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The quality of New Zealand goat meat and the effect of sous vide and microwave processing on its quality and *in vitro* protein digestibility

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

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Riddet Institute
ADVANCING FRONTIERS IN FOOD SCIENCE

Abstract

New Zealand has an untapped goat meat industry that has yet to be developed as a significant source of healthier red meat. However, this meat is known for being tough and requiring longer cooking times to achieve tenderness- preferred for household and industrial use. Currently, there are no pertinent data describing the quality of goat meat produced in New Zealand (NZ). Furthermore, processing conditions significantly affect meat quality in terms of protein digestibility and, consequently, nutritional value. This thesis investigated the quality of NZ goat meat and the impact of sous vide and microwave processing on its quality and *in vitro* protein digestibility.

The goat meat produced in NZ are mainly coming from wild goats known as feral goats. In this study, the differences between the quality characteristics of goat meat from NZ feral goats and Boer crosses a popular meat breed were investigated. Our data show that tenderness is highly affected by meat pH, collagen content, and muscle type. Meat from different breeds can be differentiated by colour and feral goat meat had higher collagen content but with lower solubility compared to Boer crosses. Further evaluation of goat meat ultrastructure revealed that the current practice in handling goat meat carcasses results in supercontracted muscle. Regardless of breed, the challenge for goat meat quality includes its inherently high pH and susceptibility to cold shortening, which affect its technological properties.

This project also highlights the impact of processing on goat meat structure and protein digestibility. Using a static *in vitro* digestion system, the protein digestibility of sous vide (SV) cooked goat meat was initially investigated between different breeds. Meat from Boer crossbreeds had higher digestibility than meat from feral goats in terms of the degree

of hydrolysis. The level of collagen significantly affected the variability in meat digestion rate, specifically during the gastric phase. Further investigation of the effects of thermal processing on goat meat was also explored by comparing the impacts of sous vide and microwave processing. The sous vide and microwave processed goat meat varied in meat quality and protein structure. However, the difference in the vitro protein digestibility of goat meat as influenced by the processing method was observed in gastric phase. Moreover, peptidomics revealed that sous vide and microwave processing significantly affected the hydrolysis of major proteins in goat meat, but this variation was only apparent in the gastric phase complimenting, the initial protein hydrolysis data.

Overall, this research provided new information on the physicochemical properties, muscle structure, and protein digestibility of goat meat as affected by breed, muscle type, and processing conditions. The outcomes provide significant knowledge about New Zealand goat meat quality and highlight the role of muscle structure and pH for its quality improvement. Protein digestibility studies also revealed that the effect of different processing conditions on meat protein digestibility was evident only in the gastric phase.

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Table of contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

.

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of publications and conference presentations

Peer reviewed journal articles

Gawat, M., Boland, M., Chen, J., Singh, J. and Kaur, L. (2023). Effects of microwave processing in comparison to sous vide cooking on meat quality, protein structural changes, and *in vitro* digestibility. Food Chemistry (434),137442

Gawat, M., Boland, M., Singh, J., and Kaur, L. (2023). Goat Meat: Production and Quality Attributes. Foods 12 (16), 313

Gawat, M., Kaur, L., Singh, J. and Boland, M. (2022). Physicochemical and quality characteristics of New Zealand goat meat and its ultrastructural features. Food Research International, 161 (2022), Article 111736, 10.1016/j.foodres.2022.111736

Gawat, M., Mao, B., Singh, J. and Kaur, L. 2023. Chapter 3 - Food protein digestion and absorption: current assessment protocols in Processing Technologies and Food Protein Digestion. (pp. 51-80). https://doi.org/10.1016/B978-0-323-95052-7.000017

Conference presentations

Gawat, M., Kaur, L., Singh, J., Chen, J. & Boland, M. (2023, 15-17 November). Changes in protein structure and digestibly of meat processed using microwave and sous vide cooking. 7th International Conference on Food Structures, Digestion, and Health. [Poster presentation]. Queenstown, New Zealand.

Gawat, M., Kaur, L., Singh, J., Chen, J. & Boland, M. (2023, 25-27 October). Microwaving meat: its effect on meat quality, protein structure and *in vitro* digestibility. 10th International Symposium on the Delivery of Functionality in Complex Food Systems (DOF2023). [Oral Presentation]. Melbourne, Australia.

Gawat, M., Kaur, L., Singh, J., Chen, J. & Boland, M. (2022, 22 December). Microwaving Meat: its impact on meat quality and structure. 1st International Symposium on Meat Chemistry, Processing and Technology (*Virtual event).*

Gawat M., Kaur, L., Singh, J. & Boland, M. (2022, 14-16 November). Effect of microwave processing on quality, ultrastructure and digestibility of goat meat and lamb. [Oral Presentation]. Riddet Institute Student Colloquium 2022, Napier, New Zealand.

Gawat, M., Kaur, L., Singh, J., Chen, J. & Boland, M. (2022, 5-7 July). Microwaving Meat: its impact on meat quality and ultrastructure. [Oral and Poster presentation]. New Zealand Institute of Food Science and Technology Conference 2017, Rotorua, New Zealand.

Gawat M., Kaur, L., Singh, J., T., & Boland, M.. (2021, 16-19 November). Microwaving Meat: its impact on meat quality and structure. [Poster presentation]. 6th International Conference on Food Structures (*Virtual event).*

Gawat, M., Kaur, L., Singh, J., & Boland, M. (2021, 6-8 July). Meat Quality characteristics of New Zealand goat meat. [Poster presentation and 3-Minute-Pitch competition]. New Zealand Institute of Food Science and Technology Conference 2021, Palmerston North, New Zealand.

Gawat M., Kaur, L., Singh J., & Boland, M. (2021, 7-9 April). Meat Quality characteristics of goat meat from New Zealand breeds. [Poster presentation]. Riddet Institute Student Colloquium 2021, Wellington, New Zealand.

Gawat, M., Kaur, L., & Boland, M. (2021, 22, June). Quality of New Zealand Goat Meat. [Poster & oral presentation]. Food Industry Enabling Technologies Symposium 2021, Palmerston North, New Zealand.

List of abbreviations

*a**	Redness
ANOVA	Analysis of variance
ATR	Attenuated total reflection
*b**	Yellowness
BF	Boer female
BM	Boer male
BSA	Bovine serum albumin
DIAAS	Digestible Indispensable Amino Acid Score
DLC	Dynamic light scattering
ESEM	Environmental scanning electron microscope
FDR	False discovery rate
FF	Female feral
FFA	Free fatty acid
FT-IR	Fourier -transform infrared
GI	Gastro-intestinal
GLM	Generalized linear model ()
HCA	Hierarchical cluster analysis
HPLC	High performance liquid chromatography
HPP	High pressure processing
IAA	Indispensable amino acids
IMF	Intramuscular fat
IVD	In vitro digestion
*L**	Lightness
LT	*Longissimus thoracis*
M	Molar
MALDI-TOF-MS	Matrix-assisted laser desorption/ionisation time of flight mass spectrometry

MHC	Myosin heavy chains
MLC	Myosin light chains
MW	Microwave
MICT	Muscle intramuscular connective tissue
OPA	O-phthalaldehyde
PC	Principal component
PCA	Principal component analysis
PEF	Pulsed electric field
pHu	Ultimate pH
PSF	Peak shear force
PSM	Peptide spectral matches
PSR	Picro-Sirius red
PVDF	Polyvinylidene fluoride
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SM	*Semimembranosus*
SSF	Simulated salivary fluid
SV	Sous vide
TCA	Trichloroacetic acid
TEM	Transmission electron microscopy
WBSF	Warner-Bratzler shear force
WHC	Water holding capacity

Chapter 1

General Introduction

1.2 Research background

In an era of alternative proteins, this research is about meat. People blame the livestock industry for our rapidly changing world. But we cannot deny that meat is still a significant commodity in our diet. However, science has no black-and-white answer regarding the best diet that will not harm the earth. The knowledge built from the beginning of time through the collective efforts of scientists helped us gain an understanding of how things work in food processing, nutrition, and health. Currently, many studies aim to replace meat or find alternatives for it, but there are still a lot of questions left unanswered in meat science, and this paper sought to answer some of them. "Stand on the shoulders of giants," they say, so this work is built upon the great ideas of the past and aims to contribute to more understanding of red meat quality and processing, in particular, the research on goat meat and the effect of processing on its protein digestibility.

Goat meat, also known as chevon, is meat from adult goat (*Capra hircus*) and is considered a healthier red meat option as it is lean and low in cholesterol (Madruga & Bressan, 2011; Mazhangara et al., 2019). Goat meat production has several advantages considering our changing environment and evolving consumer preferences. The high adaptability of goats makes them ideal as another source of red meat (Alexandre & Maghoub, 2011; Devendra & Burns, 1983; Laczo et al., 2007; Menendez-Buxadera, 2006). In New Zealand, goat meat is a less known red meat than lamb and beef and is consumed in a limited amount. However, the increasing number of immigrants from Middle Eastern and Asian countries has increased the demand for this meat (Scholtens et al., 2017). Moreover, there is a strong demand for goat meat globally (Mazhangara et al., 2019).

Despite the positive demand for goat meat, its production in New Zealand is still small and poorly established compared to the beef and lamb industries. Currently, the goat meat produced in New Zealand is mainly sourced from feral goats (wild goats), followed by dairy breeds and the popular meat breed Boer (Scholtens et al., 2017). Meat from feral goats is often discriminated against the meat from Boer goats and is tagged as inferior goat meat. However, the published information regarding the quality characteristics and nutritional value of the important New Zealand goat meat sources is minimal. Additionally, the lack of facilities to slaughter and process goat carcasses is a significant constraint for goat meat production (Scholtens et al., 2017). Goats are slaughtered the same way as lambs (Scholtens et al., 2017), which might result in undesirable qualities for goat carcasses. Therefore, it is vital to determine the attributes of goat meat processed under current slaughtering conditions. The examination of goat meat muscle structure can also give a further explanation regarding goat meat qualities. Hence, regardless of the role of goat meat in fulfilling the demand for domestic and overseas markets, a study on the quality, muscle structure, and nutritional value of commercially important goat meat in New Zealand would be valuable.

Goat meat has been reported to be tough and to have a poor response to aging (Kannan et al., 2014; Shija et al., 2013). The toughness of goat meat is a challenge for its acceptability since meat tenderness is one of the most important quality characteristics for meat consumption (Destefanis et al., 2008). The toughness of goat meat has been linked to its high collagen content, low collagen solubility, muscle fiber characteristics, and low intramuscular fat, all of which vary among breeds and muscle cuts (Hwang et al., 2019; Shija et al., 2013). Moreover, unsuitable pre- and post-slaughter handling procedures further affect the quality of meat (Pophiwa et al., 2020). In terms of meat processing, tough meat requires a longer time

to cook and specific interventions to achieve acceptable tenderness (Ertbjerg & Puolanne, 2017; Tornberg, 2005). In fact, sous vide goat has been found to be tougher than beef cooked using the same method (Ismail et al., 2019a). Among the technologies that can improve the cooked quality of goat meat, exploring different time-temperature conditions is an area that still needs investigation.

Multidisciplinary research is currently being carried out to link processing methods to the nutritional quality of meat proteins in terms of protein digestibility. The most common approach in carrying out these studies is using an *in vitro* system, which is useful in predicting digestion outcomes in vivo (Bohn et al., 2018). Studies on meat protein digestibility have demonstrated that various processing conditions can affect meat *in vitro* protein digestibility (Mitra et al., 2022; Orlien et al., 2021; Yin, Zhou, et al., 2020). Meat undergoes several manufacturing processes to suit consumer demand. The factors affecting meat protein digestibility in terms of processing can be grouped into two categories: 1) The preparation or condition of meat (e.g. breed, post-mortem condition, whole vs minced meat, etc.) and 2) Cooking methods used (e.g., pan frying, sous vide, microwave, etc.). While the effect of the preparation or condition of meat before cooking has been investigated, results remain contradictory, and no definitive conclusions have been reached. This area remains unclear and is less studied. On the other hand, the effect of temperature or cooking conditions has been vastly explored over the past decade. This is probably because thermal processing temperature is identified to be the primary determinant of the overall digestibility of meat protein (Bax et al., 2012; Mitra et al., 2022), and this effect been reported in beef (Bhat et al., 2020; Oberli et al., 2015), pork (He et al., 2018; L. Li et al., 2017; Mitra et al., 2022) and in rhea

meat (Filgueras et al., 2011). Generally, high-temperature cooking heating tends to result in lower meat protein digestibility compared to low-temperature processing (Bhat et al., 2020).

Sous vide, which uses low-temperature processing, is often idealized as a better processing method that improves meat protein digestibility (Bhat et al., 2020; Mitra et al., 2017; Yin, Zhou, et al., 2020). Its mechanism is related to the mild oxidation effect of low-temperature processing on muscle proteins; mild oxidation allows protein structures to unfold exposing more peptides for enzyme action (Zhang et al., 2020; Zhang et al., 2013). Conversely, high-temperature processing induces higher protein aggregation (Deb-Choudhury et al., 2014) and significant myofibrillar proteins can form heat-induced modifications inhibiting easy access to proteolytic enzymes (Liu & Xiong, 2000). Despite the positive effects of sous vide (low-temperature processing) on meat protein digestibility, this method can also potentially result in lower meat protein digestibility. Some sous vide processes use a very long processing time, and prolonged heating has been reported to result in further aggregation of proteins (Cai et al., 2018; Mitra et al., 2017). These more aggregated protein structures can rebury exposed structures, limiting enzyme activity and leading to longer digestion times. Ultimately, the effect of thermal processing conditions on the digestibility of meat proteins is related to the resulting meat protein molecular structure, which depends on the time and temperature conditions used.

In goat meat processing, achieving the desired texture through thermal processing is essential. As demonstrated in the literature, sous vide is a good option for cooking goat meat (Ismail et al., 2019a, 2019b). However, the use of long-time cooking is not always practical for household or industry applications due to the length of the process. Hence, a fast-cooking method that involves high-temperature processing is sometimes used to achieve the desired

meat texture, and its use in goat meat processing can be investigated. In this aspect, the applicability of microwave processing on goat meat processing can be evaluated. Currently, evaluating the effect of using low-temperature cooking in comparison to high-temperature processing on goat meat protein digestibility has not been explored. Advances in scientific techniques can be used for fundamental research to elucidate the precise mechanisms of muscle protein structural modification and the implications the technological and nutritional quality of meat.

1.3 Overall goal and research questions

1.3.1 Overall goal

This research generally aims to determine the quality characteristics of goat meat produced in New Zealand and determine the effect of sous vide and microwave processing on its structure and protein digestibility.

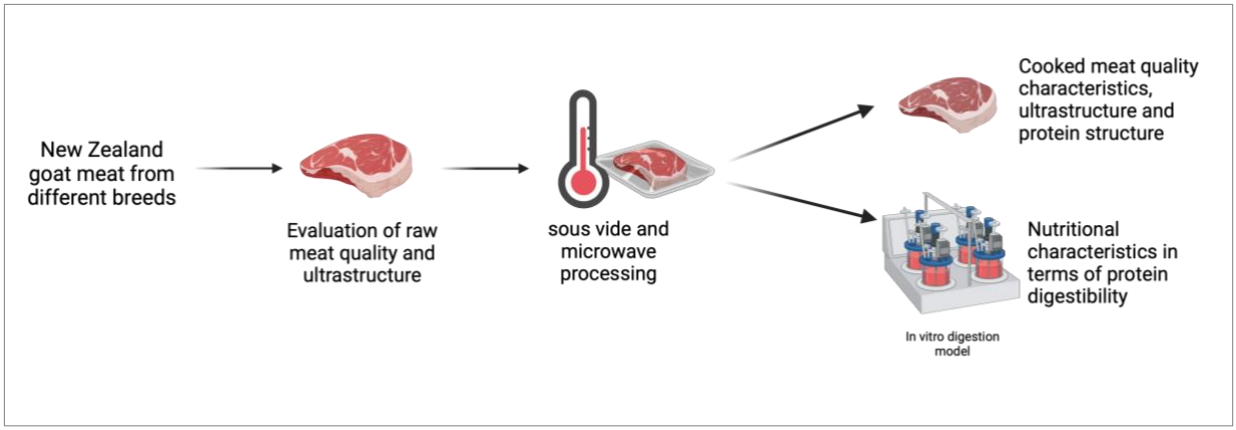

Figure 1. 1 The overall illustration of the PhD project. *Created with biorender.com.*

1.2.1 Research questions (RQs)

RQ1: What is the quality of meat from Boer and feral goats produced in New Zealand? Do breed, sex, and muscle type influence its properties regarding physicochemical characteristics such as pH, colour, cooking loss, collagen content, and collagen solubility?

RQ2: Is there a difference in the protein digestibility of goat meat between breeds and muscle types with varying textures?

RQ3: What is the quality of goat meat in terms of tenderness, cooking loss and colour when cooked under sous vide and microwave methods? Then how does goat meat respond to these cooking processes in comparison to other red meats?

RQ4: How can sous vide and microwave processing methods modify goat meat's ultrastructure and protein molecular structure?

RQ5: Is there a difference in overall protein digestibility of goat meat when processed using sous vide versus microwave methods?

1.3 Research Approach

This thesis has five experimental parts to answer the identified research questions. The experiments in **Chapter 3** were done to determine the characteristics of goat meat produced in New Zealand. This chapter explored the meat quality differences in terms of physicochemical properties and cooked meat quality among commercially significant goat breeds in New Zealand, and the effect of breed, sex, and muscle type on its quality was evaluated. Meat quality is described in terms of goat meat's physicochemical and cooking properties (colour, texture, and cooking loss). Additionally, **Chapter 3** examined the muscle ultrastructure of goat meat before and after sous vide processing.

