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Manufacture of cooked meat reaction flavour from mixed hydrolysates of lambskin and wool

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

The proposition was explored that woolly lambskin could be a useful substrate for cooked meat flavour production, in admixture with a suitable sugar source, both acting as Maillard precursors. This thesis explored making a thermal hydrolysate of collagen from the lambskin and an enzymatic hydrolysate from wool then combining the two extracts in a reaction mixture for flavour production and assessment. Proximate analysis of raw dewooled lambskin indicates its major components as moisture (75%) and crude protein (21%). Thermal extraction was initially conducted in steam at 5 psi (0.34 bar.g) pressure at various pH values. The maximum degree of solubilisation of solids was obtained at a pH of 3.55. A pH value of 4.5 was chosen for subsequent thermal hydrolysis, as further reduction in pH to 3.55 consumed large amounts of sulphuric acid. Thermal hydrolysis of lambskin was further explored at pH 4.5 to obtain collagen hydrolysates at three industrially convenient temperatures, 90°C, 108°C, and 121°C. The maximum degree of solubilisation of 89.3% was obtained for the lambskin treated at 121°C for 10 hours. Degree of solubilisation did not increase greatly with increases in temperature and time from 90°C to 121°C and from 2 hours to 10 hours. The optimal treatment condition from a process perspective was judged to be 108°C for 2 hours with a degree of solubilisation of 85%. The thermal hydrolysis itself was successful in producing collagen hydrolysates with low viscosity in the range of 2.2 to 5.6 mPa.s, which was measured using a Modular Compact rheometer at 40°C and 11°Brix. The viscosity of the extract decreased as expected with an increase in treatment time. The degree of hydrolysis (DH), an indirect measure of the peptide chain length of the hydrolysate produced, was estimated based on the ninhydrin test. The DH increased from 26% to 37% with an increase in the processing times tested. The increase in the degree of hydrolysis indicates that smaller molecular peptides are formed, which in turn reduces the viscosity of the sample.

Proximate analysis indicates that wool removed from lambskin is composed of 94.6% crude protein. Almost thrice the stoichiometric amount of sodium metabisulphite was used for sulphite pretreatment to soften the wool. Sulphite-treated wool was hydrolysed using the enzymes Neutrase® 0.8L, Alcalase® 2.4L FG, and Protamex®. The maximum degree of solubilisation of wool solids (50%) was obtained using Alcalase® 2.4L FG at an enzyme-to-substrate ratio of 3:100 at pH 7.5. Reaction mixtures were then prepared by mixing an organic sulphur source, ribose, and lambskin extract concentrated to 60°Brix. The ratio of organic nitrogen to organic sulphur was

kept constant across all the reaction mixtures at about 10.6, whereas the ribose to collagen ratio was kept constant at 1:8. The organic sulphur sources trialled were cysteine, cysteic acid, sulphite-assisted hydrolysate from wool, and alkali-assisted hydrolysate of wool (as a source of lanthionine). Sensory analysis of the samples by 25 untrained panellists confirms that cysteine is important in creating a meaty flavour profile. Sulphite-assisted hydrolysate from wool had the most pungent and animal-like flavour profile. Alkali-assisted hydrolysate of the wool, and hence lanthionine is not useful in creating flavours as the flavour note was very mild. Cysteic acid resulted in a burnt and caramel flavour note. This work did not liberate sufficient reactive cysteine from wool to indicate that woolly lambskin offers a useful meat flavour substrate.

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List of abbreviations

$Ca(OH)_2$	Calcium hydroxide
DAL	Dehydroalanine
DH	Degree of hydrolysis
DKP	2,5-diketopepiperazine
DS	Degree of solubilisation
HCl	Hydrochloric acid
HDSF	High-density steam flash-explosion
H_2SO_4	Sulphuric acid
ICSE	Instant catapult steam explosion
KABP	Keratinous animal body parts
KH	Keratin hydrolysates
LAL	Lysinoalanine
LAN	Lanthionine
MCR	Modular Compact Rheometer
MRP	Maillard-reacted peptides
NaMBS	Sodium metabisulphite
NaOH	Sodium hydroxide
N:S	Organic nitrogen: organic sulphur ratio
OH^-	Hydroxide ion
OPA	o-phthaldialdehyde
PCL	Peptide chain length
psi	Pounds per square inch
RO	Reverse osmosis

rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate
SN-TCA	Trichloroacetic acid soluble nitrogen
T_m	Melting temperature
TNBS	Trinitrobenzene sulfonic acid
TS%	Percentage of total solids
Wt. or wt.	Weight

Chapter 1: Introduction

Collagen is a valuable industrial raw material with versatile applications for the food and cosmetic industries, as well as in biotechnology and pharmaceuticals Lewandowska et al. (2021). Exploring nature-friendly methods for extracting collagen has received a lot of attention in recent years, with the skin and bones of cows, pigs, and fish, proving attractive sources. Sheepskin also presents a promising opportunity as a valuable source of collagen (Silvipriya et al., 2015). New Zealand is well known for having an active sheep farming sector, which plays a major part in the production and export of raw hides and skins of sheep or lambs globally (FAOSTAT, 2021). Thus, sheep or lambskin is an especially promising raw material for collagen production in New Zealand. Thermal hydrolysis, which involves controlled heating, offers a potential method to break down collagen-rich materials like lambskin into a soluble collagen broth. Heating lambskin can cause the hydrogen bond that maintains the triple helix structure of collagen to break, causing the collagen fibres to unwind. By dissolving the tissue matrix and releasing collagen fibres, the denaturation process increases the accessibility of collagen for extraction (Wright & Humphrey, 2002).

The food industry is always looking for new and creative ways to improve flavour profiles while implementing sustainable practices. Because of its unique composition, woolly lambskin presents itself as a creative tool for building distinctive meaty tastes. Wool is a rich source of cysteine, an amino acid that is essential for producing meaty flavour sensations (Morton et al., 1960; Shavandi et al., 2017). Hence, woolly lambskin hydrolysates containing both collagen peptides and cysteine offer the potential for creating meaty flavours. The characteristic meaty flavour caused by the Maillard reaction requires carbohydrates (particularly ribose) plus amino acids (especially cysteine) as significant meat flavour precursors (Elmore & Mottram, 2009; Mottram, 1998). However, releasing free cysteine from the keratin in wool is not easy and requires the breaking of disulphide bonds of cystine followed by peptide hydrolysis. Different pretreatments yield cysteic acid, lanthionine or cysteine-bearing peptides (Giteru et al., 2023). Whether these three compounds react similarly to free cysteine in meat flavour reaction mixtures is unknown.

The research objectives are:

(i) Investigate the effectiveness of simple thermal extraction in hydrolysing lambskin

(ii) Evaluate the efficiency of heat treatment in creating low-viscosity collagen broth

(iii) Determine whether hydrolysing wool with a sulphite pretreatment and enzyme treatment is feasible

(iv) Determine whether the byproducts of hydrolysed wool, lanthionine, and cysteic acid, can impart meaty flavours similar to cysteine

1.1. Thesis Overview

This thesis explores the above-mentioned objectives. The thesis is divided into 4 chapters which are the literature review, materials and methods, results and discussion, and conclusions and recommendations. The literature review summarises the available data and past work reported which aligns with the research objectives of the study. The materials and methods section explains laboratory methods used for hydrolysing lambskin, testing the viscosity of collagen broth produced thereby, hydrolysing wool fibres and creating reaction mixtures for meaty flavours. The results and discussion section explains the details of data obtained through trials. Conclusion and recommendations summarise what was learnt as well as suggest the improvements available for future works.

Chapter 2: Literature Review

2.1. Introduction

This study seeks to investigate the potential of using thermal hydrolysis as a method of producing low-viscosity collagen hydrolysates. It also investigates whether the woolly lambskin can act as a base for manufacturing cooked meat flavour for industrial use in food such as congee or ramen noodles. Considerable related knowledge has been published in a wide variety of literature. This chapter will summarise key existing knowledge in the manufacture of gelatin and hydrolysed collagen from lambskin, the structure of wool, the hydrolysis of keratin, and the formation of cooked meat flavours.

2.2. Lambskin

2.2.1. Structure of skin

The skin, which is the body's outermost layer, acts as a barrier between the animal's body and its surroundings. At around sixteen per cent of the overall body weight, the skin is the heaviest organ in animals. The epidermis and dermis, which make up skin, are composed of epithelial cells derived from ectoderm and connective tissue derived from mesoderm (Hejazi et al., 2013). Two to three per cent of the total thickness of the skin is made up of the epidermis. The pilar and reticular layers are the two layers that make up the dermis, or skin, itself. The pilar layer makes up between 60 and 70 per cent of the skin. The skin's foundation, the reticular layer, is its densest layer. Collagen fibres form the fundamental structure of the reticular layer. The proportion of the reticular to pilar layers is a measure of skin strength, the smaller the ratio, the stronger the skin. The overall quality of the sheepskin is determined by the structure of the reticular layer, which includes the collagen fibres' thickness and the type of bond between them. Primary and secondary follicles are found on lamb skin. The ratio of secondary to primary follicles is also an indicator of skin thickness (Pogodaev et al., 2021).

2.2.2. Composition of sheep skin

The skin accounts for 10-12% of live animal weight in sheep (Baldi et al., 2021). The major components of sheep skin are moisture and protein, which account for about 60-77% and 16-25%

respectively (Murugan et al., 2013; Wodzicka, 1958). It is reported that the fat content of sheep skin is between 5.5%-7.1% (Wodzicka, 2012). The fat that is specific to sheep, lanolin is formed as a layer below the epidermis and might be contributing to the total fat content of the sheep skin rather than the subcutaneous fat (Fourneau et al., 2020). The minor components in the skin are ash and fibre which account for 0.7% and 0.4% respectively. The ash content of animal skin is reported to be 0.8-0.9 % and the crude fibre content is about 1% (Osobamiro et al., 2023).

2.2.3. Sheep and lamb skin production and consumption statistics

The global production of raw hides and skins of sheep or lambs stood at 1.9 million metric tonnes in the year 2021 (FAOSTAT, 2021). Table 2.1 shows the top countries in the global market for trade in sheep and lambskin.

Table 2. 1. Country-wise share in sheep and lambskin production, consumption, exports, and imports in 2024

Indicators	Countries with a major share	% share
Largest consumers	China	41
	Turkey	5.86
	New Zealand	3
Largest producers	China	30
	Australia	10
	New Zealand	4.3
Largest exporters	Australia	47
	Spain	9.5
	New Zealand	8.1
Largest importers	China	61
	Turkey	17
	Spain	4.9

Source: (Indexbox, 2024)

The average export and import prices of sheep and lambskin in the year 2022 are NZ$1,262 per ton and NZ$979 per ton respectively (Indexbox, 2024).

2.3. Preliminary processing of lambskin

After pelt removal, the lambskin undergoes preliminary processing. Cleansing using water aids in the removal of manure, other waste, blood, and lymph proteins that are water-soluble, and salts applied for curing. Additionally, this process aids in water absorption and returns the fibres to their original size and shape. A serrated knife may be used for scraping the flesh that has become affixed to the inside surface of the skin. This is carried out to improve the permeation of chemicals during subsequent processing. Pelts may be submerged in vessels containing a saturated solution of lime. This aids in detaching keratinized tissues, and a knife with a serrated edge is used to cut off the hair follicles. To get rid of lime and hair, the skins are cleansed. By immersing in mild acid, the remaining lime is eliminated. Proteolytic enzymes used in this process soften and make the hides pliable. To help prepare the skins for tanning, these are pickled in sulphuric acid and salt. The pickling process homogenizes the acidity of the skin and helps clean it. In recent times, lime-free processing methods have been developed involving detergents to remove lipids and dirt and enzymatic depilation to remove wool, then chilling or freezing for the preservation of the de-wooled clean skin (Mandal, 2014).

2.4. Sheep farming in New Zealand

Just behind Australia, New Zealand is the second-biggest lamb meat exporter in the world. It is also among the largest wool exporters. According to Hall (2023), each year, lamb meat and wool exports from New Zealand total around NZ$3.8 billion (US$2.6 billion) and NZ$540 million (US$370 million) respectively. Sheep farming has grown in New Zealand since the entry of the first European migrants, and thanks to the value of wool and meat exports, it was the economy's cornerstone up until about 1990. Due to the complimentary pastoral feeding activities of the two species, sheep and beef cattle farming in New Zealand have collaborated. Numerous farms combine cattle and sheep. Now, there are over 23,400 sheep and beef farms, accounting for 45% of all farms (Hall, 2023). Figure 2.1 represents the total number of sheep in New Zealand from 1960 to 2020.

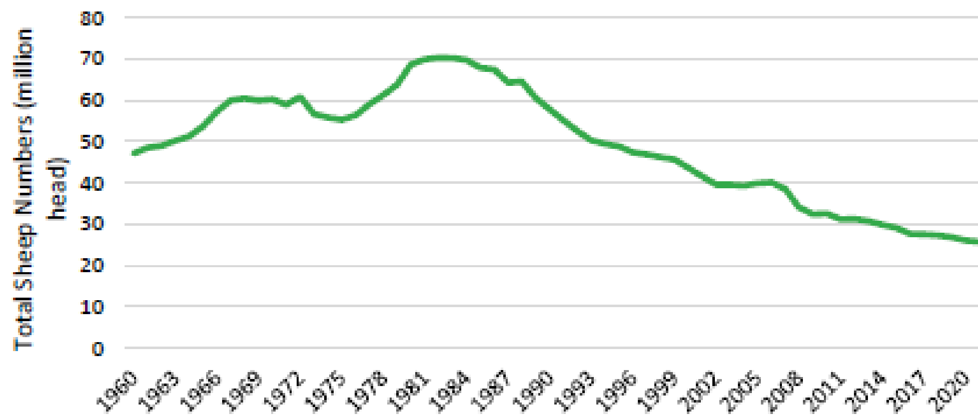

Figure 2. 1. Total number of sheep in New Zealand (Hall, 2023)

The peak number of sheep exceeded 70 million between 1980-1985. The total number of sheep has decreased over four decades from 70 million to 27 million.

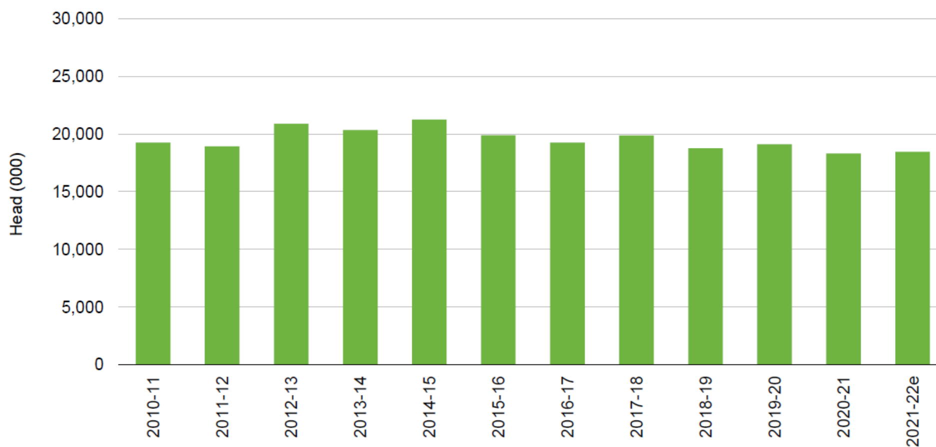

Figure 2. 2. Statistics of lamb slaughter in New Zealand (Service, 2022)

Figure 2.2 represents the statistics of lamb slaughter in New Zealand. The number of lambs slaughtered has remained consistent throughout the past decade, regardless of the steady decline in sheep numbers on farms. Figure 2.3 represents data on the export of lamb from New Zealand.

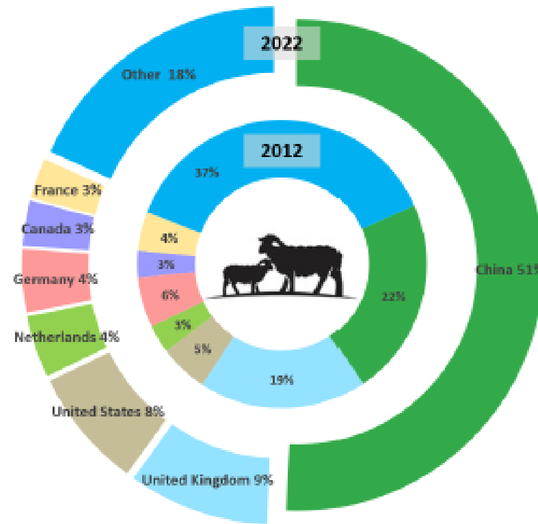

Figure 2. 3 Export of lamb from New Zealand (Hall, 2023)

The export destinations of lamb from New Zealand changed significantly between 2012 and 2022. Half of the total export of lamb now goes to China and a considerable proportion still to the UK and the US. Netherlands, Germany, Canada, and France are minor importers of lamb from New Zealand.

2.5. Collagen

Collagen is a term that comes from the Greek words "kola" meaning gum and "gen" meaning-making. Animal connective tissue and extracellular matrix both include the fibre-like structure-building protein collagen. In mammals, collagens typically account for 30% of the total protein (Alam et al., 2022; Silvipriya et al., 2015).

The major sources of collagen are the skin and bones from cows, pigs, freshwater and saltwater fish, as well as other marine organisms like starfish, jellyfish, sponges, sea urchins, squid, cuttlefish, sea anemones, and prawns. Additional non-commercial sources comprise chicken, kangaroo tails, rat tails, duck feet, horse tendons, alligator bone and skin, sheep skin (ovine source), frog skin, and occasionally even human tissue (Silvipriya et al., 2015).

The food and cosmetic industries, as well as the disciplines of biotechnology and pharmaceuticals, all use collagen as a key biomaterial because of its good biological compatibility, mild antigenicity, and limited biodegradability (Lewandowska et al., 2021). When collagen is held at a boiling

temperature in water, gelatin, which is highly water-soluble and produces viscous liquor, can be made. This compromises the stability of collagen's triple helix shape. To help solubilise a portion of collagen with native structure intact, trypsin or proteolytic enzymes are recommended with partial digestion at a lower temperature (Alam et al., 2022).

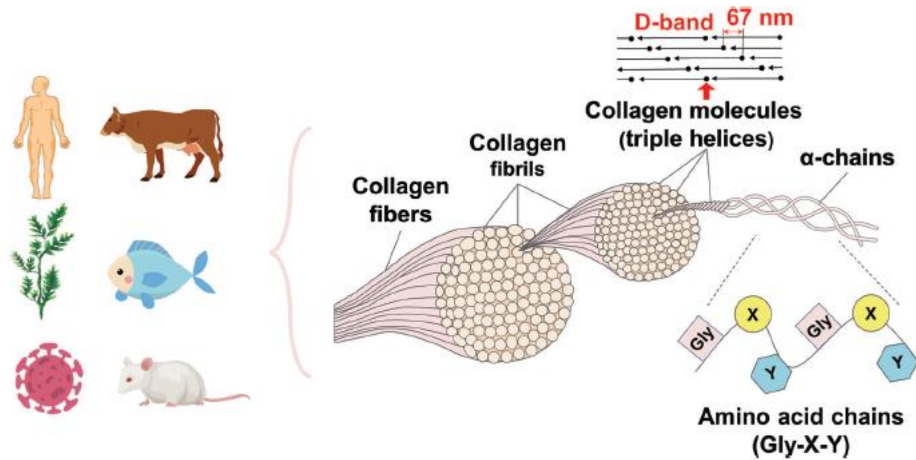

Figure 2. 4 Collagen structure and sources (Zheng et al., 2023)

2.5.1. Structure and functions

Collagen's fundamental structural component is made up of three polypeptide chains grouped in the shape of a triple helix, two of which are similar and the third of which has a slightly different chemical composition. Each chain is typically made up of 1050 amino acids that are coiled into a 300 nm long right-handed spiral. Its diameter is about 1.4 nm. Gly-x-y is the recurring motif in its structure, with proline and hydroxyproline serving as the most common examples of x and y (Alam et al., 2022; Silvipriya et al., 2015). The presence of glycine, which occurs in every third residue, the abundance of hydroxyproline and proline, allowing hydrogen bonds between chains, and electrostatic bonds between lysine and aspartate, all contribute to the stability of the triple helix (Fallas et al., 2009; Persikov et al., 2005). In older skins, other forms of crosslink contribute strength as well (Ricard-Blum, 2011). The structure of collagen is presented in Figure 2.4.

Collagens are the primary structural component of every animal connective tissue and also exist in almost all parenchymal organs and tissues, where they support physical stability and preserve the integrity of their structures (Gelse et al., 2003). Collagen provides structure to the cells and

extracellular matrix, which supports the majority of tissues. Its high tensile strength is demonstrated by the fact that it is found in tendons, bones, cartilage, fascia, etc. It gives the skin flexibility and firmness and aids in tissue and the formation of an organ. Skin is protected by collagen preventing microorganisms and poisons from being absorbed. Collagen facilitates cells' biological processes, aids in bone or blood injury recovery as well as protects the structural integrity of blood vessels (Ricard-Blum, 2011; Silvipriya et al., 2015).

2.5.2. Different types of collagens

Collagen II was discovered by Miller and Matukas (1969) in vertebrates. The collagen superfamily now consists of 29 members, numbered I through XXVIII (Ricard-Blum, 2011). The most prevalent and thoroughly researched collagen is type I collagen. More than 90% of bone's organic content is made up of it (Alam et al., 2022; Gelse et al., 2003; Shoulders & Raines, 2009).

The 5 most common types are collagen I, II, III, IV, and V. Collagen I is found in skin, tendons, bone, ligaments, teeth, organs, etc. Collagen II, III, IV, and V are found in cartilage and eyes, skin and muscles, basement membrane, hair, etc. respectively (Silvipriya et al., 2015).

2.6. Gelatin

Gelatin, a biopolymer, is a water-soluble high molecular weight protein material. Gelatin is denatured collagen. The commercial preparation of gelatin, an important hydrocolloid, involves the degradation of the collagens' tertiary, secondary, and, to a certain extent, primary structures (Alipal et al., 2021; Mariod & Fadul, 2013; Zilhadia et al., 2018)

Gelatin is composed of 18 different amino acids, with glycine, proline, and hydroxyproline making up 57%. Other amino acids especially glutamic acid, alanine, arginine, and aspartic acid make up the balance. Gelatin has an elemental makeup of 25.2% oxygen, 6.8% hydrogen, 50.5% carbon, and 17% nitrogen. It is a combination of single and double unfolded chains with a hydrophilic nature (Alipal et al., 2021). Upon removal of water from gelatin, the protein chains will reassociate randomly, reforming bonds. This behaviour gave gelatin its long history as a glue.

2.6.1. Sources and applications of gelatin

The cartilage, bones, tendons, and skin of mammals like pigs and cows are used to make about 95% of the consumable gelatin. For Muslims and Jews, gelatin made from pork is unacceptable, and for Hindus, gelatin made from cows is unacceptable. Due to epidemics in pigs, public interest in the health hazards of porcine gelatin has increased. Fish and its byproducts are an additional significant source of gelatin. In contrast to mammalian gelatin, fish gelatin has less desirable physicochemical and functional characteristics (Sha et al., 2019; Zilhadia et al., 2018).

Gelatin is widely employed in the culinary, cosmetic, and medical sectors due to its special qualities. It has long been a component of jellies, sweets, milk products like yoghurt, ice cream, desserts, etc. (Zilhadia et al., 2018). Gelatin is commonly used in the food industry as a gelling agent, whipping agent, binding agent, clarifying agent, thickener, stabiliser and emulsifier. It is a common agent for making capsules for drug delivery and casings for sausages (Mariod & Fadul, 2013).

