposed that this is directly related to the water binding capacity of the collagen preparations. Noted also is that the collagen hydrolysate carrier had an effect on flavour, resulting in some deterioration of the overall product score. If it is the hydrophilic nature of the collagen preparations this may enable this to be leveraged across a range of nutritional fortifying products, especially with meat products which provide a rich supply of the amino acids which are deficient in collagen with respect to human nutrition. Before progressing, however, it would be prudent to better understand the flavour deterioration observed.

Further investigation by the same author found collagen fibre boosts rosemary's antioxidant activity as evaluated with peroxide value and thiobarbituric acid reactive substances (TBARS).[73] This occurred in both liver and werner-type sausages whether stored in air or under vacuum. Conversely collagen hydrolysate had a masking effect reducing antioxidative impact. This also may relate to water binding. However, the term 'collagen hydrolysate' is likely as broad a definition as 'gelatine' and possibly it is time for a grading system that fits the applications it is put to. It is possible that the interest they have generated may be partially tarnished through lack of information and/or awareness.

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[70] *Ibid*.

[71] Lorenzo, G., G. Checmarev, et al. (2011). "Linear viscoelastic assessment of cold gel-like emulsions stabilized with bovine gelatin." LWT - Food Science and Technology 44(2): 457-464.

[72] Waszkowiak, K. (2007). "Effect of collagen preparations used as carriers of potassium iodide on retention of iodine and thiamine during cooking and storage of pork meatballs." Journal of the science of food and agriculture 87(8): 1473-1479.

[73] Waszkowiak, K. and W. Dolata (2007). "The application of collagen preparations as carriers of

rosemary extract in the production of processed meat." Meat Science 75(1): 178-183.

An investigation of the collagen peptides derived by enzyme hydrolysis of bovine gelatine, reported a certain molecular size range, between 600 – 2700 Da that showed inhibition of ice re-crystallisation.[74] The hydrolysis, using Alcalase, is carried out for 30 min at pH 9 and 45 °C. A group of cationic peptides with molecular size 1600 – 2400Da, were found to be more effective than anionic. While 'antifreeze proteins' have been isolated previously from a diverse range of organisms that live in sub-zero conditions, these can be harvested *en masse* and hence should find application very quickly. These can include protecting crops in severe weather, to protecting the textural properties of a wide range of frozen foods or storage of tissue and blood products. This is an opportunity that should be assessed.

6.3.3 Alternatives to collagen and gelatine

Both religious and health risk aspects present challenges and opportunities for the hide processing industry. Since the BSE scare in the U.K. and the U.S. a significant body of work has been carried out on alternatives to hide collagen and gelatine. Some of this is worth review for potential application with hide products, because it may impact existing and future markets for mammalian collagen and gelatine.

Halal

Annual sales of halal products is estimated at AU$580 billion.[75] Islam is the largest and fastest growing religion; in 2006 25% of the world was Muslim. For the red meat industry in Australia this market is likely to be positive as swine products are prohibited (haram). However, as a result of this, any product where it is not clearly halal, like collagen and gelatine, is also haram.[76]

There may be an opportunity here to supply globally halal gelatines entirely based on bovine hides, not to a niche market but to food manufacturers, that may switch to starch based products, who seek halal markets to get their products additional growth potential. This is perhaps a mindset change that will happen as growth shifts to the developing countries and their per capita income starts to rise.

Alternatives

In the time frame covered with this review, there have been more investigations into alternatives to gelatine than on gelatine, mostly involved marine gelatine. Following are a few samples of the work being done and a few dot points worth noting.

[74] Wang, S. (2009). "Ice-structuring peptides derived from bovine collagen." Journal of agricultural and food chemistry 57(12): 5501-5509.

[75] Berry, B. (2008). Global halal food market brief. Agri-Food Trade Services, Agriculture Agri-Food Canada.

[76] *Ibid*.

An investigation into the structural and functional properties of films prepared from cod gelatine with soy protein isolate (SPI) was assessed over the range of 0 to 100%.[77] The optimum level was 25% SPI: 75% cod gelatine which had a maximum force at breaking point of 1.8 times the 100% gelatine (good) with better deformation and reduced water vapour permeation (good). SPI introduced a slight yellow colour and modified the gelatine structure. Use of SPI may assist bovine collagen also.

A tea polyphenol-loaded chitosan nanoparticle (TCPN) was incorporated into fish gelatine film and tested on packaged fish oil. The TCPN impacted film structure and transparency but decreased oxygen permeability and increased water vapour permeability (not good). TCPN film had lower peroxide values. There is some functionality but most appears due to tea polyphenol not being encapsulated. Bovine collagen may perform better; however, it is likely an alternative form of nanoparticle may be required.

An investigation into the physical, mechanical, and barrier properties of carp skin gelatine (aqua-cultured in India) has shown significantly lower water vapour permeability (WVP) and oxygen permeability than mammalian skin gelatine and comparable gel strength. The bloom and tensile strength tended to be lower; however, they have potential for extending the shelf-life of chilled and frozen products.[78]

Fish and marine species gelatine grew from 0.7-1.3% of total world production between 2003 and 2008.[79] While this equates to only 0.12% per year as research investment continues, as observed through the amount of published material, manufacturing knowledge also builds.

Volumes of fish waste are large and under utilised, but vary in both mechanical and chemical properties significantly across species and genus. The total global fishery production from capture fisheries and aquaculture reached 140 million tonnes in 2004.[80] The fish gelatine price tends to be 4 to 5 times more expensive than mammalian gelatine with a niche market in kosher/halal applications.[81]

Other examples of alternatives to gelatine:

- Transgenic recombinant collagen type I alpha 1, produced with corn.[82] Initial work was a big step. However, expectation of $50/kg may be overly optimistic, as it is almost 10 times
  the price of gelatine.
- Use of yellow fin tuna gelatine. The bloom figures are significantly higher than mammalian, although it gels at a lot lower temperatures. The catch volume figures of yellow fin tuna are likely an editing mistake, as they are out by approximately 2000 times.

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[77] Denavi, G. A., M. Pérez-Mateos, et al. (2009). "Structural and functional properties of soy protein isolate and cod gelatin blend films." Food Hydrocolloids 23(8): 2094-2101.

[78] Ninan, G. (2010). "Physical, Mechanical, and Barrier Properties of Carp and Mammalian Skin Gelatin Films." Journal of food science 75(9): E620-E626.

[79] *Ibid*.

[80] Anonymous (May 2006). "FAO Fact Sheet: The international fish trade and world fisheries." from http://www.fao.org/newsroom/common/ecg/1000301/en/enfactsheet2.pdf.

[81] Karim, A. A. and R. Bhat (2008). "Gelatin alternatives for the food industry: recent developments, challenges and prospects." Trends in Food Science and Technology 19(12): 644-656.

[82] Zhang, C., J. Baez, et al. (2009). "Purification and Characterization of a Transgenic Corn Grain-Derived Recombinant Collagen Type I Alpha 1." Biotechnology Progress 25(6): 1660-1668.

- Nestle was considering rolling out a range of novel food products after signing a deal with UK company BioProgress Technology International, which has developed an alternative to gelatine.[83] If there was an opportunity and there was a product, It does not appear to have been acted on.

Below are a few examples of alternative products to gelatine in the market in 2006 and their claims.[84]

- MIRA-QUIK MGL Tate & Lyle - can replace up to 30% gelatine with no change
- ELIANE from Avebe - can replace all gelatine in jellies and wine gums
- ELIANE VC 120 from Avebe - can replace all gelatine in compressed tablets
- Amylogum from Avebe - can replace all gelatine in liquorice

6.3.4 Some new products and opportunities

- Acid based marshmallows can now be made with citrus flavours.[85] Another composite system where pectin is used to stabilise the gelatine network - note this uses acid-based gelatine. No market information as yet.

- Low fat products can utilise gelatine to maintain mouth feel while reducing fat, for example, chocolate now using 'Instant Gel Schoko,' a new gelatine product, can replace up to 39% of cocoa butter reducing fat content of chocolate by 25%.[86] Total annual sales of chocolate in 2009 were US$65.7 billion.[87] Here, the concept of adding value to an existing product market creates the opportunity for a step in sales growth that does not impact existing markets.

- Cosmeceuticals and collagen hydrolysate. A situation assessment recently published deals essentially with the general aspects of collagen hydrolysate regarding bioavailability and results of preclinical studies, relating to the effects of collagen hydrolysate oral intake on skin.[88] It identifies that to comprehensively study the different benefits of collagen hydrolysate on skin, controlled clinical trials are needed.[89] Food and ingredients that counter aging have showed growth, with the number of stock keeping units (sku's) that make a beauty related claim rising from 72 in 2004 to 140 in 2007 and down to 78 in 2009.[90] Consumer research conducted by Datamonitor® in 2008 showed that nearly half

[83] (2001). "Nestle to target meat-free sector with gelatin-substitute food range." Marketing Week 23(47): P.5.

[84] Scully. Carla, Z. (2006). Bench Strength. Candy Industry. Trends and Applications in Ingredient Technology. August: 36-37.

[85] Stevens, P. (2010). Marshmellow-type aerated confectionery and method of preparation. U. S. P. T. Office. United States of America. US 2010/0260906.

[86] Teppner, M. (2006) Pleasure and health with Less Fat: The New Generation of Chocolate. Gelita AG: Corporate Comunications

[87] Anonymous (2009). Global Confectionery. Industry Profile, Datamonitor.