Chapter 4 extends the investigation in **Chapter 3** by evaluating the *in vitro* protein digestibility of the selected breeds and two muscle types. The samples chosen in **Chapter 4** are the meat having significant variability in cooked texture to determine if these affect the meat protein digestibility. Throughout the study, digestion was carried out using a static digestion system using jacketed glass reactors. The simplicity and usefulness of *in vitro* gastrointestinal digestion models in predicting the outcomes of digestion in vivo were the reason for choosing the model for this experiment.

The succeeding experimental chapters (**Chapters 5-7**) focus on the effect of thermal processing on goat meat quality, muscle structure, protein structure, and digestibility. The experiments were done using two modes of heating: low-temperature, slow-cooking using

sous vide, and high-temperature, fast-heating involving dielectric heating via microwave processing.

The resulting meat texture was a significant parameter used to determine the processing conditions in these chapters. **Chapter 5** explores the impact of using microwave compared to sous vide cooking on goat meat quality and ultrastructure. **Chapter 6** was set up to examine the modification of meat protein molecular structure by microwave and sous vide and how these changes influence the *in vitro* gastrointestinal digestion of meat proteins in terms of the free amino nitrogen release and the protein profile of the digest. **Chapter 5** and **Chapter 6** were conducted, where experiments on goat meat were carried in comparison to lamb as another meat source to understand if goat meat behaves differently from more commonly consumed red meat. Finally, **Chapter 7** focuses on quantifying and characterizing goat meat-derived peptides released during *in vitro* digestion of microwave and sous vide cooked goat meat using a peptidomic approach. An overview of the thesis structure is presented in **Figure 1.1**

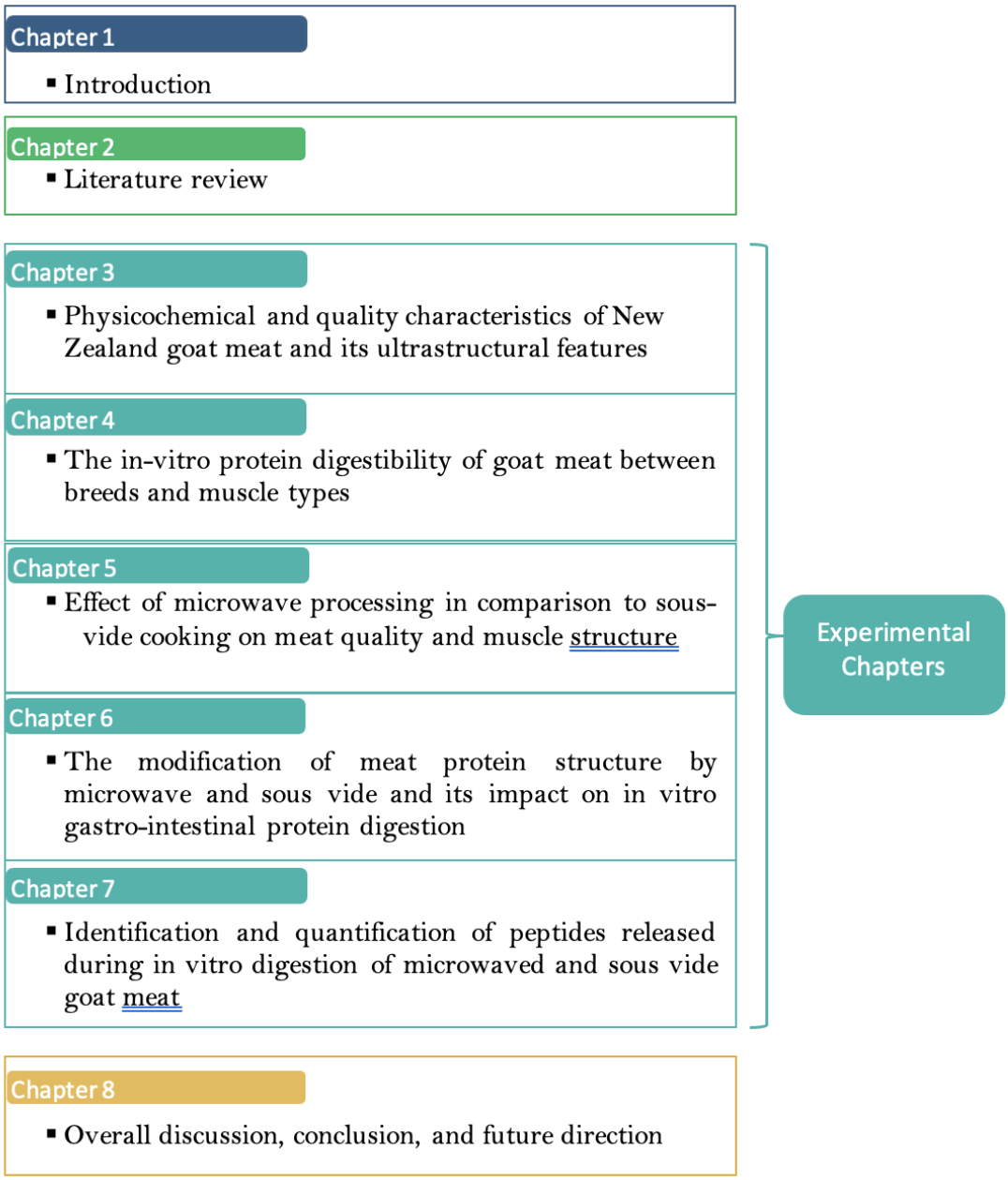

Figure 1. 1 Overview of the thesis structure.

Chapter 2

Review of Literature

This literature review aims to provide an overview of the status of goat meat production in New Zealand and a comprehensive summary of the existing literature on goat meat quality and processing. This review also includes the implications of processing on protein structure and the fundamentals of meat protein digestibility.

2.1 Goat meat

Goat meat is a general term for meat produced from a goat (*Capra hircus*). A goat is a multifunctional animal and an untapped meat source suitable for sustainable red meat production. Goats are highly adaptive to extreme environments, considering production, reproduction, and disease resistance (Alexandre & Mandonnet, 2005; Devendra & Burns, 1983; Laczo et al., 2007; Menendez-Buxadera, 2006). For example, goats can survive heat stress, prolonged water deprivation, and require little land space (Aziz, 2010; Eisler et al., 2014; Rosa García et al., 2012) (Eisler et al., 2014). Goats are both grazers and browsers and eat some grasses and shrubby plants, which other ruminants will not; this makes them efficient when raised with other ruminants for diversification (Van Niekerk & Casey, 1988). Generally, goats can be farmed with low inputs (no vitamins and antibiotics), which is essential to satisfy the emerging and growing consumer group seeking free-range and antibiotic-free meat for "the lifestyle" markets. With the changing environment and evolving consumer preferences, goats will play a vital role as an alternative red meat option, sustaining the meat demand worldwide.

There are approximately 300 breeds and types of goats, primarily found in the tropics and subtropics (Devendra & Burns, 1983). Goats are commonly reared without a specific product goal and with less breeding control, specifically for meat production (Paim et al.,

2019). Thus, compared to the other popular red meats, such as lamb and beef, there is a less formal structure for goat meat production regarding breed selection strategies. However, some breeds are considered superior in meat production and are used as breeding stocks for countries that farm goats for meat.

For meat production, goats are sold based on their live weight and its relationship to their carcass yield (Kadim & Maghoub, 2011). The carcass characteristic determines the meat quality and value of the animal before it reaches the consumer, and a breed that can produce high carcass yield and saleable meat is more valuable. Among goat breeds, South African Boer or Boer, Spanish, Kalahari, and Savannah are the known meat breeds. Boer is the most popular among the meat breed goats known to have the size, body weight, bone structure, and growth rate that fulfill all the market requirements (Malan, 2000; Pratiwi et al., 2004; Van Niekerk & Casey, 1988). Culled dairy goats comprise a large portion of the goat meat market since culled dairy goats are sold as meat (Scholtens et al., 2017). Dairy goats have the advantage of large body sizes suitable for meat production. The popular dairy breeds are Saanen, British Saanen, Sable, Alpine, and Anglo-Nubian. On the other hand, the known fibre breeds are unsuitable for meat production because of their low carcass yield (Devendra & Burns, 1983; Oman et al., 2000; Paim et al., 2019). In Australia and New Zealand, feral goats play a significant role as a primary source of meat for export because of their availability. Feral goats are wild goats from the dairy or fibre breed goats that escaped or were freed and established their habitat in islands and mountainous areas (Rolls, 1977).

Generally, goats' phenotypic characteristics and production potential vary greatly, affecting carcass and meat quality. Research shows contrasting reports on dressing out percentage values among breeds (Dhanda, Taylor, Murray, et al., 2003; Tahir et al., 1994;

Tshabalala et al., 2003). But, when different goat breeds have the same frame size (small vs. large framed goats), their carcass' quality is often highly influenced by their size more than by breed type (Dhanda, Taylor, & Murray, 2003; Johnson et al., 1995). At present, there is an increase in the popularity of goat meat as an alternative red meat option. The exploration of indigenous breeds of a country is becoming in demand because of their adaptability. The idea of food production driven by sustainability and environmental concerns gives goat meat production an advantage as a less explored red meat.

2.2 Types of goat meat in the market

The export and domestic markets generally classify goat meat into two types, the Capretto/Cabrito and Chevon, differentiated mainly by age and carcass weight (Dhanda, Taylor, & Murray, 2003). **Table 2.1** presents the description and use of the two main types of goat meat in the market. Cabrito or Capretto is the meat from suckling kids popular in various European regions. Cabrito meat is a significant goat meat product in Portugal and is part of their culinary tradition. It is also popular in the Mediterranean lifestyle (Santos et al., 2007b; Teixeira et al., 2011). Together with lamb, goat meat is a popular source of meat for traditional Mediterranean dishes that typically use lean meat. On the other hand, Capretto is the term used in Italy, France, many parts of Latin America, and the Caribbean (Borgogno et al., 2015; Vincent, 2018). This meat's distinct flavour and texture make it a premium goat meat product, and its light carcass makes it ideal to be consumed entirely by a family on significant occasions.

Chevon is the popular term for goat meat around the world, and it is the general term used to describe goat meat from adult or mature goats. It is a standard meat product traded for export and consumed in various developing countries and a popular choice of meat in India

(Naude & Hofmeyr, 1981; Vincent, 2018). In European countries, most of this meat is used for processed products, which makes it more appealing to a broader range of consumers. Chevon is often discriminated against capretto/cabrito since fresh suckling-kid meat is regarded to have higher edible quality (Santos et al., 2007b). Moreover, chevon is also known to be a tough meat characterized by its "goaty aroma/ sweaty aroma" (Wong et al., 1975), a flavour that resembles that of mutton or lamb (Sheridan et al., 2003).

In this thesis, goat meat is generally used to refer to meat from goats regardless of the classification of goat meat in the market unless explicitly stated in the discussion.

Table 2. 1 Goat meat types, their description, and their use.

Types of meat	Description	Uses
Capretto/Cabrito	Meat from milk-fed kids, up to 12 weeks, and a carcass weight of 4-12 kg. The meat is described to be light/ pale in colour and has a fine texture, tender, and lean.	Roasted like a lamb or, in the form of chops, may be broiled or fried.
Chevon	Meat from young (1-2 years old) to adult goat (2-6 years old). Typical carcass weight ranges from 14-22 kg.	In curries, braises, and stews, use moist, long, slow-cooking methods.

Sources: (Dhanda, Taylor, Murray, et al., 2003; Murray et al., 1997; Santos et al., 2007a; Teixeira et al., 2011; Vincent, 2018).

2.3 Status of goat meat production in New Zealand

New Zealand is known for its meat production and is among the world's top exporters of lamb and beef. Although New Zealand also exports goat meat, its production is not popular in the country. Figures for goat meat show that it has a tiny share of the export revenues of

the New Zealand meat industry. On average, from 2012-2017, goats contributed only 0.4% of the total slaughtered animals in New Zealand, which supplied 933 Tons of exported goat meat, with an export value of 5.08 million USD (Statistics New Zealand, 2020).

Scholtens et al. (2017) estimated the volume of slaughtered goat meat based on the existing populations of feral, Boer goats, dairy, and Angora around the country (**Table 2.2**). The relatively low volume of goat meat mostly comes from domesticated feral goats (wild goats), farmed together with sheep and cattle as weed control (Gillingham, 2008; Lopez-Lozano et al., 2017). The feral goat meat is produced in high volume (60%) because it is readily available and a cheap source for slaughter and breeding stock (Vincent, 2018). Other sources of goat meat were dairy goats; approximately 85% of these were Saanen breeds (Scholtens et al., 2017). Also, some fibre goats, specifically Angora, contribute to the production of goat meat (Lopez-Lozano et al., 2017). New Zealand also has two meat breed goats, Kiko and Kikonui, known as an improved Kiko, but these goats are not commonly bred in the country.

Most of the goat meat produced in the country is exported and these were mainly sourced from feral goats (Lopez-Lozano et al., 2017). On the other hand, in the domestic market, the goat meat sold is primarily from Boer. Among the sources of goat meat, carcasses from Boer had the highest value at an average of 4.5 NZD per kg of carcass weight (CW) since Boer meat is considered the premium goat meat, while feral meat is priced the lowest. The values suggest that the breed type significantly dictates the value of goat meat. However, no current study proves that meat from Boer is superior to New Zealand feral goats. The research done for the quality evaluation of NZ feral goat meat was in the 1970s (A. Kirton, 1970) and the qualities of commercial goat meat in the country were reported by Hogg et al. (1992), where they used Saanen x Angora crosses. This area needs to be understood further

to establish information regarding the quality characteristics of goat meat produced in New Zealand.

Table 2. 2 Estimate of New Zealand goat meat production and value (Scholtens et al., 2017)

Source	Number slaughtered	Average carcass weight (kg)	Total Carcass weight (kg)	Average Value ($/kgCW)	Total Value ($/yr)
Feral (60.2%)	91,200	9	821,000	2.5	2,052,000
Boer (11.7%)	6,380	25	160,000	4.5	718,000
Dairy (24.0%)	8,180	40	327,000	3	982,000
Angora (4.1%)	2,240	25	55,900	3	168,000
Total	108,000		1,400,000		3,920,000

Although goat meat in produced New Zealand is mainly exported, the local consumption of goat meat in the country is increasing (Statistics New Zealand, 2020); this can be explained by the increasing number of immigrants with ethnic preferences for goat meat. The pattern is similar to that in Australia, where immigrants from Middle Eastern and Asian origins have driven the increase in goat meat consumption domestically (Dhanda, Taylor, & Murray, 2003; Vincent, 2018). Even with the increased demand, the goat meat industry in the country is still small and not well established compared to the beef and lamb industries. The lack of facilities to slaughter and process goat carcasses is a significant constraint for goat meat production (Scholtens et al., 2017). Moreover, the current information regarding the quality characteristics of the important NZ goat meat sources is minimal. Also, based on personal communication from the Ministry for Primary Industries of New Zealand, there are no regulated classification schemes for goat meat; this means there is no standardized definition of goat carcass in the country. If NZ wants to exploit its capabilities for goat meat production, there's a lot of work and opportunities for its improvement.

2.4 The uniqueness of goat meat

Goat meat is widely distributed around the world. In Western countries, goat meat is not as popular as lamb and beef, is considered exotic meat, commonly consumed by migrants, and not widely farmed (Dhanda, Taylor, Murray, et al., 2003; Webb et al., 2005). But in Africa, Asia, and Arab countries, goat meat is favoured mainly because of cultural traditions and its availability. But what are the composition and characteristics of goat meat that make it unique from other red meat?

2.4.1 Chemical composition

Among the popular meat sources, goat meat is a source of lean meat with ~18-25 % protein content for raw meat (A. Kirton, 1970; Tshabalala et al., 2003; Webb et al., 2005) (**Table 2.3**). It has a favourable protein-to-fat ratio due to lower intramuscular fat (IMF) content (Tshabalala et al., 2003). The fat content of goat meat is comparable to poultry meat and significantly less than beef and lamb (Babiker et al., 1990; Johnson et al., 1995; Okuskhanova et al., 2017; Sheridan et al., 2003). However, in a study by Tshabalala et al. (2003), Boer contained a comparable amount of fat to lamb, and this is consistent with various studies (Solaiman et al., 2011; Van Niekerk & Casey, 1988), confirming that among goat breeds, Boer tends to have high-fat content (**Table 2.4**). Aside from Boer, dairy breed goats had the highest fat content. On the other hand, the feral goat was identified to be a very lean meat with fat content ranging from 0.9 to 1.5 % (Colomer-Rocher et al., 1987; N. M. Werdi Pratiwi et al., 2007).

Table 2. 3 Comparison of the composition of goat meat with meat from other species.

Type of Meat	Moisture	Protein	Fat (%)	Ash
Deer Meat	74.15	24.76	0.41	0.68
Lamb	67.20	19.12	12.79	0.89
Goat meat	75.34	22.07	1.13	1.45
Turkey Meat	67.66	24.95	5.02	2.37
Turkey meat	70.45	25.84	2.06	1.65
Beef	70.1	22.3	7.3	1.12

Sources: Okuskhanova et al., 2017; Smith et al., 2011

Goats having very low IMF (below 2 %) could be related to the maturation rate in combination with their grass diet. As frequently reported for cattle, late-maturing breeds raised on pastures had lower IMF (Bessa et al., 2015). Moreover, goats develop poor subcutaneous fat at a slow rate (Mahgoub et al., 2011; Warmington & Kirton, 1990); they tend to deposit more fat in the viscera rather than in the subcutaneous region (Gaili & Ali, 1985; Sen et al., 2004). Generally, the proximate composition of goat meat was significantly affected by genotype (Cameron et al., 2001; Dhanda, Taylor, & Murray, 2003; Peña et al., 2009; Todaro et al., 2004). Although the low IMF values for goat meat are ideal from a nutrition perspective, there are consequences when it comes to the technological properties of the meat.