2.6.2. Manufacture of gelatin

Gelatin is made from animal sources via heat denaturation of collagen commonly by either acidic (type A gelatin) or alkaline hydrolysis (type B gelatin) (Zilhadia et al., 2018). Collagen being insoluble, requires an acid or alkaline pretreatment to become soluble, making the native collagen swell and lose its ordered structure. During extraction using heat, the hydrogen and covalent bonds that help to stabilize the collagen helix are broken, allowing the collagen fibres and fibrils to dissociate into tropocollagen units. Breaking the intramolecular connections linking each of the three chains of the helix is the final step in the hydrolysis of collagen (Alipal et al., 2021; Mariod & Fadul, 2013; Samatra et al., 2022)

The isoelectric points for types A and B are, respectively, pH 6 to 9 and 4.8 to 5.5. Unlike native type A gelatin nanoparticles, native type B has a higher degree of crosslinking, indicating a gradual process of deterioration (Alipal et al., 2021; Samatra et al., 2022). The animal's age, the raw materials employed, and the particulars of the extraction such as the pretreatment, pH, temperature, and duration, may all affect the length of the polypeptide chains extracted, and the

gelatin's functional qualities, which determine the yield and quality of the gelatin (Al-Hassan, 2020).

Typical processes in extracting gelatin are degreasing, pre-treatment, extraction, evaporation, drying, and grinding (Alipal et al., 2021).

2.6.2.1. Degreasing

Degreasing is the procedure of lowering fat by roughly 2% by immersing it in hot water after cleaning it, followed by roasting for no less than 30 minutes at 100°C. Degreasing can also be performed using food-grade detergents such as sodium dodecyl sulfate (SDS) (Alipal et al., 2021).

2.6.2.2. Pretreatment

There is very little published about the pretreatment of ovine skin and more on bovine skin and hide. Cut pieces of lambskin immersed in 0.1 mol L^{-1} NaOH solution at a 1:10 mass ratio for 24 h remove all proteins other than collagen (Wang et al., 2024). Animal hides are commonly pretreated with an acid, such as HCl, at various concentrations for considerable time, such as 4% for 5 days. Buffalo hide can be used to extract gelatin using different NaOH and $Ca(OH)_2$ concentrations (0.3-0.7 mol L^{-1}), for 6-24 hours. For the biocatalysis method, proteolytic enzymes were used instead of acid and lime to hydrolyse native collagen from the skin of animals. Pepsin is now typically the enzyme that makers of gelatin choose to utilize for this purpose. Actinidin, zingibain, papain, and other reasonably priced enzymes have also been utilized (Samatra et al., 2022).

2.6.2.3. Extraction

In camel skin, a laboratory extraction procedure was carried out by placing the skins in 1.3 M calcium hydroxide for 48 hours at room temperature (25°C), then washing them in water, and neutralizing them with 4% ammonium sulfate (Al-Hassan, 2020). Goatskins that had been acid-treated were washed in distilled water until the liquid was clear. The final extraction was performed in distilled water for nine hours at 60°C. The resultant clear extract was filtered using Whatman No. 1 filter paper (Zilhadia et al., 2018). In another study on camel skin, extraction was carried out with distilled water (1:3, w/v) at 75°C for 3 h, and then at 90°C for 2 h. The extracted gelatin was

centrifuged at 15,000 rpm and 30°C for 5 minutes after being filtered through cheesecloth (Al-Hassan, 2020).

2.6.2.4. Evaporation

Evaporation helps concentrate gelatin solutions to make them suitable for industrial applications. Controlled evaporation is done under a vacuum or at atmospheric pressure. The extracted liquid passes through filters to remove any remaining fragments of bone, tissue, or skin (Alipal et al., 2021). The filtrate obtained from goatskins was dried in a 60°C oven for two hours. Once evaporated liquid containing gelatin had been produced, the filtrate was chilled in a refrigerator at 5°C to allow it to solidify (Zilhadia et al., 2018).

2.6.2.5. Grinding

After being pressed into sheets, the solid gelatin is ground into a fine powder (Alipal et al., 2021).

2.6.3. Thermal extraction of gelatin

Each of the hide sections curls when heated and then exudes gelatin as the temperature rises. Depending on their initial shape and size, the fragments keep contracting and curling into ever-tinier spirals or balls until the mass becomes a highly mobile gelatin solution with the remaining solid particles as a dispersed phase. According to the theories of colloidal behaviour, gelatin particles have an elastic fibrillar structure when they are in the gel state. The individual fibres swell due to immersion until they come together to create a flexible gel. The fibres contract in response to heat, expelling the absorbed water that causes swelling. The threads become more coiled and separate as the temperature rises, eventually contracting into spirals that resemble spherulites but are not quite as tight. Gelatin-water mixes have a reversible phase wherein the fibrils regain their original form upon cooling, provided that the temperature has not been maintained excessively high or is prolonged (Briefer & Cohe, 1927).

Heating collagen can have either irreversible or "reversible" outcomes. Proteins that are moderately heated may undergo a localized unfolding, which then seem to return to their original structure when normal temperatures are reached. A few consecutive hydrogen bonds

getting broken may be the cause of this unfolding. The original triple helical structure is irreversibly transformed into a more erratic (coiled) structure over time by extreme heating. Breaking extended sequences of hydrogen bonds that stabilize the triple helix is assumed to be the main cause of the later transition (Wright & Humphrey, 2002).

Analysis of the gelatin's protein pattern revealed that an increase in extraction temperature led to hydrolysis within the backbone of gelatin, namely the α-, β-, and γ-chains, and a rise in the amount of low molecular weight peptide. As the temperature of extraction rose, gelatin's surface hydrophobicity and surface activity both increased (Tan et al., 2020).

2.6.4. Extraction kinetics for thermal hydrolysis

Hydrolysis of collagen is assumed to follow the first-order kinetics as the reaction rate is directly proportional to the concentration of collagen. The rate equation for a first-order reaction can be written as follows (Earle, 2003):

$$-\frac{d(R)}{dt} = k(R)$$

The integrated rate equation is:

$$\ln(R) = \ln(R_{\circ}) - kt$$

Where (R) is the reactant concentration, ($R_{\circ}$) is the initial reactant concentration, k is the rate constant, and t is the time.

Arrhenius's equation indicating the relation between the rate constant and temperature of the reaction is represented as:

$$k = Ae^{-Ea/RT}$$

The integrated form is:

$$\ln(k) = \ln(A) - \frac{Ea}{RT}$$

Where k is the rate constant, A is the preexponential factor, E_a is the activation energy, R is the universal gas constant (8.314 J/mol.K) and T is the temperature (K)

2.6.5. Gel formation mechanism

Collagen depends on imino acids (proline and hydroxyproline) for its heat stability. Helix stability is increased with more imino acids. Above 40°C, collagen starts to denature and split into single, double, and triple strands. A 4 KJ/mol energy barrier for helix reformation results from controlled cooling below the melting temperature (T_m) of collagen. According to Wong (2018), Gly-I-I regions are involved in the quick initial refolding that results in a left-handed helix that is supported by water bridging. It is through "nucleation" that loops are formed, and triple strands are aligned. Triple helical collagen molecules are created via interchain alignment at high concentrations. Renaturation begins, with cooling affecting the rate. Rapid chilling causes collagen to be poorly renatured. Gelatin is created when disorganized gelatin molecules rearrange into collagen-like frameworks, although processing parameters can change the structure and makeup of the gelatin. In the process of forming junction sites for the gel network, the imino-rich sections nucleate. Through intra- and interchain interactions, additional cooling induces ordering. The collagen structure is strengthened in specific locations by recoiling and refolding, resulting in a firm gel with imino-rich joints (Dhakal et al., 2017). The diagrammatic representation of gel formation from collagen is given in Figure 2.5.

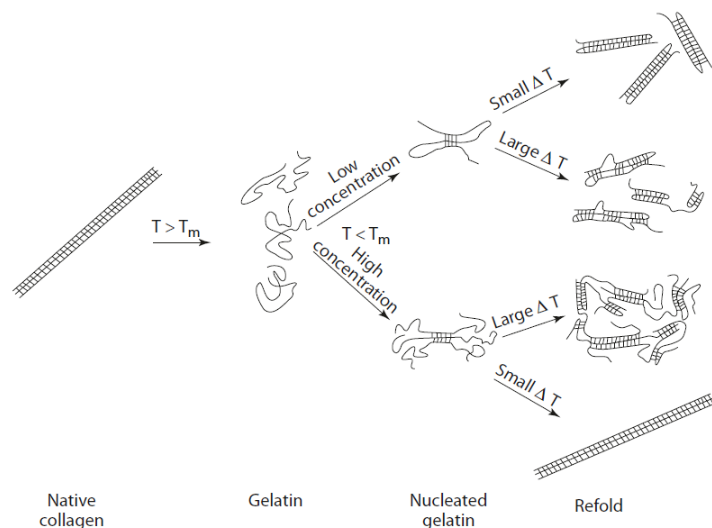

Figure 2. 5 Gel formation from collagen (Wong, 2018)

2.7. Effects of pressure cooking

2.7.1. Changes in the boiling point of a liquid

The temperature at which a liquid's vapour pressure matches the ambient pressure is known as the substance's boiling point. The boiling point varies with the pressure of the surrounding atmosphere. A liquid exhibits a higher boiling point at elevated pressure than it does at atmospheric pressure, whereas a liquid in a vacuum has a boiling point that is lower than at atmospheric pressure. This principle is used in the working of pressure cookers, which allows cooking at elevated temperatures in a short time (Speight, 2020).

2.7.2. Kinetics of reaction with temperature

Reactions are faster at higher temperatures, but the rate sensitivity to temperature varies between reactions. For some desirable reactions, temperature sensitivity exceeds competing undesirable reactions. Hence, high-temperature short-time treatments are good. For collagen solubility, the desired change is the deterioration of chains along with the breakage of crosslinks but not the hydrolysis of backbone peptide bonds (X. Zhang et al., 2020).

2.8. Rheometry

The internal resistance of a fluid due to friction or its propensity to oppose flow is known as viscosity, or more precisely dynamic viscosity. Viscosity is typically regarded as a crucial physical characteristic of the quality of liquid foodstuffs because it is so easy for humans to discern by observing or during eating or swallowing. Viscometric information is also crucial for the design and assessment of equipment used in the preparation of food, including pumps, pipes, heat exchangers, evaporators, etc. (Saravacos, 1970). Dynamic viscosity is shown by the equation:

$$\mu = \frac{shear\ stress}{shear\ strain}$$

Where shear stress is the stress element that is applied tangentially to the plane upon which the force operates and the shear strain is the resulting velocity gradient in a fluid viewed normal to that plane (Bourne & Rao, 2017).

2.8.1. Different types of viscometers

Different categories can be created according to the operating principle of each viscometer type. Capillary flow viscometers, orifice-type viscometers, falling ball viscometers, and rotating viscometers are the most frequently used viscosity measurement tools (Abbas et al., 2010). There are other viscometers such as tube viscometers, coaxial rotational viscometers, cone and plate and parallel plate viscometers, and paddle viscometers (Abbas et al., 2010; Bourne & Rao, 2017).

2.8.2. Coaxial rotating rheometers

In a coaxial rotating double gap rheometer, the sample is placed in a cup having the shape of an annular trough. A measuring spindle in the shape of a hollow cylinder is lowered into the trough such that a fluid-filled gap is formed between the cylindrical spindle and the cup. One or the other of the cylindrical spindle or cup is spun and the drag of the liquid is measured via the other (Abbas et al., 2010).

2.8.3. Influence of temperature on viscosity measurements of gelatin

Gelatin-water mixtures can continuously change from a gel form to a sol state and vice-versa. Essentially, gelatin is constantly setting or melting. In the setting phase formation outpaces the breakdown of solid aggregates generally at low temperatures. The melting phase occurs when breakdown surpasses the development at high temperatures. However, there has to be a certain narrow range of concentration and temperature where the real forces controlling viscosity are most apparent. Ideally, for gelatin solutions with low concentrations, the temperature where a solution melts or sets is approximately 37°C. This is the range where viscosity measurements are conducted. This is consistent with findings in other research that the equilibrium stage is between 35 and 38°C; for gelatin with pH = 4.7 (Briefer & Cohe, 1927).

2.8.4. Reduction of gelatin viscosity

It is known that elevated temperature over time can cause a gelatin-water mixture to permanently lose some or much of its viscosity. When the temperature and time are right, some of the spherulites will no longer be able to return to their original thread shape upon cooling since their elastic limit

has been reached due to either excessive local heating or innate weakness. The impacted spherulites will no longer fully contribute to the solution's viscosity as hydrolysis of the peptide backbone occurs. Consequently, a decrease in viscosity will be observed, the higher the temperature and the longer the duration, the greater the decrease. This is true for solutions at low concentrations (Briefer & Cohe, 1927).

2.9. Hydrolysis of peptide bond

The term "hydrolysis" describes the process by which a molecule reacts with water to break a chemical bond. A protein may undergo hydrolysis of one or more of its peptide bonds forming amino acids and peptides (Elnajjar et al., 2019). The term "hydrolysed collagen" refers to collagen that has undergone the process of hydrolysis. Collagen generally undergoes partial hydrolysis in becoming gelatin, which has an amino acid makeup that is identical to that of the original collagen (Elnajjar et al., 2019). When a peptide bond is broken by the inclusion of a molecule of water in a hydrolysis reaction; one amino acid receives a hydrogen atom, to release a free amine and its neighbour acquires a hydroxyl group to release a carboxylic acid. The carboxylic acid thus formed can be determined by titrating against a base. One important pathway for the covalent breakdown of proteins is the hydrophilic breakdown of peptide bonds on the polypeptide backbone. The catalytic hydrolysis of the peptide bonds can be done using acids such as sulphuric acid or bases such as sodium hydroxide. This process is faster and less specific than enzymatic hydrolysis and can affect the chemical groups of amino acids. Enzymatic hydrolysis of the peptide bonds uses proteases or peptidases for the cleavage of peptide bonds. This process happens under milder conditions without affecting the integrity of the amino acids released (Pan et al., 2011).

2.9.1. Degree of hydrolysis

The percentage of broken peptide bonds in a protein hydrolysis product is known as the degree of hydrolysis (DH). There are numerous ways to determine DH; the most popular ones are the pH-stat, trinitrobenzene sulfonic acid (TNBS), trichloroacetic acid soluble nitrogen (SN-TCA), o-phthaldialdehyde (OPA), and formol titration methods. The number of peptide bonds broken and, consequently, the mean size of all the peptides present are indicators of how much a protein source has undergone hydrolysis (Rutherfurd, 2010). The equation of degree of hydrolysis (Adler-Nissen, 1976) is represented as:

$$DH = \frac{no. of\ peptide\ bonds\ cleaved}{total\ no. of\ peptide\ bonds} * 100\%$$

2.9.2. Ninhydrin reaction

At specific reaction conditions, amino acids and ninhydrin react to produce a purple-blue chemical that is the ammonium salt of diketohydrindylidene-diketohydrindamine. When amino acids react with ninhydrin, they often produce carbon dioxide, and an aldehyde that has one fewer carbon atom than the starting amino acid, apart from the coloured complex. Additionally, ninhydrin interacts with primary amines and ammonia to produce coloured compounds (Toro & García-Carreño, 2002).

2.9.3. Mechanism of peptide bond hydrolysis

Three different molecular pathways can theoretically lead to purely chemical cleavage of the peptide backbone. The synthesis of 2,5-diketopiperazine (DKP) is the most well-studied and characterized process (pathway A). The second carbonyl carbon in the peptide backbone is attacked by the nucleophilic N-terminal amino group, forming DKP in the subsequent steps. In pathway B, an intermolecular pathway, a carbonyl oxygen can function as a nucleophile, attacking the next carbonyl atom to generate an oxazole moiety at the N-terminal peptide segment and an unbound peptide at the C-terminal peptide. At high temperatures via pathway C, hydrolysis occurs with either ambient water or with water from the food matrices and can break any one of the N-amide bonds in the peptide, yielding two oligopeptides. In biological systems, various proteases such as exopeptidase and endopeptidase can catalyze the hydrolysis of peptide bonds (Bikaki et al., 2021).

2.10. Wool

Sheep's skin contains follicles where wool fibres are produced. The process of keratinization causes the wool fibre to harden. During this process, one cystine molecule is formed from two cysteine molecules through a disulphide linkage. Three main variables influence wool's characteristics: diet, physiological condition, and genetic makeup. Wool's cystine content is very sensitive to dietary modifications. It is the high cysteine content in keratins that gives wool

its structural stability and structure that confers many of its useful physical properties (Rippon, 2013)

2.10.1. Composition of wool

Keratin is the major protein present in wool. Clean wool comprises 82-95 % keratinous proteins, with high cystine content. Because of their comparatively low cystine content, other proteins, referred to as "non-keratinous", make up about 17% of wool. About one per cent of the mass of wool fibre is a non-proteinaceous substance, mostly waxy lipids with a trace of carbohydrates. Twenty amino acids are found in pure wool fibres. However, when wool is completely hydrolysed with acid, 18 amino acids are observable (Rippon, 2013). About 25-70% of the total weight of raw dirty wool is comprised of impurities, which are grease, dirt, suint, and organic matter from farms (Stewart, 1985; Teasdale, 1988; Truter, 1956).

2.10.2. Structure of wool

Wool is formed from dead cells. The cells that make up the outermost layer (called the cuticle, approximately 10% of the hair shaft) bind the hair securely in the follicle. Pigmented and keratinized cells make up the intermediate layer (cortex, approximately 90%). The medulla, or hollow center, is absent from fine hair. A portion of the cells in the epidermis keratinize, die, and are forced outward to form the cortex and outer cuticle during the growth phase. The lipid-rich cell membrane structure holds the keratin fibres in place within the cells (McKittrick et al., 2012).

Wool has a substantial amount of polypeptide chain shaped like an α-helix. The sulphur in wool is primarily in the form of cystine, except for a trace amount of methionine. Cystine disulphide bonds create crosslinks, either between distinct protein chains or between segments of a single protein chain. The primary linkages that stabilize the fibre, especially when it's wet, are the interchain cystine linkages which are comparable to steps on a ladder (Rippon, 2013). The multi-scale structure of a wool fibre is represented in Figure 2.6.

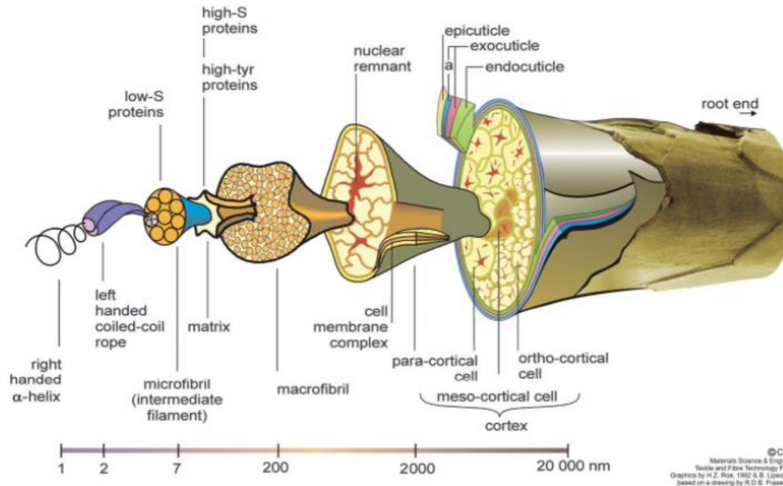

Figure 2. 6 Structure of wool fibre (Rippon, 2013)

2.10.3. Properties of wool

The fibres of wool possess an average density of 1.3 g/cm3 and an average diameter ranging from 15 to 50 µm. They are slightly elliptical. They grow at around 10 cm per year and disintegrate at about 130°C. Wool fibres can absorb 14–18% moisture at a relative humidity (RH) of 65% (McKittrick et al., 2012). Sulphur-containing cystine is a crucial component of wool fibres. Better handling qualities, increased resistance to chemical changes, and improved physical and mechanical characteristics are all associated with higher sulphur content in wool. Superior absorption of moisture, odour, and noise, flame resistance, warmth, biodegradable properties, recycling capacity, durability, softness, safety, and ease of handling are some of the qualities attributed to this intricate framework (Allafi et al., 2020).

2.11. Keratin

In vertebrates, keratin is a structural protein in the integument or outer coating. It is the second-most significant biopolymer found in animals. Different forms of keratinised materials exist, depending on their intended use. These vary from an impact-proof, structurally solid material (horn) to a basic water-resistant layer (turtle shell) (McKittrick et al., 2012).

2.11.1. Structure of keratin

Keratin protein has two forms: β-pleated sheets and α-helices. Two polypeptide chains (α-keratin) in keratin twist together to create a coiled form. The cysteine residues in keratin have a thiol group (-SH) each, which can contribute to a potent covalent disulphide bond that connects the polypeptide chains and the matrix molecules (McKittrick et al., 2012). Keratin has limited amounts of lysine, histidine, and methionine, and it contains very little tryptophan. Keratin is abundant in cystine, glycine, proline, and serine. Nonetheless, ionic, hydrophobic, and hydrogen bonding also have an impact on the stability of the keratin in wool (Shavandi et al., 2017).

Keratins have been categorized as "soft" or "hard" based on how they feel to the touch. A higher sulphur content (over 3%), compared to soft keratins (like those within the skin), is a distinguishing trait of hard keratins, such as wool, hair, hooves, horns, claws, beaks, and feathers. Most of the sulphur within keratins is found as cystine due to crosslinks formed by pairs of cysteine residues (Rippon, 2013).

2.11.2. Physical and chemical properties

Due to the intermolecular bonding of cystine, along with the intra- and intermolecular linkages of polar and nonpolar amino acids, keratin is extremely stable (Allafi et al., 2020). Most of the chemical and physical stressors in the environment do not affect wool proteins. These proteins resist conventional proteolytic enzymes like pepsin and trypsin, and they are insoluble in water, many weak acids, alkali solutions, and organic solvents (Shavandi et al., 2017).

2.11.3. Hydrolysis of keratin

Since so much of the structural stability of keratin-based biological molecules like wool, hair, and feathers is attributed to the disulphide crosslinks within the cysteine, this is the locus of primary attack for industrial hydrolysis techniques. Mechanisms include oxidation, reduction, acid attack, alkaline attack, or physical destruction with or without thermal assistance. Once the spiral structures are disrupted, proteases can gain access to peptide bonds. Few practical mechanisms leave both cysteine residues intact. Reported techniques are summarised in Table 2.2 (Shavandi et al., 2017).