[88] Zague, V. (2008). "A new view concerning the eVects of collagen hydrolysate intake on skin properties." Arch Dermatol Res 300: 479–483.

[89] Ibid.

[90] Vierhile, T. (2010). "Cosmeceuticals for the Ages." Prepared Foods 179(6): 25-33.

of all consumers surveyed globally found beauty benefit claims by oral beauty supplements to be “not credible” or “not at all credible.”[91] This is an area where there is a limited amount of specific knowledge and a collection of inferred findings, which require the expertise of those more familiar with markets to scope a potential opportunity. The cosmetic market in the US for 2007 was US$50 billion.[92]

- Gelatine boosts collagen synthesis.[93] In 2003 a laboratory study, *Cell Tissue Res 311:393-399, March 2003* was reported as indicating that collagen hydrolysate promotes freshly isolated chondrocytes from cartilage to significantly stimulate the synthesis of collagen in cartilage. A recent review of this, and other matters, has stated that “collagen hydrolysate demonstrated a suggestive evidence of efficacy.”[94] It should be noted that this narrative review is an ‘article in press’ and the corrected proof was not available prior to completion of this review.

In summary, the food sector is a large volume market that has numerous opportunities, which are accessible and involve moderate further processing. The understanding of hide components benefits with respect to food structure is only just being realised.

6.4 Medical

It is believed the growth in the medical biomaterials market is related to interest in personal healthcare, increase in the aging population, and the innovation of broad purpose products in areas such as implantable devices, tissue engineering, bone grafting substance and facial aesthetic dermal implants and it is these which will ensure the industry’s continued success.[95] Products utilised in this market sector, originating from hides, can be divided across 3 broad areas which are bone and periodontal, wounds and organ tissue engineering, and plastic surgery.

6.4.1 Bone and Periodontal

Collagen material has been frequently used in dentistry due to its biocompatibility and wound healing promotion. Collagen as an absorbable membrane has been found to be comparable to non absorbable membranes in relation to probing depth reduction, bone fill, clinical attachment gain and guided bone regeneration.[96]

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[91] Ibid.

[92] De Guzman, D. and J. Chang (2007). "Global cosmetics demand surges." ICIS Chemical Business Americas 271(16): 6-6.

[93] Oesser, S. (2003). Gelatine boosts collagen synthesis. Food Navigator. Science & Nutrition.

[94] Henrotin, Y., C. Lambert, et al. "Nutraceuticals: do they represent a new era in the management of osteoarthritis? - a narrative review from the lessons taken with five products." Osteoarthritis and Cartilage In Press, Uncorrected Proof.

[95] Anonymous (2010, 4th of October). "Global Collagen and HA-Based Biomaterials Market ". from http://www.strategyr.com/Collagen_and_HA_Based_Biomaterials_Market_Report.asp.

[96] Bunyaratavej, P. and H. L. Wang (2001). "Collagen membranes: A review." Journal of

Periodontology 72(2): 215-229.

Bone is the organ that is replaced most in the human body, with over 500,000 procedures per year[97] occurring in the United States alone. The majority of these repairs are autografts utilising the iliac crest from the pelvis, with an 80 to 90 % success rate.[98]

Lately tissue engineered bone grafts have emerged as feasible alternatives, especially with the ability to accelerate the formation of an apatite layer on a biocomposite surface.[99] This has been achieved through the use of either bovine hide powder at up to 10% of the biocomposite, copolymerised polyethylene and bovine or bird bone gelatine with either copolymerised polyethylene or low density polyethylene *in vitro*. In each of these, the biocomposite has been shown to be the stimulator of the apatite formation following immersion in a simulated body fluid (SBF) for 7 days.[100]

Recently it has been reported that a uni-axial oriented collagen hydroxyapatite composite has been self assembled *in vitro* by appropriate control of the method.[101] A 3.21% collagen gel at pH
7.5 was contacted for 24 h, drop wise with a suspension $Ca(OH)_2$, then with H_3PO_4. The hydroxyapatite (HA) is then formed by air drying at 37 °C and pH 9. Through slow drying there is
sufficient time for the fibres to reorient forming morphology similar to compact bone, bringing composite materials another step closer to actual bone. The ratio of collagen, sourced from calf hide, to HA is 20:80 and the final concentration of collagen before drying is 1.66%.

The challenge with this is the engineering of materials that can equal both the mechanical and biological aspects of real bone matrix and support vascularization functions.[102] The potential for collagen and gelatine products appears good for specialized products that meet both regulatory and biocompatibility requirements. The future of collagen use would appear to be positive with current research which focuses on the hierarchical mineralization of collagen type I at the nanoparticles level which hints at simplicity in natures biomineralisation design principles.[103]

The dental market alone for bone graft and other biomaterials in 2008 was US$62.5 million and projected to be US$160 million by 2015 for Asia-Pacific,[104] primarily including Japan, South Korea, and Australia. In 2009, the total U.S. market for dental bone graft substitutes and other biomaterials was valued at nearly $210 million.[105]

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[97] Langer, R. and J. P. Vacanti (1993). "Tissue engineering." Science 260(5110): 920-926.

[98] Haroun, A. A. (2010). "Preparation, characterization, and In Vitro application of composite films based on gelatin and collagen from natural resources." Journal of applied polymer science 116(4): 2083-2094.

[99] *Ibid*.

[100] Kokubo, T. and H. Takadama (2006). "How useful is SBF in predicting in vivo bone bioactivity?" Biomaterials 27(15): 2907-2915.

[101] Ficai, A. (2010). "Self-assembled collagen/hydroxyapatite composite materials." Chemical engineering journal 160(2): 794-800.

[102] Stevens, M. M. (2008). "Biomaterials for bone tissue engineering." Materials Today 11(5): 18-25.

[103] Liu, Y., Y.-K. Kim, et al. (2011). "Hierarchical and non-hierarchical mineralisation of collagen." Biomaterials 32(5): 1291-1300.
, Teo, W. E., S. Liao, et al. (2011). "Fabrication and characterization of hierarchically organized nanoparticle-reinforced nanofibrous composite scaffolds." Acta Biomaterialia 7(1): 193-202.

[104] Anonymous (2009). "Asia Pacific Market for Bone Graft Substitutes and Other Biomaterials 2009 (3 Countries)." November 1. from http://www.marketresearch.com/product/display.asp?productid=2529363.

[105] Anonymous (2010). "U.S. Market for Dental Bone Graft Substitutes and Other Biomaterials 2010." from http://www.reportsandreports.com/reports/34545-us-market-for-dental-bone-graft-substitutes-and-other- biomateri.html.

6.4.2 Wounds and organ tissue engineering

Wound healing involves a number of physiological and biological processes at the cellular and molecular level. Wound management usually starts with immediate coverage, for severe or widespread wounds, skin substitutes are often used to promote healing by stimulating the environment to produce cytokines enabling fibroblasts to help promote the generation of new tissue and assist in epidermal differentiation.[106]

Skin substitutes can be prepared as scaffolds which are often generated through a lyophilised liquid suspension or solution of collagen, gelatine or other components, which when done correctly results in a porous structure in which cells can grow and attach. Fibroblasts are often used to accelerate the dermal healing process, the addition of keratinocytes have been explored to better mimic normal human skin.[107] Cross-linking is often necessary with collagen scaffolds as *in vivo* native, lysyl oxidase mediated, cross-linking does not occur *in vitro.*[108]

Gelatine has been shown to have very good cell attachment and proliferation properties which are dependant on contact angle, the charge on the material surface and the presence of a sequence for cell connecting like arginine-glycine-aspartic acid (RGD).[109] Use of bovine collagen type I particles have been reviewed and it is proposed that they "can play a vital role in wound healing and should be considered over more expensive dressings."[110]

Collagen is non-toxic, non-antigenic, and favours cell adhesion, proliferation and differentiation. However, it is mechanically deficient when constructed as a gel for tissue generation and is often cross-linked for stabilisation.

Some extra cellular matter (ECM) such as collagen type III and (GAGs) has been assessed for their involvement in regulating collagen organisation.[111] Collagen III has been shown to alter fibril diameter, network structure and gel stiffness. Chondroitin sulphate (CS) also decreases fibre diameter. These impacts are being investigated on how they affect cell behaviour and may be utilised toward the design of tissue engineered materials.

A few type I collagen-glycosaminoglycan scaffolds (CGSs) have been reviewed for their ability to induce partial regeneration of organs in adult mammals by blocking wound contraction and scar

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[106] Lee, S. B., H. W. Jeon, et al. (2003). "Bio-artificial skin composed of gelatin and (1-->3), (1-->6)-[beta]- glucan." Biomaterials 24(14): 2503-2511.

[107] *Ibid*.

[108] Zeugolis, D. I. (2010). "The influence of a natural cross-linking agent (Myrica rubra) on the properties of extruded collagen fibres for tissue engineering applications." Materials science & engineering. C, Biomimetic materials, sensors and systems 30(1): 190-195.

[109] Lee, S. B., H. W. Jeon, et al. (2003). "Bio-artificial skin composed of gelatin and (1-->3), (1-->6)-[beta]- glucan." Biomaterials 24(14): 2503-2511.

[110] Rudnick, A. (2006). "Advances in tissue engineering and use of type 1 bovine collagen particles in wound bed preparation." Journal of wound care 15(9): 402-404.