Table 2. 4 Reported proximate composition of raw goat meat from various breeds.

Breeds	**Breed**	**Age and sex**	**Muscle type**	**Moisture (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Source**
Meat breeds	African Boer	Castrated males without any permanent incisors	Soft tissue	69.4	22.8	10.5	0.95	Tshabalala et al. 2003
	Boer	Bucks, 9-12 months	loin	76.3	20.0	2.2	0.9	Van Wyk et al. 2020
Dairy breeds	Alpine	Four years	*Gluteus superficialis*	74.6	19.52	3.9	1.0	Ivanović et al. 2020
	Saanen	Four years	*Gluteus superficialis*	74.8	19.82	4.1	1.0	Ivanović et al. 2020
	Guanzhong dairy breed	6-10 mos (sex, unspecified)	*Longissimus thoracis*	75.5	19.58	4.82	1.0	Ding et al. 2010
Indigenous	Balkan goat	Four years old, female	*Longissimus dorsi*	74.5	20.6	3.9	1.0	Ivanović et al. 2014
	Serbian white goat	Four years old, female	*Longissimus dorsi*	75.4	20.0	3.6	1.1	Ivanović et al. 2014

	East African Tanzania indigenous goats	1.5-2 years old, male and female	*Longissimus thoracis et lumborum*	70.6	23.45	2.5	4.4	Shija et al. 2013
	Sarda goats	Kids (42 days) male and female	*Longissimus dorsi*	75.6-75.9	21.9-22.2	1.0	1.2	Vacca et al. 2014
	Indigenous Veld goat	Male and female, 9-12 mos	Loin	75.6-76.8	19.6-20.1	1.6-2.7	1.0-1.1	Van Wyk et al. 2020
Feral Goat	Australian feral goat	Unspecified age, slaughter weight 5-70 kg	*Longissimus thoracis*	75.9	20.7	1.25	1.1	Werdi Pratiwi et al. 2007

2.4.2 Physicochemical properties

2.4.2.1 Meat pH

The inherent ultimate pH (pHu) is a critical quality determinant influencing the processing and sensory attributes of meat (Farouk et al., 2019; Pulford et al., 2009b). The ultimate pH level can be grouped into three: high (pH > 6.3), intermediate (pH > 5.7 to pH < 6.3), and low (pH < 5.7) (Pulford et al., 2009b). The typical ultimate pH for red meat ranges from 5.4 to 5.9 (Astruc, 2014a). Goat meat, on the other hand, is extensively reported to have an intermediate pH with an optimal pH ranging from 5.5 to 6.2. The relatively high pH of goat meat has been attributed to the tendency of goats to be prone to perimortem stress (Webb et al., 2005), supported by the perimortem concentrations of glycolytic metabolites in goat muscles with low glycogen concentration (Kannan et al., 2014; Simela & Frylinck, 2004b; Vincent, 2018). The normal pH of goat meat (intermediate pH, ~5.7-6.3) plays a significant role in the biochemical processing taking place post-mortem, and it is not an ideal pH for red meat. With an intermediate pH level, lysosomes are stable throughout the meat aging process (Lomiwes, Hurst, et al., 2014), thus lysosomal enzymes cannot exert their proteolytic activity on meat muscles. It is possible lysosomal enzymes in goat meat had a very low its tenderizing effect during aging. In goat meat studies, there are only limited studies conducted to

determine the pH temperature window for post-mortem storage, which is critical in optimizing goat meat production and processing systems.

2.4.2.2 Water holding capacity (WHC) and cooking loss

Water holding capacity (WHC) is the property of meat to retain its naturally occurring water when applied with external force. It is primarily influenced by the ability of the myofibrillar system to immobilize tissue water (Kadim & Maghoub, 2011). Goat meat has been reported to have superior water-holding capacity and less cooking losses compared to lamb (Babiker et al., 1990; Okuskhanova et al., 2017; Sen et al., 2004; Swan et al., 1998) and poultry (Okuskhanova et al., 2017), which can be explained by the influence of meat pH on WHC. As the pH declines, proteins have a reduced capacity to retain water and a subsequent increase of water intracellularly (Hughes et al., 2020; Warriss, 2010). Thus, meat with a higher pH exhibits significantly higher WHC.

2.4.2.3 Collagen content and solubility

Proteins from meat are also rich in collagen, the main composition of muscle intramuscular connective tissue (MICT), which plays a significant role in the "background toughness" of meat (Purslow, 2014; Veiseth et al., 2004). The amount and extent to which collagen crosslinks depends on muscle type, species, genotype, age, sex, and level of physical exercise (Warriss, 2010). Generally, the higher the insoluble collagen content, the tougher the meat (Tshabalala et al., 2003). Goat meat has been reported to have lower collagen solubility compared to mutton (Hocquette et al., 2012; Schönfeldt et al., 1993; Tshabalala et al., 2003) and beef (Ismail et al., 2019a).

Goat meat has been reported to contain significantly higher collagen levels than sheep meat (Schönfeldt et al., 1993; Teixeira et al., 2017). The high collagen content in goat muscles can be explained by how goats forage for food; they are both grazers and browsers, and getting more woody vegetation requires their muscles to be used more, leading to more developed tissue. Among breeds, mountain goats such as the white Carpathian goats in Poland, generally have higher collagen content than the Saanen (Migdał et al., 2021). When it comes to meat toughness, both the collagen content and its solubility are vital. The high level of collagen with low solubility would render goat meat tough. Hence, goat meat would highly benefit from a process that can solubilize collagen during cooking or the application of a process or enzyme that targets significant structural weakening of connective tissues to achieve acceptable tenderness.

2.4.3 Sensory properties

2.4.3.1 Meat Colour

Colour is considered an essential intrinsic parameter for meat and indicates freshness, taste, and texture (Henchion et al., 2014). It is primarily affected by the level and state of myoglobin and meat composition (Du & McCormick, 2009). Additionally, it is also highly influenced by meat pH. In fact, muscle pH is linked to the quality grades of meat for USDA standards (Page et al., 2001). High pH prevents the decrease of mitochondrial activity, and higher concentration of hydrogen competes with myoglobin in binding with oxygen, consequently limiting the increase of oxymyoglobin levels post-mortem (McKeith et al., 2016; Zhang et al., 2018). Additionally, the amount and distribution of intramuscular fat (IMF) in a

muscle also affect its colour. High IMF tends to have high lightness (L^*) values due to the light-scattering effect of lipids (Hughes et al., 2020; Listrat et al., 2016a).

Goat meat tends to be a darker red than pork or lamb (Casey et al., 2003; Kadim et al., 2010; Schönfeldt et al., 1993), which can be explained by its high pH and low IMF. The effect of genotype on the colour properties of goat meat is still debatable. Although, there are authors that reported no observed differences in meat colour between breeds such as between Kiko and Boer (Solaiman et al., 2012; Solaiman et al., 2011) and among Batina, Dhofari, and Jabal Akdha breeds (Kadim et al., 2004), many studies have also presented the effects of breed on meat colour (Dhanda, Taylor, Murray, et al., 2003; Santos et al., 2007a). The colour differences are mainly observed for the redness values that correlate to the amount of haem pigment in a muscle (Peña et al., 2009). Haem concentration is directly influenced by many factors, such as species, age, type of muscle, and physical activity (Dhanda et al., 1999; Dhanda, Taylor, Murray, et al., 2003; Santos et al., 2007a).

2.4.3.2 Instrumental tenderness

The instrumental measurement of tenderness is expressed in terms of peak shear force (PSF), measured in Newtons (N) or kilograms (Kg). The higher the shear force values, the tougher the meat. Although there is a high correlation in using shear force for describing the toughness of meat, direct comparisons between reported values in the literature are difficult due to differences in the methods used. **Table 2.5** shows some reported values for the instrumental tenderness of goat meat, assessed using Warner-Bratzler shear force (WBSF). WBSF has been a widely used method for evaluating meat texture, and its values are highly correlated to meat sensorial tenderness (Battaglia et al., 2020; Huffman et al., 1996; Purslow, 2014). PSF values of 40 N or 4.1 Kg are used as the threshold for acceptable tenderness

(Huffman et al., 1996). Goat meat is widely reported to have WBSF values higher than 5 Kg, so it is mainly described as tough.

The high shear force values of goat meat have been linked to high collagen content, low collagen solubility, muscle fibre characteristics, and low intramuscular fat (Shija et al., 2013). Kadim & Maghoub (2011) explained that goat meat is tough because most of the goat meat sold is from slaughtered adult goats with decreased collagen solubility. In addition, goats have a thin subcutaneous fat layer that predisposes it to cold shortening during rapid chilling (Abhijith et al., 2021; Kannan et al., 2014), which happens due to a small goat carcass with less fat cover, facilitating a fast decrease in temperature that induces cold shortening (Abhijith et al., 2021; Webb et al., 2005). Furthermore, the inherent intermediate pH of goat meat can also hinder the endogenous proteolytic enzymes that could exert a tenderizing effect during aging.

Table 2. 5 Reported shear force values of goat meat chilled for 24 h and cooked at an internal temperature ranging from 70-85 °C.

Breed	Age and Sex	Muscle	Cooking method	Shear Force (kg/cm^2)	Source
Guanzhong dairy breed and its crosses	6-10 mos, unspecified sex	LT	Water bath to an internal temperature of 70 °C	4.9-6.3	Ding et al. 2010
Serrana Transmontana	Male and female kids	*longissimus*	Water bath to 70 °C internal temperature	6.95	Teixeira et al. 2011
Australian feral	Kids to adult entire and castrated male	LTL	Water bath at 85 °C for 45 min	7.48	Werdi Pratiwi et al. 2004
Omani goats	One year male	LD	Water bath 70 °C for 90 min	7.2-7.7	Kadim et al. 2004
South African indigenous goats	Castrated and female, young to adult	SM	roasted to 72 °C internal temperature	7.6	Simela & Frylinck et al. 2004a
Crossbreeds	Male and female Kids	LTL	Water bath at 70 to 75 °C internal temperature	7.8	Dhanda et al. 2003
	Male and female Kids	GB	Water bath at 70 to 75 °C internal temperature	10.2	Dhanda et al. 2003
Boer	Kids	SM	Roasted to 72 °C internal temperature	11.1-14.3	Sheridan et al. 2003

SM- *M. semimembranosus*, BF-*M. biceps femoris*, LD-*M. longissimus dorsi*, GB- *Gluteobiceps*, LTL- *longissimus thoracis et lumborum*, LT- *longissimus thoracis*, QF- *quadriceps femoris*, and GB- *gluteobiceps*, kids: 8-20 kg live weight, young: 1-2 years old goat; adult 2 to 6 years old.

Upon examining the breed type, differences are apparent when comparing animals from different groups, such as dairy breed, fibre breed, and meat breed. However, the effect of genotype on goat meat tenderness is not apparent, and it is also because meat tenderness is a complex trait resulting from multiple factors that include pre- and post-mortem handling, genetics, and rearing conditions. Additionally, tenderness depends on the structural proteins in intramuscular connective tissue and myofibrillar proteins, which vary among breeds (Hwang et al., 2019; Purslow, 2017). Boer goats, having elevated levels of IMF compared to other goat breeds, have an advantage when it comes to having more tender meat due to the effect of IMF on the perceived texture of the meat. While Boer is widely studied among goat breeds, there is no information about the comparability of New Zealand feral goats regarding texture. This area needs to be investigated.

2.4.3.3 Juiciness

Juiciness greatly contributes to the acceptability of meat, and it is directly related to meat intramuscular lipids and moisture content (Smith et al., 1974). There is a high consensus in the literature that goat meat and its products tend to be less juicy than mutton and mutton products (Gaili & Ali, 1985b; Griffin et al., 1992; Schönfeldt, Naude, Bok, Van Heerden, Sowden, et al., 1993; R Sheridan et al., 2003; R. Sheridan et al., 2003). Less juicy goat meat can be explained by its low intramuscular fat content compared to sheep. Kirton (1970), on the other hand, did not find any significant difference in juiciness between meat from sheep and New Zealand feral goats; this result is surprising given that feral goats are lean compared to some domestic goat breeds. The juiciness of goat meat may vary with genotype. For

example, Tshabalala et al. (2003) reported that indigenous goat meat patties were the least juicy and greasy and differed significantly from meat from Boer goats. Meat from crossbred goats (Boer x French Alpine) got higher tenderness and juiciness scores than meat from pure French Alpine goats (Brzostowski et al., 2008).

2.4.3.4 Flavour

The flavour of goat meat resembles that of mutton or lamb (Sheridan et al., 2003b), with flavour acceptability values ranging from medium to high (Madruga et al., 2008; Peña et al., 2009), comparable to acceptability values for lamb (Babiker et al., 1990; Griffin et al., 1992; Swan et al., 1998). Although goat meat is always compared to lamb, Schoenfeld et al. (1991) clearly stated that the flavour of goat meat is unique and not interchangeable with meat from sheep. However, goat species-specific flavour is described to be weaker (less perceptible) compared to lamb or mutton (Babiker et al., 1990; Brand et al., 2018; Madruga et al., 2009); explained by its low fat content since fat greatly contributes to the flavour development in meat (Arshad et al., 2018).

The effects of sex, age, and breed are evident in many studies. The effect of breed on goat meat flavour is contrasting. Swan et al. (1998) and Tshabalala et al. (2003) reported that the breed type affects the sensory scores for aroma intensity. While for young goats, Peña et al. (2009) showed that breed type did not affect flavour. Slaughter age is a significant factor for flavour; young goats have less flavour intensity and lower acceptability than adult goats (Griffin et al., 1992; Sheridan et al., 2003; Smith et al., 1974). Castration, age, and slaughter weights also affect the flavour of goat meat. Castrated males are more acceptable than intact males (Dhanda et al. 2003; Pratiwi et al., 2004), and the intensity of goat meat flavour is inversely proportional to slaughter weight (Werdi Pratiwi et al., 2004, 2007). Hence,

Devendra and Burns (1983) suggest that the most appropriate age for slaughtering a goat is young (1-2 years). Therefore, goats should be slaughtered at an ideal age (1-2 years old) and should be castrated males or females to have an acceptable flavour profile.

2.5 General characteristics of goat meat

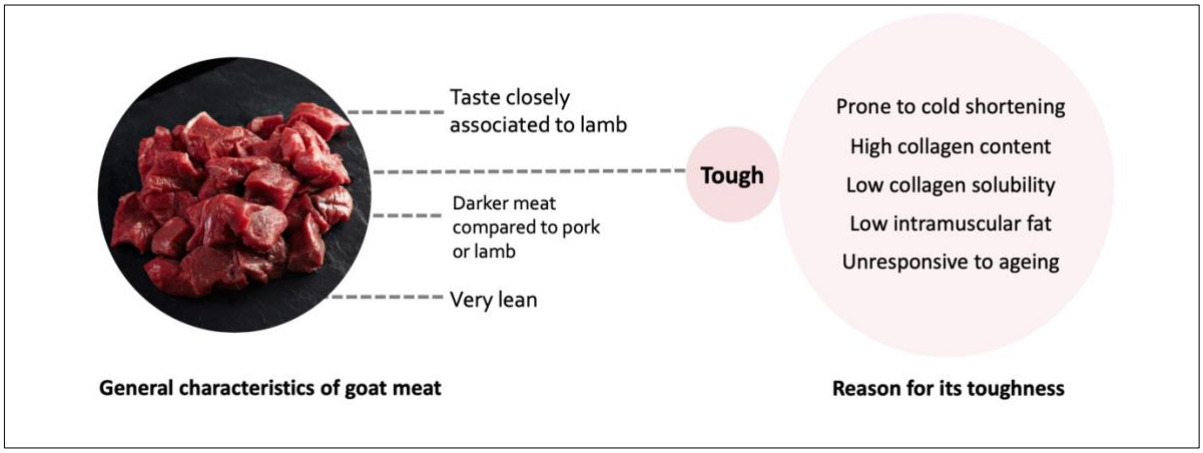

Figure 2. 1 The general characteristics of goat meat and the underlying mechanisms of its toughness.

Figure 2.1 summarizes the general characteristics of goat meat. From a technological perspective, the toughness of goat meat appears to be a challenge, especially because tenderness is a critical meat quality attribute assessed by consumers (Listrat et al., 2016b). Among the major sources of goat meat in New Zealand, it is valuable to determine the factors that contribute to the toughness of a muscle and potentially address the cause. There are pre-slaughter, slaughter, and post-slaughter conditions that can be designed to improve the quality of goat meat, which is well presented in the review paper by Pophiwa et al. (2020). Regarding cooking conditions, processing goat meat might require intensive techniques to achieve the desired tenderness. Tough goat meat is mainly processed for curries, braises, and stews that use moist, long, slow-cooking methods. However, the intensity of these processes could alter

muscle structural characteristics and have consequences on the nutritional quality of meat proteins.

2.6 The effects of thermal processing conditions on goat meat quality and muscle structure

In this section, the various studies on cooking methods and innovative processing technologies on goat meat have been reviewed, highlighting their impact on meat quality and muscle structure.