Table 2. 2. Different methods of keratin extraction

Methods	Description	Drawbacks	References
Reduction	• Cysteine disulphide bonds are broken by using reducing chemicals such as thiols (mercaptoethanol, for example), which maintain keratin structure. • A less expensive substitute is sodium sulphide, which produces cysteine and cysteine-sulfonate. • There is only disulphide bond cleavage. • The process is based on nucleophilic displacement reactions, where air quickly oxidises thiol groups or cysteine residues.	• The drawbacks of mercaptoethanol include its potential for toxicity and high cost. • Requires the use of significant urea concentrations as a protein denaturant, which can alter the resulting keratin's physicochemical characteristics.	Rajabinejad et al. (2018); Shavandi et al. (2017)
Oxidation	• Usage of oxidising agents, the most common acids to create a sulfonic acid being formic or peracetic acids • Disulphide bonds are only broken. • Cysteine completely transforms into cysteic acid. • The consequence is water-soluble keratoses that cannot form cross-links. • Cu(II)–wool and other wool-metal complexes are involved in the mechanism. • Reactive species dramatically change the characteristics of keratin by breaking down complexes and changing disulphide bonds to sulfonic acid.	• Typically, the treatment takes a long period because a reasonable yield requires a reaction time of longer than 24 hours.	Giteru et al. (2023); Rajabinejad et al. (2018); Shavandi et al. (2017)
Alkali extraction	• Peptide length is tailored via controlled alkaline hydrolysis. • 75%–80% of the materials produced by wool's alkaline hydrolysis are soluble in water. • Wool fibres that swell at high pH levels carry a negative charge. • Alkali treatment improves enzyme accessibility and hydrolysis index	• Needs a substantial amount of acids for neutralization and alkaline substances for hydrolysis • During the hydrolysis process, the primary protein chain sustains damage	Giteru et al. (2023); Rajabinejad et al. (2018); Shavandi et al. (2017)

Steam explosion	• Conventional protease with high-density steam flash-explosion (HDSF) assistance may break down 95% of the feather substrate in two hours without the requirement for keratinases. • Instant catapult steam explosion (ICSE) is superior to general proteolysis in that it breaks down over 90% of chicken feather powder into soluble peptides by keratinolysis in 3 hours, with over 90% of the peptides being <3 kDa.	• Requires safe and complicated equipment that can reach high pressures of about 1-2 MPa	Guo et al. (2020); Qin et al. (2022); C. Zhang et al. (2020)
Sulfitolysis	• Only disulphide bonds are cleaved. • Sulfitolysis by sulphite produces thiol and s-sulfonate. • Urea disrupts hydrogen bonds, swelling keratin fibrils. • Sulfitolysis yields s-sulfonated keratin intermediates. • Urea and SDS promote cysteine formation, protecting against re-oxidation. • Sodium sulphite reacts, forming hydrosulphite, OH−, Dehydroalanine (DAL), perthiocysteine, cysteine, and sulphur. • Dehydroalanine is an extremely reactive compound that can generate lanthionine (LAN) and lysinoalanine (LAL) by crosslinking with cysteine and lysine. LAN and LAL have the potential to be poisonous and non-nutritive, and animals are unable to convert them back into cysteine or lysine.	• High urea concentration (7-8 mol L^{-1}) increases production costs. • Lowering urea concentration to 1 mol L^{-1} reduces keratin extraction. • Sulfitolysis converts cystine to cysteic acid and keratoses. • Keratoses are heat-stable, water-soluble, nonreactive products formed after disulphide bond cleavage by sulfitolysis.	Giteru et al. (2023); Rajabinejad et al. (2018); Tasaki and Kaneoka (2019)
Ionic liquids	• Employing environmentally friendly solvents • Because of their capacity to solvate keratin fibrils, been effectively used for extracting keratin protein with a high yield	• Process needs exact temperature control and a nitrogen atmosphere. • Incorporating raw material into hot liquid • The keratin that results is water-insoluble	Shavandi et al. (2017)

		• The toxicity of ionic liquids and their high recovery costs prevent this technology from being widely used	
Superheated water hydrolysis	• Green and solvent-free process • Superheated water can be produced in a microwave reactor	• Cystine destroyed	Rajabinejad et al. (2018); Zoccola et al. (2012)
Enzymatic hydrolysis	• Solvent-free, environmentally friendly technique • Keratinases are a unique class of proteolytic enzymes that hydrolyse wool. These enzymes are generated by naturally occurring microorganisms and degrade keratin. The stiff and highly crosslinked keratin polypeptide chains that exist in wool are hydrolytically cleaved by keratinases, which are serine endopeptidases	• Time-consuming • Cost-prohibitive	Giteru et al. (2023); Rajabinejad et al. (2018); Tasaki and Kaneoka (2019)
Microbial extractions	• Capable of extracting keratin from keratinous animal body parts (KABP)	• In four days, naturally occurring bacteria extract less than 10% of the keratin. • Other research shows about 70% extraction in the same amount of time • Prolonged incubation reduces the efficiency of keratin extraction.	Tasaki and Kaneoka (2019)
Microwave extraction	• In a microwave reactor, wool fibres were hydrolysed using superheated water. • Protein material can be extracted from keratin wastes by cleaving the cystine disulphide bonds without using dangerous, hazardous chemical agents. • Free amino acids, peptides, and low molecular weight proteins are present in the liquid fraction obtained, along with trace amounts of cystine and lanthionine.	• Large-scale use optimisation is challenging	Zoccola et al. (2012)

2.12. Flavour formation in meat

The taste and aroma of any food, including meat, combine to create its unique flavour. Uncooked meat merely tastes like blood and has little to no fragrance. Fresh raw meat has a dull, metallic, and mildly salty flavour (Bailey, 1994). Cooking causes a lot of intricate thermally induced interactions between the non-volatile elements of lean and fatty tissues, producing volatile chemicals, and contributing to the meat flavour. The characteristic cooked meat flavour is caused by the Maillard reaction. Compounds produced by lipid breakdown give cooked meat its fatty smell. Significant meat flavour precursors are amino acids, peptides, and carbohydrates, particularly ribose. Additional water-soluble substances involved in the formation of meat flavour include thiamine, nucleotides, sugar phosphates, organic acids, and other amino compounds like creatine, carnosine, and creatinine (Elmore & Mottram, 2009; Mottram, 1998). Heterocyclic compounds containing sulphur, nitrogen, and oxygen such as furans, pyrazines, pyrroles, thiophenes, thiazoles, imidazoles, pyridines, oxazoles, and cyclic ethylene sulphides are among the chemicals that give meat its final unique flavour (Bailey, 1994; Sohail et al., 2022). The lean tissues carried the building blocks for the meaty flavour that characterizes all cooked meats, whereas the fatty tissues gave species-specific flavour upon heating (Mottram, 1998).

2.12.1. Maillard reaction

Typically, there are three steps in the Maillard reaction. First, an amino group and reducing sugar condense to form an N-glycosylamine when an aldose sugar is involved, which subsequently reorganizes into the Amadori product (or Heyns product when a ketose sugar is involved). The second step involves the release of the amino group and the formation of sugar fragmentation compounds from the Amadori/Heyns product. In the last step, various types of dehydration, fragmentation, cyclization, and polymerization processes happen involving the amino groups formed in the second step (van Boekel, 2006).

The process known as "Strecker degradation," in which amino acids are broken down by dicarbonyls produced in the Maillard reaction, is crucial for the development of flavour. This process results in the deamination and decarboxylation of the amino acid. The reactant type has a significant impact on the different reaction pathways that can occur, and hence on the final flavour

formed. The reaction kinetics are determined by factors such as temperature, pH, amount of moisture, and reaction time (Cui et al., 2021; van Boekel, 2006). A diagrammatic representation of Maillard reaction products is presented in Figure 2.7

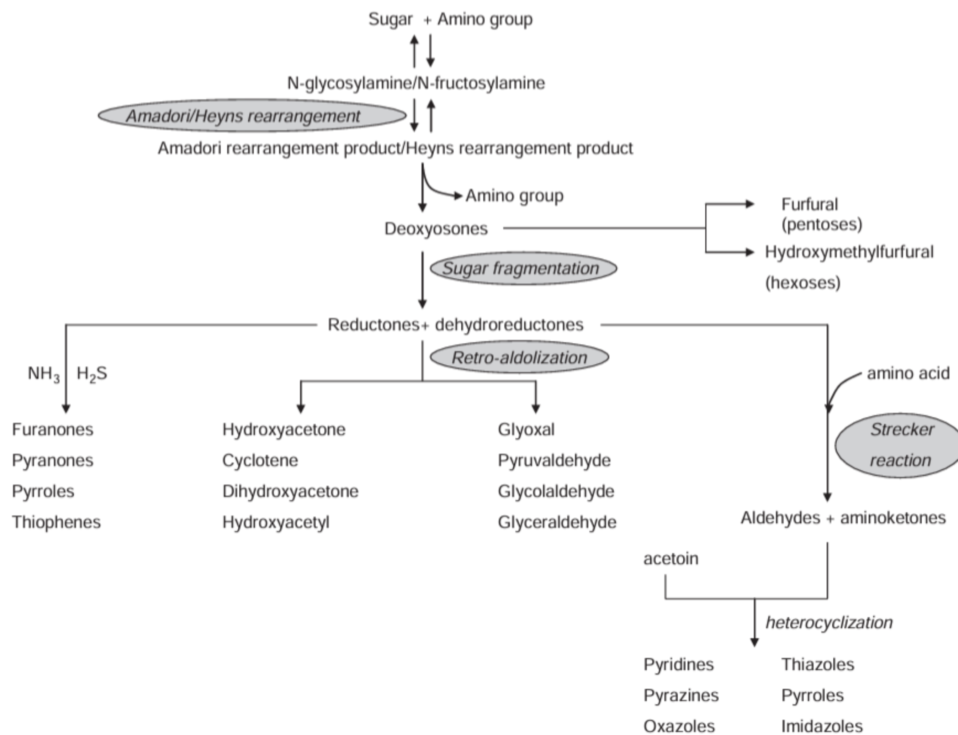

Figure 2. 7 Maillard reaction products (van Boekel, 2006)

2.13. Meaty flavour

2.13.1. Reactions involving meat-like flavour

The distinct flavours present in cooked meat are created by a variety of chemical processes that fall into various categories: Maillard reaction; lipid oxidation; thermal degradation of amino acids and peptides; sugar, and thiamine degradation; and reactions among the various products of both the Maillard reaction and lipid oxidation. Of these, amino acid degradation, and sugar caramelisation happen at temperatures above 125°C to form flavour compounds such as aldehyde, amines, aromatic compounds, and other caramelisation products. Meat contains thiamine, which is primarily composed of nitrogen and sulphur, and it readily breaks down in neutral and alkaline environments. Lipids, especially phospholipids oxidise during heating and ultimately transform

into volatile carbonyl molecules including furans, alcohols, ketones, aldehydes etc. (Sun et al., 2022). Usually, more than fifty per cent of the volatile chemicals in cooked meat are caused by lipid oxidation and breakdown. The aroma of meat that is specific to a species is typically thought to be influenced by these volatile chemicals. Polyunsaturated fatty acids (PUFA), one of which is found in some phospholipids, are easily oxidised (Sohail et al., 2022).

Heating has been shown to decrease the amount of amino acids and carbohydrates, with cysteine and ribose exhibiting the greatest losses. Studies on the aromas released when combinations of sugars and amino acids are heated have shown how crucial a part cysteine and ribose play in the development of cooked meat flavour (Morton et al., 1960). The predominant presence of compounds containing sulphur is a noteworthy characteristic of the volatiles obtained from cooked meat.

Most are found in tiny concentrations, but because of their extremely low odour thresholds, they are powerful aroma compounds that play a significant role in the cooked meat's flavours. Hence sulphur has been employed in meat flavouring reaction mixtures, typically in the form of hydrogen sulphide, cysteine, or other amino acids that contain sulphur (Mottram, 1998).

2.13.2. Effect of enzyme hydrolysis on flavour formation

The Maillard reaction can involve peptides with a molecular weight of 1000–5000 Da and enhance the food flavour. The most common technique for creating Maillard-reacted peptides (MRPs) is enzyme hydrolysis. Protein hydrolysates prepared via enzymatic hydrolysis can then be subjected to the Maillard reaction to produce MRPs. It has been demonstrated that a range of food-grade proteases can be used to enzymatically hydrolyse animal protein to produce MRPs. According to certain research, flavour-enhancing MRPs can be extracted from a variety of protein hydrolysates (Sun et al., 2022).

2.13.3. Sheepy flavour

The comprehensive flavour characteristics of lamb after thermal processing are largely influenced by aldehydes, carboxylic acids, pyrazines, alcohols, and lactones, which are the products of fat oxidation (Sun et al., 2022; Young et al., 1994). Due to the presence of several methyl-branched saturated fatty acids (such as 4-methyloctanoic acid and 4-methylnonanoic acids) in sheep meat

that are not found in any other meats, cooked sheep meats are quite recognizable and are unappealing to consumers in some countries. These compounds originate from the fats in sheep as a result of specific metabolic activities taking place in the rumen (Mottram, 1998).

2.14. Rate-all-that-apply (RATA) sensory test

Sensory science offers unbiased data on how consumers perceive a product, whether they accept or reject stimuli, and what feelings are elicited. Using the rate-all-that-apply (RATA) sensory test, customers score the intensity of every attribute they identify in the evaluated product (Marques et al., 2022). This method can distinguish between samples that are comparable to each other concerning sensory attributes and can also distinguish between samples based on how strong a sensory response is. These techniques are frequently quick, simple for customers to understand, adaptable, and sometimes cost-effective (Ruiz-Capillas et al., 2021).

2.15. Opportunities for raw materials:

By 2050, it's anticipated that meat consumption will reach 455 MMT globally (Alexandratos & Bruinsma, 2012). As a result, an increasing number of by-products such as wool and animal skin would also be produced. All of these low-valued wastes are candidates for conversion into value-added products for environmental and economic reasons.

2.15.1. Opportunity for lambskin

Numerous byproducts of animal slaughtering can be turned into high-end goods. Skins have always been regarded as a byproduct of sheep all around the world, except for breeds like Gotland and Karakul which are raised primarily for their pelts. The majority of the livestock in New Zealand's national flock are nowadays maintained and bred primarily for meat production supported by wool. Leather is the most common product made from animal skin. Animal hides or skins are transformed into leather by the chemical process of "tanning" (Mandal, 2014). Skins are turned into a variety of leather items after slaughter and, in the case of ovine leather have a low market value (McRae et al., 2022). Hence, there is a need for value addition to these by-products.

One way of adding value to animal skin is to convert it to a higher-valued food product, gelatin. Food-grade gelatin can be prepared from the skin of pigs and sheep. Foods can be made more

elastic, consistent, and stable by adding gelatin made from animal skins. It may also be used as sausage casings (Shen et al., 2019).

2.15.2. Opportunity for wool

Globally, over 2.5 million tonnes of wool are produced each year, with the leading five producers being Australia, China, New Zealand, Iran, and Argentina. Wool is a valuable commodity for the textile business, however, some low-quality trimmings from slaughter facilities cannot be utilized in the wool sector and end up as waste (Shavandi et al., 2017).

The majority of low-value animal source materials are at present disposed of at the renderer's expense. The rendering business could profit from new commercial uses of keratin made from animal waste in expanding markets, and it would also gain from reduced environmental impact from less landfill waste. In some countries, a considerable amount of feather is rendered thermally to feather meal used as stock food, but most of the cysteine is lost in the process. Keratin, a highly valued product, is extracted from wool and has various applications. Keratin hydrolysates (KHs) have been more and more popular in hair, skin, and nail care products lately. KHs are recognized for their ability to restore, rejuvenate, and prevent ageing in skin and hair (Tasaki & Kaneoka, 2019).

2.16. Application of collagen stock

2.16.1. As a congee base

Rice porridge known as congee is made by slowly cooking white rice in a large amount of water till the rice is broken down and the resulting liquid becomes thick and creamy (Erlewein, 2010). It typically employs millet and rice as its base ingredients, with the inclusion of corn grits and certain flours in some places (Yang et al., 2023). Congee stands out for its ease of digestion, making it the ideal nutritious and therapeutic diet for the ill, aged, disabled, and infants. It is a popular breakfast food in many places (Erlewein, 2010).

Most frequently, polished (white) rice is used to make congee. This dish can be enhanced with products or herbs that have the appropriate flavours or qualities to make it more palatable and to produce therapeutic effects. These can be introduced at the final stage of cooking or right before

serving, substituted for either a portion or all the liquid, or cooked into the congee from the very start. Meat products and broths, dried herbs, vegetables, seafood, eggs, crisp toppings like roasted seeds or nuts, stir-fried vegetables, or cooked meats, fish, or seafood, and sweet toppings like nuts, seeds, raisins, or dried fruit can be added (Erlewein, 2010).

2.16.2. Application to instant noodles

Instant noodles are considered a convenience food worldwide. Meat broth or stock is widely used in instant noodles, especially in Asian countries. The addition of meat stock to instant noodles helps enhance the flavour of the noodles by adding a savoury or umami note to them. It also adds certain nutrients such as minerals and protein from meat into the otherwise nutrient-deficient instant noodles. Consumer preference for a richer serving of instant noodles can be fulfilled by the addition of meat broth. Among all meat broths or stocks, chicken stock is the most preferred noodle soup stock (Choi, 2016).

2.17. Research gap

From the literature, it is understood that the proposition to create a cooked meat flavour through the Maillard reaction using wool hydrolysate, collagen hydrolysate and ribose is unexplored. The task is to produce collagen hydrolysates and wool hydrolysates through simple, industrially viable techniques. Hydrolysing the lambskin by using only thermal treatment as a sustainable method is promising but not researched enough. Since wool hydrolysis produces lanthionine and cysteic acid apart from cysteine-bearing peptides, the effect of these agents on cooked meat flavour formation needs to be studied.

Chapter 3: Materials and Methods

3.1. Materials

The raw materials and equipment used in the current study are summarised in Table 3.1.

Table 3. 1 Raw materials and equipment used in the current study

Sl. No.	Raw materials/equipment	Manufacturer
1	Lambskin (less than one year old)	Silverfern Farms, Hawera, New Zealand
2	Dynasty HL-G12 mincer	Hsiao Lin Machine Co., Ltd., Taichung, Taiwan
3	All-American 921 pressure cooker	Wisconsin Aluminum Foundry Co., Manitowoc, Wisconsin, United States
4	RSD500 handheld refractometer	Hangzhou West Tune Trading Co., Ltd., Hangzhou, Zhejiang Province, China
5	Orion Dual Star pH meter	ThermoFisher Scientific, Waltham, Massachusetts, United States
6	i-buttons	iButtonLink Technology, Wisconsin, United States
7	Multifuge X Pro Series centrifuge	Thermo Scientific, Thermo Electron LED GmbH, Osterode am Harz, Germany
8	Sulphuric acid (CAS No. 7664-93-9)	Thermo Scientific Chemicals, Waltham, Massachusetts, United States
9	Anton Paar Physica MCR 301 rheometer	Anto Paar GmbH, Graz, Austria
10	L-Leucine (CAS No. 61-90-5)	Sigma-Aldrich, Buchs, Switzerland
11	L-Proline (CAS No. 147-85-3)	Sigma-Aldrich, Buchs, Switzerland
12	Ninhydrin reagent (CAS No. 485-47-2)	Sigma-Aldrich, Buchs, Switzerland
13	Minolta Chroma Meter CR-400	Konica Minolta, Tokyo, Japan
14	Wool	WRONZ, Lincoln, New Zealand
15	Sodium metabisulphite (CAS No. 7681-57-4)	Sigma-Aldrich, Buchs, Switzerland
16	SDS (CAS No. 151-21-3)	Sigma-Aldrich, Buchs, Switzerland
17	Neutrase® 0.8L (CAS No. 9080-56-2)	Novozymes North America, Inc., Franklinton, North Carolina, United States
18	Zinc sulfate heptahydrate (CAS No. 7446-20-0)	Ajax Finechem, Sydney, Australia
19	NaOH (CAS No. 1310-73-2)	Thermo Scientific Chemicals, Waltham, Massachusetts, United States
20	Alcalase® 2.4L FG (CAS No. 9014-01-1)	Novozymes North America, Inc., Franklinton, North Carolina, United States
21	Protamex® (CAS No. 9014-01-1)	Novozymes North America, Inc., Franklinton, North Carolina, United States
22	L-Cysteine (CAS No. 52-90-4)	United States Biochemical Group, Cleveland, Ohio, US
23	D-Ribose (CAS No. 50-69-1)	Tasman Health, Te Atatu Peninsula, Auckland

24	L-cysteic acid monohydrate (CAS No. 23537-25-9)	Sigma-Aldrich, Buchs, Switzerland
25	Dihan Scientific oil bath	SciLab Korea Co. Ltd, Daejeon, Korea

3.2. Thermal hydrolysis of lambskin

3.2.1. Preparation of minced lambskin

Randomly cut pieces of lambskins at a pH of 8.33±0.02 were obtained from an external provider. The lambskin had been enzyme depilated, washed in water, then treated with 3% hydrogen peroxide solution and frozen at -20°C until use. For each experimental trial, the required amount of lambskin was thawed overnight in the chiller at 4°C. The thawed lambskin was washed with RO water and the remaining free water was squeezed out by hand using a cheesecloth. Later, the skin was fine minced using a mincer with a 2 mm round-hole spaghetti plate. The raw minced lambskin is represented in Figure 3.1.

Figure 3. 1. Raw minced lambskin

3.2.2. Proximate analysis of the lambskin

Proximate analysis of the raw lambskin was done at the Nutrition Laboratory at Massey University, Palmerston North, New Zealand. The samples were milled fine before analysis for moisture, ash, fat, protein, and crude fibre. The amount of carbohydrates was obtained by difference from the

total percentage. Ash measurement was done following AOAC 920.153 and AOAC 923.03 using a muffle furnace at 550°C. The sample's moisture content was measured using the AOAC method for meat, 950.46B. Dumas method was done following the AOAC 968.06 protocol using an N-P factor of 6.25 for protein content measurement. AOAC method 991.36 using Soxtec was used for fat analysis. The crude fibre was measured using a modified method using the AOAC protocols 962.09/978.10. All the AOAC methods are adapted from G. W. Latimer, Jr. (2023).

3.2.3. Kinetics of thermal extraction of lambskin

The thermal extraction of minced lambskin was performed according to the method described by Wang et al. (2013) with slight modifications. The extraction was solely based on heat, no chemical additives were added to the skin samples. The test samples were prepared by mixing thawed skin (57 %) and water (43 %) followed by adjusting pH to 4.5 using 0.52 M H_2SO_4. The pH value for extraction was fixed at 4.5 after balancing the degree of solubilisation (DS) at various pH values against the titration curve indicating the demand for sulphuric acid reagent. The samples were kept in a 250 mL glass beaker, covered with aluminum foil to reduce evaporation. Thermal extractions were done at 90°C (atmospheric pressure), 108°C (5 psi or 0.34 bar.g), and 121°C (15 psi or 1.0 bar.g) for 2,4,6,8 and 10 hours. The extractions at 90°C were conducted using a water bath, whereas the extractions at 108°C and 121°C were done in a pressure cooker. To record temperatures, the i-buttons, sealed in a retort pouch were kept in the beaker along with the samples to ensure the temperature remained consistent throughout the extraction process. All air in the pressure cooker was purged before pressure was allowed to build and the temperature-pressure correlation was checked to ensure a 100% steam atmosphere.

The temperature profile of the samples was plotted at 121°C to find out how stable the temperature is throughout the extraction process and has been represented in Figure 3.2. The i-button readings show that the temperature was kept almost constant throughout the trials. The expected temperature was 121ºC at 15 psi (1.0 bar.g) but the actual temperature was found to be slightly less than 120ºC.

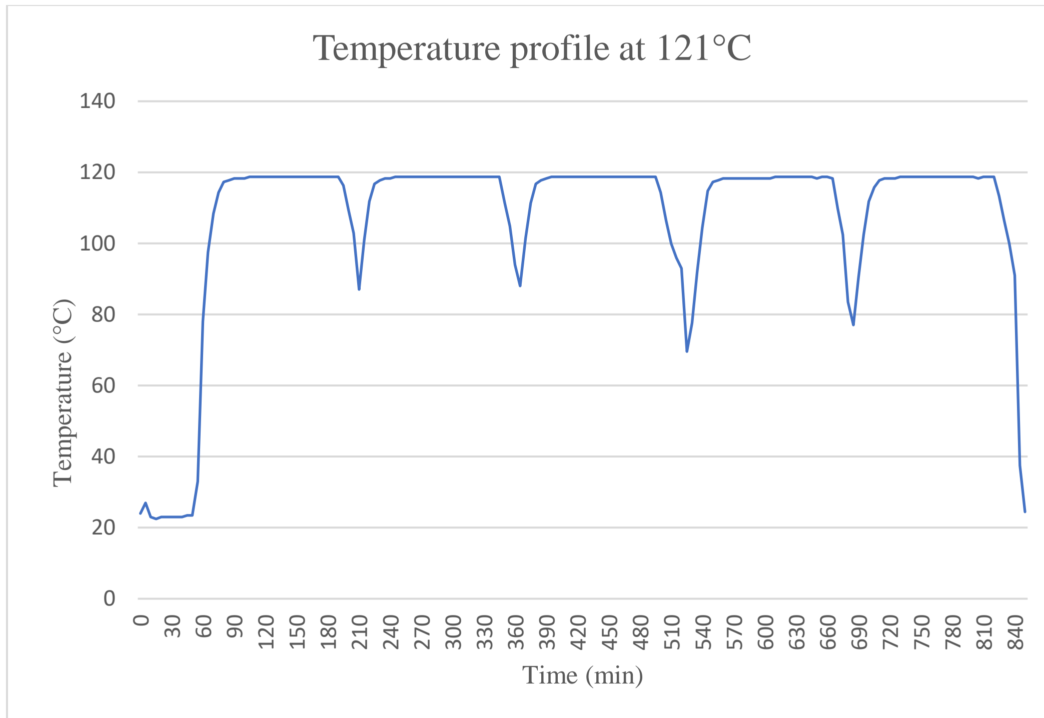

Figure 3. 2 Temperature profile of the lambskin samples plotted at 121°C temperature

*The dips in the graph show the samples were taken out at intervals

All the trials were carried out in duplicate with separate pressure cooker runs. The samples after extraction were centrifuged twice when still hot to separate the fat, liquid part, and residue. The first centrifugation was done at 4000xg (4137 rpm), 30°C for 20 minutes, using 50 mL centrifuge tubes. The fat was decanted off and the extract was drained into another centrifuge tube for the second centrifugation at 4000xg (4137 rpm), 30°C for 10 minutes. The degree of solubilisation of the process was calculated on a dry basis using the formula given below, and the detailed calculations are mentioned in Appendix D. The degrees Brix and pH of the final liquid extract were measured using a handheld refractometer and a pH meter. The lambskin sample before and after thermal extraction are represented in Figure 3.3.

$$\text{DS } \% = \left(1 - \frac{(TS\,\%\ in\ residue * wt. of\ residue) + (TS\,\%\ in\ liquid\ extract * wt.\ of\ liquid\ extract)}{(TS\,\%\ in\ skin * wt. of\ skin)}\right) * 100$$

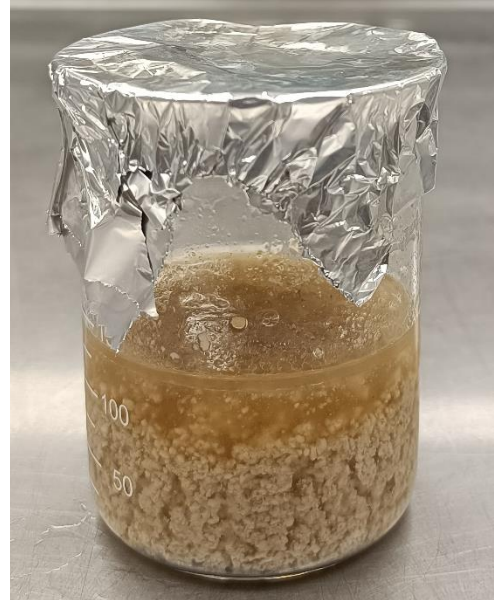

Figure 3. 3 (a): The lambskin sample before thermal extraction and, (b) The lambskin sample after thermal extraction

3.2.4. Titration curve

50.05 g of wet lambskin was mixed with 35 mL of RO water. The initial pH of the sample containing lambskin was noted. Aliquots (0.5 mL) of H_2SO_4 were repeatedly added to the sample; each time it was mixed well and equilibrated for 15 minutes before the final pH values were taken. This process was repeated till the pH was reduced to 3.37.