[111] Stuart, K. (2009). "Characterization of gels composed of blends of collagen I, collagen III, and chondroitin sulfate." Biomacromolecules 10(1): 25-31.

formation.[112] A number of structural determinants were identified, including presence of ligands for fibroblasts binding, average scaffold pore size for ligand density and degradation rate. The processing variables which can impact these were found to include the kinetics of collagen fibre swelling in acetic acid, freezing of the collagen GAG suspension and cross-linking of the final scaffold. Other recent scaffold changes identified as significant were a variation of pore sizes within a range, gradients of physiochemical properties and inclusion of a mineral component.[113]

Collagen must be modified both for mechanical strength and reduced degradation for use as bioprosthesis.[114] Collagen was successfully cross-linked with 2-methacryloylethyl phosphorylcholine (MPC) to make an MPC-immobilised collagen gel through several stages of cross-linking, resulting in a collagen-phospholipid polymer hybrid. This biosynthetic hybrid biomaterial is more resistant to collagenase as a result of both intra- and interhelical cross-links and intermolecular cross-links which makes the network a lot denser.[115] Repeated cross-linking with additional polymer resulted in addition of more MPC groups resulting in further decrease in swelling and delay in collagenase degradation. Hence, it can be used as an alternative collagen- based gel for an implantable biomedical device. This product is now proposed for *in vivo* assessment.

A number of natural cross-linking agents have been assessed including glutaraldehyde, genipin and transglutaminase but none have yet yielded a general solution.[116] The natural cross-linking agent Myrica ruba, used on extruded collagen fibres produced using a syringe, has resulted in fibres that match native tissues like tendon and anterior cruciate ligaments for thermal, structural, physical and mechanical properties. This cross-linking agent will need to be assessed for its biocompatibility.

Use of scaffolds in regenerative medicine has been increasing quite rapidly. Integra®, Neuragen®, Matriderm®, Aplicraft® and Orcel® are collagen based scaffolds commercially available.[117] Integra launched in 1996 had already been used over 100,000 times on burns, chronic skin wounds or plastic surgery by 2008.[118] The future of regenerative tissue engineering appears to have enormous potential that has only just begun to be realised, despite initial unrealistic commercial and clinical expectations.[119] However, there is a need for quicker transfer from laboratory to market, meeting safety requirements while adapting to ~~changes in regulatory require~~ments.[120]

[112] Yannas, I. V., D. S. Tzeranis, et al. (2010). "Biologically active collagen-based scaffolds: Advances in processing and characterization." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 368(1917): 2123-2139.

[113] *Ibid*.

[114] Nam, K., T. Kimura, et al. (2007). "Preparation and characterization of cross-linked collagen- phospholipid polymer hybrid gels." Biomaterials 28(1): 1-8.

[115] *Ibid*.

[116] Stuart, K. (2009). "Characterization of gels composed of blends of collagen I, collagen III, and chondroitin sulfate." Biomacromolecules 10(1): 25-31.

[117] Böttcher-Haberzeth, S. (2010). "Tissue engineering of skin." Burns 36(4): 450-460.

[118] Yannas, I. V., D. S. Tzeranis, et al. (2010). "Biologically active collagen-based scaffolds: Advances in processing and characterization." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 368(1917): 2123-2139.

[119] Böttcher-Haberzeth, S. (2010). "Tissue engineering of skin." Burns 36(4): 450-460.

[120] *Ibid*.

In 2001 the cost of keratinocytes sheets ranged from US$1,000 to US$1,300 per 1% of body area covered and this would effectively double with addition of a dermal component.[121] Given the normal adult body surface area (BSA) is 1.73 m²,[122] a market estimate for Integra® alone over its 100,000 uses, assuming a 1% average BSA and single sheet use, would be US$100 million.
The global market for organ replacement therapies was in excess of $350bn in 2006, while the projected US market for regenerative medicine was estimated at $100bn[123].

6.4.3 Plastic surgery

The demand for, and variety of, dermal fillers have increased dramatically over the last few decades from bovine collagen being approved in 1981 by the Food and Drug Administration (FDA) to the recent Hyaluronic acid products.[124] The most commonly used products up to 2008 were autologous fat, collagens, hyaluronic acid (HAu) and biosynthetic polymers.[125] Because the risk of allergic reaction is around 3% for collagen fillers, the need for prior testing, and the limited efficacy of 2 to 6 months, it is proposed that HAu products are now the most commonly used facial filler worldwide, although it is more expensive than collagen products, it lasts longer.[126]

It is projected that the Collagen and Hyaluronic acid (HAu) based biomaterials market will exceed US$2.0 billion in 2015.[127] In 2001 the U.S. HAu based biomaterials market alone was US$411 million and was being projected to be US$903 million by 2008.[128]

This market appears to be sensitive to economic conditions which are cited as having a significant impact on recent market performance, “with sales plunging in 2009, and the decline continuing in 2010” due to the elective nature of the utilisation.[129] While these market figures are identified as being collected from both primary and secondary sources, these figures will require more rigor to be conclusive.

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[121] Stocum, D. L. (2006). Regenerative Medicine of Skin, Hair, Dental Tissues, and Cornea. Regenerative Biology and Medicine. Burlington, Academic Press: 63-88.

[122] Mattar. JA (1989 Aug;). "A simple calculation to estimate body surface area in adults and its correlation with the Du Bois formula." Crit Care Med. 17(8): 846-847.

[123] Shakesheff, K. (2010, 1 April). "Tissue Engineering: the Next Regeneration." from http://www.medicaldevice-network.com/features/feature81032/.

[124] Cockerham, K. (2009). "Collagen-based dermal fillers: Past, present, future." Facial plastic surgery 25(2): 106-113.

[125] Buck Ii, D. W., M. Alam, et al. (2009). "Injectable fillers for facial rejuvenation: a review." Journal of Plastic, Reconstructive & Aesthetic Surgery 62(1): 11-18.

[126] *Ibid*.

[127] Anonymous (2010, 4th of October). "Global Collagen and HA-Based Biomaterials Market ". from http://www.strategyr.com/Collagen_and_HA_Based_Biomaterials_Market_Report.asp.

[128] Anonymous (2002). Ageing population sparks growth in biomaterials market, Decision News Media SAS.

[129] Anonymous (2010, October 4). "Global Collagen and HA-Based Biomaterials Market." from http://www.prweb.com/releases/prwebbiomaterials_collagen/hyaluronic_acid/prweb4590214.htm.

In summary the medical sector as a market place for hide products, is not a volume market but a specialised bio-products market that has a high cost of entry to participate in. however it is very likely that opportunities for products, like collagen fibres, in other market segments may find utility in this market sector.

6.5 Packaging

Interest in hide components in the packaging sector, other than within the food sector as edible films and the pharmaceutical sector for drug delivery, appears mainly related to biodegradability. This is most likely due to the high growth rate in this sector.[130]

Biodegradable films

Gelatine is a natural, biodegradable, renewable biopolymer that is edible and is available in large supply at a reasonable price. As a protein film former it has very good oxygen and carbon dioxide barrier properties, including aroma trapping. However, it does tend to age, becoming brittle, and in high humidity environments it will swell due to its hydrophilic nature and lose mechanical strength[131].

Over the last ten years more interest has been directed toward those raw materials that are actually sustainable, not just recyclable, but they must be functional, and this is what will drive the opportunities in biodegradable packaging. Following are some of the explorations that have happened in the last ten years that may help hide products occupy part of the potentially significant market of sustainable packaging.

An investigation of the biodegradability of packaging made from enzymatic hydrolysate of collagen (H) sourced from leather or meat casings and processed with dialdehyde starch (DAS) to make hydrogels has shown it to depend on the amount of DAS.[132] With 25 – 30% H and 15 – 20% DAS, thermo-reversible hydrogels result which can be processed as soft gelatine capsules. Concentrations of DAS over 20% results in thermo-irreversible hydrogels.

Recently, an investigation of collagen hydrolysate cross-linked with dialdehyde starch found that aging problems with dipping or casting to make biodegradable packaging material could be resolved by heating it for 1 hour at 60 – 90 °C thereby reducing the moisture content by around 12%.[133] This should enable a significant increase in the use of these products. Extending comments made earlier in this review, there is a need for a way to grade collagen hydrolysates which takes into account source, processing history, molecular weight and intended application. This will aid in selection and ensure a good fit of product to purpose.

[130] Anonymous (2010, January 4). "Sustainable Packaging Market to Reach $170 Billion Worldwide by 2014." from http://www.pikeresearch.com/newsroom/sustainable-packaging-market-to-reach-170-billion- worldwide-by-2014.

[131] Carvalho, R. A. and C. R. F. Grosso (2005). "Properties of chemically modified gelatin films " Brazilian Journal of Chemical Engineering 23(01): 44-53.

[132] Langmaier, F., P. Mokrejs, et al. (2008). "Biodegradable packing materials from hydrolysates of collagen waste proteins." Waste Management 28(3): 549-556.

[133] Langmaier, F. (2009). "Hydrogels of collagen hydrolysate cross-linked with dialdehyde starch." Journal of thermal analysis and calorimetry 98(3): 807-812.

In a series of three investigations into polyvinyl alcohol blends, the third of which is the focus of this exploration, the first explored the minor components of H and glycerol, and the second the optimisation of blends with H.[134] This investigation details water sensitivity with glycerol and H.[135] The concentration range investigated for glycerol was 11 – 23% and for H 14 – 30%. The H was found to speed up biodegradation at 5 °C, countering the effects of the glycerol. However, there was no significant difference for degradation rates at 20 °C. This work is targeted at providing biodegradable packaging that will break down rapidly in aqueous solutions, and in the soil, making them suitable for hospital laundry bags, containers of water soluble substances, water and waste treatment additives, fertilizers, and sanitary products.