Liu et al. (2013) evaluated the effect of retorting the *semimembranosus* muscle at various endpoint temperatures for 30 min. A significant increase in tenderness was attributed to the increased solubility of collagen, which significantly increases when heated between 50 and 60 °C. This trend follows the tenderizing effect of cooking meat at low temperatures where toughness decreases between 50 °C and 60 °C in beef muscles (L. Christensen et al., 2011; Christensen et al., 2000). On the contrary, goat meat cooked at higher temperatures (75-80 °C) became tough. The increase in toughness at higher temperatures has been attributed to the increased strength of single muscle fibres and further denaturation of proteins at temperatures 60-80 °C (Chinzorig & Hwang, 2018; Christensen et al., 2000; Combes et al., 2004). Significant increases in the cooking loss were observed during high-temperature processing, and the meat became paler with increasing cooking temperature. The effect of heating on goat meat ultrastructure was examined under a scanning electron microscope (SEM). The SEM revealed that gaps between the fibres and the surrounding endomysium widened when the meat was heated to 50–70 °C (Liu et al., 2013). At 90 °C, significant breakage of the sarcomere was observed. Structural examination showed that heating at 60–

65 °C can result in significant transverse and longitudinal shrinkage of meat fibres; this result is in agreement with findings in the literature for other red meat (Astruc et al., 2010; Supaphon et al., 2021; Tornberg, 2005).

Cooking goat meat at low temperatures for prolonged duration has been investigated by Ishmael et al. (2019) using the sous vide process. Sous vide is a popular process of cooking vacuum-packed food in controlled heating conditions (Baldwin, 2012). The long cooking time during sous vide makes this process popular for tough meat cuts (Karki et al., 2022; Uttaro et al., 2019). Ismail et al. (2019a) introduced a 2-step sous vide treatment (two-temperature and two-time combinations) that improved water-holding capacity and reduced cooking loss for goat *semitendinosus* muscle. Also, a significant reduction of toughness was successfully achieved using 2-step cooking temperatures (6 hrs at 45 and 60 °C) compared with sous vide using single temperatures (60, 65, and 70 °C). Among the single-stage sous vide processes, cooking at 60 °C resulted in the lowest shear force for goat *semitendinosus* muscle. Ismail, Hwang, & Joo (2019b) further investigated the effect of 2-step sous vide cooking on goat gluteus medius and bicep femoris muscles; again, the lowest shear force values were achieved at a combined temperature of 45 °C and 60 °C for 6 hrs of cooking. The tenderizing effect of low-temperature processing on goat meat is attributed to some types of collagen that solubilize during prolonged cooking (Purslow, 2014). Additionally, at low temperatures, collagen fibres will have a decreased breaking strength due to partial denaturation and shrinkage of collagen fibres (Christensen et al., 2000; Tornberg, 2005), and endogenous enzymes such as cathepsins B and L and serine proteases still have an effect in degrading muscle proteins (L. Christensen et al., 2011; Purslow, 2014; Zielbauer et al., 2016). Hence, based on these findings, sous vide would be a good option for cooking goat meat, and the optimal

cooking temperature should be around 50-60 °C. The effect of high collagen content and low solubility that contribute to goat meat toughness can be overcome by this process. However, the safety concerns regarding the use of low temperature processing should also be considered when using sous vide.

Unlike the slow cooking in sous vide, microwave processing uses a dielectric heating that is a fast-heating process (Abea et al., 2023; Soni et al., 2020). Microwave frequencies ranges from 300 MHz to 300 GHz and the frequencies for industrial and domestic applications are typically assigned at 915 to 2450 MHz. In food applications, microwave heating has been around since 1949, and it is now a common domestic application (Jiang et al., 2018). The advantage of microwave heating is its volumetric heating effect that allows for efficient and fast heating (Abea et al., 2023). Current trends in food processing have also explored the application of microwave processing in sterilization.

The effects of microwave heating on goat meat have been studied. Yarmand & Homayouni (2009) compared microwave cooking to conventional oven heating. The frequency used for microwave heating was 2450 MHz with two wattage levels of 700 W (domestic microwave) and 12000 W (industrial microwave), and the meat was heated to an internal temperature of 70 °C. Microwave heating induced a higher expulsion of fat globules, resulting in higher fat loss. Between the processing methods, goat *semimembranosus* showed a 26.61 % cooking loss for conventional cooking, which increased to 29.29 % (700 W) and 30.70 % (1200 W) at two power levels for microwave cooking, respectively. This study showed the negative effect of microwaving on the moisture content of cooked meat due to increased fat and water loss. The higher wattage used in the study resulted in higher electromagnetic wave penetration efficiency, that resulted in more intense processing. When chevon leg chop was

microwave-processed at a power level of 900 W for 3-4 min and compared to broiled sample cooked at 232 °C for 25 min (James & Berry, 1997), the microwave (MW) was a faster method of producing cooked meat, but both processes resulted in no difference in tenderness. However, the broiled chevon was darker, had lower fat content, and had higher moisture content than the MW-cooked sample. Hence, MW treatment offers a fast method of cooking but can result in higher cooking loss, which is a typical observation for meat cooked at high temperatures (Bax et al., 2012; Dominguez-Hernandez et al., 2018; Roldán et al., 2013).

Yarmand & Homayouni (2010) also evaluated the effect of microwave and conventional cooking on goat meat semimembranosus muscle. Electron micrographs revealed that microwave heating caused more structural damage, including shrinkage and breakdown oof connective tissue and myofibrillar elements, in the semimembranosus muscle. The use of SEM in both studies by Liu et al. (2013) and Yarmand & Homayouni (2010) revealed the structural changes in muscle after cooking. However, the result is only limited to the meat structural changes seen from the surface of the meat. Their images do not reveal the breakage of the M-line or Z-disk, which have been cited as significant changes in meat structure after processing shown in beef muscle ultrastructure (Astruc, 2014b; Chian et al., 2019).The SEM mainly shows surface damage, such as granulation and separation of the meat structure.

High pressure processing (HPP) is an innovative technology identified as a green technology and a non-thermal process. It is often applied with elevated temperature processing to improve meat tenderness (Sikes & Tume, 2014). Jalarama-Reddy (2015) evaluated the effect of HPP on goat meat; pre-packed meat pieces were subjected to HPP at selected pressures of 300 and 600 MPa for 5 and 10 min at 28 °C. The result showed an increase in meat hardness with the application of HPP. Although they used an elevated

temperature of 28 °C, this temperature is not the optimal condition to induce significant shear force reduction. The optimum temperature for tenderizing meat using a pressure-heat process was reported to be 55–60 °C (Sikes et al., 2010). When high pressure is applied at temperatures below 60 °C, a hardening effect occurs (Ma & Ledward, 2004), which was observed in HPP-treated goat meat. What has been achieved in this study is the positive effect of HPP in controlling the microbial profile in processing goat meat, resulting in an increased shelf-life of pre-packed meat due to HPP.

Jiao et al. (2020) investigated the effects of different processes (steaming, braising, boiling, pan frying, deep-frying, roasting, and drying) and studied the quality of goat meat cooked using a common thermal processing method. The direct comparison of the effect of cooking on meat quality is challenging to point out since all the processes are very varied in terms of the heating mode; nonetheless, all these processes can be classified under high-temperature processing (100-228 °C). Steaming, boiling, and braising resulted in a noticeable increase in lightness and a decrease in redness. The redness values reported in this paper can be likened to goat meat at a temperature higher than 70 °C (Liu et al., 2013). For texture, the dried sample with the least moisture content was the hardest. Although the study was intended to provide information regarding the effect of processing, there are a lot of questions that their findings can't answer, and the study also did not give a definitive explanation for the observed variations.

The effects of steaming, boiling, and roasting on goat meat tenderness were investigated using a proteomics approach (Jia et al., 2022). Their results showed that moist heat cooking (boiling and steaming) is a better option than roasting in improving the texture of goat meat's *longissimus* muscle. The proteomics data correlated improved goat meat tenderness to the

relative abundance of troponin T, a significant structural protein. The use of proteomics in understanding the effect of processing on goat meat was valuable in visualizing the muscle structural changes when exposed to thermal treatment. With the available data, it can be inferred that moist heat cooking is a better option for cooking goat meat.

This section has presented the effects of thermal processing on goat meat. However, many innovative technologies have been used for meat processing (Bhat et al., 2018; Gong et al., 2022) with a focus on improving the quality of beef or lamb. So far, goat meat has not been tested using these technologies to address its toughness. There is an extensive research gap in this area. A comparative study on the use of goat meat and other types of meat would be critical in understanding the effect of a process on goat meat. As for the literature reporting the effect of thermal processing in achieving acceptable meat quality, it appears that sous vide cooking would be an appropriate method for tough goat meat. However, this process requires a long cooking time impractical for many homes and industrial applications; some interventions using exogenous enzymes are another option to speed the process (Christensen et al., 2009; Maqsood et al., 2018). The use of sous vide processing in cooking various muscle cuts from specific breeds can be further explored to understand its applicability in goat meat processing. Furthermore, the structural examination of goat meat has been mainly done using SEM. It would be necessary to use other techniques, such as transmission electron microscopy (TEM), to reveal muscle structural changes.

Various thermal processing conditions are essential to make the meat palatable and safe for consumption. But the choice of a process can also modify meat structure, affecting not only the meat's technological properties but also its nutritional quality, particularly its protein quality. Further understanding the effect of processing on goat meat protein quality would be

relevant, particularly for a process-specific condition required for the unique goat meat. The next part of this review will focus on the relationship between processing methods and the meat protein nutritional quality.

2.7 Meat protein digestibility

2.7.1 The gastrointestinal digestion of dietary proteins

Dietary proteins from different food sources must first be transformed into single amino acids or very short peptides (di- and tripeptides) before the body can efficiently utilize them; this is achieved through the process of gastrointestinal (GI) digestion, which is precisely controlled and coordinated by the gut endocrine and enteric nervous systems (Stipanuk & Caudill, 2012).

Food digestion starts in the mouth, where food is mechanically broken down and incorporated with saliva through a process called mastication. The interaction between saliva and food forms new food complexes and structures due to the mechanical and biochemical processes (Humphrey & Williamson, 2001). Food breakdown and bolus formation depend largely on the sensory characteristics of the food as well as the physiological condition of the individual. For example, the hardness of meat, can affect the number of chewing cycles and the degree of fragmentation before ingestion (Peyron et al., 2004). During the oral phase, the break down of protein foods is mainly physical since proteins have not yet been acted upon by hydrolysing enzymes. Hence, proteins may exist intact biological molecules with a stable conformation.

Gastric digestion is compartmentalized in the stomach, and the interplay of stomach acid, enzymes, and temperature facilitates protein hydrolysis. The mechanical breakdown of dietary proteins in the stomach is facilitated by gastric motility (Bornhorst & Singh, 2012). Pepsin is the enzyme responsible for the hydrolysis of proteins in the stomach. It is highly active and stable in an acidic environment around pH 1.8-3.5 and is inactivated irreversibly at pH levels above 7.2 (Binder & Mansbach, 2016; Zhirong Fu et al., 2021). The acidic

environment in the stomach denatures proteins and enhances pepsin activity, resulting in the formation of oligopeptides. The varying protein structures in food result in different rates of pepsin hydrolysis. For example, pepsin has high activity on denatured collagen, thus facilitating the gastric digestion of meat more effectively than plant-based proteins (Chang & Leung, 2014). Some proteins in food are stable at a very low pH, which makes them more resistant to pepsin activity (Zhirong Fu et al., 2021). Moreover, a polypeptide with intramolecular disulfide bonds has also been found to resist pepsin activity (Momma, 2006). Despite the varying efficiency of pepsin in hydrolysing dietary protein, it performs a significant role in breaking down bulky protein molecules into shorter peptide chains in preparation for small intestinal digestion.

The long peptides from the stomach are further digested in the small intestine into their absorbable forms by the pancreatic enzymes and enterocyte peptidases. The pancreatic enzymes are endopeptidases (trypsin, chymotrypsin, elastase) and exopeptidase carboxypeptidase which are initially produced as zymogens (inactive enzyme precursors). Trypsin is important in stimulating the digestion of proteins in the small intestine. Trypsin activates the conversion of trypsinogen to trypsin. Consequently, trypsin converts chymotrypsinogen to chymotrypsin, procarboxypeptidase to carboxypeptidase, and proelastase to elastase. Each enzyme has a particular specificity, and their combined action results in a very efficient breakdown of proteins in the small intestine, producing free amino acids and oligopeptides bioavailable for multiple body functions (Binder & Reuben, 2009). However, proline-containing peptides mainly resist pancreatic enzyme hydrolysis primarily because of their structure. Proline has a distinctive cyclic structure that makes it rigid, inhibiting the access of proteolytic enzymes. Furthermore, the presence of proline in a peptide

can disrupt the typical sequence and structure that enzymes recognize, making the peptide less susceptible to hydrolysis.

The enterocyte peptidases are peptidases within the apical surface of the enterocyte plasma membrane where they further break down small peptides resulting from pancreatic hydrolysis. Enterocyte peptidases are protein-specific and can be aminopeptidases, carboxypeptidases, endopeptidases, and dipeptidases. Unlike pancreatic enzymes, these enzymes can cleave proline-containing peptides, complementing the action of pancreatic proteases. Hence these peptidases are vital in the hydrolysis of proteins from a wide variety of foods. In addition, the protein breakdown by brush-border enzymes is necessary to prepare the luminal digestion products to enter the circulation inside the enterocytes. For protein digestion and absorption, the coordinated action of the gastrointestinal system is very efficient; approximately only <4% of ingested nitrogen is excreted in the faeces (Binder & Mansbach, 2016).

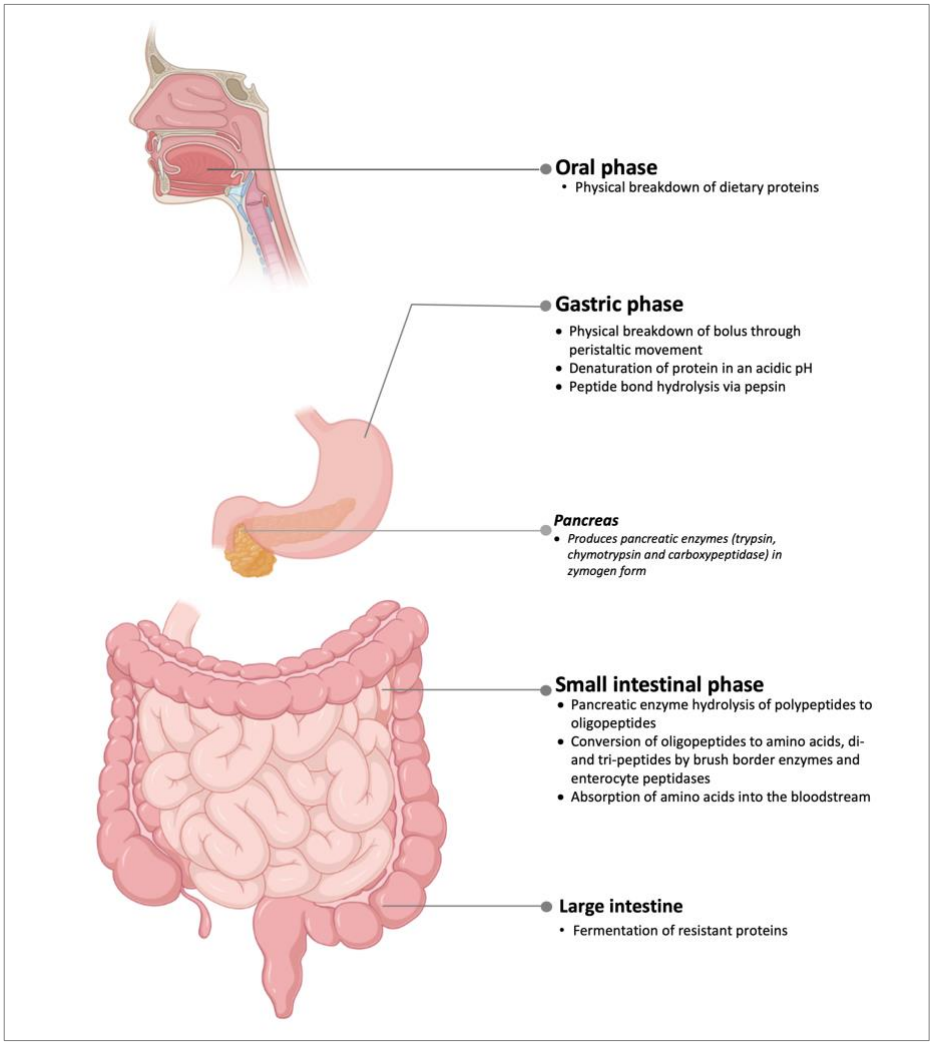

Figure 2. 2 Main mechanical and biochemical processes during gastrointestinal digestion of dietary protein. Adapted from: Gawat et al. (2023).

2.7.2 The nutritional quality of meat protein

Meat derived from animal skeletal muscle is an excellent source of protein. For this review, the discussion on meat will mainly focus on the skeletal muscle from livestock and significant papers on this aspect. Highly ordered lean muscle contains 17–23 % protein, primarily composed of myofibrillar, sarcoplasmic, and stromal proteins in varying levels (Kaur et al., 2022). Proteins in meat are approximately 40-45 % indispensable amino acids (IAA)—the nutrients needed to synthesize bodily proteins (Astruc, 2014b). Since the quality of dietary

proteins is determined by the extent to which their constituent amino acids match the amino-acid requirements of the consumer (Marinangeli & House, 2017), the presence of a high amount of IAA in meat makes it a very valuable protein source that is superior to plant-derived proteins.