3.2.5. Viscosity measurements

Viscosity was measured according to the protocol described by Parker (2014) using a rheometer with a double gap geometry (DG 26.7) at a temperature of 40°C. In duplicates, 5 samples were processed at 108°C for 2 to 10 hours. Frozen skin extracts were thawed and then heated to about 40°C, and then the concentration was adjusted to 11°Brix, before rheometry. The sample (4.5 mL) was loaded into the equipment. The rheometer was set at controlled shear mode, between 2 and 120 s^{-1}, shearing up to 120 s^{-1} for 200 seconds, holding for 15 seconds at 120 s^{-1}, and then shearing down to 2 s^{-1} in 200 seconds. The viscosity readings were taken at intervals of 5 seconds. The picture of the geometry used is shown in Appendix K.

3.2.6. Degree of hydrolysis

Degree of hydrolysis (DH) was assayed following the protocol described by (Toro & García-Carreño, 2002) using ninhydrin reagents with slight modifications. In duplicates, 5 samples were processed at 108°C for 2 to 10 hours. The reagents used were L-Leucine, L-Proline and 2% ninhydrin reagent. Calibration standards (1 mL) were separately prepared from 1.5 mmol L^{-1} solutions of proline and leucine and were diluted to 4 standard solutions having a concentration ranging from 0.1-0.3 mmol L^{-1} for L-Proline and 0.05-0.15 0.01 mmol L^{-1} for L-Leucine. Ninhydrin solution (2% w/v%, 0.2 mL) was added to a 1 mL aliquot of each of the blanks, the samples, and the calibration standards. The mixtures were held in a water bath at 100°C for 12 minutes and then cooled to room temperature before measuring the absorbance at 570 nm for leucine and at 440 nm for proline. The number of equivalents of peptide bonds hydrolysed (h), expressed as meq/g protein was calculated from the following equation:

$$h = (A \times b)/m$$

where A is the absorbance at 570 or 440 nm and b and m are the y-intercept and slope of the calibration curves. To calculate h, the values of h from 570 nm and 440 nm were summed.

The degree of hydrolysis was determined for each sample from the following equation:

$$DH = h/h_{tot} \times 100\%$$

where h_{tot}, taken as 8 amino meq/g protein, is the total amount of peptide bonds (Toro & García-Carreño, 2002).

3.2.7. Total solids measurements

The moisture loss on drying was measured following the AOAC Official method 950.46 (G. W. Latimer, 2023). About 2 g of sample was spread on dried Aluminum dishes with a dimension of 50*40 mm. The dishes were kept open in a hot air oven at 102°C for 17 hours, then cooled in a desiccator, before taking the final weight readings.

3.2.8. Colour measurement

Colour measurements were carried out using a Chroma Meter, standardized using ceramic tiles. The samples previously equilibrated to room temperature, were filled into a clear Petri dish of 35mm*10mm size before the readings were taken for L*, a*, and b* values.

3.3. Flavour improvement on the skin extract

3.3.1. Proximate analysis of wool fibres

The wool samples used were a gift from WRONZ (Lincoln, New Zealand) and were of New Zealand strong wool, scoured and machine chopped to about 1 mm length giving the appearance of wool powder. Proximate analysis of the finely chopped scoured wool fibres was done at the Nutrition Laboratory at Massey University, Palmerston North, New Zealand, covering moisture, ash, fat, crude protein, and crude fibre, as described in 3.1.2.

3.3.2. Wool hydrolysis using sulphite treatment

Wool hydrolysis was done using a sulphite pre-treatment followed by enzyme treatment according to the method described by (Eslahi et al., 2013; Savolainen, 1993) with modifications. Sodium metabisulphite was preferred for the sulphite treatment as it is a food-grade option. According to the stoichiometry, the amount of sodium metabisulphite required to disrupt the disulphide bonds in cystine was calculated as 4.7 g per 100 g wool, assuming 6% cystine in wool. About thrice the stoichiometric amount of sodium metabisulphite was mixed with SDS (1 g/L) in a small amount of warm water using a vortex mixer. One part powdered wool was mixed with 4 parts water, to which the sulphite-SDS mixture was added. The mixture was heated in a water bath at 60°C for 3 hours and stirred continuously with an attached magnetic stirrer. The pH was then adjusted to 7.5 using a 4 mol L^{-1} NaOH solution. The enzyme Alcalase® 2.4L FG was added to the mixture with an enzyme-to-substrate ratio of 3:100, and incubated for 2 hours at 60°C, with continuous stirring. Samples were held at 100°C for 15 minutes to deactivate the enzyme, then cooled to 25°C and centrifuged at 4000xg for 10 minutes. The extract is separated from the residue by decanting the centrifuge tubes. The calculations and details of preliminary trials are described in Appendix H.

3.3.3. Preparation of alkali-treated wool extract

Alkali-treated wool extract was prepared according to the method described by (Blackburn & Lee, 1956; Lindley & Phillips, 1945). Wool (60 g) was mixed with 3 parts of water and adjusted to a pH of 10.08 with 4 mol L^{-1} NaOH. The samples were transferred into Duran bottles and heated in a water bath at about 100°C with constant stirring using a magnetic stirrer. The samples were held at 100°C for 45 minutes. The samples were then centrifuged at 4000xg for 10 minutes at 25°C. The extract was separated from the residue by decanting the centrifuge tubes.

3.3.4. Preparation of skin extract hydrolysate

Skin extract was prepared according to the method described by Ge (2024). Skin was minced according to the method described in section 3.1. The minced lambskin was mixed with 12% water and kept in a 250 mL glass beaker, covered with aluminium for thermal extraction in the pressure cooker at 5 psi (0.34 bar.g) pressure. Thermal extraction was carried out for 2 hours. Neutrase® 0.8L in amounts of 0.3% lambskin (wet basis) along with zinc sulfate heptahydrate in amounts of 25% of enzyme quantity is mixed in lukewarm water. The thermal extract of lambskin was heated to 65°C and then mixed with the enzyme and zinc sulfate heptahydrate and held at that temperature for 1 hour. Then the sample was held at 100°C for 15 minutes to deactivate the enzyme. All trials were carried out in duplicate with separate beakers in separate pressure cookers. The samples after extraction were centrifuged twice when still hot to separate the fat, liquid part, and residue. The first centrifugation was done at 4000xg (4137 rpm), at 30°C for 20 minutes, using 50 mL centrifuge tubes. The fat was decanted off and the extract was drained into another centrifuge tube for the second centrifugation at 4000xg (4137 rpm), 30°C for 10 minutes. The degree of solubilisation of the process was calculated on a dry basis. The liquid extract was then freeze-dried for 24 hours.

3.3.5. Reaction mixtures for flavour development

The reaction mixtures for flavour development were created according to the method described by (Ge, 2024; Morton et al., 1960) with modifications. The freeze-dried extract of lambskin was reconstituted to 60° Brix with water under continuous stirring in a water bath set at 50°C. The reference reaction mixture was prepared by mixing the reconstituted lambskin extract, L-cysteine,

and D-ribose. Reaction mixtures were prepared by replacing cysteine in the reference mixture with alkali-treated wool extract, L-cysteic acid monohydrate, and sulphite-treated wool extract. All the reaction mixtures were prepared such that the molar ratio of total amino nitrogen to organic sulphur content was kept constant. The ideal ratio of total amino nitrogen to organic sulphur content was calculated to be approximately 10.6 from the fixed protein-to-cysteine ratio used by Ge (2024). The calculations are shown in Appendix G. The dry ingredients were mixed with lukewarm water in a 6:4 ratio with continuous stirring in a magnetic stirrer. The ingredients mixture was then combined with reconstituted lambskin hydrolysate and mixed well under constant stirring in a water bath set at 50°C. The final reaction mixtures were transferred to cylindrical steel tube reactors of dimensions 9 mm *205 mm to fill them. The mixture is then heated to 120°C in an oil bath for 1 hour. The flavours thus formed were transferred to air-tight glass bottles and kept in the chiller at 4°C until sensory testing. Table 3.2 represents the amount of reactants required per 100 grams of reconstituted lambskin. Figure 3.4 represents the reaction flavours developed using reconstituted lambskin extract. Detailed calculations have been shown in Appendix I and the pictures of the experiment have been shown in Appendix K.

Table 3. 2. The amount of reactants required per 100 g of reconstituted lambskin extract

Sl. No.	Sample name	Cysteine (g)	Cysteic acid (g)	Alkali wool extract (g)	Sulphite wool extract (g)	Ribose (g)
1	186	7.42	-	-	-	7.42
2	628	-	10.47	-	-	7.54
3	461	-	-	-	277.8	7.78
4	239	-	-	6.94	-	7.50
5	347	-	-	-	277.8	277.8

Figure 3. 4. Reaction flavours developed using reconstituted lambskin extract

3.3.6. Sensory testing

The rate-all-that-apply (RATA) sensory testing method was used for all 5 samples. As a part of sensory testing, a panel of 25 untrained members was selected. Sensory panellists were volunteers from staff and students at Massey University. Samples (1g) were given in 3 mL air-tight plastic bottles. Participants were asked only to unscrew the cap and sniff the samples and not to taste or eat them. The panellists were asked to identify if the below-mentioned attributes were present in the samples and rate them on a scale of 5. Approval from the Human Ethics Committee, Massey University has been obtained with a notification number: 4000029226. Table 3.3 represents the sensory attributes used in sensory testing and its description.

Flavour intensity rating scale:

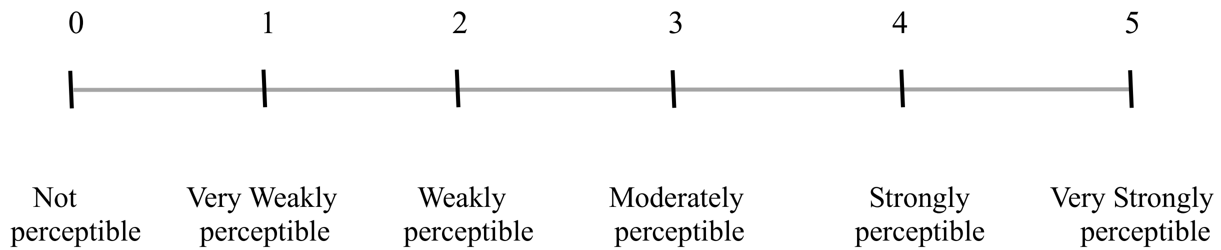

Table 3. 3. Sensory attributes and description

Sl. No.	Attributes	Descriptors
1	Roast meat	Roast pork or beef
2	Boiled meat	Pork or beef soup or curry
3	Fatty	Fried beef or pork
4	Pungent	Unpleasant strong, sharp smell
5	Caramel	Caramelised sugar
6	Burnt	Burnt food like
7	Animal-like	The smell of farm or sheep
8	Ammonia-like	Sweat or urine

3.4. Statistical Analysis

Statistical analysis was carried out using Microsoft Excel 365 (version: 2407) and Minitab 21. The functions used in Excel are Average, Max, and Min. Since some of the trials were done in duplicate, calculating half of the range between the two values was considered to be the most appropriate method of expressing variability in the data rather than using the standard deviation or standard error. The Max and Min functions were used to find the range of values. Statistical analysis performed using Minitab 21 included One-way ANOVA, and Tukey's pairwise comparison procedures for grouping, with the confidence interval kept at 95%. Interval plot was also plotted using Minitab 21.

Chapter 4: Results and Discussion

4.1. Thermal hydrolysis of lambskin

4.1.1. Proximate analysis of the lambskin

Analysis of the lambskin was done to quantify major components. The major components in lambskin are moisture (ca. 75%), protein (ca. 21%), and fat (ca. 4.5%), with traces of ash and fibre. The results of the proximate analysis are represented in Appendix A and summarised in Table 4.1. This composition is generally consistent with published values summarised in section 2.2.2.

Table 4. 1 Proximate composition of lambskin

Proximate contents	**Percentage Value (%)***
Moisture	74.8
Ash	0.7
Crude protein	20.8
Fat	4.5
Crude fibre	0.4
Carbohydrate	Nil

**The maximum possible variation of the values is 5%, according to the Massey Nutrition Lab.*

4.1.2. Lambskin extraction at various pH

The degree of solubilisation achieved of dry matter present in the skin at various pH values and 108°C is given in Table 4.2. The degree of solubilisation is expressed as the fraction of the original starting solids content of lambskin recovered in the soluble fraction of the extract on a dry basis. The highest degree of solubilisation of 90.2% is found at a pH of 3.5, whereas the lowest value of 85.6% is found at a pH of 6.03. Protein, mainly collagen is the major solid component in the skin, which is potentially soluble in water. As is the case for other proteins, the solubility of native collagen varies according to different pH levels. It is highly soluble in acidic conditions but less soluble in neutral to alkaline pH ranges (Vate et al., 2023). As the solubility increases, the degree of solubilisation also increases. This explains the results showing a higher degree of solubilisation

of lambskin and hence collagen at a pH of 3.5 compared to 4.5 or 6. The calculations are shown in Appendix B.

Table 4. 2 Degree of solubilisation of lambskin samples at various pH values

Initial pH values	Degree of solubilisation (% dry basis)*	Final pH	Final concentration (°Brix)
2.55 ± 0.02	$(87.13 \pm 0.0)^{C}$	3.55 ± 0.01	15.73 ± 0.03
3.55 ± 0.01	$(90.23 \pm 0.1)^{A}$	4.27 ± 0.03	20.35 ± 0.16
4.53 ± 0.02	$(88.64 \pm 0.0)^{B}$	5.04 ± 0.02	19.80 ± 0.01
6.03 ± 0.03	$(85.62 \pm 0.1)^{D}$	6.79 ± 0.02	15.80 ± 0.04

The data are presented as mean ± half the range of values measured (n = 2).

**Means that do not share a letter are significantly different from each other*

The increased solubility of collagen in water at acidic conditions can be explained based on its isoelectric point. The isoelectric point of native collagen ranges from 7 to 8 and is hence least soluble at that pH range (Leon-Lopez et al., 2019). The results showing the lowest degree of solubilisation at pH 6, followed by pH 4.5 can be justified on this basis. An interval plot showing the degree of solubilisation at various initial pH values is shown in Figure 4.1.

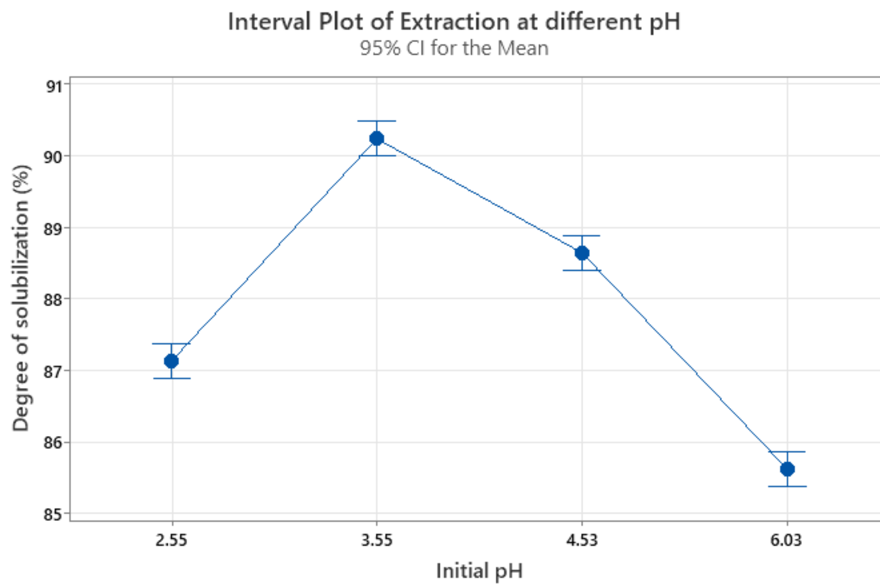

Figure 4. 1. Interval plot showing the degree of solubilisation of lambskin samples at various initial pH values

The degree of solubilisation of collagen at pH 2.5 was lower than that at 3.5 and 4.5 but higher than at 6.03. A considerable amount of sulphate ion was added to the sample in the form of sulphuric acid to reduce its pH. A large amount of sulphate ion present in the sample might have contributed to more precipitation than solubilisation. The decrease in the solubility of collagen might be due to added sulphate ions, similar to sodium chloride ions reported, but to a lesser degree (Ibrahim et al., 2023).

The final pH of all the samples was found to increase slightly after extraction. The final concentration measured as °Brix of the liquid extract increased with an increase in the degree of solubilisation of solid matter. The more the solids get solubilized, the greater the dissolved solids content in the extract, impacting the refractive index.

4.1.3. Titration curve

A titration curve was plotted to estimate the actual amount of sulphuric acid needed to decrease the pH of the skin sample and is represented as amounts needed per kilogram of wet lambskin in Figure 4.2. The supporting calculations are presented in Appendix C. Relatively little acid is required to reduce the pH to about 6.5, after which the curve gets flatter indicating the requirement for increasing amounts of acid to reduce the pH further. About 60 mL of acid solution was used to decrease the pH from 8.33 to 6.15, whereas another 45 mL was used to decrease the pH further down to 4.5. A further 60 mL of sulphuric acid was consumed to reduce the pH from 4.5 to 3.5.

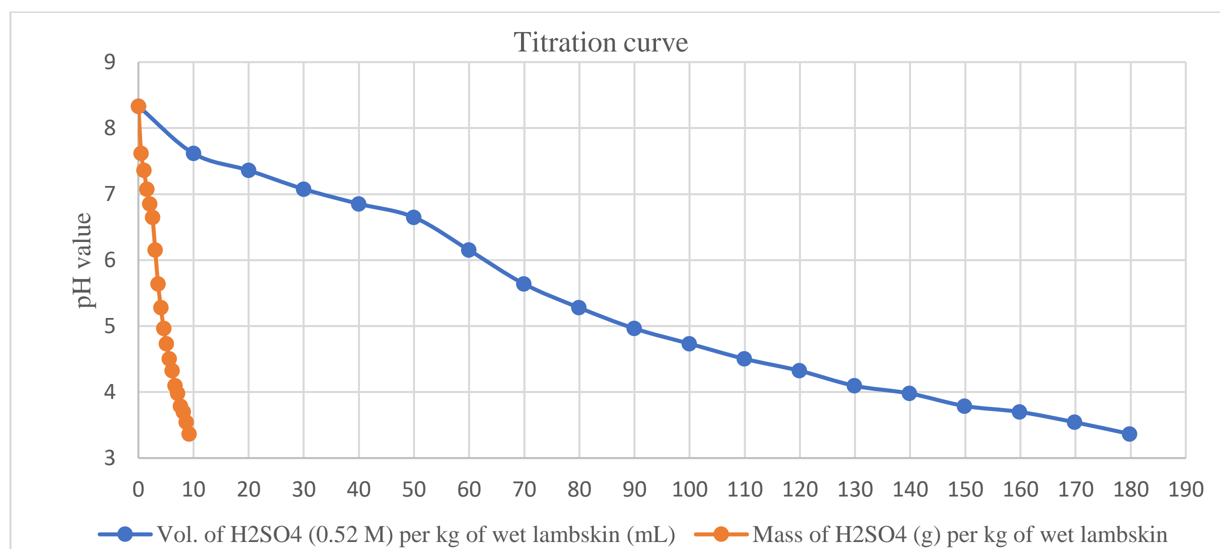

Figure 4. 2. Titration curve for reducing pH of lambskin

From the titration curve and degree of solubilisation of solids in the lambskin, it was judged that the thermal extraction is optimum near a pH of 4.5. Beyond this pH, additional sulphuric acid used doesn't account for a considerable increase in the degree of solubilisation of protein.

4.1.4. Degree of solubilisation of thermal extraction of lambskin

The lambskin with an initial pH of 4.5 was heated at three different temperatures of 90ºC, 108ºC, and 121ºC for 2,4,6,8 and 10 hours. The degree of solubilisation observed is represented in Table 4.3. The calculations are shown in Appendix D.

Table 4. 3. The degree of solubilisation of lambskin samples at various thermal treatments

Trial	Temp-erature	Time	TS of residue	TS of liquid extract	Degree of solubilisation *	Final pH	Final Brix
Unit	ºC	h	%	%	%	-	°Brix
T3 A	90	2	15.30 ± 0.07	11.75 ± 0.04	80.42 ± 0.40^{F}	4.65 ± 0.02	10.59 ± 0.01
T3 B	90	4	17.33 ± 0.02	14.45 ± 0.04	83.30 ± 0.00^{E}	4.78 ± 0.02	12.74 ± 0.04
T3 C	90	6	19.37 ± 0.10	16.27 ± 0.02	85.41 ± 0.29^{CD}	4.83 ± 0.02	15.10 ± 0.04
T3 D	90	8	19.66 ± 0.12	16.87 ± 0.10	86.73 ± 0.50^{BC}	4.95 ± 0.03	15.83 ± 0.02
T3 E	90	10	20.24 ± 0.07	17.75 ± 0.02	87.97 ± 0.19^{AB}	4.96 ± 0.05	17.44 ± 0.04
T1 A	108	2	18.91 ± 0.08	14.07 ± 0.05	85.01 ± 0.01^{D}	4.81 ± 0.01	12.88 ± 0.03
T1 B	108	4	24.62 ± 0.31	17.01 ± 0.08	86.71 ± 0.51^{BC}	4.86 ± 0.01	16.36 ± 0.04
T1 C	108	6	28.40 ± 0.16	20.91 ± 0.06	87.88 ± 0.24^{AB}	4.87 ± 0.02	19.63 ± 0.04
T1 D	108	8	29.25 ± 0.02	21.11 ± 0.14	88.71 ± 0.11^{A}	4.94 ± 0.03	20.47 ± 0.46
T1 E	108	10	30.60 ± 0.15	22.82 ± 0.16	88.93 ± 0.03^{A}	4.96 ± 0.01	21.09 ± 0.03
T2 A	121	2	30.95 ± 0.04	20.86 ± 0.02	86.96 ± 0.04^{BC}	5.07 ± 0.03	18.61 ± 0.04
T2 B	121	4	30.95 ± 0.15	21.54 ± 0.03	88.07 ± 0.16^{AB}	5.12 ± 0.01	19.14 ± 0.01
T2 C	121	6	31.08 ± 0.06	22.67 ± 0.02	88.80 ± 0.25^{A}	5.15 ± 0.01	20.18 ± 0.02
T2 D	121	8	31.82 ± 0.02	23.68 ± 0.01	89.07 ± 0.06^{A}	5.18 ± 0.01	21.71 ± 0.02
T2 E	121	10	32.28 ± 0.63	24.62 ± 0.03	89.33 ± 0.63^{A}	5.23 ± 0.04	23.51 ± 0.48

The data are presented as mean ± half the range of values measured (n = 2)

**Means that do not share a letter are significantly different*

The maximum degree of solubilisation of 89% occurred at 121ºC treated for 10 hours, while a minimum of 80.5% was for lambskin treated at 90ºC for 2 hours. The maximum and minimum degrees of solubilisation occurred as expected, with the severe treatment having the highest and the mild treatment having the lowest. The degree of solubilisation consistently increased from

80.5% to 88% for the treatment at 90ºC with an increase in time. At 108ºC (5 psi or 0.34 bar.g) only a 3.7% increase in solubilisation was observed with a considerable increase in time. At 121ºC the increase was only 2.4% between 2 and 10 hours. The degree of solubilisation at 2 hours was the highest at 121ºC followed by 108ºC and 90ºC, which is as expected that higher temperature leads to greater solubilisation of solids content. The maximum possible degree of solubilisation ranged from 88-89% for all the trials, indicating that increasing temperature doesn't have a significant effect on the maximum solubilisation degree for the period considered for the trials. These results also indicate that a similar degree of solubilisation can be attained with higher temperatures with less time. This is also evident from Tukey's comparison (Table 4.3) that the degree of solubilisation % was not significantly different after 8 hours at 108ºC or after 6 hours at 121ºC.