An evaluation of modifying the structural network of gelatine film using formaldehyde and gloxal was achieved through assessing mechanical properties, water vapour permeability, solubility and colour.[136] Formaldehyde and gloxal levels explored were 3.8 – 8.8 mmoles/100mls and 6.3 –
26.3 mmoles/100mls respectively. Both cross-linking agents resulted in higher strengths, lower water vapour permeability, were more opaque and had higher colour than the untreated film. These results also infer that at around 7 mmoles/100ml concentration, the films seem to reach a saturation that may be a function of spatial arrangement. This effect was more noticeable with gloxal where it had negative effects on the assessed parameters. While biodegradability was given as the reason for choosing gelatine, this evaluation was primarily focused on exploring the limits to cross-linking, which has been successful.

A characterisation of collagen fibres from bovine hide for biodegradable film production has been achieved through evaluation of collagen solubility with pH, thermal analysis with pH, and chemical composition.[137] This simple analysis provides a clear and concise set of experimental procedures to avail the basic physicochemical properties of a starting raw material. This is fundamental and useful for an understanding of the basics of a raw material before forming composite products with it.

The above work was extended at a later date to provide a physicochemical characterisation of both collagen fibres and powder for self-composite film production. When hide is wet milled for a long time both fibres and powder are recovered, it was proposed that the fibres could provide structure as a 'filler' and the powder would become the film matrix. Compositionally, the two materials were the same; however, micro-structurally they were very different, the powder appearing as agglomerates while the fibres showed an internal axis. Composite films prepared with a 50:50 ratio, were produced with a fixed width of 180µm while the fibres appeared to remain as an integral part of the film. There were only subjective assessments with respect to the film's characteristics.

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134 Alexy, P., D. Bakos, et al. (2003). "Poly(vinyl alcohol)-collagen hydrolysate thermoplastic blends: I. Experimental design optimisation and biodegradation behaviour." Polymer Testing 22(7): 801-809.

135 Alexy, P., D. Bakos, et al. Ibid."Poly(vinyl alcohol)-collagen hydrolysate thermoplastic blends: II. Water penetration and biodegradability of melt extruded films." 811-818.

136 Carvalho, R. A. and C. R. F. Grosso (2005). "Properties of chemically modified gelatin films " Brazilian Journal of Chemical Engineering 23(01): 44-53.

137 Wolf, K. L., P. J. A. Sobral, et al. (2006). "Characterisation of collagen fibres for biodegradable film production." IUFoST. from

http://iufost.edpsciences.org/index.php?option=com_toc&url=/articles/iufost/abs/2006/01/contents/contents
.html.

An evaluation of the effect of the nanostructures developed by various gelatine/montmorillonite (GE-MMt) composites was carried out using thermal stability.[138] The concentrations of MMt explored were 1, 5, 10 and 15%w/w, however, only concentration between 3 and 10% resulted in improved thermal stability. Higher concentrations showed lower stabilising effect fitting with the agglomerated structures observed. It appears the systems thermal stability is firmly fixed with the MMt concentration. Again this work looks at the fundamentals to build a model understanding to develop laminates with applications in the biodegradable packaging field.

A biodegradable, three layer film, consisting of two outer layers made by heat compression of piled (DAS)–cross-linked and plasticized to gelatine films (Ge-10DAS) and an inner layer made of sodium-(MMt) plasticized–gelatine film (Ge-5MMt) was constructed.[139] The multi-layer film was compact, uniform and the lamination reduced moisture absorption. The tensile strength and elastic modulus were 8.0 MPa and 14.7 MPa respectively, which were due to the (Ge-5MMt). The water vapour pressure was 0.8 ± 0.1x 10^{-13} kg m Pa^{-1} s^{-1} m^{-2} which is less than any of the single components. All three layers being based on gelatine simplified the lamination process as they can be compressed with mild heat. A slightly modified version of this film was later assessed for biodegradation by soil burial indoors and monitoring water absorption and weight loss. The film was found to have modulated biodegradability, with some layers like the DAS being more resistant than others.

The market for sustainable packaging is a fast growing market with the projections for global growth totalling 93% over a 5 year period compared with 23% for the global packaging industry over the same time frame.

Global packaging industry revenues are forecast to increase from $429 billion in 2009 to $530 billion by 2014.[140]
Global sustainable packaging market is forecast to increase from $88 billion to $170 billion 2009 – 2014[141].

6.6 Pharmaceuticals

6.6.1 Drug delivery

Both collagen and gelatine are used across a range of drug delivery systems. Their biocompatibility, modification through cross-linking, non-toxicity and availability all assist in this application. The following are recent examples of their exploitation.

An investigation to optimize parameters for the fabrication of gelatine nanoparticles for a drug carrier system, explored the four key parameters of temperature, gelatine concentration, agitation speed and quantity of acetone.[142] Particles of size above 230 nm have been shown to

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[138] Martucci, J. F., A. Vázquez, et al. (2007). "Nanocomposites based on gelatin and montmorillonite: Morphological and thermal studies." Journal of thermal analysis and calorimetry 89(1): 117-122.

[139] Martucci, J. F. and R. A. Ruseckaite (2010). "Biodegradable three-layer film derived from bovine gelatin." Journal of Food Engineering 99(3): 377-383.

[140] Anonymous (2010, January 4). "Sustainable Packaging Market to Reach $170 Billion Worldwide by 2014." from http://www.pikeresearch.com/newsroom/sustainable-packaging-market-to-reach-170-billion- worldwide-by-2014.

[141] Ibid.

[142] Jahanshahi, M., M. H. Sanati, et al. (2008). "Optimization of parameters for the fabrication of gelatin nanoparticles by the Taguchi robust design method." Journal of Applied Statistics 35(12): 1345-1353.

accumulate in the spleen as a result of capillary sizes. Hence, for effective delivery, particles need to be smaller. Temperature and acetone quantity were found to have the most impact on particle size, at the optimised conditions the nanoparticles size produced was 174 nm.[143]

One of the issues with gelatine, especially in both hard and soft capsules, is that it cross-links over time, thereby changing its delivery through changed dissolution properties. This was resolved through introducing a cross-linking-reducing combination of citric acid and glycerine into the film mass or solution during its preparation.[144]

Gelatine hydrogels have been stabilised by several different cross-linking agents of which genipin is one. Investigation of a marker drug 4,4'-bis(2-sulfostyryl) biphenyl (DSBP) for controlled release in a genipin cross-linked gelatine thin film was carried out experimentally and numerically for drug delivery by transdermal patches.[145] It was elucidated that the diffusivity of DSBP was independent of the gel water concentration. Additionally, the release of DSBP could be controlled by the concentration of genipin, increasing genipin concentration delayed DSBP release.

Liposomes have the capability of carrying both hydrophilic and lipophilic drugs, unfortunately they are not stable in storage.[146] It has been shown that significant effort has gone into enhancing their stability.[147] As an observation, it appears that none of the authors' previously identified studies have explored the use of gelatine and the authors have made no mention why they have chosen to use it now. The object of this investigation was to prepare and characterise liposomes of camptothecin with chitosan, gelatine and a mixture of both as coating materials while also increasing its circulation time maintaining its active lactone state. Gelatine was found to be an ideal candidate to improve the mechanical and biological properties of a chitosan membrane.[148] Additionally camptothecin showed an increase in its circulation endurance making it a suitable option for liposome encapsulation.

As with the medical field both collagen and gelatine continue to perform a role very few other products can fulfill, being non-toxic, biodegradable, freely cross-linked, and readily available.

Total global pharmaceutical growth for 2008 was projected to be $735-745 billion.[149]
Global sales of over the counter (OTC) products totalled US$106,532 billion in 2008.[150]
US drug delivery system industry was worth $80.2 billion in 2008 but includes all types (oral, parenteral, inhalation, transdermal, implantable …).[151]

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[143] *Ibid*.

[144] Rao, K. V. R., S. P. Pakhale, et al. (2003). "A Film Approach for the Stabilization of Gelatine Preparations Against Cross-Linking." Pharmaceutical Technology 27(4): 54.

[145] Abbasi, A., M. Eslamian, et al. (2008). "Controlled release of DSBP from genipin-crosslinked gelatin thin films." Pharmaceutical Development and Technology 13(6): 549-557.

[146] Shende, P. and R. Gaud (2009). "Formulation and Comparative Characterization of Chitosan, Gelatin, and Chitosan-Gelatin-Coated Liposomes of CPT-11-HCl." Drug Development & Industrial Pharmacy 35(5): 612-618.

[147] *Ibid*.

[148] *Ibid*.

[149] (2007). "Global pharma market expected to slow down in 2008." Chemical Business 21(11): 89-89.

[150] (2009). Global OTC Pharmaceuticals. Industry Profile, Datamonitor.

The total global pharmaceutical market is difficult to break down to any one segment as each of the leading companies structure their businesses differently. It is very clear, however, that these companies benefit from patent ownership and it appears that this enables the large investments required to get new products through the regulatory framework into the market place. Most of these companies are also involved in the medical markets as well. Given the global OTC sales figures, the drug delivery market will be substantial.