In the past 100 years, many methods have been developed for assessing the quality of dietary proteins and protein quality is currently best described using the Digestible Indispensable Amino Acid Score (DIAAS) (Moughan, 2021; Wolfe et al., 2016), a scoring system where the protein quality is calculated based on the essential amino acid composition of food and the extent of digestion of these proteins at the ileum terminal (Leroy et al., 2023). Protein sources with a DIAAS ≥ 100 have excellent quality protein, and a DIAAS of 75 – 99 contains high-quality protein. Compared to other protein sources, meat has a higher DIAAS than plant-based proteins, with some exceptions, such as soy protein and potato protein (Kaur et al., 2022). In general, the (DIAAS) systems position meat proteins as good to excellent protein sources.

Aside from the amino acid composition, protein quality can also be assessed by the rate of protein digestion and absorption that affect postprandial protein synthesis and muscle protein turnover (Bax et al., 2012; Dangin et al., 2001; Pereira & Vicente, 2013). Protein digestibility describes the extent of digestion and the availability of circulating amino acids (Bohn et al., 2018; Dangin et al., 2001; Fuller & Tomé, 2005). The concept of slow and fast proteins related to protein digestibility was established by Boirie et al. (1997). Fast protein provides readily available amino acids for protein synthesis, which is significant in preventing sarcopenia in elderly individuals (Dangin et al., 2001).

In terms of protein digestibility, Remond et al. (2007) reported that meat proteins could be categorized as fast proteins since their digestion rates are closer to those observed with whey proteins (fast proteins) than with casein (slow proteins). Bax et al. (2013) also reported very high digestibility (about 95%) of meat proteins in an in vivo experiment, and meat was highly digestible under *in vitro* conditions (Farouk et al., 2014). It can be concluded that with its high protein content, the rapid digestion of meat makes it a good source of protein. With its nutritional value, high density, and highly bioavailable nutrients, meat has a critical dietary role in securing the health of a population. However, the rate of meat protein digestion is not an intrinsic factor, and the effects of processing and other treatments can affect its digestibility (Farouk et al., 2019).

2.7.3 The evaluation of meat protein digestibility

Over the past decade, extensive research has been conducted on the impact of processing on the digestive characteristics of meat, and advances in evaluating protein digestibility have led to significant knowledge about the various factors affecting protein digestibility. In food digestion research, the fate of protein in the gastrointestinal tract has been investigated using both in vivo and *in vitro* methods. Each method has advantages or disadvantages over the other.

2.7.3.1 In vivo system

The in vivo system is ideal for conducting a digestion study because it generally includes all stages and biochemical interactions during digestion (Astruc, 2014b). Even though studying human digestion is the most accurate way, ethical issues and the complexity of involving human subjects complicate an in vivo digestion study (Montoya et al., 2019). Thus, other species like growing pigs and rats, are commonly used as animal models instead of

humans (Gan et al., 2018; Xu, Shrestha, Pre át, & Beloqui, 2021). A pig model is ideal among other species since its stomach and small intestine are more comparable to the human GI tract and pigs consume meals similarly to humans, unlike grazers (Roura et al., 2016). However, there are also disadvantages to animal trials, such as high costs, subject variability, sampling restrictions, lengthy experimental duration, and ethical concerns (Astruc, 2014b; Bohn et al., 2018; Montoya et al., 2019). When using in vivo studies, the protein digestibility is measured indirectly by quantifying residual nitrogen at the extremity of the digestive tract. However, the measurement of residual nitrogen is quite challenging due to the presence of endogenous nitrogen (Fuller & Tomé, 2005). Hence, the determination of the true digestibility of a specific protein source can be more complicated for in vivo experiments. However, the relevance of conducting in vivo experiments, primarily if the experiment's goal is geared towards the specific effect on human health, should be valuable.

2.7.3.2 In vitro system

Simple *in vitro* digestion models mimicking the GIT system have been widely used as an alternative to in vivo experiments (Bohn et al., 2018). The *in vitro* method is a straightforward method of assessing protein digestibility that allows the separate set-up for gastric and small-intestinal phases by having individual reactors for each stage (Gatellier & Santé-Lhoutellier, 2009; Kaur et al., 2010). The setup ranges from simple enzyme reactions, such as the hydrolysis of proteins by pepsin in controlled conditions in a beaker, to the complex sophistication of the TNO Gastro-Intestinal Model (TIM) and other similar dynamic models (Verhoeckx et al., 2015). In vitro systems have been a significant tool for understanding the mechanisms of how food structure and composition can affect human health (Bordoni et al., 2014), they are widely applied in assessing food digestion profiles such as peptides, fatty

acids, and simple sugars, and evaluating release of micronutrients from the food matrix (Brodkorb et al., 2019). Despite the simplicity of *in vitro* models, they are often instrumental in predicting outcomes of digestion in vivo (Bohn et al., 2018; Sousa et al., 2023). In assessing protein digestibility, an advantage of using *in vitro* models over in vivo is the absence of endogenous protein secretion, and the values obtained from data generated should be comparable to true digestibility (Bohn et al., 2018; Brodkorb et al., 2019). Hence, when it comes to understanding the impact of food structure on digestibility and release of food components during digestion, an *in vitro* system is a good option.

The INFOGEST network of interdisciplinary specialists has established *in vitro* digestive protocols to obtain standard *in vitro* conditions based on physiological data (Sousa et al., 2020). In vitro systems use static or dynamic models. Dynamic models can mimic GI motility, allowing sequential secretion of digestive fluids, gradual changes in pH, gastric emptying, and the inoculation of luminal microbiota (Dupont et al., 2019). Static *in vitro* digestion set-up is relatively more straightforward and uses a fixed temperature, enzyme concentration, pH, reaction time, and agitation speed (mimicking gastric motility) to distinguish between oral and GI tract digestion conditions (Alegría et al., 2015). Although static *in vitro* models are simple, they are often very useful in predicting the outcomes of in vivo digestion.

In meat protein digestibility, recent studies have used *in vitro* systems to examine the effects of processing, structure, and post-mortem treatment on the digestibility of various meat sources (Bhat et al., 2020; Chian et al., 2019; Farouk et al., 2019; Kaur et al., 2023b; Mitra et al., 2022; Wang et al., 2022). A quantitative estimation of amino or carboxyl groups using spectrophotometric methods can determine the degree of hydrolysis of meat proteins. These

assays can be performed using ninhydrin, o-phthalaldehyde (OPA), 2,4,6-trinitrobenzenesulphonic acid (TNBS), or fluorescamine (Chalabi et al., 2014; Rutherfurd, 2019). The degree of hydrolysis of significant muscle proteins is also monitored using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). For low molecular weight proteins generated during digestion, Tricine SDS-PAGE is efficient for its separation with high resolution (Haider et al., 2012). However, the interpretation of SDS-PAGE data is often descriptive, and care should be taken during sample preparation and loading, and destaining, since these steps affect protein band intensity. In most studies, the SDS-PAGE technique and quantification of free-amino nitrogen release are often used at the same time.

2.7.3.3 Peptidomics for assessing protein digestion

Profiles of meat digests from *in vitro* systems can be further analysed using an advanced omics approach such as peptidomics. In fact, "digestomic" was coined as an analysis involving the quantitative mapping of peptides and amino acids (Bingeman et al., 2017). Peptide characterization is carried out using highly advanced mass spectrometry (MS) analysis coupled with liquid chromatography (LC) for protein separation; hence, the whole set-up is known as LC-MS. Some have used mass spectrometry with other ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) that can also provide comprehensive peptidomics data (Nadler et al., 2017). Currently, peptidomics has been extensively used to monitor the proteolysis of major proteins from food in the gastrointestinal tract during digestion (He et al., 2018; L. Li et al., 2017; Portmann et al., 2023). It identifies protein domains that are easily cleaved or sequences that resist digestion (Dupont, 2017). The efficiency of the peptidomics approach is best suited for tracking the evolution of digestive processes in both in vivo and *in*

vitro systems; this is demonstrated by monitoring peptide digestion profiles for muscle protein, dairy, and plant proteins in multiple laboratories (Portmann et al., 2023). With peptidomics, it is possible to characterize peptides and to track if they are resistant to hydrolysis. Using an *in vitro* set-up, the peptide profiles of meat digests have been investigated using different cooking methods. This was carried out for beef (Yin, Pereira, et al., 2020; Zhao et al., 2020), pork (L. Li et al., 2017; Zou et al., 2018), chicken and fish (Martini et al., 2019; Wen, Zhou, Song, et al., 2015).

Despite the usefulness of peptidomics, this method has limitations in giving the overall picture of peptide release during digestion. Very small peptides (1-4 amino acid length), the typical end product of small-intestinal digestion, are difficult to recover during the protein separation stage, and peptide profiles of the digest can be underestimated (De Cicco et al., 2019). Only dietary peptides with a molecular weight of 500–3500 Da (>5 amino acids in length) can be unambiguously identified using LC–MS–MS. Nonetheless, using peptidomics plus quantifying free-amino nitrogen release and muscle protein profiling is a powerful tool when investigating protein digestibility.

When conducting digestibility experiments, one should be specific about the type of analysis to be carried out and the target substance to be determined. In the case of meat protein digestibility, the significant enzyme involved, and the phase and conditions of the GI tract being investigated should be highly considered (Verhoeckx et al., 2015). More importantly, the complexity of *in vitro* models should be tuned toward answering specific questions related to human digestion physiology. Furthermore, the type of analysis carried out after digestion is essential to get a comprehensive understanding of the whole process, and this can be achieved through complementary methods.

2.8 Factors affecting meat protein digestibility

The mechanism of dietary protein digestion is complex, and the protein digestion rate is influenced by many factors (Pennings et al., 2013; Stipanuk & Caudill, 2012). Although it is recognized that an individual's physiology plays a significant role in protein digestion, this thesis will only focus on the influence of meat preparation or processing used before meat consumption. Meat undergoes several manufacturing processes to suit consumer demands. All these processes can potentially affect the nutritional quality of meat. **Figure 2.3** shows an overview of the meat digestion concept, highlighting the areas in which processing interventions occur before consumption. The factors affecting meat protein digestibility in terms of processing can be grouped into two categories: 1. The preparation or condition of meat (e.g., rearing condition, breed, aging, whole vs minced meat) and 2. Cooking conditions used (e.g., pan frying, sous vide, microwave, etc.). This section presents the relevant findings on the effects of meat preparation or background and thermal processing on meat protein digestibility.

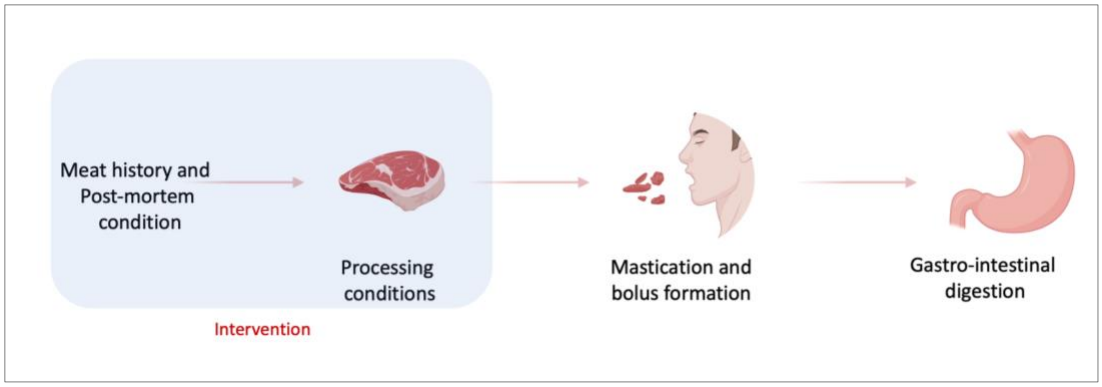

Figure 2. 3 Preparation and meat cooking conditions before consumption that affect protein digestion.

2.8.1 The effects of meat preparation method and post-mortem conditions on meat protein digestibility

The effect of aging meat on its digestibility has been investigated. Bax et al. (2012) initially reported that the impact of aging is low, while Farouk et al. (2014) reported a significant effect of aged beef (high pH) in protein digestibility. It is possible that the impact of aging was more apparent in Farouk et al.'s study, which investigated a more extended aging period compared to Bax et al.'s set-up, which was held only for 4 days. The findings of Lee et al. (2020, 2021) also supported the results of Farouk et al. (2014) that aging can improve the digestibility of red meat. However, in the *in vitro* system used by Lee et al. (2023), the digestion was carried out using an infant GI *in vitro* model, with reduced pepsin secretion and increased gastric pH compared to the normal or adult *in vitro* set-up. It can be inferred that if aging is done for a prolonged period (> 4 days), it will significantly impact meat protein digestibility due to the endogenous breakdown of muscle proteins, highlighting the importance of muscle structural integrity before exposure to digestive enzymes. Moreover, the structural changes during prolonged aging, such as breakage of transverse muscle fibres, would likely facilitate the access of digestive enzymes to myofibrillar proteins and consequently enhance their hydrolysis (Astruc, 2014b). However, the muscle structure changes before heat treatment of processing must be significant enough for its effect to be significant during digestion. Furthermore, the impact of aging will be apparent for less efficient GI conditions, such as those in infants or older people. Still, these areas need to be investigated further, mainly when aging is linked to meat pH, tenderness, and the use of different muscle types.

Table 2. 6 The effect of meat condition before processing on the protein digestibility of meat.

Meat preparation condition	Meat	Digestion method	Results	Reference
Aging	Pork	In vitro	Slightly slowed pepsin digestion rate but no impact on maximal degradation at the end of digestion.	Bax et al., 2012
Aging	beef	In vitro	Improvement in overall *in vitro* protein digestibility	Lee et al., 2020
Aging	Lamb myofibrils	In vitro	No significant effect	Santé-Lhoutellier et al. 2008
Mincing	Pork myofibrils	In vitro	No significant effect	Bax et al., 2013
Mincing	beef	In vivo	Improved digestibility	Pennings et al., 2013
Mincing	beef	In vitro	Mild effect	Farouk 2019
pH	beef	In vitro	Digestibility increased with increasing pHu	Farouk et al. 2014
Freezing	Beef puree		Freezing at -50 improved protein digestibility compared to -20 and -70 °C	Lee et al. 2023
Addition of exogenous enzyme	Beef	In vitro	Improved digestibility	Zhu et al., 2018
Muscle type	Pork	In vitro	In vitro, protein digestion of pork cuts differs with muscle type	Zou et al. 2018 (2018)

Mincing, a very common step in preparing meat, was reported not to significantly affect protein digestibility *in vitro* (M. L. Bax, C. Buffière, et al., 2013). Farouk et al. (2019) reported a minor effect of ground meat in gastric protein digestibility. However, in an in vivo study, minced beef was more rapidly digested and absorbed than beef steak, resulting in increased amino acid availability and greater postprandial protein retention (Pennings et al., 2013). Potentially, the effect of mincing on meat protein digestibility is due to the mastication efficiency of the individual; Pennings et al. (2013) designed the study for older men, where mastication efficiency is compromised. The effect of mincing could be mild, but with altered physiological conditions, this factor can significantly modulate protein digestion rate. In most meat protein digestibility studies, the oral part is usually done by grinding meat samples (Chian et al., 2019; Mitra et al., 2022); hence, the meat samples appear to be ground samples

before digestion. However, it should be considered that the resulting meat structure after mastication or before the gastric phase is the starting structure of protein digestion that can consequently affect the succeeding digestion process (Guo et al., 2020). There is a significant gap in research considering the link between meat particle size before gastric digestion and its impact on overall protein digestibility.

Regarding the animal's background, such as collagen content, slaughter age, and meat cut, minimal data is available to get a conclusive result. Meat composition only slightly affects digestion (Bax et al., 2013). Farouk et al. (2019) reported that beef from older cattle is more digestible than beef from younger cattle. This finding is surprising and lacks support from other studies. They explained that this is probably due to the protein solubility differences that affected the assay used. The digestibility of beef was not substantially affected by the muscle state of rigor or the type of cut (Farouk et al. 2019). However, in an *in vitro* study by Zou et al. (2018), the proteomics approach showed that the digestion of pork cuts differs with muscle type. The difference in digestibility between muscle cuts is possible since different cuts could vary in composition with varying levels of muscle proteins with different thermal properties (Liu et al., 2019; Zhang et al., 2013). Differentiating the effects of breeds and muscle type on protein digestibility might only be evident if the proteomics approach is used to visualize the pattern of protein hydrolysis for a specific group of muscle proteins.

In most *in vitro* digestion models, the effects of meat preparation or condition before processing are primarily relevant to gastric phase. The method of meat preparation can significantly modulate the rate of pepsin activity during the gastric phase but may not affect the behaviour of enzymes in the small intestine. However, the available data are insufficient to support this conclusion.

2.8.2 Effect of thermal treatment on meat protein digestibility

The history of habitual fire use began approximately 350,000–320,000 years ago, and this is also the time when early humans are thought to have been involved in heating and preparing food (Shimelmitz et al., 2014). The use of heating in meat processing, not just through fire, has come a long way. Meat can be grilled, steamed, microwaved, boiled, sous vide, etc. Each of these thermal processes has different cooking conditions, such as time, temperature, and cooking medium, which can induce varying effects on meat proteins and have nutritional implications.