The TS content of the residue and extract increased with an increase in time and temperature. The same is true for the Brix value of the extract. The residue is getting more and more concentrated with insoluble materials, thus increasing the TS% per gram of residue left. As more solids content gets solubilized in the liquid extract, the TS% and Brix value of the extract increases. The maximum and minimum total solids content of the residue is found to be 32.3% and 15.3% respectively. The TS% of the extract increased from 11.8% to 24.6%, whereas the Brix value of the extract increased from 10.6°Brix to 23.5°Brix with an increase in time and temperature. The final pH of the extract also increased with time and temperature from 4.65 to 5.23, presumably implying the pH impact of liberated free amines exceeded that of the liberated carboxyl group over the pH range of relevance. The rate of increase in the TS% of the residue, TS% of the extract, and the final Brix value were less for higher temperatures. The final pH value was higher for higher temperatures and longer times because of more solubilisation of proteins, mainly collagen.

Evaporation or condensation have been reduced using Aluminum foil to tightly cover the beakers used in the extraction process. But this doesn't rule out all possibility of the effect of concentration or dilution of the extract. However, changes to total solids due to evaporation or condensation are assumed to be negligible.

4.1.5. Kinetics of thermal extraction of lambskin

Extraction kinetics for the thermal extraction at three different temperatures is plotted and is represented in Figure 4.3. Detailed calculations are summarised in Appendix D. The y-axis represents the ratio of the amount of unreacted solids available at a particular time to the initial amount of total solids. From the graph, it is observed that the ratio of the amount of available reactant to the initial amount of reactant decreases linearly with time. This aligns with first-order kinetics, in which the reaction occurs at a pace determined by the reactant's residual concentration. Over time, more collagen is extracted, resulting in a slower extraction rate.

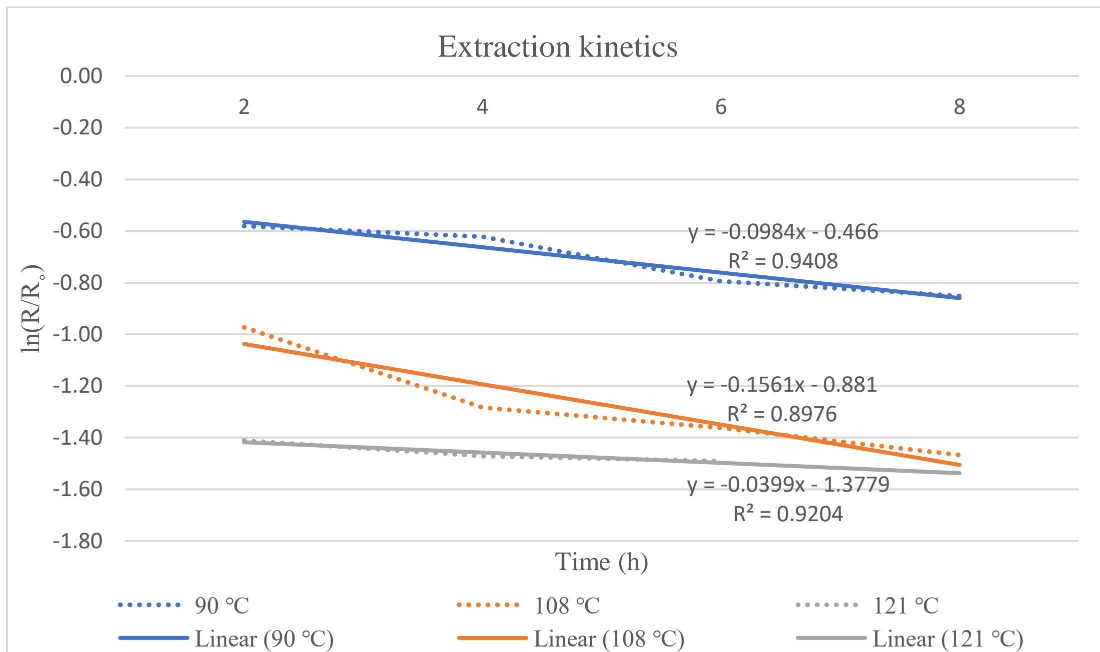

Figure 4. 3 Extraction kinetics of first-order reaction

The first-order rate constants obtained from Figure 4.3 are shown in Table 4.4.

Table 4. 4 First-order rate constants (k) at three different temperatures

Sl. No.	Temperature	First-order rate constants (k)
	°C	h^{-1}
1	90	0.10
2	108	0.16
3	121	0.04

An Arrhenius plot is shown in Figure 4.4 using the rate constants at 3 different temperatures obtained from the slope of the regression line from Figure 4.3. The preexponential factor A and the activation energy are calculated from the Arrhenius plot as 0.03 and -3.75 J/mol respectively. From the Arrhenius plot, it is observed that the rate constant and hence the overall reaction rate increases with an increase in temperature from 90℃ to 108℃ and then decreases with a further increase in temperature. The low reaction rate observed at 121℃ is probably an artefact: so much of the reaction was completed within 2 hours that the apparent rate beyond 2 hours is artifactually low. This work needs to be repeated to fully characterise lambskin thermal hydrolysis kinetics. The activation energy is a low negative value which is due to a decrease in rate of reaction with increasing temperature to 121℃.

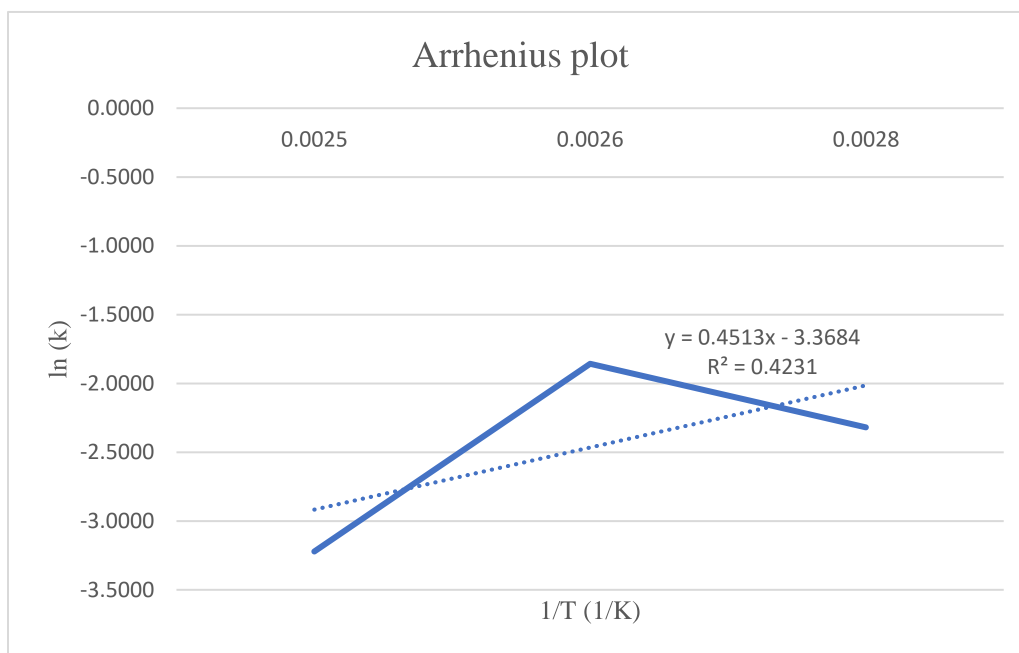

Figure 4. 4 Arrhenius plot for the rate constant

4.1.6. Colour measurements

Essentially, gelatin's colour is important since the confectionery sector uses about 60% of the global supply. Since products in this sector are frequently coloured, it makes sense that producing a homogeneous product would be easier with the less varied hue of the materials. Pale colours are typically preferred over darker ones because, in the perceptions of most people, they are linked with purity (Cole & Roberts, 1997).

The source material and extraction techniques both affect the colour of gelatin. The colour characteristic may affect the consumer attractiveness of gelatin but does not affect its functional characteristics (Rather et al., 2022).

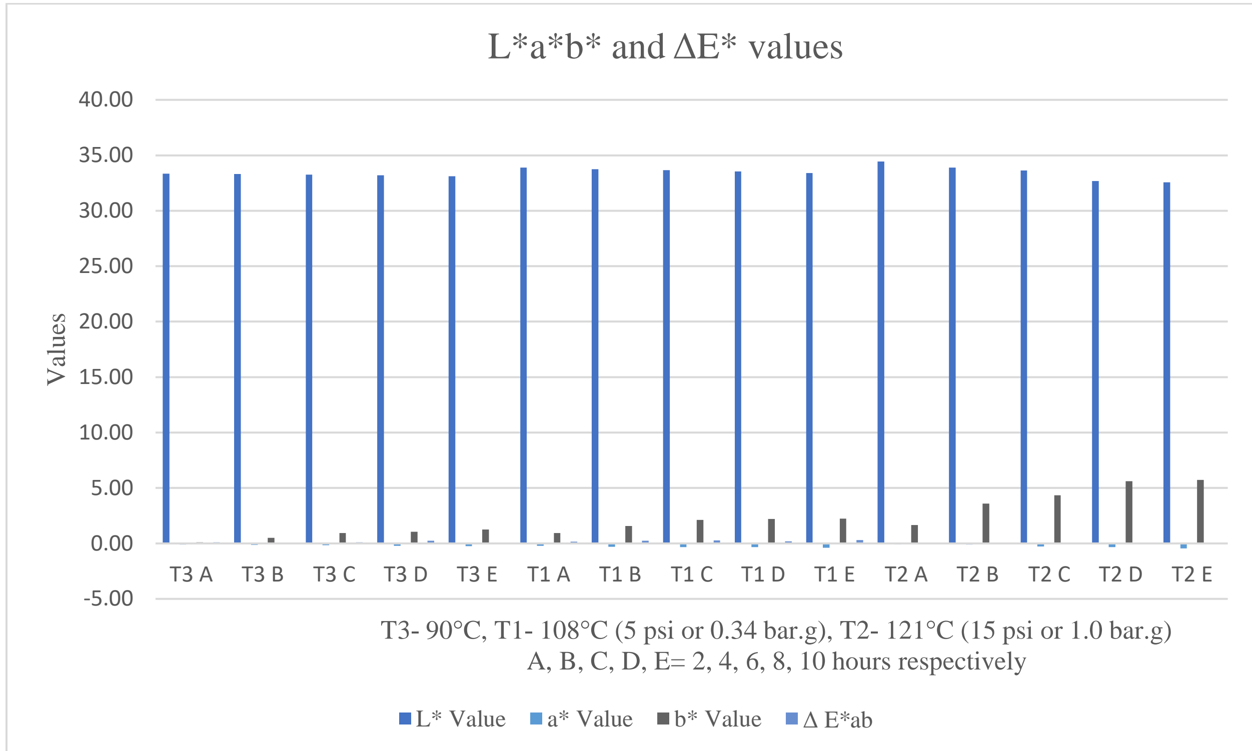

*Figure 4. 5. L*a*b* and ΔE* values of samples treated at different conditions (N=2)*

The L*a*b* values of the samples measured in the current trial are shown in Figure 4.5. The readings are shown in Appendix E. The L* values remained consistent between 33-34 throughout the trials irrespective of the time and temperature of the treatment. This indicates that the colour of the samples does not get considerably lighter due to the changes in processing conditions. The values for a* values ranged from -0.2 to -0.4. There is a slight increase in the a* values with time and temperature but the increase is almost negligible. The a* value indicates that the colour of the samples is slightly greenish. There is a considerable increase in the b* values with time and temperature from 0.10 to 5.74. This indicates that the samples are slightly yellowish and the intensity of the yellow colour increases as the processing conditions become severe. There was no considerable difference between the readings for L*, a*, and b* values in the L*a*b* colour space as the maximum value for Δ E*ab was found to be 0.31. The colour of commercial gelatin is

reported to range between very pale yellow and dark amber (Cole & Roberts, 1997). L*, a*, and b* of bovine commercial gelatins are reported to be 34.40 ± 0.03, 1.87 ± 0.12, and 1.87 ± 0.12 respectively (Abdul Rahman, 2012). L* a* and b* values for goat gelatin are reported to be 27.39 ± 0.17, -0.27 ± 0.03, and -2.08 ± 0.15 respectively (Mad-Ali et al., 2017). These results are consistent with the colour of gelatin obtained from lambskin.

4.1.7. Viscosity measurements

The viscosity measurements of extract obtained from lambskins treated at 108°C from 2 to 10 hours are shown in Figure 4.6. All the samples have shown Newtonian behaviour as the viscosity remained constant irrespective of the change in shear rate. The disturbance in viscosity measurements at lower shear rates can be explained as the time taken by the colloidal particles to rearrange under shear to a constant value. The viscosity decreased from 5.58 to 2.2 mPa.s with the increase in the extraction time of lambskin from 2 to 10 hours. From the viscosity values, it is evident that the samples have a low viscosity in general.

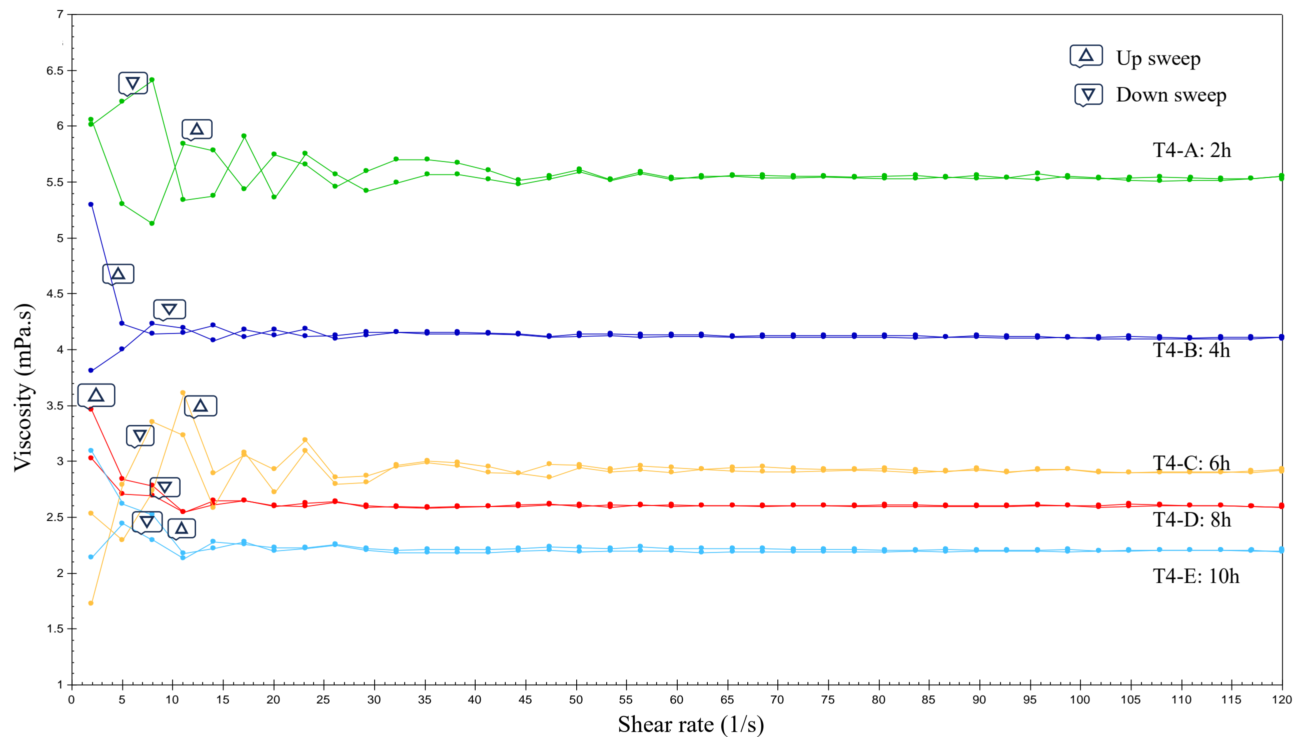

Figure 4. 6. The viscosity measurements of extract obtained from lambskins treated at 108°C from 2 to 10 hours

Viscosity values of gelatin obtained from native chicken leg skin ranged from 6.07 to 7.02 mPa.s. A range of 2.0 to 7.5 mPa.s has been mentioned for commercial gelatin by ISO standards (Sompie & Triasih, 2018). The viscosity values for the gelatin-collagen mixture solution obtained from lambskin are consistent with the reported values. A concentration of 11°Brix was chosen for viscosity measurements as the collagen molecules aggregate in higher concentrations and single molecular properties are not visible. Under Newtonian conditions, the viscosity of collagen solutions is reported to decrease with an increase in extraction temperature and time (Kahn & Witnauer, 1966). The graph showing the kinetics of viscosity reduction has been represented in Figure 4.7. The initial rate of viscosity reduction is more compared to a further increase in extraction time. This can be due to more hydrolysis of collagen occurring in the first 6 hours compared with another 4 hours, thus decreasing viscosity.

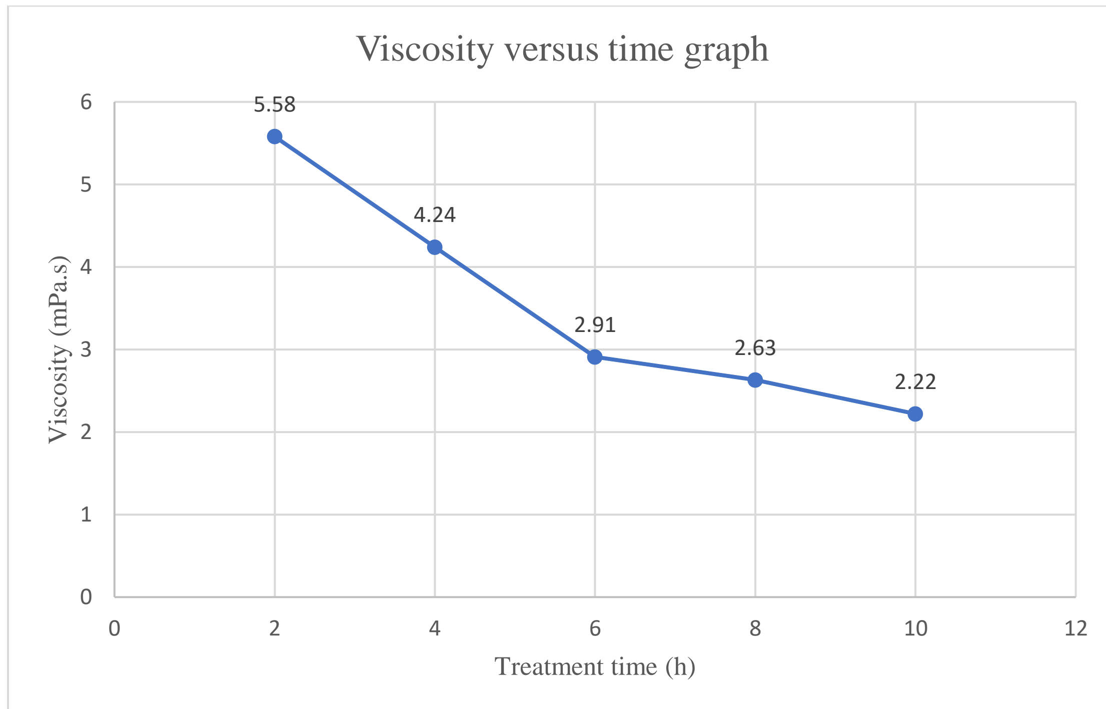

Figure 4. 7. Kinetics of viscosity reduction for lamskin extraction at 108℃. The average value of viscosity was obtained as the samples showed Newtonian behaviour.

The reduction in viscosity with an increase in extraction time can be explained based on the molecular weight of small chain segments of collagen. With the increase in extraction time, more peptide bonds are hydrolysed and smaller peptide segments with lower molecular weight are

obtained. These smaller peptides don't contribute much to electrostatic repulsion between peptide chains, thus reducing viscosity (Leon-Lopez et al., 2019).

4.1.8. Degree of hydrolysis

The degree of hydrolysis is expressed as the percentage of peptide bonds hydrolysed in the extract. It is assumed that a free amino group is released during the hydrolysis, which then reacts with ninhydrin forming a purple-blue compound. The absorbance of the common amino acids is measured at 570 nm. Proline and hydroxyproline, prominent imino acids in collagen, give a yellow product instead, whose absorbance is measured at 440 nm. The intensity of the absorbance value is directly proportional to the amount of primary amino groups present in the sample. The standard curves for proline and leucine are plotted and are represented in Figures 4.8 & 4.9 respectively. The calculations are shown in Appendix F.