6.7 Industrial Applications

Applications of hide products in this sector have primarily been around the leather industry, however over the last 10 years it appears interest has broadened and some of the functional aspects of these raw materials are being assessed more broadly

An evaluation of collagen dispersions prepared from bovine hide to coagulate, sludge-thicken and assist in sludge filtration of colloidal suspensions was shown to be effective. Used as an aid with alum it improved the turbidity, using approximately half of the alum dose and, at 1-2% of sludge dry solids, it increased the gravity settling rate by 5 times.[152] These results are very similar to the use of cationic or anionic polyelectrolyte's used as flocculent aids, for which the savings have been shown to be equivalent to 50% of the total chemicals used compared with just alum alone or $0.04/1000 litres treated.[153] In the US alone in 1997 the coagulant and flocculent market was worth US$1.25 billion.[154]

Successful recovery of Pt (IV) and Pd (II) with a bayberry tannin immobilised goat collagen fibre membrane from water has been investigated.[155] The membrane is simply prepared by tanning leather with bayberry and shaving to 0.70mm. With Pt (IV) saturation, regeneration of the membrane is easily achieved using 0.1M thiourea where full removal of Pd (II) is difficult. The membrane is capable of collecting over 2.1 kg/m^2 of Pt (IV) and over 2.5 kg of Pd (II) before any of their ions are detected in the effluent. They also exhibit selective adsorption for Pt (IV) and Pd (II) for a mixed solution of metal ions. This is a small, but potentially important, market here in Australia, especially as the membrane is principally tanned hide. Pt (IV) price is currently $1,696.49 and Pd(II) is $708.48 a troy ounce.[156]

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[151] Anonymous (2008). "Drug Delivery Systems to 2012 - Demand and Sales Forecasts, Market Share, Market Size, Market Leaders." from http://www.freedoniagroup.com/Drug-Delivery-Systems.html.

[152] Davis, J. F. and G. J. Maffia (1995). "Collagen dispersions for liquid-solid separations in water treatment and sludge dewatering." Separations Technology 5(3): 147-152.

[153] Haydar, S. and J. A. Aziz (2009). "Coagulation-flocculation studies of tannery wastewater using combination of alum with cationic and anionic polymers." Journal of Hazardous Materials 168(2-3): 1035- 1040.

[154] Kline & Company Inc (February 1, 1998). "Coagulants & Flocculants North America 1997." from http://www.marketresearch.com/product/display.asp?productid=1142724.

[155] Ma, H.-w., X.-p. Liao, et al. (2006). "Recovery of platinum(IV) and palladium(II) by bayberry tannin immobilized collagen fiber membrane from water solution." Journal of Membrane Science 278(1-2): 373- 380.

[156] Anonymous (2010). "Precious metal prices." Perth Mint Australia. Retrieved 12 December, from http://www.perthmint.com.au/investment_invest_in_gold_precious_metal_prices.aspx.

A similar application has also been investigated to recover Hg (II) from waste water where the collagen fibres were tanned with bayberry tannin and cross-linked with oxazolidine.[157] This was found to be very effective especially as a column which showed an adsorption capacity of 617 mg/g which matched the isothermal studies of 619mg/g. The column was effectively desorbed using 0.1M lactic acid recovering 98% of Hg (II). The collagen fibres would obviously lose adsorption capacity over a number of uses; however, this is seen as an effective process because the material costs are low.

The immobilisation of Pd metal nanoparticles (MNP) using epigallocatchin-3-gallat grafted to collagen fibres was investigated to ensure that the nanoparticles do not coalesce, making an effective nano-catalyst.[158] The catalyst was adapted to the hydrogenation of nitrobenzene and its derivatives for which the results are presented as outstanding for both activity and selectivity. MNPs hold promise in electro-optical devices, medical diagnostics, catalysts and biological imaging. The concept of using collagen fibres as a matrix to fix a range of adsorbents or catalysts would have potential for a number of products, with such a large surface area and a charged surface would make this worth further review.

An invention has been claimed where a thermoplastic is produced from collagen.[159] The raw material is derived from any collagen source. If previously limed hide splits, they are soaked in water until saturated then ground to 2mm cylindrical worms. These particles are then dried at between 60°C and 80 °C for 16 hours, ensuring the molecular weight is kept above 500 kD, to moisture of 4 to 8 %w/w and milled to a particle size of 50 to 100 µm. This can be stored, but is hydroscopic so would preferably be further processed. The powder can then be extruded at 75
°C to 90 °C and 30 bar to 100 bar pressure, with any of most extrusion additives either pre-mixed or feed directly to produce thermoplastic pellets.

The products that can be produced from these pellets, which are biodegradable and recyclable, include food products, any moulded product, and packaging material including heat sealable films. Being biodegradable it is claimed to be particularly suited to food service utensils, rubbish bags, golf tees and pet toys. While the process appears relatively simple the extent of knowledge displayed in the patent with respect to plastics processing adds weight to the viability of such a process.

[157] Huang, X., X. Liao, et al. (2009). "Hg(II) removal from aqueous solution by bayberry tannin-immobilized collagen fiber." Journal of Hazardous Materials 170(2-3): 1141-1148.

[158] Wu, H., L. Zhuo, et al. (2009). "Heterogeneous hydrogenation of nitrobenzenes over recyclable Pd(0) nanoparticle catalysts stabilized by polyphenol-grafted collagen fibers." Applied Catalysis A: General 366(1): 44-56.

[159] Garralda, V. E., O. Kotlarski, et al. (2006). Collagen powder and collagen thermoplastic composition for preparing conformed articles. U.S. Pat. and Trademark Office. US 2009/022657

A1.

An invention is claimed where a thermoset composition is made using biodegradable protein.[160] The process described for processing collagen involves a pre-mix fluid consisting of 8 parts xanthan gum and 92 parts anhydrous glycerol which have been mixed and heated to 40 °C which becomes very viscous and sticky. This is pumped to a single screw extruder, which is kept below 14 °C, at a rate of 60 parts, where collagen with molecular weight over 500 kD is fed at
100 parts into the extruder. A compound is formed which develops the right flowability and cohesiveness before exiting through a straight slotted die forming a thick flat horizontal sheet which is passed through a series of calendars that convey and spread the sheet until forming a finer and wider film. This film is fed into a microwave oven on a conveyor where it is heated to reach a temperature of 90 °C for a few seconds and sets giving sheet with smooth feel and leather appearance.

By extruding the material through an extended nylon pipe, and then through the microwave, the product can set as a rod and be turned into pellets which can be used for conventional thermoplastic extrusion.[161]

These two patents have one common inventor, which is apparent, however the difference small changes can make with ingredient levels and processing steps would indicate this type of application is worth consideration if just for the flexibility it would introduce.

An earlier invention specified a process where limed hides are utilised to prepare very specialised collagen powder.[162] The collagen is purified using enzymes and additives then wet pulverised using a grinder and a disintegrator, tanned then dehydrated using a centrifuge, filter press and fluid bed dryer to a moisture below 10% and pulverised again in a jet mill to a particle size between 4 to 25 µm. This powder when combined with a synthetic resin around 1:1 can be sprayed on to a synthetic surface providing a leather look and feel. The process is controlled to produce a collagen powder with particle size having less than 15% over 40 µm, a density between 0.1 and 0.3 g/cm3, water absorption between 120 and 300% and oil absorption between 1.0 and 1.5.

It is difficult to assess if it would not be more cost effective to use leather. However by spraying the product on, usage levels would be very low.

Thermoplastics are 65% of the world synthetic polymer production, which totals over 250 million tonnes annually.[163] Currently there are no protein sources that are being considered as a source for bio-based plastics, primarily as there is no current large scale pilot facility to meet what is considered a threshold for consideration.[164] Current polypropylene price is $1,180-1,280/tonne[165] which makes competing with petrochemicals as a raw material economically challenging.

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[160] Garralda, V. E. and G. I. Martinez (2007). Biodegradable protein based thermoset compositions, preparation methods and applications thereof. W.I.P.O. WO 2007/104323 A1.

[161] *Ibid*.

[162] Yoshida, E., H. Yoshida, et al. (1992). Collagen powder having good dispersion stability and use thereof a leather-like surface layer forming agent. U. S. Patent. 5,153,067.

[163] Shen, L., J. Haufe, et al. (2009). Product overview and market projection of emerging bio-based plastics. PRO-BIP 2009, University Utrecht. Final Report.

[164] *Ibid*.

[165] Lin, C. B. (2010). "Polypropylene (PP) Prices and Pricing Information." Retrieved 16 December, from http://www.icis.com/v2/chemicals/9076429/polypropylene/pricing.html.

However, if the history with packaging materials is any indication, then consumer pressure may have a similar impact in the moulded plastics market also.

7 Success in achieving objectives

A critical review of novel applications for hides and skins from the red meat industry based on their material composition, other than for leather has been successfully completed.

A review of current and recent values of hides and skins has been provided and shown to support one of the key premises for this project that being the impact that the luxury goods market has on the hides and skins market.

The potential value of opportunities has been assessed where possible and if not directly, market segments that they fall within have been sized. The material component based analysis has been used as a theme for the review and all areas relating to hide components have been review this way. However only areas where novel ideas have been presented in the last 10 years have been reviewed.

An appropriately presented report for meat processors to motivate further investigation of value adding options is being completed.

8 Impact on meat and livestock industry – Now and in five years time

The opportunities reviewed and recommended all have potential to provide value for hides and skin products over the coming 5 years, some opportunities will require development to mitigate risk.

However the grouping of the opportunities into three broad areas, around material components, should ensure that a maximum return can be achieved through a number of opportunities helping to spread risk across the options.

A strategic approach to these opportunities is recommended as there is an interconnectedness that should enable a national approach and thereby benefiting a majority of stakeholders for a minimum of risk.