Many studies have been conducted to determine the effect of heating on muscle protein digestibility. Bax et al. (2012) reported that cooking temperature greatly affects the *in vitro* meat protein digestion rate, and this has been the finding of many studies on beef (Bhat et al., 2020; Oberli et al., 2015), pork (He et al., 2018; Mitra et al., 2022), and on rhea meat (Filgueras et al., 2011). Generally, high-temperature heating resulted in lower digestibility compared to low-temperature processing; this is mainly explained by the denaturation and aggregation of proteins at varying temperatures that influence the accessibility of protein cleavage sites for hydrolysis. Promeyrat et al. (2010) demonstrated that at high-temperature processing (>80 °C), there is a rolling up of myofibrillar proteins into aggregates confirmed by a significant decrease in the aggregate size. Furthermore, higher temperatures induced more rapid aggregation rates and larger cluster sizes (Brenner et al., 2009; Cai et al., 2018). At higher temperatures, significant oxidation also contributes to the aggregation of meat proteins (Bax et al., 2012). At lower temperatures, meat proteins are partially denatured or unfolded, and proteolytic enzymes can quickly attack the initially unexposed peptide bonds. Moreover, at

lower temperatures, there is a lesser aggregation of proteins. Hence, many cleavage sites are available for enzymatic hydrolysis by gastric or small intestinal enzymes (Zhang et al., 2013).

Proteomic evaluation of meat digest from thermally treated meat has shown that varying temperatures can affect the hydrolysis of major muscle proteins (He et al., 2018; L. Li et al., 2017; Sayd et al., 2016). Sayd et al. (2016) reported that sarcoplasmic proteins were more hydrolyzed when processed at 50 °C than high-temperature processing at 70 °C and 90 °C. Li et al. (2017) reported that myofibrillar proteins from stewed pork cooked at 100 °C for 150 min were less digested than myofibrillar proteins from steamed pork cooked at 72 °C, in peptic digestion. Based on the limited studies using proteomics, the effect of thermal treatment is dependent on the type of muscle protein. The resistance of major myofibrillar proteins to high-temperature processing may be associated with the ability of certain muscle proteins to undergo heat-induced modifications, which can make them resistant to pepsin (Deb-Choudhury et al., 2014). Myosin, in particular, has ~ 42 sulfhydryl groups, and most of them are highly susceptible to oxidative agents. Myosin can be oxidized to form disulfide bonds and aggregates more resistant to pepsin (Liu & Xiong, 2000).

The digestibility of thermally processed meat also depends on the duration of heating. Using a controlled temperature, Kaur et al. (2014) demonstrated that beef cooked at 100 °C for 10 min had higher digestibility than beef cooked for 30 min. The results agree with those of Santé-Lhoutellier et al. (2008), who reported a decrease in protein digestibility (in terms of TCA-soluble peptides) with an increase in cooking time at 100 °C. However, in the results of Bhat (2020), varying heating duration at 60 °C for 4.5 or 10 h did not show different overall protein digestibility. It is possible that with a prolonged cooking using relatively low temperatures (<80 °C,) no further aggregation can occur since the heat denaturation of

proteins is usually irreversible at temperatures higher than 80 °C; under these conditions, proteins generally aggregate after heat denaturation (Matsuura et al., 2015). It shows that the effect of cooking duration on digestibility would be significant for high-temperature heating.

Although sous vide was reported and claimed to improve meat protein digestibility (Baldwin, 2012; Bhat et al., 2020), one should also consider using lower temperatures (< 80°C) and the cooking duration to avoid the adverse effects of protein aggregation in prolonged cooking. Moreover, even at very low temperatures, cooking duration is still potentially critical in preserving the digestibility of proteins. However, the negative effect of sous vide processing on meat protein digestibility concerning the cooking duration has not been demonstrated. Current studies are mainly conducted on the impact of different time-temperature conditions on myofibrillar modifications (Cao et al., 2019; Mitra, Lametsch, Greco, et al., 2018). Contrary to sous vide cooking, microwaving involves volumetric heating that rapidly heats food; hence, microwave heating uses a high-temperature, short-time process (Soni et al., 2020). However, the effect of microwaving on protein digestibility has been explored mainly in colloidal food systems (Xiang et al., 2020) and food (Dong et al., 2021) while the digestibility of intact muscle food under microwave has not been studied.

Overall, protein digestibility is affected by the intensity of heat treatment, and meat protein digestibility decreases as heating temperature and time increase (Bax et al., 2012; Kaur et al., 2014; Oberli et al., 2015). As the temperature further increases, the degree of protein denaturation increases. Additionally, as the length of cooking time increases, even at low temperatures, proteins tend to form larger aggregates. **Table 2.7** summarizes the effect of various heating conditions on protein structure and digestibility.

Although *in vitro* studies have shown that thermal processing can affect meat protein digestion, some findings in in vivo studies show that cooking temperature did not affect the overall protein digestibility but only the protein digestibility rate in vivo. Bax et al. (2013) reported that the rate of meat protein digestion can vary, but it does not affect the overall digestibility of meat. Furthermore, Prodhan et al. (2020) reported that different cooking temperatures and times did not affect the postprandial plasma amino acid concentrations in healthy adults. However, the findings of Hodgkinson et al. (2018) show that cooking conditions can affect the DIAAS of beef using the pig model, but these differences are minor. Nevertheless, current meat protein digestibility findings proved that meat protein is highly digestible.

Table 2. 7 The mechanisms of how various time-temperature conditions affect muscle protein structure and digestibility.

Cooking method	hydrophobicity	aggregation	oxidation	Overall Protein digestibility
Low temperature and shorter time (<80 °C, <20 min)	low	low	low	high
low temperature long time (sous vide) (<80 °C, >20 min)	initially low but will be higher as the duration increases; can reach a point of no further increase	low but will increase with further heating	low	low-moderate
high temperature but shorter (>80 °C, < 20 min)	high	moderate aggregation	high	low-moderate
High temperature and long time (>80 °C, >20 min)	Initially high and can decrease due to protein aggregation over time	high	high	low

References: (Bai et al., 2016; Bax et al., 2012; Bhat et al., 2020; He et al., 2018; Kaur et al., 2014; Mitra et al., 2022). The low and high-protein digestibility columns refer to the susceptibility of the resulting cooked meat protein to be hydrolysed by proteolytic enzymes during digestion and are not a definitive unit of measure.

The proteomics approach in the analysis of meat digestion shows that as a consequence of thermal processing, some muscle proteins are resistant to pepsin, affecting the rate of gastric digestion, but these proteins are efficiently hydrolyzed in the small intestinal phase (L. Li et al., 2017; Sayd et al., 2016). Hence, the thermal processing of meat can significantly affect the protein digestibility rate, mainly seen during the gastric phase. But the efficiency of the proteolytic enzymes in the small intestine can convert most meat proteins to amino acids and tri- and di-peptides at the end of digestion. But this needs to be elucidated further. Research on the digestibility of meat proteins affected by processing conditions is still relevant. The rate of meat protein hydrolysis is significant in the concept of fast and slow proteins, as established by Boirie (1997). Fast proteins are essential in improving postprandial protein anabolism for elderly individuals (Dangin et al., 2003). However, it should be recognized that the body's postprandial utilization of dietary amino acids varies according to the speed of protein digestion and the physiology of the consumer (M.-L. Bax et al., 2013; Stipanuk & Caudill, 2012).

2.9 Gaps in literature

The feral goat population is a significant source of goat meat in New Zealand, and it is often identified as an inferior meat compared to a Boer, a known meat breed. However, currently, there is no data about the quality characteristics of feral goat meat produced in New Zealand, and specifically for feral goats slaughtered for export. Furthermore, the main quality issue for goat meat is its toughness. Many authors have argued that this was mainly due to its low intramuscular fat content, leading to cold shortening, and high collagen content with low solubility, but these factors would potentially differ among breeds. Currently, there are no pertinent data describing the quality of goat meat produced in New Zealand. Hence, the evaluation of the quality of New Zealand feral goats compared to other breeds would be relevant in determining if there is a significant difference between these breeds and in understanding variation in their quality, if there is any.

Processing intervention would be the best tool to enhance the quality issues of goat meat, specifically its toughness. However, very limited studies have been done to explore innovative processing technologies such as sous vide, ultrasound, pulsed electric field, etc., to improve the quality of goat meat. The current literature regarding goat meat processing shows that sous vide might be an appropriate process for tough meat such as goat. However, there is a large research gap in understanding how sous vide, or any innovative processing technology can enhance the quality of tough goat meat. Appropriate time-temperature combinations applicable for certain muscle cuts of a certain breed can be evaluated. Moreover, the evaluation of the physicochemical properties and chemical compositions of goat meat can be done to understand the changes occurring in goat meat during processing. A lot of research can be done to fully understand how goat meat can be processed or handled and make it more

acceptable without sacrificing its nutritional composition. The effect of these processes on goat meat quality can also be further evaluated by analysing meat ultrastructure.

As reported, most studies describing the biochemical mechanisms of meat protein structural modifications in response to processing are based on beef, chicken, or pork, leaving this significant information unexplored for goat meat. However, several potential mechanisms must be understood between goat meat structural modification, processing, and digestion. More importantly, tough goat meat requires more intense processing to achieve acceptable tenderness. This can be explored using prolonged cooking or high-temperature processing. However, the implications of these processes on goat meat protein digestibility have not been evaluated. Although sous vide is reported to enhance meat protein digestibility, the effect of prolonged cooking was reported to result in further aggregation of meat proteins even at low temperature. Hence, it is still possible that the use of sous vide conditions can lead to significant molecular changes that would also decrease meat digestibility. This condition can be explored further in comparison to cooking meat using high temperature.

Although generally, meat proteins are highly digestible, processing can influence the rate of meat protein hydrolysis, and the rate of protein digestion is more relevant when looking into postprandial protein anabolism for elderly individuals. Hence, a study on the link between goat meat, processing, and digestion is essential to understand the implications of processing on the nutritional aspect of cooking goat meat.

2.10 Copyright information

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Chapter 3

Physicochemical, ultrastructure and quality of New Zealand goat meat: The effects of breed and muscle type

3.1 Introduction

New Zealand has a small goat meat industry that contributes to its meat product revenue. However, goat meat production in the country is less specialized, making it challenging for goat meat to compete with the established beef and lamb supply. Most of the goat meat produced in the country comes from feral goats (New Zealand wild goats) and Boer goats. Of the total goat meat produced, about 60 % are being exported that are mainly sourced from feral goats (Rotorua Land Use Directory, 2019). In contrast, meat from Boer goats and some from dairy goats are sold domestically (Gillingham, 2008; Lopez-Lozano et al., 2017).

The feral goat population in New Zealand initially came from goats introduced into Arapawa Island in the Marlborough Sounds during the voyage of Captain Cook in the 1770s (Gillingham, 2008). These goats were thought to be descendants of the Old English breed, a common all-purpose goat breed in Britain in the 1770s (Sutherland & Staff, 2016). The early feral herd was joined by the goats brought by whalers, sealers, and mariners, and later the domesticated fibre goats such as Angora escaped from farms (Alley, 1991; Parkes, 1990). At present, there is still a large population of feral goats, thriving in about 14% of the country's land area (Department of Conservation, 2020). The domesticated feral goats are farmed with sheep and cattle for weed control and goat meat production. Since feral goats are readily available, they have the advantage of being produced in high volume as a cheap source for both slaughter and breeding stock.

The Boer goat meat supplied in the New Zealand market comes from Boer crosses with dairy or fibre goat genetics. Meat from Boer goat is considered a premium goat meat; in fact, Boer or South African Boer is the most popular imported goat breed for meat production (Luginbuhl, 2015). On the other hand, meat from feral goats has been tagged as inferior meat.

The average value per Kg of Carcass Weight (CW) from feral is usually priced lower than carcasses from Boer and dairy goats (Scholtens et al., 2017).

While data on goat meat quality characteristics from popular goat breeds have been reported, there are no data available describing the meat quality differences between New Zealand feral goats and Boer crossbred goats. Moreover, the information about the attributes of New Zealand goat meat is very limited. Hence, this study was designed to provide valuable information regarding the meat quality characteristics of feral goats and Boer crossbred goats. Additionally, this study seeks to evaluate goat meat's chemical and physicochemical properties to explain their meat quality differences.

The main concern about goat meat is its toughness and this is mainly attributed to its low-fat content linked to the susceptibility of goat muscles to undergo cold shortening (Kannan et al., 2014; Shija et al., 2013). At present, there is no recorded data on goat muscle structure supporting the occurrence of cold shortening in goat meat carcasses. Likewise, the severity of muscle contraction due to cold shortening caused by the current commercial processing conditions has not been examined. Thus, this chapter also sought to investigate the ultrastructural characteristics of goat meat from various breeds processed under a typical commercial processing condition in New Zealand. The results of this study will be significant in understanding the intrinsic structural features of goat meat that might be important for improving the goat meat production system in the country.

3.2 Materials and methods

3.2.1 Animals and carcass selection

The Boer cross and New Zealand feral goats were supplied by New Zealand Premium Goat Meat (Central Otago, New Zealand) between November 2020 and April 2021. The animals aged 12-24 months were categorized as young goats that produce chevon. Boer goats were sourced from North and South Island farms and the feral goats were specifically sourced from the Manawatu region. The background of the animals was confirmed from the supplier, where Boer crossbreed goats were at least 75% South African Boer with a small Saanen and Cashmere percentage. The feral goats were farmed from feral bloodstock originally from New Zealand highlands. The diet of the goats was described by the supplier as being mainly grass, rosehip, broom, and weeds.

The animals were slaughtered at Venison Packers Feilding Ltd. (Feilding, Manawatu, New Zealand), following the New Zealand Code of Welfare: Commercial slaughter (MPI, 2018). The goats were allowed to rest in lairage overnight between transport and slaughter. Goats were electrically stunned (3 amps for 3 sec), exsanguinated, weighed hot, suspended by the Achilles tendon, and dry-aged at 4 ± 1 °C overnight. The carcass selection was done randomly from a slaughter line and screened based on the average empty body weight (15.37 ± 1.5 kg) and girth rib (GR) fat score of 3 to 4. The fat score was measured using the actual soft tissue depth at the GR site (110mm from the midline over the 12th rib). A total of twenty-three goats: 7 castrated male Boer crossbreed (BM), 8 female Boer crossbreed (BF), and 8 female feral (FF) goats were selected.

Figure 3. 1 Goat meat carcass within 24 hr post-mortem.

Table 3. 1 The carcass characteristics of goat meat samples.

Trait	Feral	Boer XF	Boer XM
Breed	New Zealand feral	Boer cross	Boer cross
Sex	Female	Female	Male
Age	1-2 years old	1-2 years old	1-2 years old
Average empty weight	13.74	16.37	16.0
Fat score	3,4,5	3,4	3
Source	Manawatu Region/North Island	Hakataramea Valley/South Island	Hakataramea Valley/South Island
Diet	Grass, Rosehip, broom, and weeds	Grass, Rosehip, broom, and weeds	Grass, Rosehip, broom, and weeds

3.2.2 Muscle sampling procedures

After chilling overnight (~16 hrs) at 4 ± 1°C, the carcasses were transported to a butchering facility, and *Longissimus thoracis et lumborum* (LTL) and *semimembranosus* (SM) muscles were excised, and vacuum packaged. These samples were aged (wet aging) for another 24 h at 4 °C. After 48 h postmortem, each muscle was removed from the vacuum bag, and visible fat and epimysia sheaths were uniformly removed. **Figure 3.2** shows the sampling

procedure for each muscle. For *longissimus thoracis et lumborum* (LTL), only the cranial *M. longissimus thoracis* (LT) portion was utilized; hence all samples from LTL will be referred to as *longissimus thoracis* (LT) in this paper. Only muscles from the left side were used for colour and pH evaluation for each carcass. The left and right muscles were utilized for proximate composition (section A), sous vide cooking, and measurement of collagen content (section C). Samples from section C were also used to analyse various meat quality parameters for cooked samples. Sections B and C were processed immediately, while samples from section A were vacuum-packed and stored at -20 °C until use.

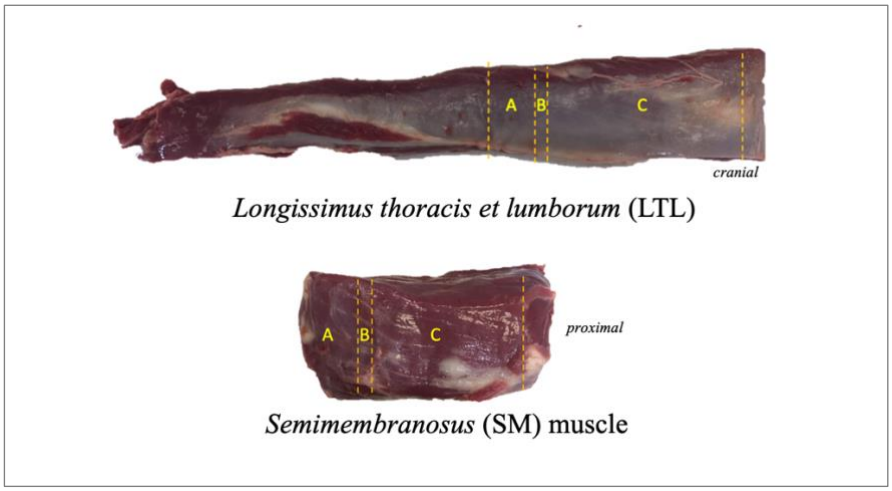


Figure 3. 2 Sampling procedure on longissimus thoracis et lumborum (LTL) and semimembranosus (SM) muscle.
(A) Proximate composition. (B) Colour, pH, and microstructure/ultrastructure. (C) Sous vide and collagen content.