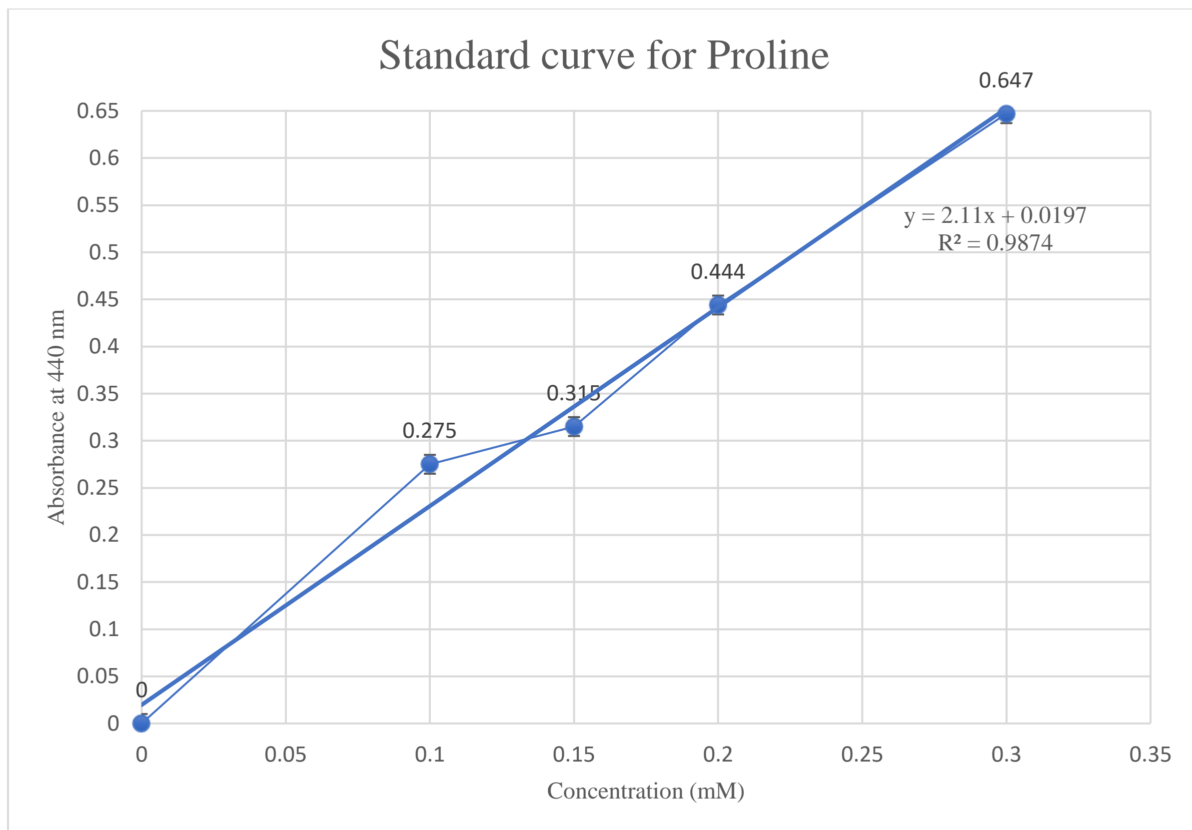

Figure 4. 8 Standard curve measured for proline

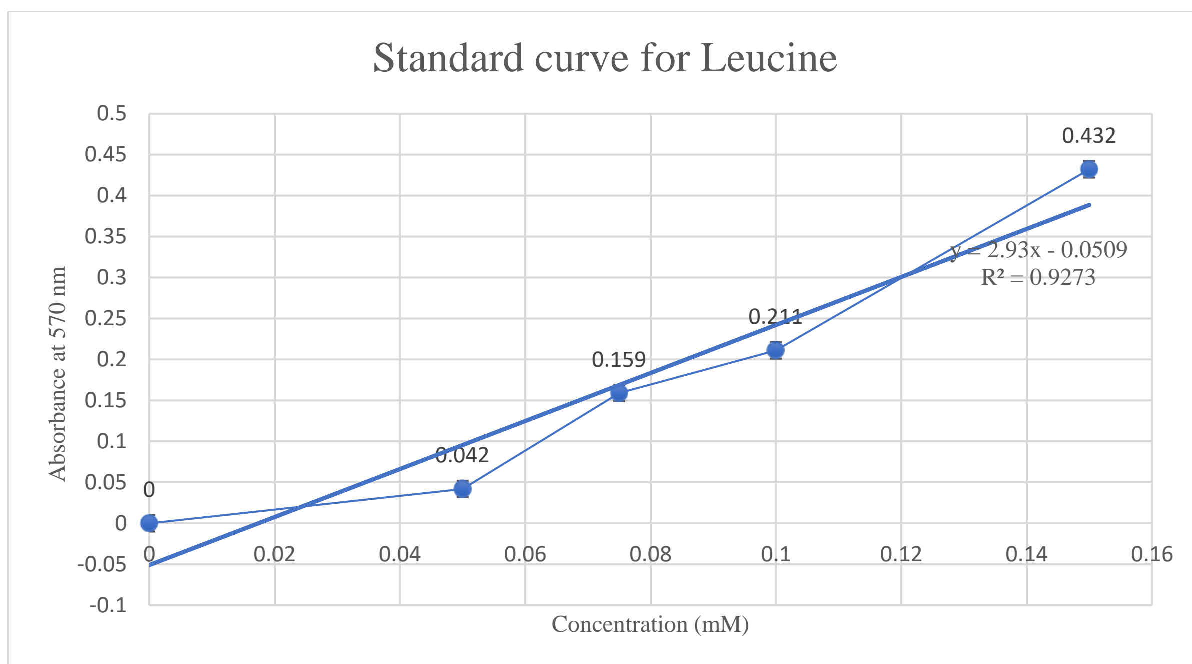

Figure 4. 9. Standard curve measured for leucine

A regression line equation has been fitted for calculating the number of equivalents of peptide bonds hydrolysed (h), expressed as meq/g protein. For the standard curves of both leucine and proline, the regression lines fit well with the R^2 values greater than 0.92. The absorbance and the degree of hydrolysis of the samples treated at 108°C are listed in Table 4.5. The detailed calculations are given in Appendix F.

Table 4. 5. The absorbance value and the degree of hydrolysis of the lambskin samples treated at 108°C

Time	Abs_{570}	h_{570}	Abs_{440}	h_{440}	h_{tot}	DH*
	-	meq/g protein	-	meq/g protein	meq/g protein	%
2	0.274 ± 0.00^{e}	1.787 ± 0.01	0.618 ± 0.01^{c}	0.288 ± 0.00	2.076 ± 0.01	25.9 ± 0.2^{d}
4	0.284 ± 0.00^{d}	1.852 ± 0.02	0.583 ± 0.00^{d}	0.272 ± 0.00	2.125 ± 0.02	26.6 ± 0.2^{d}
6	0.313 ± 0.00^{c}	2.037 ± 0.02	0.676 ± 0.00^{a}	0.315 ± 0.00	2.352 ± 0.02	29.4 ± 0.2^{c}
8	0.337 ± 0.00^{b}	2.198 ± 0.02	0.642 ± 0.00^{b}	0.300 ± 0.00	2.497 ± 0.02	31.2 ± 0.3^{b}
10	0.403 ± 0.00^{a}	2.625 ± 0.02	0.672 ± 0.01^{a}	0.314 ± 0.00	2.939 ± 0.02	36.7 ± 0.3^{a}

The data are presented as mean ± half the range of values (n = 3).
**The mean ± half the range of values (n = 3) for DH are calculated from individual DH values for each of the absorbance readings.*

The absorbance of the samples at 570 nm and 440 nm were in the reliable range of 0.2 to 0.7. The absorbance value observed at both wavelengths increased with increasing extraction time. This suggests the amount of primary amino groups in the samples increases with time, implying that more hydrolysis of peptide bonds has happened. The degree of hydrolysis (DH) values calculated for the samples varied from 25.9% to 36.7%. The DH values for the samples treated for 2 hours and 4 hours were not significantly different from each other as Tukey's comparison showed (Table 4.5). This indicates that little hydrolysis of peptide bonds happened with an increase in extraction time from 2 hours to 4 hours.

The results obtained for the extract from lambskin coincide with the reported values of DH from the literature for enzymatic hydrolysates. Collagen hydrolysate obtained using Alcalase® and Flavourzyme® provided DH values of 36.11% and 12.02% respectively (Schmidt et al., 2020). The DH values reported for mushroom protein hydrolysate ranged from 53.52–67.13 % (Ang & Ismail-Fitry, 2020).

The protein hydrolysate's DH values can be taken to calculate the average length of the protein chain (amino acids and peptides).

$$Average\ peptide\ chain\ length\ (PCL) = \frac{100}{DH\ \%} * NSI$$

NSI = nitrogen solubility index is taken as 1 (Fonkwe & Singh, 1996).

Thus, the average peptide chain length of the hydrolysate was 3.86, 3.76, 3.40, 3.20, and 2.72 amino acid residues for 2,4,6,8 and 10 hours respectively. Between 2 and 4 hours relatively few peptide bonds were hydrolysed but the impact on viscosity is great, implying that a smaller number of large peptides are being broken up in this phase. The DH measure gives no indication of molecular weight distribution of peptides beyond the average chain length.

Variation of DH with viscosity has been plotted and represented in Figure 4.10. The graph shows that DH and viscosity are inversely proportional, and far from linear. With an increase in the degree of hydrolysis, peptide chains of lower molecular weight are formed, which act as individual entities not capable of causing a strong electrostatic interaction between them or reforming into a gel structure, thus decreasing the viscosity.

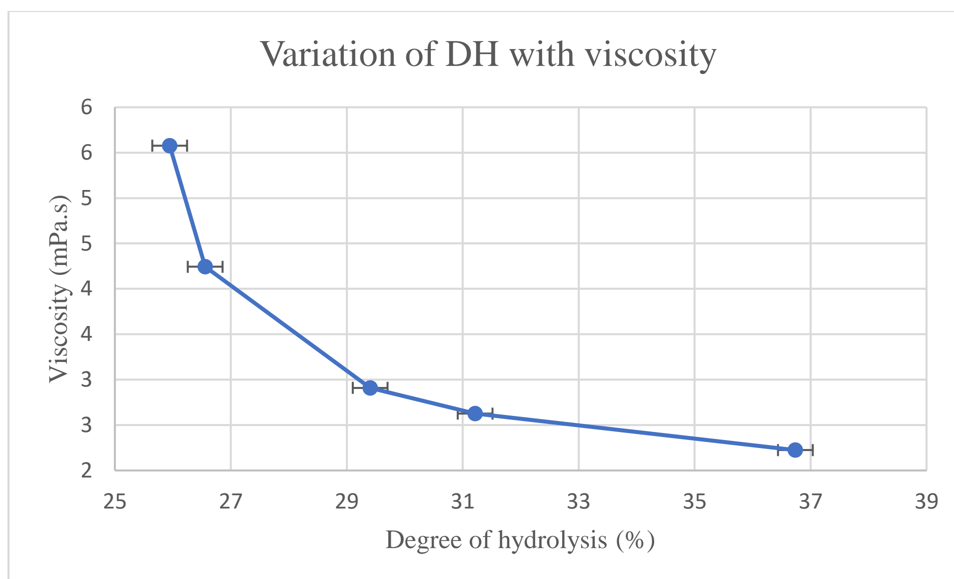

Figure 4. 10. Variation of DH with viscosity. The error bar shows an error of ±0.3 in DH values.

4.2. Flavour development with skin extract

4.2.1. Proximate analysis of the wool fibres

The results of the proximate analysis of the finely chopped wool are detailed in Appendix A and summarised in Table 4.6. The wool is composed of mainly protein which accounts for 94.6% of its total weight. Moisture was measured at about 8%. Crude fibre and fat account for 1.3% and 1% respectively. The carbohydrate content of the wool is found to be nil.

Table 4. 6. Proximate analysis of wool

Proximate contents	Percentage (%)*
Moisture	8.1
Ash	0.6
Crude protein	94.6
Fat	1
Crude fibre	1.3
Carbohydrate	Nil

**The maximum possible variation of the values is 5%, according to the Massey Nutrition Lab.*

4.2.2. Hydrolysis of wool

Wool was hydrolysed using the enzyme Alcalase® 2.4L FG with a sodium meta-bisulphite pretreatment. The degree of solubilisation of solids content in the wool from preliminary trials using various enzymes at different concentrations is summarised in Table 4.7. The calculations are shown in Appendix H.

Table 4. 7. The degree of solubilisation of solids content in the wool from preliminary trials

Sl. No.	NaMBS %	Enzyme	Enzyme %	Initial pH	Degree of solubilisation %
1	6.34 (0.1 mol L^{-1} solution)	Neutrase	1	7	19.3 ± 3
2	63.37 (1 mol L^{-1} solution)	Neutrase	1	7	32.9 ± 0.3
3	13.34	Neutrase	4	7	22.7 ± 3
4	13.34	Alcalase	4	7.5	50.0 ± 1
5	13.34	Protamex	4	7	25.1 ± 1
6	13.34	Alcalase	3	8	43.6 ± 3
7	13.34	Alcalase	3	7.5	47.2 ± 2
8	13.34	Neutrase	3	7	21.2 ± 3

The data are presented as mean ± half the range of values (n = 3).

From Table 4., it is inferred that the degree of solubilisation increased about 1.5 times with an increase in the concentration of NaMBS from 0.1 mol L^{-1} to 1 mol L^{-1}. This increase in solubilisation degree was not very high when compared with the increase in NaMBS concentration. According to the stoichiometry, the amount of sodium metabisulphite required to disrupt the disulphide bonds in cystine was calculated as 4.7 g per 100 g wool, assuming 6% cystine is present in wool. Hydrolysis using 6.3% NaMBS, which is 1.34 times higher than the stoichiometric ratio solubilises about 20% of the solids content. With a NaMBS concentration of tenfold higher, the degree of solubilization rises to 33%. A midpoint point of 2.8 times the stoichiometric ratio was chosen for further work.

Hydrolysis trials used 3:100 and 4:100 enzyme-to-substrate ratios using enzymes Neutrase® 0.8L, Alcalase® 2.4L FG, and Protamex®. Alcalase® 2.4L FG at a 3:100 enzyme-to-substrate ratio, at a pH of 7.5 with a degree of solubilisation of 50% was chosen for reaction flavour work.

4.2.3. Sensory analysis

Reaction mixtures for flavour development were created by mixing the lambskin extract reconstituted to 60°Brix and ribose with cysteine, cysteic acid, or wool hydrolysates. It was reported in recent work that meaty flavours could be created using reconstituted lambskin extract, cysteine, and ribose (Ge, 2024). The present trials were done to find out if it is possible to create Maillard reaction flavours specifically, meaty flavours by using woolly lambskin instead of cysteine. Cysteic acid is a product of the oxidation of wool keratin. The alkali extract of the wool was used to find out if lanthionine, the major compound formed due to the alkaline hydrolysis of wool can create flavours similar to that of cysteine. Cysteic acid and lanthionine were chosen to get an idea about whether it's possible to create a meaty flavour from oxidation or alkali treatment of wool. Sulphite-treated extract of wool was used to find if mild hydrolysis using sulphite is useful for creating a meaty flavour. The flavours are formed by mixing reconstituted lambskin extract with ribose (protein to ribose ratio 8:1) along with either cysteine, cysteic acid, sulphite-treated wool extract, or alkali-treated wool extract, all with a constant N:S ratio of 10.6. Sensory testing of the flavours created was done with 25 untrained panelists and the results are represented in Figure 4.11. The calculations are shown in Appendix J.

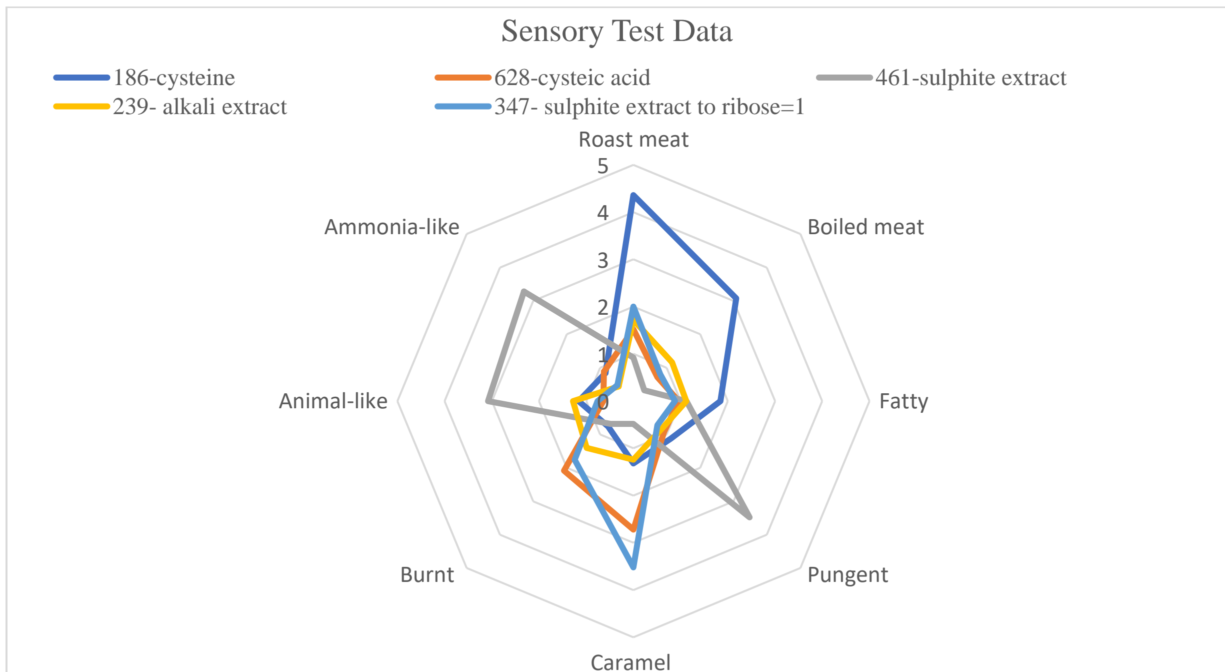

Figure 4. 11. Radar chart showing the results of sensory testing of the flavours

The amount of the reactants used in each sample is summarised in Table 3.2. The results show that roast and boiled meat flavours were most intense in reaction products using free cysteine (sample 186), with a rating of about 4.5 on a 5-point scale. While using cysteine as the sulphur source, the flavour of the product was mostly described as that of roast meat along with a slightly boiled meat flavour. The sample from cysteine also had a slightly fatty, animal-like, and caramel-like flavour.

While using cysteic acid (sample 628) as the sole organic sulphur source, the flavour is described as having a note of caramel and burnt flavour, along with a slight animal-like flavour. The flavour created using sulphite extract (sample 461) is described as pungent with ammonia-like and animal-like flavours.

The flavour created with alkali extract (sample 239) was very mild with low descriptor scoring for all the attributes considered. The sulphite extract with greatly enhanced ribose level (sample 347) was reported to give a caramel-like flavour with a burnt note but scored low on ‘meaty’ attributes.

Chapter 5: Conclusions and Recommendations

From the extraction kinetics of lambskin under acidic conditions, it can be concluded that by increasing the temperature and time of extraction from 90°C for 2 hours to 121°C for 10 hours, the degree of solubilisation increased by only 10%. This increase is not huge when considering energy use and productivity in the industry. Extraction at 108°C for 2 hours achieved a degree of solubilisation of 85%, which is judged to be optimum. Secondary hydrolysis with an enzyme such as Neutrase® is worth considering to boost the extraction yield.

This research created a clean-label product without any additives other than food-grade sulphuric acid to reduce pH. Substantial conversion of lambskin seems to be attainable by including a modest acid addition before the thermal hydrolysis. The samples had low viscosity in the range of 2 to 5 mPa.s at 11°Brix and 40°C, which makes it easy to handle in industry, especially for mixing in various applications. The DH value of about 37% shows that the extraction process is quite successful in making low molecular weight peptides or amino acids.

Sulphite-assisted enzyme hydrolysis of the wool yielded about 50% degree of solubilisation of the solids content. Alcalase® 2.4L FG is found to be the most effective enzyme in hydrolysing sulphite pretreated wool at a 3:100 enzyme-to-substrate ratio, at a pH of 7.5, yielding a degree of solubilisation of 50%. Sensory analysis of the samples indicates that alternative organic sulphur sources such as cysteic acid and lanthionine, which are potential keratin hydrolysis products, are not as effective as free cysteine in creating Maillard flavours, especially meaty flavours. Lanthionine contributed to only a mild flavour of little practical use. The sulphite-assisted enzyme hydrolysate from wool resulted in a pungent, animal-like smell. Expanding the wool fibres using a preliminary process like steam explosion may aid access of protease enzyme to keratin chains. Prior softening with sulphite would enhance steam explosion and steam may purge excess sulphur dioxide. A more complete breakdown of wool fibres to cysteine might be successful in creating a meaty flavour. The identification of compounds responsible for each of the flavours using techniques such as gas chromatography-mass spectrometry (GC/MS) would be recommended to improve the process. Reducing the pH of the reaction mixtures to about 3.5-4.5 to induce meaty flavours was considered but was practically not feasible with the reconstituted lambskin extract being sticky. The hydrolysis of lambskin with higher concentrations of enzyme in the enzyme

hydrolysis step might reduce the stickiness of the extract. Rather than rating the samples based only on smell, a comprehensive sensory study that includes tasting might be useful for capturing the full flavour profile.

It can be concluded that a combination of cysteine, ribose and lambskin hydrolysate works well in creating cooked meat flavour. The idea of releasing cysteine from wool already associated with the lambskin is attractive. It is a readily available source with a promising composition. Extracting and hydrolysing lambskin collagen using heat followed by enzymatic treatment is practicable. Softening wool enough to allow enzyme access is difficult without the loss of some cysteine to lanthionine, cysteic acid or other species. Lanthionine or cysteic acid did not appear to replace the functionality of cysteine in reaction flavour production. Introducing a physical process such as a steam explosion to break apart wool fibres to allow enzyme access could help in a more complete breakdown of wool fibres to cysteine.

References:

Abbas, K. A., Abdulkarim, S. M., Saleh, A. M., & Ebrahimian, M. (2010). Suitability of viscosity measurement methods for liquid food variety and applicability in food industry - A review. *Journal of Food, Agriculture & Environment*, *8*.

Abdul Rahman, M. N. (2012). Extractions, Physicochemical Characterizations and Sensory Quality of Chicken Feet Gelatin. *Borneo Science*, *30*, 1-13.

Adler-Nissen, J. (1976). Enzymatic Hydrolysis of Proteins for Increased Solubility. *Journal of Agricultural and Food Chemistry*, *24*(6), 1090–1093.

Al-Hassan, A. A. (2020). Gelatin from camel skins: Extraction and characterizations. *Food Hydrocolloids*, *101*. https://doi.org/10.1016/j.foodhyd.2019.105457

Alam, M. R., Shahid, M. A., Alimuzzaman, S., & Khan, A. N. (2022). Sources, extractions and applications of bio-maker collagen–A review. *Biomedical Engineering Advances*, *4*. https://doi.org/10.1016/j.bea.2022.100064

Alexandratos, N., & Bruinsma, J. (2012). *World Agriculture Towards 2030/2050: The 2012 Revision*.

Alipal, J., Mohd Pu'ad, N. A. S., Lee, T. C., Nayan, N. H. M., Sahari, N., Basri, H., Idris, M. I., & Abdullah, H. Z. (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, *42*, 240-250. https://doi.org/10.1016/j.matpr.2020.12.922

Allafi, F., Hossain, M. S., Lalung, J., Shaah, M., Salehabadi, A., Ahmad, M. I., & Shadi, A. (2020). Advancements in Applications of Natural Wool Fiber: Review. *Journal of Natural Fibers*. https://doi.org/10.1080/15440478.2020.1745128

Ang, S.-S., & Ismail-Fitry, M. R. (2020). Production of Different Mushroom Protein Hydrolysates as Potential Flavourings in Chicken Soup Using Stem Bromelain Hydrolysis. *Food Technology and Biotechnology*, *57*, 472-480. https://doi.org/10.17113/ftb.57.04.19.6294

Bailey, M. E. (1994). Maillard reactions and meat flavour development. In F. Shahidi (Ed.), *Flavor of Meat and Meat Products* (pp. 153-173). Springer US. https://doi.org/10.1007/978-1-4615-2177-8_9

Baldi, G., Soglia, F., & Petracci, M. (2021). Valorization of meat by-products. In *Food Waste Recovery* (pp. 419-443). https://doi.org/10.1016/b978-0-12-820563-1.00017-2

Bikaki, M., Shah, R., Müller, A., & Kuhnert, N. (2021). Heat induced hydrolytic cleavage of the peptide bond in dietary peptides and proteins in food processing. *Food Chemistry 357*. https://doi.org/10.1016/j.foodchem.2021.129621

Blackburn, S., & Lee, G. R. (1956). The reaction of wool keratin with alkali. *Biochimica Et Biophysica Acta*, *19*(3), 505-512. https://doi.org/10.1016/0006-3002(56)90474-7. PMID: 13315311

Bourne, M. C., & Rao, M. A. (2017). Viscosity Measurements of Foods. In *Instrumental Methods for Quality Assurance in Foods* (pp. 211-229). https://doi.org/10.1201/9780203750711-9

Briefer, M., & Cohe, J. H. (1927). A Study of Gelatin Viscosity and Related Problems. *Industrial and engineering chemistry*, *19*(2), 252-257.