9 Conclusions and recommendations.

While there is a large body of work that has been carried out over the last 10 years with respect to the application of collagen and collagen derived products, the work appears to have been overly focused around tissue engineering and nano-technology which utilises very low volumes of specialised high value products such as scaffolds, sponges and biocompatible interfaces for which collagen is well suited. The requirement is specialised enough that pharmaceutical/medical supply companies have considered setting up their own herds for raw material supply. This market could be captured as part of a larger volume market such as food.

There has also been a significant emphasis through out all application areas of seeking alternatives to bovine collagen as a result of the BSE outbreaks in both Europe and America. This has reduced in the later half of the time period.

Significant findings are being made which progress the understanding of collagen from a physicochemical, biochemical and mechanical viewpoint with advances in using composites as well as cross-linking and the ability to discern with some confidence structural occurrences.

This critical review of novel applications for hides and skins from the red meat industry based on their material composition, other than for leather products, has identified a range of opportunities which can be facilitated under three areas of focus, these being;

4) The extraction of functional 'collagen fibres' that may be utilised in food, packaging, medical and Industrial applications.

a) Hide stabilisation using new milling process b) Collagen fibres as a matrix for fixing adsorbents and catalysts
c) Collagen fibre and powder as 50:50 film components
d) 9M urea extraction process e) Collagen alginate interaction in medical and food products f) Skin casing change from gut to collagen g) Osmotic drying of meats h) Effects of ball milling i) Membrane recovery of Pt and Pd j) Raw material for thermoset and thermoplastic extrusion

5) The effective hydrolysis of collagen optimally utilising biological, chemical and/or physical means.

a) Optimised chem. and biotech. hydrolysis of collagen to gelatine and hydrolysates b) Low fat food products from specific gelatines for emulsification
c) Specific cleavage of bovine hide collagen using mutant pepsin to increase gelatine yield
d) Thermoplastic extrusion of collagen to thread e) Halal gelatine from BSE free Australia f) Use of SPI for reduction of water vapour permeability in bovine gelatine film

6) Characterisation and specification of collagen hydrolysates that accounts for origin, processing and functionality, enabling effective manufacturing utilisation.

a) Understanding collagen hydrolysates like 'antifreeze protein'
b) Hydrogels as collagen hydrolysate composites for biodegradable nappies c) Use of collagen hydrolysate for retention in nutritional fortification like iodine & thiamine d) Injected collagen hydrolysates for cartilage repair e) Thermal treatment of, DAS cross-linked collagen hydrolysate to reduce 'film aging' f) Biodegradable packaging from polyvinyl blends with collagen hydrolysate

10 Bibliography

(2001). "Nestle to target meat-free sector with gelatin-substitute food range." Marketing Week 23(47): P.5.

(2007). "Global pharma market expected to slow down in 2008." Chemical Business 21(11): 89-89.

(2009). Global OTC Pharmaceuticals. Industry Profile, Datamonitor.

Abbasi, A., M. Eslamian, et al. (2008). "Controlled release of DSBP from genipin-crosslinked gelatin thin films." Pharmaceutical Development and Technology 13(6): 549-557.

Alexy, P., D. Bakos, et al. (2003). "Poly(vinyl alcohol)-collagen hydrolysate thermoplastic blends: II. Water penetration and biodegradability of melt extruded films." Polymer Testing 22(7): 811-818.

Alexy, P., D. Bakos, et al. (2003). "Poly(vinyl alcohol)-collagen hydrolysate thermoplastic blends: I. Experimental design optimisation and biodegradation behaviour." Polymer Testing 22(7): 801-809.

Anonymous (2002). Ageing population sparks growth in biomaterials market, Decision News Media SAS.

Anonymous (2002). "TRADE WASTE MANAGEMENT PLANS." from http://www.vicwater.org.au/uploads/Downloads/TWMPG%20&%20Template04.pdf.

Anonymous (2008). "Drug Delivery Systems to 2012 - Demand and Sales Forecasts, Market Share, Market Size, Market Leaders." from http://www.freedoniagroup.com/Drug-Delivery-Systems.html.

Anonymous (2009). Annual Report & Accounts 2009, Devro plc.

Anonymous (2009). "Asia Pacific Market for Bone Graft Substitutes and Other Biomaterials 2009 (3 Countries)." November 1. from http://www.marketresearch.com/product/display.asp?productid=2529363.

Anonymous (2009). Global Confectionery. Industry Profile, Datamonitor.

Anonymous (2010). from http://au.alibaba.com/products/Chondroitin-sulphate.html.

Anonymous (2010). "Co_products." from http://www.redmeatinnovation.com.au/innovation-areas/value-adding/co-products.

Anonymous (2010, 12 Oct). "The Gelita group is world leader." from http://www.gelita.com/DGF-english/index.html.

Anonymous (2010, October 4). "Global Collagen and HA-Based Biomaterials Market." from http://www.prweb.com/releases/prwebbiomaterials_collagen/hyaluronic_acid/prweb4590214.htm.

Anonymous (2010, 4th of October). "Global Collagen and HA-Based Biomaterials Market ". from http://www.strategyr.com/Collagen_and_HA_Based_Biomaterials_Market_Report.asp
.

Anonymous (2010). "Precious metal prices." Perth Mint Australia. Retrieved 12 December, from http://www.perthmint.com.au/investment_invest_in_gold_precious_metal_prices.aspx.

Anonymous (2010). "Raw materials." Gelatin.org. from http:/www.gelatine.org/en/about-gelatine/manufacturing/raw-materials.html.

Anonymous (2010). "Raw materials & Production." GMIA. from http:/www.gelatin-gmia.com/html/rawmaterials.html.

Anonymous (2010, January 4). "Sustainable Packaging Market to Reach $170 Billion Worldwide by 2014." from http://www.pikeresearch.com/newsroom/sustainable-packaging-market-to-reach-170-billion-worldwide-by-2014.

Anonymous (2010). "U.S. Market for Dental Bone Graft Substitutes and Other Biomaterials 2010." from http://www.reportsandreports.com/reports/34545-us-market-for-dental-bone-graft-substitutes-and-other-biomateri.html.

Anonymous (May 2006). "FAO Fact Sheet: The international fish trade and world fisheries." from http://www.fao.org/newsroom/common/ecg/1000301/en/enfactsheet2.pdf.

Ashley, P. (2010). Victorian Food and Fibre Export Performance. C. Jonathan and F. Simon, Dept of Primary Industry.

Badii, F. (2006). "Enthalpy and mechanical relaxation of glassy gelatin films." Food Hydrocolloids 20(6): 879-884.

Berkhout, H. J. and J. R. Garcia Del Rio (2003). Process for treating animal skins. European patent office. W. I. P. Organisation. WO 2005/059184 A3.

Berry, B. (2008). Global halal food market brief. Agri-Food Trade Services, Agriculture Agri-Food Canada.

Böttcher-Haberzeth, S. (2010). "Tissue engineering of skin." Burns 36(4): 450-460.

BRUNO, W. (1990). Process for the production of meat products. European Patent Office. Germany. US 4910034 (A).

Buck Ii, D. W., M. Alam, et al. (2009). "Injectable fillers for facial rejuvenation: a review." Journal of Plastic, Reconstructive & Aesthetic Surgery 62(1): 11-18.

Bunyaratavej, P. and H. L. Wang (2001). "Collagen membranes: A review." Journal of Periodontology 72(2): 215-229.

Burey, P., B. R. Bhandari, et al. (2009). "Confectionery Gels: A Review on Formulation, Rheological and Structural Aspects." International Journal of Food Properties 12(1): 176-210.

Carvalho, R. A. and C. R. F. Grosso (2005). "Properties of chemically modified gelatin films " Brazilian Journal of Chemical Engineering 23(01): 44-53.

Cheng, X., U. A. Gurkan, et al. (2008). "An electrochemical fabrication process for the assembly of anisotropically oriented collagen bundles." Biomaterials 29(22): 3278-3288.

Chomarat, N., L. Robert, et al. (1994). "Comparative efficiency of pepsin and proctase for the preparation of bovine skin gelatin." Enzyme and Microbial Technology 16(9): 756-760.

Cockerham, K. (2009). "Collagen-based dermal fillers: Past, present, future." Facial plastic surgery 25(2): 106-113.

Coppola, M., M. Djabourov, et al. (2008). "Phase Diagram of Gelatin Plasticized by Water and Glycerol." Macromolecular Symposia 273(1): 56-65.

Dallora, N. L. P., J. G. D. Klemz, et al. (2007). "Partitioning of model proteins in aqueous two-phase systems containing polyethylene glycol and ammonium carbamate." Biochemical Engineering Journal 34(1): 92-97.

Davis, J. F. and G. J. Maffia (1995). "Collagen dispersions for liquid-solid separations in water treatment and sludge dewatering." Separations Technology 5(3): 147-152.

De Guzman, D. and J. Chang (2007). "Global cosmetics demand surges." ICIS Chemical Business Americas 271(16): 6-6.

de Wolf, F. A., Ed. (2003). Progress in Biotechnology, Industrial Proteins in Perspective
Chapter 5. Collagen and gelatin.

Denavi, G. A., M. Pérez-Mateos, et al. (2009). "Structural and functional properties of soy protein isolate and cod gelatin blend films." Food Hydrocolloids 23(8): 2094-2101.

Ficai, A. (2010). "Self-assembled collagen/hydroxyapatite composite materials." Chemical engineering journal 160(2): 794-800.

Figueiró, S. D., J. C. Góes, et al. (2004). "On the physico-chemical and dielectric properties of glutaraldehyde crosslinked galactomannan-collagen films." Carbohydrate Polymers 56(3): 313-320.