3.2.3 Sous vide cooking

The sous vide cooking conditions were chosen after preliminary sous vide treatments over a range of cooking times using goat meat from the supermarket. The chosen treatments consisted of 1) 60 °C for 3, 6, 12 and 14 h and 2) two-stage sous vide cooking at 45 °C for 3 h

and a second temperature at 60 °C for 3 h (45+60 °C in total 6 h) (Ismail et al., 2019b; Xiaojie Zhu et al., 2018) (Supplementary material 3.1). The time-temperature combination of 60 °C for 6 h for both LT and SM muscles showed acceptable tenderness values (Peak shear force value < 40 N) (Warner et al., 2017) and was chosen as the cooking condition. Cooking red meat for more than 4 h at 60 °C is an acceptable cooking condition for sous vide cooking in New Zealand from microbial safety aspects (MPI, 2015). Additionally, this process can be more easily and practically applied in household or commercial cooking instead of the double-step sous vide process. Six samples with a dimension of 5 × 4 × 1.5 cm (28 ± 2 g/slice) were used, totalling six sous vide bags for each muscle type per carcass. The measurements used were based on the maximum steak size that can be achieved for a given goat meat sample. After cooking, the bags were immediately placed in ice-cold water for at least 15 min. Representative cooked meat samples (~5 g and without the cooking liquid) from each bag were taken for proximate composition analysis. Additionally, the cooking liquid was also collected from each bag for collagen content determination. The meat and cooking liquid samples were stored at -20 °C until use.

3.2.4 Meat quality measurements

3.2.4.1 pH

The pH of raw and cooked samples was determined using a pH meter (Orion 3 Star, Thermo Electron Corporation, Waltham, MA, USA) equipped with an insertion glass electrode (Sensorex, Garden Grove, CA, USA) and a thermocouple that were inserted into the center part of the meat. Before use, the pH meter was calibrated using buffers of pH 4 and

7 at room temperature (17-20 °C). The probe was rinsed using reverse osmosis (RO) water between samples.

3.2.4.2 Colour

Colour measurement was done following the recommended guidelines for instrumental meat colour measurements (Hunt et al., 2012). The sample was cut to a thickness of 12–15 mm, then allowed to bloom on a tray at 4 °C for 30 min. The cutting was done across the length of the muscle. After blooming, six different locations along the cut surface of the meat were selected and served as the six measurement points. The colour values lightness (L^*), redness (a^*), and yellowness (b^*) were measured using a handheld Minolta Chroma Meter CR- 400 (aperture size of 3.18 cm, Konica Minolta Sensing, Inc., Japan) with D65 Illuminant and at 10° observer angle. Additionally, Chroma (colour intensity, $C = [a^{*2} + b^{*2}]1/2$) and hue angle (discolouration, $HA = \tan{-1}\,[b^*/a^*]$) were computed.

3.2.4.3 Cooking loss (%)

Samples subjected to sous vide cooking were used for cooking loss measurement. After cooling for approximately 20 min in an ice bath, the samples were taken from the bags, blotted with filter paper, and weighed. Cooking loss (%) was the percentage loss related to the initial sample weight.

$$\text{Cooking loss (\%)} = \frac{raw\ weight - cooked\ weight}{raw\ weight} \times 100$$

3.2.4.4 Meat texture

The texture of cooked SM and LT muscles was determined using TA.XT plus texture analyser (Stable Microsystems, Godalming, UK). The cooked meat was chilled overnight at 4 °C to obtain firm samples, then a section, 1×1 × 2 cm (1cm^2 shear area) with a

fibre direction parallel to the length of the sample was cut. The cuts were collected randomly, and 6 subsamples were obtained per muscle type, per animal. Samples were sheared perpendicularly to the muscle fibre direction using a V-notched cutting blade, with a peak force obtained using a 50 N load cell at a crosshead speed of 250 mm/min (Ismail et al., 2019b). The meat texture was reported as the peak shear force (PSF) value in Newtons (N). The values reported are the average of the 6 subsamples.

3.2.5 Chemical properties measurement

3.2.5.1 Proximate composition analysis

Before analysis, the meat was trimmed to remove connective tissues and visible fat and was minced finely. The moisture content was determined using an air-oven method 950.46 (AOAC, 2006) set at 105 ± 1 °C for 3 h. The ash content was determined using a dry ashing method at 550 °C for 5 h in a muffle furnace (Thiex et al., 2012). The total crude protein content of the raw and cooked meat samples was determined using the Kjeldahl method 984.13 (AOAC, 1990) using FOSS KjeltecTM 8200. The factor used for converting nitrogen to total crude protein was 6.25. The intramuscular fat (IMF) content analysis was done using the Soxtec method 991.36 (AOAC, 2000).

3.2.5.2 Total collagen content of raw meat and heat soluble collagen in cooking loss

The determination of the total collagen content in raw meat and the soluble collagen in the cook loss liquid sample after cooking was done using the methods of Kolar (1990). The colour development and absorbance measurements were carried out as outlined by their method, while the hydrolysis steps for the meat and cook loss samples were modified.

For the hydrolysis of fresh meat samples, 4 g of homogenized meat were treated with 30 mL of H2SO4 (3.5 M) at 105 ± 1 °C for 16 h. After hydrolysis, the hot hydrolysate was filtered using a qualitative filter paper (Whatman® qualitative filter paper, Grade 1) and diluted to 500 mL in a volumetric flask. Then, 5 mL of the filtrate was diluted with Milli-Q water to 100 mL as the final solution.

For the hydrolysis of cook loss liquid, samples were thawed and centrifuged at 3000 × g at 4 °C for 30 min (Ismail et al., 2019c). Then 5 mL of the supernatant was hydrolyzed using 30 mL of H_2SO_4 (3.5 M) at 105 ± 1 °C for 16 h and diluted with Milli-Q water to 100 mL. Then, 10 mL of this solution was diluted further to 50 mL as the final solution.

3.2.6 Meat ultrastructure

The changes in goat meat ultrastructure were examined using Transmission Electron Microscopy (TEM) analysis. The raw meat at 48 hrs postmortem and sous vide samples were cut into 10 × 3 × 3 mm using a carbon steel surgical blade. The cut samples were fixed immediately using a modified Karnovsky fixative and stored at room temperature (20 ± 2 °C) before processing. The method from post-fixing to ultra-thin section staining was carried out using the protocol established by the Massey Manawatu Imaging Centre (MMIC) (Sample Processing for TEM, unpublished) as described by Chian et al. (2019). Post-fixing was done in 1% osmium tetroxide, followed by washing and dehydration through a graded acetone series. After the pre-treatment, a 100 nm ultra-thin section was cut from the treated tissue and was stained with lead citrate for 4 min, then washed with Milli-Q water. These stained sections were stored at room temperature until used for TEM examination.

The stained ultra-thin sections were viewed under the FEI Tecnai G^2 Spirit BioTWIN transmission electron microscope (FEI Corp., Brno-Černovice, Czech Republic).

The sarcomere length for raw tissues was measured on TEM images (at x8,200 and x11,500 magnification) using the ImageJ software version 1.53a (National Institute of Health, Bethesda, MD, USA). Three images were examined for each sample, and sarcomere length from 10 muscle fibres per image was measured (Supaphon et al., 2021).

3.2.7 Statistical Analysis

Throughout the paper, the term animal group will be referred to as the group of breed × sex to avoid confounding since male feral goat samples were not available. The comparisons done were between animal groups (BF, BM, and FF), between female Boer cross and female feral goats (BF and FF) for the effects of breed, and between female and male Boer crosses (BF and BM) for the effects of sex. The determinations of each parameter were done in triplicate unless otherwise stated. Normality test was performed before analysis using the Shapiro-Wilk test, and outliers (computed using Dixon's test) were removed where applicable. Statistical evaluation was performed using the Mixed-effects model of Minitab Version 19.2020.2.0 (Minitab Inc., State College, PA, USA). The model includes animal group as fixed factor and individual animals as random factors. The estimation method for the variance component used was restricted maximum likelihood (REML) and Kenward-Roger's approximation for fixed effects analysis (Starkey et al., 2015). The statistical analyses were performed separately for each muscle type. To classify the level effects into groups when at least one group was statistically different, multiple comparisons analysis was done using the Tukey test at a 95 % confidence interval. The significance level between muscle types was assessed at $p < 0.05$.

To explain the variation in shear force, one prediction model using both muscle groups was fitted. The fitted covariate terms were pH, cook loss, total collagen, collagen solubility;

fixed terms were an animal group and muscle type, and the random term was the individual animal (Model 1). In addition, the cross-factor animal group × muscle type was added to the model. Values reported were based on the best-fitted mixed-effects model with the given sample size. Principal component analysis (PCA) was used to determine the association of the colour properties of raw meat. PCA was run using prcomp in R (Rstudio Version 1.4.1103), and PCA biplots were presented using two principal components.

3.3 Results and discussion

3.3.1 Meat composition

The summary of meat composition for raw and sous vide cooked meat is presented in **Table 3.2** Additional information for the interaction effect of muscle and animal group on meat composition and other parameters is presented in the supplementary document (**Supplementary Tables 1-3**). The total protein and ash contents did not vary (p >0.05) between genotype and sex. The obtained values are within the range reported in the literature for various goat breeds (A. H. Kirton, 1970; Ripoll et al., 2012; Tshabalala et al., 2003). Significant variation was recorded between FF and BF for moisture. BF had higher moisture than FF for both LT (p < 0.01) and SM (p < 0.05); while no difference (p < 0.05) was seen for BF and BM.

The intramuscular fat (IMF) values obtained for feral goats and Boer crossbreed goats were comparable to values reported for Boer and Australian feral goats (Werdi Pratiwi et al., 2006). These values (0.6 -1.5 %) correspond to a very low IMF (< 2%) for meat (Bessa et al., 2015) and agree with the reported low IMF for goat meat which is comparable to poultry meat and significantly less than beef and lamb (Johnson et al., 1995; Okuskhanova et al., 2017;

Sheridan & Ferreira, 2003). The IMF contents differed significantly ($p < 0.05$) between breeds or LT muscle but was similar for SM muscle. The effect of genotype on goat meat IMF also varies in the literature. Many authors have reported that IMF is a common parameter that varies between breeds in goat meat composition studies (Dhanda, Taylor, & Murray, 2003; Ripoll et al., 2012). On the other hand, a study by Van Wyk et al. (2022) showed that meat from Boer goats and Indigenous Veld goats did not differ in IMF content. The significant differences in IMF values were also apparent between sexes, where BM had lower IMF than BF for both muscles. This observation is consistent with the reports that meat from male goats contains less fat than meat from female goats (A. H. Kirton, 1970; Tshabalala et al., 2003). The variations between muscle types and animal groups can be explained by the fact that meat composition varies considerably with the breed, age, sex, weight, and the plane of nutrition (Webb et al., 2005). Our data show that meat from feral goats is not always be leaner than meat from Boer cross goats and that IMF content can vary between breeds depending on the muscle or cuts.

After cooking, both LT and SM from feral goats were consistent in having lower moisture content than those from the Boer goats. The LT muscle of FF had lower moisture ($p < 0.05$) than BF. The LT muscle of FF had higher IMF than BF and BM. Furthermore, the LT muscle of BF had higher IMF ($p < 0.01$) compared to the LT of BM for cooked samples. Hence, our results show that fat content and moisture can differentiate the samples for cooked feral goat and Boer cross meat samples. These parameters also distinguished the raw samples between breeds. Since cooking methods can affect the chemical composition of meat (Lopes et al., 2015), meat composition changes after cooking are inevitable. Sous vide cooking at 60 °C for 6 hrs showed different effects on the LT and SM muscles. The difference in cooking

losses between the SM and LT muscles can explain the differences between the moisture content of the cooked samples.

Table 3. 2 The proximate composition for raw and sous vide cooked longissimus thoracis (LT) and semimembranosus (SM) muscles.

Parameters	Muscle	Animal group			*p*-value[1]		
		FF	BF	BM	Animal group	Breeds (FF × BF)	Sex (BF×BM)
Raw							
Moisture (%)	LT	71.7 ± 0.32^{aX}	73.6 ± 0.34^{aX}	74.3 ± 0.39^{aX}	ns	**	ns
	SM	73.6 ± 0.25^{aY}	73.7 ± 0.42^{aX}	74.4 ± 0.49^{aX}	ns	*	ns
Ash (%)	LT	1.16 ± 0.02^{aX}	1.16 ± 0.02^{aX}	1.10 ± 0.04^{aX}	ns	ns	ns
	SM	1.17 ± 0.02^{aX}	1.16 ± 0.02^{aX}	1.20 ± 0.02^{aX}	ns	ns	ns
Total protein (%)	LT	23.1 ± 0.21^{aX}	23.5 ± 0.56^{aX}	22.1 ± 0.23^{aX}	ns	ns	ns
	SM	23.1 ± 0.20^{aX}	22.5 ± 0.29^{aX}	22.4 ± 0.20^{aX}	ns	ns	ns
Intramuscular Fat (%)	LT	5.12 ± 0.29^{aX}	2.54 ± 0.45^{bX}	3.81 ± 0.61^{aX}	**	*	**
	SM	3.26 ± 0.33^{bY}	3.52 ± 0.22^{abX}	3.02 ± 0.16^{abX}	**	ns	**
Sous vide							
Moisture (%)	LT	66.6 ± 0.30^{aX}	67.5 ± 0.52^{ab}	67.9 ± 0.66^{b}	*	*	ns
	SM	68.0 ± 0.32^{aY}	69.3 ± 0.50^{a}	68.9 ± 0.58^{a}	ns	ns	ns
Ash (%)	LT	1.13 ± 0.02^{aX}	1.06 ± 0.03^{aX}	1.10 ± 0.01^{aX}	ns	ns	ns
	SM	1.12 ± 0.02^{aX}	1.21 ± 0.01^{aY}	1.17 ± 0.01^{aY}	ns	ns	ns
Total protein (%)	LT	26.8 ± 0.29^{aX}	27.1 ± 0.24^{aX}	27.7 ± 0.38^{aX}	ns	ns	ns
	SM	28.1 ± 0.41^{aY}	26.7 ± 0.66^{aX}	27.9 ± 0.63^{aX}	ns	ns	ns
Intramuscular Fat (%)	LT	6.55 ± 0.37^{bY}	5.30 ± 0.53^{aY}	4.40 ± 0.54^{aX}	**	*	**
	SM	3.43 ± 0.38^{aX}	3.95 ± 0.95^{aX}	3.61 ± 0.72^{aX}	ns	ns	ns

Animal group- FF- feral female, N=8; BF- Boer cross female, N=8; BM- Boer cross male, N=7.

FF × BF- difference between female Boer cross and female Feral (Genotype effect), BF × BM – difference between female and male Boer crosses (Sex effect).

[a-c] Means ± SEM within a row with the same superscript letter are not different ($p > 0.05$) between animal group.1ns = $p > 0.05$, *= $p < 0.05$, and ** = $p < 0.01$.

[XY] Means ± SEM within a column with the same superscript letter are not different between muscle types ($p > 0.05$).

SEM- Standard error of the mean.

For all the animal groups, moisture content decreased due to cooking loss, and consequently, protein and fat content increased. The increase in protein and fat is expected due to moisture loss during the process, increasing the relative proportions of other components. Furthermore, the stable ash content for all muscles showed that the sous vide process only led to no or low leaching of minerals. Although there are reports in the literature that sous vide cooking can significantly reduce the mineral content in meat (Bhat et al., 2020; Dominguez-Hernandez et al., 2018), our results indicate that the sous vide conditions used did not cause significant mineral leaching.

3.3.2 Meat quality measures

3.3.2.1 Meat pH

Regardless of breed, the average pH values of raw goat meat ranged from 5.76 to 5.98 (**Table 3.3**) and fell under the intermediate pH category (5.7 – 6.3). This agrees with the reported optimum pH range for goat meat, which is between 5.5 and 6.2 (Herold et al., 2007; Kadim et al., 2014; Werdi Pratiwi et al., 2006). Furthermore, this supports the observation that goat meat typically has a higheer pH compared to the normal optimum pH (5.4 – 5.7) for most animals (Astruc, 2014b; Pulford et al., 2009a; Webb et al., 2005). Among Boer cross and feral goats, no significant differences ($p > 0.05$) were observed for pH. Between muscle types within a group, the pH of LT and SM muscles for FF and BF differed ($p < 0.05$). The observed variation in pH between muscles is common for fresh meat as influenced by muscle fibre composition (glycolytic and oxidative fibres) (Listrat et al., 2016b).

The high pH obtained from all samples indicates that even at 48 h postmortem, goat meat does not readily achieve the lower pH (< 5.7), which is desirable for optimum technological and organoleptic characteristics (Young et al., 2004). Additionally, goats are prone to perimortem stresses that exacerbate glycogen depletion (Pophiwa et al., 2020; Webb, 2014). Possibly, feral and Boer cross goats had low glycogen content. A high pH for goat meat, resulting from a glycogen store below the critical threshold, has been reported by Abhijith et al. (2021) and Simela & Frylinck (2004a). However, it should be noted that a low glycogen level is not always the limiting factor for glycolysis, the absence of adenosine monophosphate and the inactivation of glycolytic enzymes can also limit further glycolysis (Gagaoua et al., 2015).

3.3.2.2 Cooking loss

Sous vide cooking at 60 °C for 6 h resulted in some cook losses, supported by the changes in moisture content values of meat after sous vide cooking. Cooking loss values were not significantly different between genotypes ($p > 0.05$), suggesting that meat from female feral and female Boer goats responded equally to the sous vide treatments used. However, cooking loss was observed to vary ($p < 0.05$) only for SM muscle of BF and BM. Across animal groups, the variation in the cooking loss was mainly influenced by muscle types, with a significant main effect of $p = 0.006$ (data not shown). The LT muscle had significantly ($p < 0.05$) higher cooking loss than SM, consistent for each animal group. Higher cooking loss values for LT muscle from all samples were consistent with the low moisture content of LT after sous vide cooking. Higher cooking loss for LT agrees with those reported in literature where LT muscle had higher cooking loss than other muscle types for Lingqiu Greyback goats (Pan et al., 2021) and Australian feral goats (N. M.