Choi, S. E. (2016). Comparison of Soup Stock Preparation and Soup Product Consumption Behavior in an Ethnically Diverse Population. *Journal of Culinary Science & Technology*, *14*(3), 234-262. https://doi.org/10.1080/15428052.2015.1110544

Cole, C. G. B., & Roberts, J. J. (1997). Gelatine colour measurement. *Meat Science*, *45*(1), 23-31. https://doi.org/https://doi.org/10.1016/S0309-1740(96)00096-4

Cui, H., Yu, J., Zhai, Y., Feng, L., Chen, P., Hayat, K., Xu, Y., Zhang, X., & Ho, C.-T. (2021). Formation and fate of Amadori rearrangement products in Maillard reaction. *Trends in Food Science & Technology*, *115*, 391-408. https://doi.org/10.1016/j.tifs.2021.06.055

Dhakal, D., Shrestha, S. M., & Anal, A. K. (2017). Bioprocessing of Beef and Pork Meat Processing Industries, 'Waste to Value-Add'. In *Food Processing By-Products and their Utilization* (pp. 395-415). https://doi.org/10.1002/9781118432921.ch17

Earle, M. (2003). Product changes during processing. In V. Emerton (Ed.), *Fundamentals of Food Reaction Technology* (Vol. 1, pp. 32-72). The Royal Society of Chemistry. https://doi.org/10.1039/9781847559470-00032

Elmore, J. S., & Mottram, D. S. (2009). Flavour development in meat. In *Improving the Sensory and Nutritional Quality of Fresh Meat* (pp. 111-146). https://doi.org/10.1533/9781845695439.1.111

Elnajjar, A., Mourad, A., Brandt, M. E., & Lippelt, C. (2019). *Hydrolyzed collagen compositions and methods of making thereof* (US Patent No. US 10 , 253 , 090 B2). U. S. Patent.

Erlewein, R. (2010). Congee - Longevity Food for Life. *Journal of Chinese Medicine (JCM)*, *92*, 31–35.

Eslahi, N., Dadashian, F., & Nejad, N. H. (2013). Optimization of enzymatic hydrolysis of wool fibers for nanoparticles production using response surface methodology. *Advanced Powder Technology 24*, 416–426. https://doi.org/http://dx.doi.org/10.1016/j.apt.2012.09.004

Fallas, J., Gauba, V., & Hartgerink, J. (2009). Solution Structure of an ABC Collagen Heterotrimer Reveals a Single-register Helix Stabilized by Electrostatic Interactions. *The Journal of biological chemistry*, *284*, 26851-26859. https://doi.org/10.1074/jbc.M109.014753

FAOSTAT. (2021). *Raw hides and skins of sheep or lambs*. Retrieved from https://data.un.org/Data.aspx?q=sheep+skin&d=FAO&f=itemCode%3a995

Fonkwe, L. G., & Singh, R. K. (1996). Protein recovery from mechanically deboned turkey residue by enzymic hydrolysis. *Process Biochemistry*, *31*(6), 605-616. https://doi.org/https://doi.org/10.1016/S0032-9592(95)00101-8

Fourneau, M., Canon, C., Van Vlaender, D., Collins, M. J., Fiddyment, S., Poumay, Y., & Deparis, O. (2020). Histological study of sheep skin transformation during the recreation of historical parchment manufacture. *Heritage Science*, *8*(1). https://doi.org/10.1186/s40494-020-00421-z

Ge, X. (2024). *Manufacture of Meaty Reaction Flavours from Lamb Skin and Various Sugars*. Singapore Institute of Technology-Massey University.

Gelse, K., Poschl, E., & Aigner, T. (2003). Collagens--structure, function, and biosynthesis. *Advanced Drug Delivery Reviews*, *55*(12), 1531-1546. https://doi.org/10.1016/j.addr.2003.08.002

Giteru, S. G., Ramsey, D. H., Hou, Y., Cong, L., Mohan, A., & Bekhit, A. E. A. (2023). Wool keratin as a novel alternative protein: A comprehensive review of extraction, purification, nutrition, safety, and food applications. *Compr Rev Food Sci Food Saf*, *22*(1), 643-687. https://doi.org/10.1111/1541-4337.13087

Guo, L., Lu, L., Yin, M., Yang, R., Zhang, Z., & Zhao, W. (2020). Valorization of refractory keratinous waste using a new and sustainable bio-catalysis. *Chemical Engineering Journal*, *397*. https://doi.org/10.1016/j.cej.2020.125420

Hall, R. (2023). *New Zealand Sheep Production and Trends*. New Zealand

Hejazi, S., Yaghoubi, S., Delghandi, M., & Javid, F. (2013). Histogenesis study of skin in sheep. *Life Science Journal 10(5s)*(35), 194-198. http://www.lifesciencesite.com

Ibrahim, N. I., Oslan, S. N. H., Shapawi, R., Mokhtar, R. A. M., Md. Noordin, W. N., Nurdiani, R., & Huda, N. (2023). Isolation and Characterization of Acid-Soluble Collagens from the Bone and Fins of the Barracuda (Sphyraena spp.) as Marine Collagen Sources. *International Journal on Advanced Science, Engineering and Information Technology*, *13*(2), 553-563. https://doi.org/10.18517/ijaseit.13.2.18074

Indexbox. (2024). *World - Sheep or Lamb Skins (Without Wool) - Market Analysis, Forecast, Size, Trends and Insights* https://www.indexbox.io/store/world-sheep-or-lamb-skins-without-wool-market-report-analysis-and-forecast-to-2020/

Kahn, L. D., & Witnauer, L. P. (1966). The Viscometric Behavior of Solubilized Calf Skin Collagen at Low Rates of Shear. *The Journal of Biological Chemistry*, *241*, 1784-1789.

Latimer, G. W. (2023). Meat and Meat Products In *Official methods of analysis of AOAC international*. https://doi.org/https://doi.org/10.1093/9780197610145.003.515

Latimer, G. W., Jr. (2023). Official Methods of Analysis of AOAC INTERNATIONAL. In G. W. Latimer, Jr. (Ed.). Oxford University Press. https://doi.org/10.1093/9780197610145.001.0001

Leon-Lopez, A., Morales-Penaloza, A., Martinez-Juarez, V. M., Vargas-Torres, A., Zeugolis, D. I., & Aguirre-Alvarez, G. (2019). Hydrolyzed Collagen-Sources and Applications. *Molecules*, *24*(22). https://doi.org/10.3390/molecules24224031

Lewandowska, K., Szulc, M., & Sionkowska, A. (2021). Effect of Solvent on the Hydrodynamic Properties of Collagen. *Polymers (Basel)*, *13*(21). https://doi.org/10.3390/polym13213626

Lindley, H., & Phillips, H. (1945). The action of alkalis on wool: 2. The identity of the lanthionine-forming and bisulphite-reactive fractions of the combined cystine of wool. *Biochemical Journal*, *39*(1), 17-23. https://doi.org/10.1042/bj0390017

Mad-Ali, S., Benjakul, S., Prodpran, T., & Maqsood, S. (2017). Characteristics and gelling properties of gelatin from goat skin as affected by drying methods. *Journal of Food Science and Technology*, *54*(6), 1646-1654. https://doi.org/10.1007/s13197-017-2597-5

Mandal, P. K. (2014). Animal by-products utilization. In P. K. Mandal & A. K. Biswas (Eds.), *Animal Products Technoloy* (pp. 586-608). ResearchGate. https://www.researchgate.net/publication/301794214

Mariod, A., & Fadul, H. (2013). Review: Gelatin, source, extraction and industrial applications. *ACTA Scientiarum Polonorum Technologia Alimentaria*, *12*, 135-147.

Marques, C., Correia, E., Dinis, L. T., & Vilela, A. (2022). An Overview of Sensory Characterization Techniques: From Classical Descriptive Analysis to the Emergence of Novel Profiling Methods. *Foods*, *11*(3). https://doi.org/10.3390/foods11030255

McKittrick, J., Chen, P. Y., Bodde, S. G., Yang, W., Novitskaya, E. E., & Meyers, M. A. (2012). The Structure, Functions, and Mechanical Properties of Keratin. *Jom*, *64*(4), 449-468. https://doi.org/10.1007/s11837-012-0302-8

McRae, K. M., Cooper, S. M., McEwan, J. C., Anderson, R., Bain, W. E., Baird, H. J., Dodds, K. G., Clarke, S. M., Pickering, N. K., & Holmes, G. (2022). Genetic variation in skin traits in New Zealand lambs. *Journal of the Science of Food and Agriculture*, *102*(11), 4813-4819. https://doi.org/https://doi.org/10.1002/jsfa.11844

Miller, E. J., & Matukas, V. J. (1969). Chick cartilage collagen: a new type of alpha 1 chain not present in bone or skin of the species. *Proc Natl Acad Sci U S A*, *64*(4), 1264-1268. https://doi.org/10.1073/pnas.64.4.1264

Morton, I. D., Akroyd, P., & May, C. G. (1960). *Flavoring substances and ther preparation* (US 2,934,437). U. S. P. Office.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, *62*(4), 415-424

Murugan, D., Rai, C. L., & Sivarajan, M. (2013). Moisture desorption characteristics of raw sheep and goat skins : A tool for eco-friendly method of preservation. *Journal of the american leather chemists association*, *108*, 32-40.

Osobamiro, T. M., Oluwole, O. T., & Abdullah, S. O. (2023). Proximate Analysis and Determination of Polycyclic Aromatic Hydrocarbons in Processed Animal Skin Sold in Major Markets in South West Nigeria. *Journal of Chemical Society of Nigeria*, *48*(3). https://doi.org/10.46602/jcsn.v48i3.887

Pan, B., Ricci, M. S., & Trout, B. L. (2011). A Molecular Mechanism of Hydrolysis of Peptide Bonds at Neutral pH Using a Model Compound. *The Journal of Physical Chemistry B*, *115*(19), 5958-5970. https://doi.org/10.1021/jp1076802

Parker, M. E. (2014). *Design of a process for manufacture of beef stock*. Massey University.

Persikov, A., Ramshaw, J., Kirkpatrick, & Brodsky, B. (2005). Electrostatic Interactions Involving Lysine Make Major Contributions to Collagen Triple-Helix Stability †. *Biochemistry*, *44*, 1414-1422. https://doi.org/10.1021/bi048216r

Pogodaev, V. A., Arilov, A. N., Mangutov, S. N., & Khechev, E. V. (2021). Product properties and histostructure of young sheepskin of the Dorper Breed. *IOP Conference Series: Earth and Environmental Science*, *624*(1), 012132. https://doi.org/10.1088/1755-1315/624/1/012132

Qin, X., Xu, X., Guo, Y., Shen, Q., Liu, J., Yang, C., Scott, E., Bitter, H., & Zhang, C. (2022). A sustainable and efficient recycling strategy of feather waste into keratin peptides with antimicrobial activity. *Waste Management*, *144*, 421-430. https://doi.org/10.1016/j.wasman.2022.04.017

Rajabinejad, H., Zoccola, M., Patrucco, A., Montarsolo, A., Rovero, G., & Tonin, C. (2018). Physicochemical properties of keratin extracted from wool by various methods. *Textile Research Journal 88(21)*, 2415–2424. https://doi.org/10.1177/0040517517723028

Rather, J. A., Majid, S. D., Dar, A. H., Amin, T., Makroo, H. A., Mir, S. A., Barba, F. J., & Dar, B. N. (2022). Extraction of Gelatin From Poultry Byproduct: Influence of Drying Method on Structural, Thermal, Functional, and Rheological Characteristics of the Dried Gelatin Powder. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.895197

Ricard-Blum, S. (2011). The collagen family. *Cold Spring Harbour Perspective in Biology*, *3*(1). https://doi.org/10.1101/cshperspect.a004978

Rippon, J. A. (2013). The Structure ofWool. In D. M. Lewis & J. A. Rippon (Eds.), *The Coloration of Wool and other Keratin Fibres* (1 ed., pp. 1-42). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118625118.ch1

Ruiz-Capillas, C., Herrero, A. M., Pintado, T., & Delgado-Pando, G. (2021). Sensory Analysis and Consumer Research in New Meat Products Development. *Foods*, *10*(2). https://doi.org/10.3390/foods10020429

Rutherfurd, S. M. (2010). Methodology for Determining Degree of Hydrolysis of Proteins in Hydrolysates: A Review. *JOURNAL OF AOAC INTERNATIONAL*, *93*(5), 1515-1522. https://doi.org/10.1093/jaoac/93.5.1515

Samatra, M. Y., Noor, N., Razali, U. H. M., Bakar, J., & Shaarani, S. M. (2022). Bovidae-based gelatin: Extractions method, physicochemical and functional properties, applications, and future trends. *Comprehensive Reviews in Food Science and Food Safety*, *21*(4), 3153-3176. https://doi.org/10.1111/1541-4337.12967

Saravacos, G. D. (1970). Effect of Temperature on Viscosity of Fruit Juices and Purees. *Journal of Food Science*, *35*(2), 122-125. https://doi.org/10.1111/j.1365-2621.1970.tb12119.x

Savolainen, J. E. T. (1993). *Procedure for hydrolyzing keratin*. U. S. Patent.

Schmidt, M., Fontoura, A., Vidal, A., Prestes Dornelles, R., Kubota, E., Mello, R., Cansian, R., Demiate, I., & Soltovski, C. (2020). Characterization of hydrolysates of collagen from mechanically separated chicken meat residue. *Food Science and Technology*, *40*. https://doi.org/10.1590/fst.14819

Service, B. L. N. Z. E. (2022). *Export Lamb Slaughter*. Beef+Lamb New Zealand.

Sha, X.-M., Hu, Z.-Z., Ye, Y.-H., Xu, H., & Tu, Z.-C. (2019). Effect of extraction temperature on the gelling properties and identification of porcine gelatin. *Food Hydrocolloids*, *92*, 163-172. https://doi.org/10.1016/j.foodhyd.2019.01.059

Shavandi, A., Silva, T. H., Bekhit, A. A., & Bekhit, A. E. A. (2017). Keratin: dissolution, extraction and biomedical application. *Biomaterials Science*, *5*(9), 1699-1735. https://doi.org/10.1039/c7bm00411g

Shen, X., Zhang, M., Bhandari, B., & Gao, Z. (2019). Novel technologies in utilization of byproducts of animal food processing: a review. *Critical Reviews in Food Science and Nutrition*, *59*(21), 3420-3430. https://doi.org/10.1080/10408398.2018.1493428

Shoulders, M. D., & Raines, R. T. (2009). Collagen structure and stability. *Annual Review of Biochemistry*, *78*. https://doi.org/10.1146/annurev.biochem.77.032207.120833

Silvipriya, K., Kumar, K., Bhat, A., Kumar, B., John, A., & Lakshmanan, P. (2015). Collagen: Animal Sources and Biomedical Application. *Journal of Applied Pharmaceutical Science*, 123-127. https://doi.org/10.7324/japs.2015.50322

Sohail, A., Al-Dalali, S., Wang, J., Xie, J., Shakoor, A., Asimi, S., Shah, H., & Patil, P. (2022). Aroma compounds identified in cooked meat: A review. *Food Research International*, *157*. https://doi.org/10.1016/j.foodres.2022.111385

Sompie, M., & Triasih, A. (2018). Effect of extraction temperature on characteristics of chicken legskin gelatin. *IOP Conference Series: Earth and Environmental Science*(102). https://doi.org/10.1088/1755-1315/102/1/012089

Speight, J. G. (2020). The properties of water. In *Natural Water Remediation*. https://doi.org/10.1016/B978-0-12-803810-9.00002-4

Stewart, R. G. (1985). Wool Scouring and Allied Technology. In WRONZ (Ed.), (Vol. 2). Christchurch, New Zealand.

Sun, A., Wud, W., Soladoye, O. P., Aluko, R. E., Bak, K. H., Fu, Y., & Zhang, Y. (2022). Maillard reaction of food-derived peptides as a potential route to generate meat flavor compounds: A review. *Food Research International*, *151*. https://doi.org/10.1016/j.foodres.2021.110823

Tan, C.-C., Karim, A. A., Uthumporn, U., & Ghazali, F. C. (2020). Effect extraction temperature on the emulsifying properties of gelatin from black tilapia (Oreochromis mossambicus) skin. *Food Hydrocolloids*, *108*. https://doi.org/10.1016/j.foodhyd.2020.106024

Tasaki, K., & Kaneoka, L. (2019). *Process to extract and recover Keratin and keratin associated protein from animal body parts* (US Patent No. US 2019 / 0194297 A1).

Teasdale, D. C. (1988). *Wool Testing and Marketing Handbook*. University of New South Wales, New South Wales, Australia.

Toro, M. A. N. d., & García-Carreño, F. L. (2002). Evaluation of the Progress of Protein Hydrolysis. *Current Protocols in Food Analytical Chemistry*.

Truter, E. V. (1956). Wool Wax. *UK: Cleaver-Hume Press, London*.

van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, *24*(2), 230-233. https://doi.org/https://doi.org/10.1016/j.biotechadv.2005.11.004

Vate, N. K., Strachowski, P., Undeland, I., & Abdollahi, M. (2023). Structural and functional properties of collagen isolated from lumpfish and starfish using isoelectric precipitation vs salting out. *Food Chemistry: X*, *18*. https://doi.org/https://doi.org/10.1016/j.fochx.2023.100646

Wang, W., Li, Z., Liu, J., Wang, Y., Liu, S., & Sun, M. (2013). Comparison between Thermal Hydrolysis and Enzymatic Proteolysis Processes for the Preparation of Tilapia Skin Collagen Hydrolysates. *Czech Journel of Food Science*, *31*(1), 1-4. https://doi.org/10.17221/49/2012-CJFS

Wang, Y., Li, Y., Chen, Y., Huang, X., Zuo, D., Tian, X., Li, Y., & Wang, W. (2024). Insight into the self-assembly process, microrheological properties and microstructure of acid-soluble collagen from lamb skin under different environmental conditions. *Food Bioscience*, *61*, 104978. https://doi.org/https://doi.org/10.1016/j.fbio.2024.104978

Wikipedia. (2024, July 5). *Collagen*. Wikipedia, The Free Encyclopedia. Retrieved July 19, 2024 from https://en.wikipedia.org/w/index.php?title=Collagen&oldid=1232733115

Wodzicka, M. (1958). Studies on the thickness and chemical composition of the skin of sheep. *New Zealand Journal of Agricultural Research*, *1*(4), 582-591. https://doi.org/10.1080/00288233.1958.10431543

Wodzicka, M. (2012). Studies on the thickness and chemical composition of the skin of sheep. *New Zealand Journal of Agricultural Research*, *1*(4), 582-591. https://doi.org/10.1080/00288233.1958.10431543

Wong, D. W. S. (2018). Proteins. In *Mechanism and Theory in Food Chemistry, Second Edition* (pp. 55-122). https://doi.org/10.1007/978-3-319-50766-8_2

Wright, N. T., & Humphrey, J. D. (2002). Denaturation of collagen via heating: an irreversible rate process. *Annual Review of Biomedical Engineering*, *4*, 109-128. https://doi.org/10.1146/annurev.bioeng.4.101001.131546

Yang, Y., Wu, Y. N., Ce, L. G. E., Ge, X. G. B. R., Shuang, Q., & Zhang, F. M. (2023). Analysis of microbial community and its correlation with flavor compounds during Congee fermentation. *Food Bioscience*, *51*. https://doi.org/10.1016/j.fbio.2022.102261

Young, O. A., Reid, D. H., Smith, M. E., & Braggins, T. J. (1994). Sheepmeat odour and flavour. In F. Shahidi (Ed.), *Flavor of Meat and Meat Products*.

Zhang, C., Xia, L., Zhang, J., Liu, X., & Xu, W. (2020). Utilization of waste wool fibers for fabrication of wool powders and keratin: a review. *Journal of Leather Science and Engineering*, *2*(1). https://doi.org/10.1186/s42825-020-00030-3

Zhang, X., Xu, S., Shen, L., & Li, G. (2020). Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. *Journal of Leather Science and Engineering*, *2*(1). https://doi.org/10.1186/s42825-020-00033-0

Zheng, M., Wang, X., Chen, Y., Yue, O., Bai, Z., Cui, B., Jiang, H., & Liu, X. (2023). A Review of Recent Progress on Collagen-Based Biomaterials. *Advanced Healthcare Materials*, *12*(16). https://doi.org/10.1002/adhm.202202042

Zilhadia, Yahdiana, H., Irwandi, J., & Effionora, A. (2018). Characterization and functional properties of gelatin extracted from goatskin. *International Food Research Journal*, *25*(1).

Zoccola, M., Aluigi, A., Patrucco, A., Vineis, C., Forlini, F., Locatelli, P., Sacchi, M. C., & Tonin, C. (2012). Microwave-assisted chemical-free hydrolysis of wool keratin. *Textile Research Journal*, *82*(19), 2006-2018. https://doi.org/10.1177/0040517512452948

Appendices:

Appendix A

1/1

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SUBJECT: Final Report DATE: 11/12/23 ON: PN96312MATRO

TRIAL: TN23-848 *SAMPLES RECEIVED:* 21/11/23

Number of pages in this report: 1 Client Reference:

Testing initiated: *22/11/23* Testing completed: *11/12/23*

TN23-848 Lambskin Results are on an as received basis

NutLab ID	Sample Name	Moisture %	Ash %	Crude Protein %	Fat %	Carb %	Crude Fibre %
TN23-848-01	Raw Neutral Lambskin	74.8	0.7	20.8	4.5	Not Detected	0.4

Methodology

Ash : Furnace 550°C AOAC 920.153, 923.03 (Meat)
Moisture : (Meat), AOAC 950.46B
Crude protein : AOAC 968.06 (Dumas method). N-P = 6.25
Fat : Soxtec, (Meat), AOAC 991.36
Crude fibre : AOAC 962.09/978.10 (modified)
* *Carbohydrate (Carb) : By difference*

*Tests marked with an asterix are currently outside the scope of the Nutrition Laboratory's accreditation

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TO:	Richard Archer	AT:	Riddet Institute		
SUBJECT:	Final Report	DATE:	6/05/24	ON:	PN96312 MATRO
TRIAL:	TN24-279	SAMPLES RECEIVED:	9/04/24		

Number of pages in this report: 1 Client Reference:

Testing initiated: *16/04/24* Testing completed: *6/05/24*

TN24-279 Wool powder Results are on an as received basis

NutLab ID	Sample Name	Moisture %	Ash %	Crude Protein %	Fat %	Crude Fibre %	Nitrogen %
TN24-279-01	Powered Wool	8.1	0.6	94.6	1.0	1.3	15.1

Methodology

Moisture : (Meat), AOAC 950.46B

Ash : Furnace 550°C AOAC 920.153, 923.03 (Meat)

Crude protein : AOAC 968.06 (Dumas method). N-P = 6.25

Fat : Soxtec, (Meat), AOAC 991.36

Crude fibre : AOAC 962.09/978.10 (modified)

Total Nitrogen : AOAC 968.06 (Dumas method)

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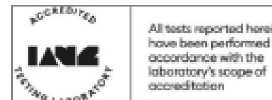

Appendix B

The degree of solubilisation at different pH

Treatment: 5 psi, 8 hours, pH adjusted using 0.526 mol L^{-1} H_2SO_4

Sample contains lambskin - 68.4 %, water- 31.6 %

Actual Wt. of skin= Wt. of sample *0.684 (same batch of sample was used throughout the experiment)

Moisture content of the residue and liquid extract were found using AOAC 950.46B (G. W. Latimer, Jr., 2023)

$$TS\% = 100 - moisture\ content\ \%$$

Initial pH Values	Avg. pH Values	Wt. of Sample	Actual Wt. Of Skin	wt. of Residue	TS % of Residue	Wt. of liquid extract	TS % of Liquid Extract	DS %
-	-	g	g	g	%	g	%	%
2.53	2.55	50.48	34.53	8.65	26.16	6.39	17.87	87.11
2.57		50.07	34.25	8.55	26.22	6.31	17.95	87.15
3.56	3.55	50.00	34.20	5.79	30.34	4.03	22.45	90.12
3.54		50.18	34.32	5.71	30.27	3.98	22.42	90.34
4.51	4.53	50.40	34.47	6.75	29.24	4.78	20.74	88.68
4.55		50.47	34.52	6.71	29.48	4.73	20.83	88.59
6	6.03	111.00	75.92	21.64	25.14	16.20	16.75	85.75
6.06		109.04	74.58	21.37	25.25	15.97	16.71	85.49

wt. of liquid extract= $wt.of\ residue - (TS\%\ in\ residue * wt.of\ residue/100)$

DS % =$(1 - \frac{(TS\ \%\ in\ residue*wt.of\ residue) + (TS\%\ in\ liquid\ extract*wt.of\ liquid\ extract)}{(TS\%\ in\ skin*wt.of\ skin)}) * 100$