Galea, C. A., B. P. Dalrymple, et al. (2000). "Modification of the substrate specificity of porcine pepsin for the enzymatic production of bovine hide gelatin." Protein Science 9(10): 1947-1959.

Garralda, V. E., O. Kotlarski, et al. (2006). Collagen powder and collagen thermoplastic composition for preparing conformed articles. U.S. Pat. and Trademark Office. US 2009/022657 A1.

Garralda, V. E. and G. I. Martinez (2007). Biodegradable protein based thermoset compositions, preparation methods and applications thereof. W.I.P.O. WO 2007/104323 A1.

Haroun, A. A. (2010). "Preparation, characterization, and In Vitro application of composite films based on gelatin and collagen from natural resources." Journal of applied polymer science 116(4): 2083-2094.

Haydar, S. and J. A. Aziz (2009). "Coagulation-flocculation studies of tannery wastewater using combination of alum with cationic and anionic polymers." Journal of Hazardous Materials 168(2-3): 1035-1040.

Henrotin, Y., C. Lambert, et al. "Nutraceuticals: do they represent a new era in the management of osteoarthritis? - a narrative review from the lessons taken with five products." Osteoarthritis and Cartilage In Press, Uncorrected Proof.

Huang, X., X. Liao, et al. (2009). "Hg(II) removal from aqueous solution by bayberry tannin-immobilized collagen fiber." Journal of Hazardous Materials 170(2-3): 1141-1148.

Jahanshahi, M., M. H. Sanati, et al. (2008). "Optimization of parameters for the fabrication of gelatin nanoparticles by the Taguchi robust design method." Journal of Applied Statistics 35(12): 1345-1353.

Jarvis, L. (2002). "Gelatin Market Stable as BSE Moves From the Spotlight." Chemical market reporter 261(6): 8.

Karim, A. A. and R. Bhat (2008). "Gelatin alternatives for the food industry: recent developments, challenges and prospects." Trends in Food Science and Technology 19(12): 644-656.

Kasapis, S., I. M. Al-Marhoobi, et al. (2003). "Gelatin vs polysaccharide in mixture with sugar." Biomacromolecules 4(5): 1142-1149.

Kasapis. S, Mitchell. J, et al. (2004). "Rubber-to-glass transitions in high sugar biopolymer mixtures." Trends in Food Science & Technology 15(6): 293-304.

Kline & Company Inc (February 1, 1998). "Coagulants & Flocculants North America 1997." from http://www.marketresearch.com/product/display.asp?productid=1142724.

Kokubo, T. and H. Takadama (2006). "How useful is SBF in predicting in vivo bone bioactivity?" Biomaterials 27(15): 2907-2915.

Langer, R. and J. P. Vacanti (1993). "Tissue engineering." Science 260(5110): 920-926.

Langmaier, F. (2009). "Hydrogels of collagen hydrolysate cross-linked with dialdehyde starch." Journal of thermal analysis and calorimetry 98(3): 807-812.

Langmaier, F., P. Mokrejs, et al. (2008). "Biodegradable packing materials from hydrolysates of collagen waste proteins." Waste Management 28(3): 549-556.

Lavers, B. (2009). "Collagen cash cow." Food Manufacture(February): 37-38.

Lee, S. B., H. W. Jeon, et al. (2003). "Bio-artificial skin composed of gelatin and (1-->3), (1-->6)-[beta]-glucan." Biomaterials 24(14): 2503-2511.

Li, D., C. Mu, et al. (2009). "Ultrasonic irradiation in the enzymatic extraction of collagen." Ultrasonics Sonochemistry 16(5): 605-609.

Lima, A. M., M. A. Cerqueira, et al. (2010). "New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits - Influence on fruits gas transfer rate." Journal of Food Engineering 97(1): 101-109.

Lin, C. B. (2010). "Polypropylene (PP) Prices and Pricing Information." Retrieved 16 December, from http://www.icis.com/v2/chemicals/9076429/polypropylene/pricing.html.

Liu, Y., Y.-K. Kim, et al. (2011). "Hierarchical and non-hierarchical mineralisation of collagen." Biomaterials 32(5): 1291-1300.

Lorenzo, G., G. Checmarev, et al. (2011). "Linear viscoelastic assessment of cold gel-like emulsions stabilized with bovine gelatin." LWT - Food Science and Technology 44(2): 457-464.

Ma, H.-w., X.-p. Liao, et al. (2006). "Recovery of platinum(IV) and palladium(II) by bayberry tannin immobilized collagen fiber membrane from water solution." Journal of Membrane Science 278(1-2): 373-380.

Madhan, B., C. Muralidharan, et al. (2002). "Study on the stabilisation of collagen with vegetable tannins in the presence of acrylic polymer." Biomaterials 23(14): 2841-2847.

Martucci, J. F. and R. A. Ruseckaite (2010). "Biodegradable three-layer film derived from bovine gelatin." Journal of Food Engineering 99(3): 377-383.

Martucci, J. F., A. Vázquez, et al. (2007). "Nanocomposites based on gelatin and montmorillonite: Morphological and thermal studies." Journal of thermal analysis and calorimetry 89(1): 117-122.

Maser, F., C. Ströher-Glowienka, et al. (1991). "Collagen film as a new pervaporation membrane." Journal of Membrane Science 61: 269-278.

Maser, F. and O. Tuerk (2004). Multi-perforated collagen film. U. S. P. T. Office. United States of America. US 2004/0161500 A1.

Mattar. JA (1989 Aug;). "A simple calculation to estimate body surface area in adults and its correlation with the Du Bois formula." Crit Care Med. 17(8): 846-847.

Meyer, M. (2010). "Collagen fibres by thermoplastic and wet spinning." Materials science & engineering. C, Biomimetic materials, sensors and systems 30(8): 1266-1271.

Meyer, M., R. Mühlbach, et al. (2005). "Solubilisation of cattle hide collagen by thermo-mechanical treatment." Polymer Degradation and Stability 87(1): 137-142.

Mokrejs, P., F. Langmaier, et al. (2009). "Extraction of collagen and gelatine from meat industry by-products for food and non food uses." Waste Management and Research 27(1): 31-37.

Morgan, T. (2010). "Trends in Australian co-product values in 2009." MLA market information, Co-products Brief.

Mysker, T. (2005). Apparatus and method to net food products in shirred tubular casing. European Patent Office. U.S. Pat. and Trademark Office. USA.

Nam, K., T. Kimura, et al. (2007). "Preparation and characterization of cross-linked collagen-phospholipid polymer hybrid gels." Biomaterials 28(1): 1-8.

Nicoleti, J. and V. Telis (2009). "Viscoelastic and Thermal Properties of Collagen–Xanthan Gum and Collagen–Maltodextrin Suspensions During Heating and Cooling." Food Biophysics 4(3): 135-146.

Ninan, G. (2010). "Physical, Mechanical, and Barrier Properties of Carp and Mammalian Skin Gelatin Films." Journal of food science 75(9): E620-E626.

Oesser, S. (2003). Gelatine boosts collagen synthesis. Food Navigator. Science & Nutrition.

Raghava Rao, J. and B. U. Nair "Novel approach towards recovery of glycosaminoglycans from tannery wastewater." Bioresource Technology In Press, Corrected Proof.

Rao, K. V. R., S. P. Pakhale, et al. (2003). "A Film Approach for the Stabilization of Gelatine Preparations Against Cross-Linking." Pharmaceutical Technology 27(4): 54.

Raspanti, M., M. Viola, et al. (2008). "Glycosaminoglycans show a specific periodic interaction with type I collagen fibrils." Journal of Structural Biology 164(1): 134-139.

Rito-Palomares, M. (2004). "Practical application of aqueous two-phase partition to process development for the recovery of biological products." Journal of Chromatography B 807(1): 3-11.

Rito-Palomares, M., C. Dale, et al. (2000). "Generic application of an aqueous two-phase process for protein recovery from animal blood." Process Biochemistry 35(7): 665-673.

Roldan (2009). "The rise of pharmaceutical gelatin plant - uncertainty of the market." from http://news.frbiz.com/the_rise_of_pharmaceutical-227675.html.

Rudnick, A. (2006). "Advances in tissue engineering and use of type 1 bovine collagen particles in wound bed preparation." Journal of wound care 15(9): 402-404.

Sang, L., X. Wang, et al. (2010). "Assembly of collagen fibrillar networks in the presence of alginate." Carbohydrate Polymers 82(4): 1264-1270.

Santana, R. C., F. A. Perrechil, et al. "Emulsifying properties of collagen fibers: Effect of pH, protein concentration and homogenization pressure." Food Hydrocolloids In Press, Corrected Proof.

Saravanan, S., J. R. Rao, et al. (2006). "Recovery of value-added globular proteins from tannery wastewaters using PEG-salt aqueous two-phase systems." Journal of Chemical Technology and Biotechnology 81(11): 1814-1819.

Saravanan, S., J. R. Rao, et al. (2007). "Partition of tannery wastewater proteins in aqueous two-phase poly (ethylene glycol)-magnesium sulfate systems: Effects of molecular weights and pH." Chemical Engineering Science 62(4): 969-978.

Saravanan, S., J. R. Rao, et al. (2008). "Aqueous two-phase poly(ethylene glycol)-poly(acrylic acid) system for protein partitioning: Influence of molecular weight, pH and temperature." Process Biochemistry 43(9): 905-911.