Avg. pH Values	Avg DS	Half The Range of DS	Final pH	Avg Final pH	Half Range of Final pH	Final conc. (°Brix)	Avg Final conc. (°Brix)	Half Range of Final conc.
2.55	87.13	0.02	3.56	3.55	0.01	15.70	15.73	0.03
			3.54			15.75		
3.55	90.23	0.11	4.24	4.27	0.03	20.50	20.35	0.15
			4.30			20.19		
4.53	88.64	0.05	5.06	5.04	0.02	19.78	19.80	0.01
			5.02			19.81		
6.03	85.62	0.13	6.77	6.79	0.02	15.75	15.80	0.04
			6.81			15.84		

Appendix C

Titration curve

The volume of 0.52 mol L^{-1} H_2SO_4 required to reduce the pH of 50.05 g skin was represented as the volume required for 1000 g of lambskin using the formula:

Vol. of H_2SO_4 (0.52 mol L^{-1}) per kg of wet lambskin (mL) = $\frac{\text{Acid vol. (mL)} * 1000}{50.05}$

No. of moles= molarity*volume

Mass of H_2SO_4= molarity*volume*molecular mass

Molarity= 0.52 mol L^{-1}, Molecular weight= 98.079 g

Sl. No.	Final pH	Acid vol. (mL)	Vol. of H_2SO_4 (0.52 M) per kg of wet lambskin (mL)	Mass of H_2SO_4 (g) per kg of wet lambskin
1	8.33 ± 0.02	0	0.00	0.00
2	7.62 ± 0.06	0.5	9.99	0.51
3	7.36 ± 0.01	1	19.98	1.02
4	7.08 ± 0.01	1.5	29.97	1.53
5	6.85 ± 0.04	2	39.96	2.04
6	6.65 ± 0.00	2.5	49.95	2.55
7	6.15 ± 0.02	3	59.94	3.06
8	5.64 ± 0.05	3.5	69.93	3.57
9	5.28 ± 0.04	4	79.92	4.08
10	4.97 ± 0.03	4.5	89.91	4.59
11	4.74 ± 0.01	5	99.90	5.10
12	4.51 ± 0.02	5.5	109.89	5.60
13	4.33 ± 0.01	6	119.88	6.11
14	4.10 ± 0.03	6.5	129.87	6.62
15	3.98 ± 0.03	7	139.86	7.13
16	3.79 ± 0.03	7.5	149.85	7.64
17	3.70 ± 0.12	8	159.84	8.15
18	3.54 ± 0.04	8.5	169.83	8.66
19	3.37 ± 0.04	9	179.82	9.17

Appendix D

Degree of solubilisation calculation

Trial	Wt. of residue (g)	TS % in residue	Avg TS % in residue	Half range of TS % residue	TS % in liquid extract	Avg TS % in liquid extract	Half range of TS % liquid extract
	g	%	%	%	%	%	%
T1 A	8.57	18.83			14.02		
	8.46	18.99	18.91	0.08	14.12	14.07	0.05
T1 B	4.80	24.93			16.92		
	4.78	24.31	24.62	0.31	17.09	17.01	0.08
T1 C	3.83	28.25			20.97		
	3.85	28.56	28.40	0.16	20.85	20.91	0.06
T1 D	3.38	29.23			20.97		
	3.34	29.28	29.25	0.02	21.25	21.11	0.14
T1 E	3.19	30.75			22.98		
	3.20	30.45	30.60	0.15	22.66	22.82	0.16
T2 A	3.41	30.99			20.84		
	3.41	30.91	30.95	0.04	20.87	20.86	0.02
T2 B	3.21	30.80			21.57		
	3.21	31.11	30.95	0.15	21.51	21.54	0.03
T2 C	3.03	31.01			22.68		
	3.14	31.14	31.08	0.06	22.65	22.67	0.02
T2 D	3.02	31.80			23.67		
	2.98	31.84	31.82	0.02	23.69	23.68	0.01
T2 E	2.91	31.65			24.64		
	2.96	32.91	32.28	0.63	24.59	24.62	0.03
T3 A	16.00	15.24			11.79		
	16.02	15.37	15.30	0.07	11.71	11.75	0.04
T3 B	13.42	17.31			14.41		
	13.46	17.35	17.33	0.02	14.48	14.45	0.04
T3 C	10.03	19.26			16.25		
	10.08	19.47	19.37	0.10	16.29	16.27	0.02
T3 D	9.40	19.78			16.76		
	9.37	19.55	19.66	0.12	16.97	16.87	0.10
T3 E	8.54	20.16			17.73		
	8.59	20.31	20.24	0.07	17.76	17.75	0.02

DS % $=(1 - \frac{(TS\ \%\ in\ residue * wt.of\ residue) + (TS\%\ in\ liquid\ extract * wt.of\ liquid\ extract)}{(TS\%\ in\ skin * wt.of\ skin)}) * 100$

Moisture content of the residue and liquid extract were found using AOAC 950.46B (G. W. Latimer, Jr., 2023)

$$TS\% = 100 - moisture\ content\ \%$$

TS% in skin= 25.2%

Wt. of liquid extract= $\text{Wt. of residue} - (\text{TS\% in residue} * \text{Wt. of residue}/100)$

Trial	Wt. of skin (initial)	DS %	Avg. DS % (dry basis)	Half range of DS %	Final pH	Avg. Final pH	Half range of final pH	Final conc.	Avg. conc.	Half range of final conc.
	g	%	%	%	-	-	-	°Brix	°Brix	°Brix
T1 A	16.90	85.03	85.01	0.01	4.80	4.81	0.01	12.90		
	16.90	85.00			4.82			12.85	12.88	0.025
T1 B	16.90	86.20	86.71	0.51	4.85	4.86	0.01	16.40		
	16.90	87.22			4.87			16.32	16.36	0.04
T1 C	16.90	88.12	87.88	0.24	4.88	4.87	0.02	19.66		
	16.90	87.64			4.85			19.59	19.63	0.035
T1 D	16.90	88.60	88.71	0.11	4.92	4.94	0.02	20.93		
	16.90	88.82			4.96			20.01	20.47	0.46
T1 E	16.90	88.89	88.93	0.03	4.95	4.96	0.01	21.06		
	16.90	88.96			4.97			21.11	21.09	0.025
T2 A	17.16	86.92	86.96	0.04	5.05	5.07	0.02	18.56		
	17.16	86.99			5.09			18.65	18.61	0.045
T2 B	17.16	88.23	88.07	0.16	5.12	5.12	0.00	19.13		
	17.16	87.91			5.11			19.14	19.14	0.005
T2 C	16.89	89.05	88.80	0.25	5.14	5.15	0.01	20.16		
	16.89	88.55			5.15			20.20	20.18	0.0205
T2 D	17.08	89.01	89.07	0.06	5.17	5.18	0.00	21.73		
	17.08	89.13			5.18			21.69	21.71	0.0195
T2 E	17.04	89.96	89.33	0.63	5.20	5.23	0.03	23.03		
	17.04	88.70			5.26			23.98	23.51	0.475
T3 A	17.37	80.83	80.42	0.40	4.66	4.65	0.02	10.60		
	17.37	80.02			4.63			10.57	10.59	0.015
T3 B	17.22	83.30	83.30	0.00	4.76	4.78	0.02	12.70		
	17.22	83.30			4.79			12.78	12.74	0.04
T3 C	17.10	85.70	85.41	0.29	4.84	4.83	0.02	15.05		
	17.10	85.13			4.81			15.14	15.10	0.045
T3 D	17.16	86.23	86.73	0.50	4.93	4.95	0.02	15.80		
	17.16	87.22			4.97			15.85	15.83	0.025

T3 E	17.19	88.16	87.97	0.19	4.92	4.96	0.04	17.40	17.44	0.035
	17.19	87.79			4.99			17.47		

Extraction kinetics:

TS %			Time (h)	Wt. of residue			Total unused available protein (R)			Initial solids (R_0)			$\log_e R/R_0$		
%			h	g			g			g			-		
90 °C	108 °C	121 °C	-	90 °C	108 °C	121 °C	90 °C	108 °C	121 °C	90 °C	108 °C	121 °C	90 °C	108 °C	121 °C
15.3	18.91	30.95	2	16.01	8.51	3.41	244.95	161.01	105.48	437.79	425.93	432.46	-0.58	-0.97	-1.41
17.33	24.62	30.95	4	13.44	4.79	3.21	232.95	118.02	99.31	433.94	425.93	432.46	-0.62	-1.28	-1.47
19.37	28.4	31.08	6	10.06	3.84	3.08	194.80	109.12	95.87	430.81	425.93	425.74	-0.79	-1.36	-1.49
19.66	29.25	31.82	8	9.39	3.36	3.00	184.53	98.22	95.46	432.37	425.93	430.32	-0.85	-1.47	-
20.24	30.6	32.28	10	8.57	3.19	2.93	173.39	97.75	94.71	433.09	425.93	429.46	-0.92	-1.47	-1.51

$$Total\ unused\ available\ protein\ (R)\ =\ TS\%*Wt.of\ residue$$

$$Initial\ solids\ (R0)\ =\ Wt.of\ skin*TS\%\ in\ skin$$

Rate constants:

Sl. No.	Temperature	k	ln(k)	T	1/T
	°C	h^{-1}	h^{-1}	K	K^{-1}
1	90	0.0984	-2.3187	363.1500	0.0028
2	108	0.1561	-1.8573	381.1500	0.0026
3	121	0.0399	-3.2214	394.1500	0.0025

Appendix E

Colour measurements

Sl No.	Trials	L* value	a* Value	b* Value	ΔE^*_{ab}
1	T1 A	33.89 ± 0.02^{BC}	-0.22 ± 0.04^{CDEF}	0.93 ± 0.02^{G}	0.15
2	T1 B	33.75 ± 0.01^{BC}	-0.3 ± 0.04^{EFGH}	1.57 ± 0.16^{E}	0.25
3	T1 C	33.67 ± 0.03^{BCD}	-0.33 ± 0.02^{FGH}	2.11 ± 0.08^{D}	0.27
4	T1 D	33.55 ± 0.04^{CDEF}	-0.34 ± 0.01^{GHI}	2.20 ± 0.02^{D}	0.17
5	T1 E	33.39 ± 0.03^{DEFG}	-0.39 ± 0.01^{HI}	2.24 ± 0.06^{D}	0.31
6	T2 A	34.45 ± 0.09^{A}	-0.07 ± 0.03^{A}	1.66 ± 0.01^{E}	0.05
7	T2 B	33.89 ± 0.06^{B}	-0.11 ± 0.01^{AB}	3.60 ± 0.01^{C}	0.03
8	T2 C	33.62 ± 0.17^{BCDE}	-0.29 ± 0.02^{EFGH}	4.34 ± 0.04^{B}	0.07
9	T2 D	32.67 ± 0.12^{H}	-0.34 ± 0.03^{GHI}	5.60 ± 0.03^{A}	0.08
10	T2 E	32.57 ± 0.21^{H}	-0.44 ± 0.01^{I}	5.74 ± 0.04^{A}	0.07
11	T3 A	33.35 ± 0.04^{DEFG}	-0.1 ± 0.04^{A}	0.10 ± 0.03^{I}	0.09
12	T3 B	33.32 ± 0.02^{EFG}	-0.14 ± 0.02^{ABC}	0.50 ± 0.04^{H}	0.07
13	T3 C	33.27 ± 0.04^{FG}	-0.16 ± 0.03^{ABCD}	0.93 ± 0.05^{G}	0.09
14	T3 D	33.19 ± 0.01^{G}	-0.21 ± 0.03^{BCDE}	1.07 ± 0.16^{FG}	0.23
15	T3 E	33.12 ± 0.01^{G}	-0.26 ± 0.02^{DEFG}	1.26 ± 0.02^{F}	0.04

*N=3

Appendix F

Degree of hydrolysis (DH)

Treatment	Abs.570	Avg Abs.570	Dil. Factor	Predicted Abs.	h_{570}	Avg h_{570}
5 psi, 2hrs	0.276	0.274	375	103.50	1.80	1.79
	0.273		375	102.38	1.78	
	0.274		375	102.75	1.78	
5 psi, 4hrs	0.281	0.284	375	105.38	1.83	1.85
	0.285		375	106.88	1.86	
	0.287		375	107.63	1.87	
5 psi, 6hrs	0.31	0.313	375	116.25	2.02	2.04
	0.313		375	117.38	2.04	
	0.315		375	118.13	2.05	
5 psi, 8hrs	0.334	0.337	375	125.25	2.18	2.20
	0.341		375	127.88	2.22	
	0.337		375	126.38	2.20	
5 psi, 10hrs	0.4	0.403	375	150.00	2.61	2.63
	0.402		375	150.75	2.62	
	0.407		375	152.63	2.65	

Treatment	Abs.440	Avg Abs.	Dil. Factor	Predicted Abs.	h_{440}	Avg. h_{440}
5 psi, 2hrs	0.624	0.618	50	31.2	0.29	0.29
	0.613		50	30.65	0.29	
	0.617		50	30.85	0.29	
5 psi, 4hrs	0.584	0.583	50	29.2	0.27	0.27
	0.584		50	29.2	0.27	
	0.582		50	29.1	0.27	
5 psi, 6hrs	0.677	0.676	50	33.85	0.32	0.32
	0.674		50	33.7	0.31	
	0.676		50	33.8	0.32	
5 psi, 8hrs	0.639	0.642	50	31.95	0.30	0.30
	0.645		50	32.25	0.30	
	0.641		50	32.05	0.30	
5 psi, 10hrs	0.681	0.672	50	34.05	0.32	0.31
	0.661		50	33.05	0.31	
	0.674		50	33.7	0.31	

Treatment	htot	Avg htot	DH	Avg DH
5 psi, 2hrs	2.09	2.08	26.12	25.95
	2.06		25.81	
	2.07		25.91	
5 psi, 4hrs	2.10	2.12	26.29	26.56
	2.13		26.62	

	2.14		26.77	
5 psi, 6hrs	2.34	2.35	29.19	29.40
	2.35		29.42	
	2.37		29.60	
5 psi, 8hrs	2.47	2.50	30.93	31.21
	2.52		31.53	
	2.49		31.18	
5 psi, 10hrs	2.92	2.94	36.55	36.74
	2.93		36.59	
	2.97		37.08	

Treatment	Half of range of Abs.440	Half of range of h440	Half of range of Abs.570	Half of range of h570	Half of range of htot	Half of range of DH
5 psi, 2hrs	0.01	0.00	0.00	0.01	0.01	0.15
5 psi, 4hrs	0.00	0.00	0.00	0.02	0.02	0.24
5 psi, 6hrs	0.00	0.00	0.00	0.02	0.02	0.20
5 psi, 8hrs	0.00	0.00	0.00	0.02	0.02	0.30
5 psi, 10hrs	0.01	0.00	0.00	0.02	0.02	0.26

Appendix G

Stoichiometry for sulphite

1 molecule of sodium metabisulphite reacts with 2 molecules of cysteine.

$$Na_2S_2O_5 + 2 * cysteine = cysteine\ sulphur\ bond\ formation$$

Considering the molecular weight of sodium metabisulphite and cysteine, 190.107 g/mol of sodium metabisulphite reacts with 242.32 g/mol of cysteine. Therefore, 1 g of cysteine needs about 0.784g of sodium metabisulphite to break the disulphide bonds.

100 g of wool is assumed to have 6g of cysteine. Hence, the amount of sodium metabisulphite was calculated as 4.704g for 100g of wool.

Appendix H

Wool hydrolysis

Sl. No.	NaMBS %	Enzyme	Enzyme %	Initial pH
1	6.34 (0.1 mol L^{-1} solution)	Neutrase	1	7
2	63.37 (1 mol L^{-1} solution)	Neutrase	1	7
3	13.34	Neutrase	4	7
4	13.34	Alcalase	4	7.5
5	13.34	Protamex	4	7
6	13.34	Alcalase	3	8
7	13.34	Alcalase	3	7.5
8	13.34	Neutrase	3	7

Enzyme	NaMBS conc.	TS%	Avg. TS%	Half of range of TS%	Wt. of residue	Wt. of raw material	TS % of liquid extract	DS %	Avg. DS %	half of range of DS
	%	%	%	%	g	g	%	%	%	%
Alcalase, 3% (pH=8)	13.34	24.82	25.53	0.68	15.81	6.04	7.98	46.39	43.65	2.66
		26.17			15.85	5.97	7.82	41.06		
		25.59			15.78	6.02	7.76	43.49		
Alcalase, 3% (pH=7.5)	13.34	24.77	24.86	0.32	15.58	6.09	8.10	48.00	47.23	1.73
		24.59			15.51	6.05	8.16	48.57		
		25.22			15.60	5.92	8.13	45.11		
Neutrase, 3% (pH=7)	13.34	30.22	30.98	0.76	16.50	6.06	6.21	23.32	21.17	2.56
		30.98			16.42	6.11	6.24	21.98		
		31.74			16.54	6.04	6.29	18.21		
Neutrase, 1% (pH=7)	63.37	34.67	34.45	0.21	15.78	7.51	7.89	32.51	32.89	0.30
		34.46			15.81	7.53	7.86	33.03		
		34.24			15.77	7.46	7.84	33.12		

Neutrase, 1% (pH=7)	6.34	32.69	31.61	0.91	20.43	7.48	6.15	15.14	19.29	3.46
		31.26			20.44	7.58	6.15	20.67		
		30.87			20.37	7.57	6.17	22.07		
Neutrase, 4% (pH=7)	13.34	31.64	31.96	0.31	7.70	3.01	6.29	23.84	22.68	2.79
		31.99			7.65	3.06	6.46	24.89		
		32.26			7.96	2.97	6.79	19.30		
Alcalase, 4% (pH=7.5)	13.34	24.20	24.00	0.17	7.68	2.92	8.02	48.13	49.99	1.43
		23.96			7.55	3.00	7.96	51.00		
		23.85			7.77	3.05	8.02	50.83		
Protamex, 4% (pH=7)	13.34	30.18	30.21	0.03	8.01	3.00	6.02	24.54	25.58	1.04
		30.24			7.98	3.08	6.03	26.62		

Appendix I

Reaction mixtures for flavour development

Amino acid composition in mammal skin (Wikipedia):

Type of amino acid	g of aminoacid/g of protein	Molecular wt. (g/mol)	No. of moles
glycine	0.33	75.07	0.004
proline	0.13	115.13	0.001
alanine	0.11	89.09	0.001
hydroxyproline	0.10	131.13	0.001
glutamic acid	0.07	147.13	0.001
arginine	0.05	174.20	0.000
aspartic acid	0.05	133.10	0.000
serine	0.04	105.09	0.000
lysine	0.03	146.19	0.000
leucine	0.02	131.17	0.000
valine	0.02	117.15	0.000
threonine	0.02	199.12	0.000
phenylalanine	0.01	165.19	0.000
isoleucine	0.01	131.17	0.000
methionine	0.01	149.21	0.000
hydroxylysine	0.01	162.19	0.000
histidine	0.01	155.15	0.000
tyrosine	0.00	181.19	0.000
cysteine	0.00	1216	0.000
tryptophan	0.00	204.23	0.000
total	1.00		0.010

Grams of amino acids in 1 g collagen = 1.004
Total No. of moles in 1 g protein = 0.010

Amino acids composition in wool (Giteru et al., 2023):

aa$^-$	Min %	Max %	Avg %	Molecular Wt. (g)	No. of Moles
arginine	6.90	10.40	8.65	174.20	0.05
cysteine	11.30	11.70	11.50	1216	0.09
histidine	0.90	0.90	0.90	155.15	0.01
isoleucine	3.10	3.80	3.45	131.17	0.03
leucine	6.80	8.40	7.60	131.17	0.06
lysine	3.50	3.80	3.65	146.19	0.02
methionine	0.40	0.60	0.50	149.21	0.00
phenylalanine	2.10	3.50	2.80	165.19	0.02
threonine	5.80	6.40	6.10	119.12	0.05
valine	5.30	5.80	5.55	117.15	0.05

alanine	4.10	5.20	4.65	89.09	0.05
aspartic acid	7.00	7.30	7.15	133.10	0.05
glutamic acid	12.80	16.00	14.40	147.13	0.10
glycine	4.10	8.00	6.05	75.07	0.08
proline	6.40	6.40	6.40	115.13	0.06
serine	7.70	10.80	9.25	105.09	0.09
tryptophan	0.00	0.00	0.00	204.23	0.00
tyrosine	3.40	4.00	3.70	181.19	0.02
total			102.30		0.83

Wt. of cysteine in 100g keratin = 11.5

Wt. of methionine in 100g keratin= 0.5

Molecular mass of cysteine= 1216 g/mol
Molecular mass of methionine= 149.21 g/mol

Moles of cysteine in 100 g keratin= 0.095
Moles of methionine in 100 g keratin= 0.003

Moles of S in 1 g keratin= 0.001

% of protein in wool= 94.6 %
% keratin in wool= 78.045 %

Moles of S in 1 g wool= 0.0007
Moles of amino N in 100 g keratin = 0.827
Moles of amino N in 1 g keratin = 0.008
Moles of amino N in 1 g wool= 0.006

Cysteine ($C_3H_7NO_2S$):

Molar mass: 1216 g/mol
Atomic mass of S= 32.065 u
% of S in cysteine= 26.465
Moles of S in 1 mole of cysteine =1
Moles of S in 1 g cysteine = 0.008
Moles of amino N in 1 mole of cysteine =1
Moles of amino N in 1 g cysteine= 0.008

Cysteic acid ($C_3H_7NO_5S$):
Molecular wt.= 169.16 g/mol

Mole of S in 1 mol cysteic acid= 1
Moles of S in 1 g cysteic acid= 0.006

Moles of N in 1 g cysteic acid= 0.006

Preparation of reaction mixtures:

Moles of amino N in 8 g collagen= 0.079
Moles of amino N in 1 g cysteine= 0.008
Moles of S in 1 g cysteine= 0.008
Molar ratio of amino N to S in 8 g collagen and 1 g cysteine= 10.6

Moles of a N in 1 g collagen= 0.009
Protein to sugar ratio= 8 to 1

Assumptions:
1 g sulphite wool extract (S.E)= 100% keratin
1 g alkali wool extract (A.E)= 50 % Lanthionine + 50% Cysteine (Lindley & Phillips, 1945)

Ingredient along with reconstituted skin extract and ribose	Moles of amino N in 8g collagen	Moles of amino N in 1g of other amino N sources	Total amino N	moles of sulphur in 1 g of S source	Molar ratio of N to SS	Total amino N for N:S= 10.6	Moles of collagen
L-Cysteine	0.079	0.008	0.087	0.008	10.596	0.087	0.079
L-Cysteic Acid	0.079	0.006	0.085	0.006	14.397	0.063	0.057
S.E	0.079	0.008	0.087	0.001	89.013	0.010	0.002
A.E	0.079	0.009	0.088	0.009	9.870	0.095	0.086

Ingredients	Amount of collagen	Reconstituted skin extract	Cysteine	Cysteic acid	Sulphite wool extract	Alkali wool extract	Ribose
	g	g	g	g	g	g	g
Cysteine	8.00	13.33	1	0	-	-	1
Cysteic acid	5.73	9.55	-	1	-	-	0.72
S.E	0.22	0.36	-	-	1	-	0.027
A.E	8.65	14.42	-	-	-	1	1.08

Sample No.	Reconstituted collagen (g)	Cysteine (g)	Cysteic acid (g)	Alkali wool extract (g)	Sulphite wool extract (g)	Ribose (g)
186	8.89	0.66	-	-	-	0.66
628	9.55	-	1	-	-	0.72
461	2.7	-	-	-	7.5	0.21
239	7.208	-	-	0.5	-	0.54
347	1.8	-	-	-	5	5

Appendix J

Sensory testing

Attributes/Sample	186	628	461	239	347
Roast meat	4.36	1.52	0.92	1.72	2.00
Boiled meat	3.08	0.72	0.32	1.16	0.80
Fatty	1.84	0.96	1.13	1.12	0.88
Pungent	1.12	0.92	3.48	0.84	0.72
Caramel	1.32	2.72	0.48	1.24	3.52
Burnt	0.76	2.08	0.68	1.40	1.76
Animal-like	1.16	0.64	3.08	1.28	0.76
Ammonia-like	0.84	0.88	3.28	0.44	0.48

Appendix K

Images of equipment used in the current study

The double gap geometry used for viscosity measurements

The apparatus used for creating reaction flavours

See Table 3.1 for manufacturers’ details.

Appendix L

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