Scully. Carla, Z. (2006). Bench Strength. Candy Industry. Trends and Applications in Ingredient Technology. August: 36-37.

Shakesheff, K. (2010, 1 April). "Tissue Engineering: the Next Regeneration." from http://www.medicaldevice-network.com/features/feature81032/.

Shen, L., J. Haufe, et al. (2009). Product overview and market projection of emerging bio-based plastics. PRO-BIP 2009, University Utrecht. Final Report.

Shende, P. and R. Gaud (2009). "Formulation and Comparative Characterization of Chitosan, Gelatin, and Chitosan-Gelatin-Coated Liposomes of CPT-11-HCl." Drug Development & Industrial Pharmacy 35(5): 612-618.

Sivasubramanian, S., B. M. Manohar, et al. (2008). "Mechanism of enzymatic dehairing of skins using a bacterial alkaline protease." Chemosphere 70(6): 1025-1034.

Sobral, P. J. A., F. C. Menegalli, et al. (2001). "Mechanical, water vapor barrier and thermal properties of gelatin based edible films." Food Hydrocolloids 15(4-6): 423-432.

Stevens, M. M. (2008). "Biomaterials for bone tissue engineering." Materials Today 11(5): 18-25.

Stevens, P. (2010). Marshmellow-type aerated confectionery and method of preparation. U. S. P. T. Office. United States of America. US 2010/0260906.

Stocum, D. L. (2006). Regenerative Medicine of Skin, Hair, Dental Tissues, and Cornea. Regenerative Biology and Medicine. Burlington, Academic Press: 63-88.

Stuart, K. (2009). "Characterization of gels composed of blends of collagen I, collagen III, and chondroitin sulfate." Biomacromolecules 10(1): 25-31.

Suirami. Jean and Lhoutellier. Pascal (2007). Method for continuos production of minced meat dried and restored to thin slabs. European Patent Office. France. EP 1406501 (A1)

Suzuki, F., H. Kimura, et al. (2000). "Formation having a tanning gradient structure of collagen membrane by the pervaporation technique." Journal of Membrane Science 165(2): 169-175.

Teo, W. E., S. Liao, et al. (2011). "Fabrication and characterization of hierarchically organized nanoparticle-reinforced nanofibrous composite scaffolds." Acta Biomaterialia 7(1): 193-202.

Teppner, M. (2006) Pleasure and health with Less Fat: The New Generation of Chocolate. Gelita AG: Corporate Comunications

Ustunol, Z. (2009). Edible Films and Coatings for Meat and Poultry. Edible Films and Coatings for Food Applications. K. C. Huber and M. E. Embuscado, Springer New York: 245-268.

Valeika, V., K. Beleška, et al. (2009). "An approach to cleaner production: from hair burning to hair saving using a lime-free unhairing system." Journal of Cleaner Production 17(2): 214-221.

Ventre, M., F. Mollica, et al. (2009). "The effect of composition and microstructure on the viscoelastic properties of dermis." Journal of Biomechanics 42(4): 430-435.

Vierhile, T. (2010). "Cosmeceuticals for the Ages." Prepared Foods 179(6): 25-33.

Wang, S. (2009). "Ice-structuring peptides derived from bovine collagen." Journal of agricultural and food chemistry 57(12): 5501-5509.

Waszkowiak, K. (2007). "Effect of collagen preparations used as carriers of potassium iodide on retention of iodine and thiamine during cooking and storage of pork meatballs." Journal of the science of food and agriculture 87(8): 1473-1479.

Waszkowiak, K. and W. Dolata (2007). "The application of collagen preparations as carriers of rosemary extract in the production of processed meat." Meat Science 75(1): 178-183.

Windholz, M., Ed. (1976). The Merck Index, Merck & Co Inc.

Wolf, K. L., P. J. A. Sobral, et al. (2006). "Characterisation of collagen fibres for biodegradable film production." IUFoST. from http://iufost.edpsciences.org/index.php?option=com_toc&url=/articles/iufost/abs/2006/01/contents/contents.html.

Wu, H., L. Zhuo, et al. (2009). "Heterogeneous hydrogenation of nitrobenzenes over recyclable Pd(0) nanoparticle catalysts stabilized by polyphenol-grafted collagen fibers." Applied Catalysis A: General 366(1): 44-56.

Xiong, X., R. Ghosh, et al. (2009). "A new procedure for rapid, high yield purification of Type I collagen for tissue engineering." Process Biochemistry 44(11): 1200-1212.

Yannas, I. V., D. S. Tzeranis, et al. (2010). "Biologically active collagen-based scaffolds: Advances in processing and characterization." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 368(1917): 2123-2139.

Yoshida, E., H. Yoshida, et al. (1992). Collagen powder having good dispersion stability and use thereof a leather-like surface layer forming agent. U. S. Patent. 5,153,067.

Zague, V. (2008). "A new view concerning the eVects of collagen hydrolysate intake on skin properties." Arch Dermatol Res 300: 479–483.

Zeugolis, D. I. (2010). "The influence of a natural cross-linking agent (Myrica rubra) on the properties of extruded collagen fibres for tissue engineering applications." Materials science & engineering. C, Biomimetic materials, sensors and systems 30(1): 190-195.

Zeugolis, D. I., S. T. Khew, et al. (2008). "Electro-spinning of pure collagen nano-fibres - Just an expensive way to make gelatin?" Biomaterials 29(15): 2293-2305.

Zhang, C., J. Baez, et al. (2009). "Purification and Characterization of a Transgenic Corn Grain-Derived Recombinant Collagen Type I Alpha 1." Biotechnology Progress 25(6): 1660-1668.

Zohuriaan-Mehr, M., A. Pourjavadi, et al. (2009). "Protein- and homo poly(amino acid)-based hydrogels with super-swelling properties." Polymers for advanced technologies 20(8): 655-671.

11 Appendices

11.1 Appendix 1 Databases Utilised

11.1.1 Scopus

About this resource
Abstracting and indexing database of scientific, technical, medical and social science literature. Includes peer-reviewed titles from international publishers, Open Access journals, conference proceedings, trade publications, patent records and quality web sources. Seamless links to full text sources where the library holds a subscription.
Updates: Updated daily.
Subject headings

- Science—Abstracts & Indexes.
- Social sciences—Abstracts & Indexes.
- Medicine—Abstracts & Indexes.
- Technology—Abstracts & Indexes.
- Physical sciences—Abstracts & Indexes.
- Life sciences—Abstracts & Indexes.

11.1.2 ScienceDirect

ScienceDirect contains over 25% of the world's science, technology and medicine full text and bibliographic information. Apart from online eBooks, Reference Works, Handbooks and Book Series, ScienceDirect offers a rich journal collection of over 2,000 titles. In addition, the Backfiles program offers the ability to search a historical archive of over 6.75 million articles directly from

your desktop, back to Volume 1, Issue 1. The collections contain 4 million articles prior to 1995, and 2.75 million articles from after 1994.
Data Format: Text (HTML and PDF)

Subject headings
- Science--Handbooks, manuals, etc.
- Physical sciences--Handbooks, manuals, etc.
- Social sciences--Handbooks, manuals, etc.
- Life sciences--Handbooks, manuals, etc.
- Medical sciences--Handbooks, manuals, etc.
- Technology--Handbooks, manuals, etc.
- Science--Periodicals--Indexes.
- Science--Periodicals--Abstracts.
- Physical sciences--Periodicals--Indexes.
- Physical sciences--Periodicals--Abstracts.
- Social sciences--Periodicals--Indexes.
- Social sciences--Periodicals--Abstracts.
- Life sciences--Periodicals--Indexes.
- Life sciences--Periodicals--Abstracts.
- Medical sciences--Periodicals--Indexes.
- Medical sciences--Periodicals--Abstracts.
- Technology--Periodicals--Indexes.
- Technology--Periodicals--Abstracts.

11.1.3 Proquest

Proquest is a leading electronic database providing indexing and abstracting of over 7,000 scholarly and general interest publications. It includes full text or full image coverage of more than 3,000 of these publications. It provides access to the combined information from a number of leading online databases which cover subjects including: business, law, education, computing, science, technology, engineering, arts, medicine, and religion.

Data Format: Text (HTML and PDF)

Subject headings
- Periodicals--Indexes.
- Periodicals--Abstracts.
- Newspapers--Indexes.
- Newspapers--Abstracts.

11.1.4 SpingerLink

Provides full text access to Springer journals and book series. Subject coverage includes: chemical sciences, computer science, economics, engineering, environmental sciences, geosciences, law, life sciences, mathematics, medicine, and physics and astronomy.

Data Format: Text (abstracts and full text) of electronic journals and books in HTML and PDF file formats.

Subject headings
- Science--Databases.
- Science--Periodicals--Databases.
- Economics--Databases.

- Economics--Periodicals--Databases.
- Law--Databases.
- Law--Periodicals--Databases.
- Medicine--Databases.
- Medicine--Periodicals--Databases.

11.1.5 Business source premier

Business Source Premier provides full text for more than 2,300 business and economics journals, including full text for more than 1,100 peer-reviewed titles. Covering all disciplines of business, it also includes country economic reports and Datamonitor company and industry profiles from around the world.
Data Format: Database of full text journals; tables of contents, citations and abstracts available in HTML format; full text articles and company profiles available in HTML and PDF formats.
Frequency of updates: Updated daily
Subject headings

- Business.
- Business--Periodicals--Indexes.
- Business--Periodicals--Abstracts.
- Management.
- Management--Periodicals--Indexes.
- Economics.
- Economics--Periodicals--Indexes.