Werdi Pratiwi et al., 2007), but in contrast to the results for Omani goats (Kadim et al., 2004) and Spanish does (Kannan et al., 2001). It is generally accepted that water loss during cooking is related to thermal denaturation and subsequent changes in the protein conformation of a specific protein type (Zielbauer et al., 2016). Hence, the specific differences in muscle composition for each muscle type can also explain such differences.

Compared to other muscle types that were sous vide cooked in the same conditions, the cooking loss for SM and LT differed from some values reported values in the literature. For example, compared to Korean native black goats, SM and LT in this study had higher cooking loss than bicep femoris (BF) (19.43 %) and gluteus medius (GM) (19.57 %) (Ismail et al., 2019b) and lower than semitendinosus (~25 %) (Ismail et al., 2019a). Cooking loss differences between muscles show that muscle types affect the ability of meat to retain moisture. Individual muscles from different anatomical positions have different myofibrillar and connective tissue profiles (Hwang et al., 2019), which undergo physical changes such as shrinkage (Offer, 1989) and swelling (Baldwin, 2012). These changes are crucial factors for cooking loss, primarily influenced by meat pH, muscle fibre composition, sarcomere length, and cooking conditions (M. Christensen et al., 2011; Listrat et al., 2016b).

Table 3. 3 Meat quality measures for raw and sous vide cooked longissimus thoracis (LT) and semimembranosus (SM) muscles.

Parameters	Muscle	Animal group			*p*-value[1]		
		FF	BF	BM	Animal group	Breed (FF × BF)	Sex (BF × BM)
pH (48 h)	LT	5.79 ± 0.21^{aX}	5.87 ± 0.04^{aX}	5.80 ± 0.06^{aY}	ns	ns	Ns
	SM	5.98 ± 0.02^{aY}	5.83 ± 0.48^{aX}	5.76 ± 0.06^{aX}	ns	ns	ns
Cooking loss (%)	LT	24.6 ± 0.51^{aY}	23.5 ± 0.40^{aY}	25.2 ± 0.63^{aY}	ns	ns	ns
	SM	22.7 ± 0.33^{abX}	20.7 ± 0.41^{aX}	24.2 ± 0.54^{bX}	*	ns	*
Collagen content (mg/g)	LT	16.2 ± 2.75^{bX}	6.9 ± 0.61^{aX}	7.6 ± 0.92^{aX}	**	**	ns
	SM	12.0 ± 1.72^{aY}	10.2 ± 2.88^{aY}	7.8 ± 0.63^{aX}	ns	ns	ns
Collagen solubility (%)	LT	0.8 ± 0.11^{aY}	0.9 ± 0.18^{abY}	1.6 ± 0.25^{bX}	*	ns	ns
	SM	0.8 ± 0.19^{aX}	0.8 ± 0.15^{aX}	1.4 ± 0.19^{bX}	**	ns	ns
Peak Shear force (N)	LT	87.3 ± 4.73^{aY}	71.4 ± 4.29^{aY}	76.1 ± 7.59^{X}	ns	*	ns
	SM	66.7 ± 1.70^{aX}	62.1 ± 4.66^{aX}	71.5 ± 7.33^{aX}	ns	ns	ns

Animal group- **FF**- feral female, *N*=8; **BF**- Boer cross female, *N*=8; **BM**- Boer cross male, *N*=7.

FF × BF- difference between female Boer cross and female Feral (Genotype effect), **BF × BM** – difference between female and male Boer crosses (Sex effect).

[a-c] Means ± SEM within a row with the same superscript letter are not different ($p > 0.05$) between animal group.[1]ns = $p > 0.05$, *= $p < 0.05$, and ** = $p < 0.01$.

[XY] Means ± SEM within a column with the same superscript letter are not different between muscle types ($p > 0.05$).

SEM- Standard error of the mean.

3.3.2.3 Collagen content and collagen solubility

Meat from feral goats consistently had a higher collagen content than meat from Boer crosses, although significant variation was observed only for LT muscles ($p < 0.05$). The LT from FF had significantly higher ($p < 0.01$) collagen content than BF. The higher collagen from feral goats could be an acquired trait as a result of how they forage for food in hilly slopes and mountainous regions, leading to more developed collagen. Surprisingly, even though SM was reported to have the highest collagen content among goat muscles (Bakhsh et al., 2019; Hwang et al., 2019) it was not evident in our results. Within animal groups, the LT muscle of FF had higher ($p < 0.05$) collagen than SM, while SM of BF had higher ($p < 0.05$) collagen content than LT. Since the level of connective tissue in a muscle is dictated by its functional requirements (Purslow, 2014), possibly, the cranial end of the longissimus muscle used was the portion with developed connective tissues when goats forage for shrubs and trees as their preferred diet (Chebli et al., 2020).

Collagen solubility varied significantly between LT ($p < 0.05$) and SM ($p < 0.01$) across animal groups. Notably, the muscles from feral goats were consistent with having the lowest collagen solubility across animal groups. Although statistically, variation was not significant when examining female feral and female Boer, the differences in value across the three animal groups is worth noting. Low collagen solubility of meat from feral is possibly due to higher collagen crosslinking. The effects of breed on collagen characteristics is a common observation reported in the literature (Purslow, 2014). The differences in collagen content and solubility among genotypes can be explained partly by the growth characteristics of each breed, where

early-maturing breeds tend to have higher collagen content with less solubility (Campo et al., 2000; M. Christensen et al., 2011). Unfortunately, growth characteristic data for Boer crosses and feral goats are not available to link collagen characteristics to their stage of maturity.

The collagen solubility values obtained for SM and LT muscles from feral goats and Boer crossbreed goats were comparatively lower than those of goat meat from Korean native black goats cooked under the same conditions (Ismail et al., 2019b), with collagen solubility around 2.0-6.3 % for semitendinosus, gluteus medius, and biceps femoris. Although the level of collagen solubility is highly dependent on cooking conditions (Roldán et al., 2013; Tornberg, 2005), the degree of collagen cross-linking and the organization of the endomysium and perimysium also play significant role in the process of solubilizing collagen, which are highly influenced by genotype, muscle type, age, and diet (Lepetit, 2008; Listrat et al., 2016b). While collagen denaturation occurs between 56 °C and 62 °C (Martens et al., 1982), the collagen from SM and LT muscles from Boer crosses and feral goats possibly had little physical changes. The shrinking, swelling, and softening of collagen could be less due to more heat-stable bonds that retain intermolecular linkages even during cooking (Tornberg, 2005).

3.3.2.4 Meat texture

The peak shear force (PSF) values from all animal groups for both LT and SM muscles were high (62-87 N), showing that sous vide cooking at 60 °C for 6 h was not enough to achieve even a moderately acceptable PSF value of < 40-60 N (Warner et al., 2017; Webb et al., 2005). Meat texture after cooking depends on the extent of heat-induced modifications to meat structure

(Dominguez-Hernandez et al., 2018). Although muscle protein modifications have been reported to occur below 60 °C (Sun et al., 2006), significant structural changes for myofibril and collagen happen at temperatures higher than 60 °C (Bertola et al., 1994; Brüggemann et al., 2010; Tornberg, 2005). In this study, sous vide cooking at 60 °C resulted in fewer structural alterations, insufficient for a significant tenderizing effect. Furthermore, the high PSF values can also be attributed to lower desmin degradation at 60 °C sous vide cooking process, reported by Ismail et al. (2019b) for Korean native black goat.

Among animal groups, The LT and SM muscles of BF consistently recorded the lowest PSF value. The effect of genotype was observed for LT muscle only, where FF had a higher ($p < 0.05$) PSF value than BF. The inconsistent trend for the differences in PSF values cannot give conclusive results for the differences in texture profile of Boer cross and feral goat meat. Published results on goat meat texture as influenced by genotype also shows a varying trend. No significant differences among genotypes were reported (Kadim et al., 2004; Santos et al., 2007a; Van Wyk et al., 2022) while significant differences were observed by some authors (Gadiyaram et al., 2008; Peña et al., 2009; Simela & Frylinck, 2004a).

Muscle type significantly affected tenderness, where PSF values for LT muscle were consistently higher than SM for all samples. However, it is not statistically higher ($p < 0.05$) for BM. It appears that LT muscles of goat meat tend to have higher PSF values, similar to what has been reported by Werdi Pratiwi et al. (2007) for Australian feral goats. The PSF value for LT muscle was significantly higher compared to *triceps* (shoulder) and *vastus* (thigh) muscles. Additionally, Van Wyk et al. (2022) reported that LT had the highest shear force value among six muscle types. In our results, at higher PSF value for LT can be explained by its collagen content

and higher cook loss for LT muscle. Moreover, the sensitivity of the loin muscle to cold shorten compared to other muscles might have contributed to its toughness.

3.3.2.5 Variations in meat texture

Since the texture is a significant determinant of meat quality, a model predicting the variation of meat texture was generated using the meat quality characteristics (**Table 3.4**). From the generated Model 1 (R^2 = 79 %) using both muscles, the meat quality measures that significantly affected texture were pH ($p < 0.01$) and collagen content ($p < 0.05$). Neither cooking loss nor collagen solubility had a significant effect on the predictability of texture. The effect of pH was demonstrated in our results. A scatter plot showing the relationship of pH with texture from both muscle types across the animal groups is shown in **Figure 3.3**.

Table 3. 4 Meat quality measures (covariates) and animal group and muscle type effects (fixed) that explains the variation in shear force for LT and SM muscles (Model 1, R2=79.15).

	P-value
Intercept	0.000
Meat quality measures (covariates)	
pH	0.002
Total collagen	0.042
Cook loss	0.165
Collagen solubility	0.759
Fixed Effect	
Animal group (FF, BF, BM)	0.248
Muscle (LT and SM)	0.002
Animal group*Muscle	0.443

The samples followed the typical relationship of pH and PSF values for meat, where the toughness of red meat tends to be higher from 5.5 - 6 and will decrease beyond pH 6 (Ertbjerg & Puolanne, 2017). It can be inferred that the reported toughness of goat meat can be explained by its pH level. The pH of goat meat (intermediate pH, ~5.7-6.3) plays a significant role in the biochemical processing taking place postmortem. Meat toughness in the intermediate pH range can be explained by less proteolytic activity of lysosomal enzymes (cathepsins) and lesser degradation of myofibrillar proteins as exhibited by some intermediate pH meat (Lomiwes, Hurst, et al., 2014). The intermediate pH of the goat meat samples possibly led to the up-regulation of small heat shock proteins (sHSP), inhibiting apoptosis and the action of calpain in cleaving myofibrillar proteins (Lomiwes, Hurst, et al., 2014). The up-regulation of sHSP at intermediate pH would mean that myofibrillar proteins of goat meat samples are more stable to proteolytic degradation as observed in beef (Balan et al., 2014). Hence, even after 48-h postmortem, there may be minimal proteolytic activity for SM and LT muscles, leading to tough goat meat.

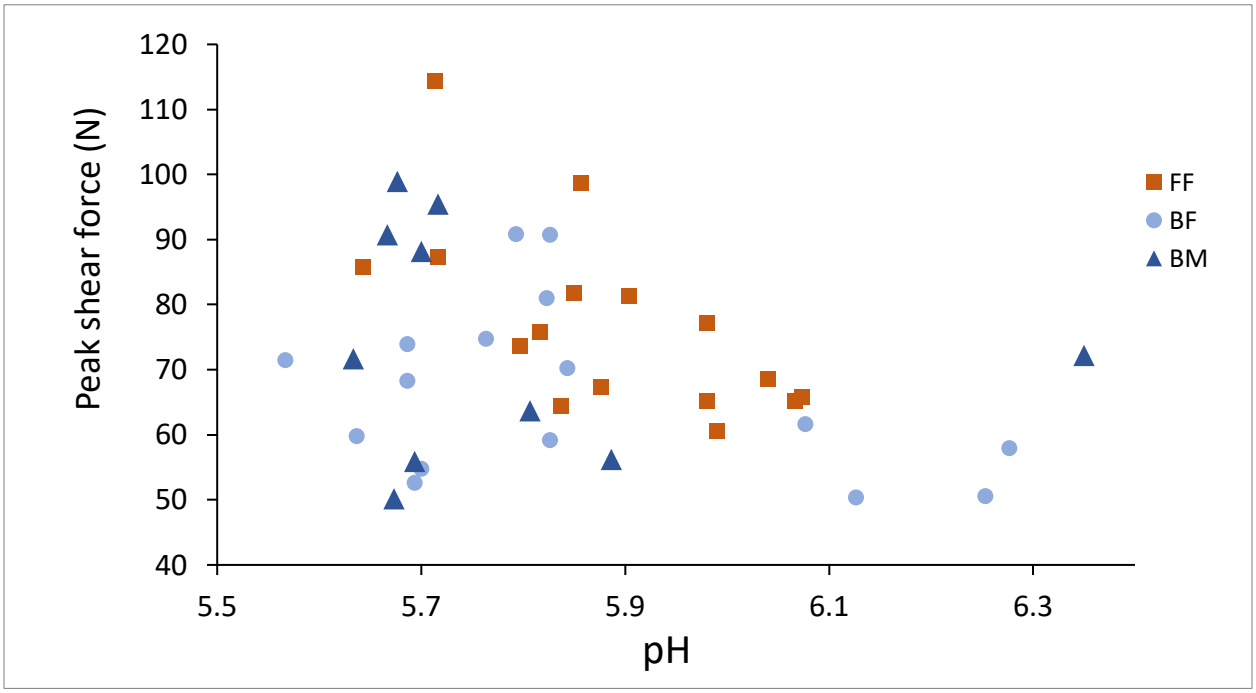


Figure 3. 3 Scatter plots showing the relationship between peak shear force (N) and pH (48 h). Values are from the *longissimus thoracis et lumborum* (LT) and *semimembranosus* (SM) muscles of three animal groups (FF, BF, BM). From the generated Model 1, pH is a highly significant (p <0.01) covariate that explains the variation of shear force in the studied goat meat samples regardless of genotype. High shear force values cluster within the intermediate pH zone.

The collagen content was positively correlated to texture and had a significant effect (p < 0.05) on texture variability. The higher PSF values for LT muscle for both Boer crossbreed goats and feral goats can be explained by the higher collagen content of LT than SM. On the other hand, collagen solubility showed no significant effect (p > 0.05) on the variation of shear force. This observation was not surprising since the tenderness of sous vide cooked meat is often associated with a higher degree of collagen solubility (L. Christensen et al., 2011; Ismail et al., 2019c). In this study, the collagen solubility values were low, and even if they varied among animal groups, their effect was not enough to affect the variation in tenderness. The sous vide conditions

applied only resulted in minimal collagen solubility for all samples. Furthermore, our results agree with the findings of Ismail et al. (2019b), who concluded that 6 hr sous vide cooking over a range of temperatures (60-80 °C), collagen solubility had less or no role in tenderizing sous vide goat muscles.

Muscle type had a significant ($p < 0.01$) main effect on the texture variability, in agreement with the reports that muscle type significantly affects texture due to the variability of muscle fibre types and degree of collagen crosslinking for each muscle (Chriki et al., 2013; Listrat et al., 2016b). Although the predictability of meat tenderness is complex and can be explained in various perspectives (sarcomere length, degree of protein degradation, muscle fibre types) in this study, it is limited only based on known meat quality parameters that generated an R^2 = 79 %. To enhance the predictability of the model, other predictors can be added, such as desmin degradation (Starkey et al., 2015) and protein solubility (Starkey et al., 2017). Further validation using other parameters reported to significantly affect goat meat tenderness can be done in future studies.

3.3.3 Meat colour

The colour values for fresh meat were within the acceptable range for redness (a^*) from 9.5-19 and lightness (L^*) >34 (Holloway & Wu, 2019) (**Table 3**). Meat from Boer crosses and feral goats differ in a^* for both LT ($p < 0.01$) and SM ($p < 0.01$) muscles. The results demonstrated the significant effect of genotype for a^* values, and that meat from feral goats and Boer crosses can be differentiated by its redness. This finding agrees with the reported higher a^* for Australian

feral goats than Boer crosses (Dhanda, Taylor, & Murray, 2003). Varying values for a^* consequently affected the value of chroma (colour intensity), which differed for LT ($p < 0.05$) and SM ($p < 0.01$) across animal groups.

PCA biplots for raw muscles are presented in **Figure 3.4** (A and B) to visualize the association of individual colour properties for each animal group. Raw LT and SM are well-approximated by a two-dimensional set of principal components, explaining 94 % variation for LT and 93 % for SM. For both muscles, the most important component is in PC1, in which the variability is explained mainly by a^*, b^*, chroma, and hue (discolouration). Additionally, the variability in PC2 is primarily explained by L^*. For LT muscle in PC1, FF clusters in the direction of a^* and chroma and separate itself from BF and BM. Data for BM and BF sat close on the plane because no significant differences ($p > 0.05$) exist for colour parameters between Boer crossbreeds. The lower L^* and higher a^* show a darker red colour for feral goat LT muscle compared to Boer crosses. For SM muscle, in PC1, FF SM can be distinguished as having a lower a^* compared to BF that positively correlated with a^* and chroma. A significant effect of genotype on a^* was reported in published papers and correlated the difference with the levels of heme pigment content (Dhanda, Taylor, & Murray, 2003; Dhanda et al., 1999; Santos et al., 2007a). Additionally, varying levels of redness can be explained by the differences in muscle fibre composition between breeds. Redness values in goat meat were reported to increase with a high number of type I fibres and a low number of type IIB fibres (Bakhsh et al., 2019; Hwang et al., 2010). Likewise, the differences in colour properties between LT and SM can be explained by the muscle fibre composition, responsible for the varying rates of myoglobin oxidation that is also pH-dependent (Bakhsh et al., 2019; Hughes et al., 2020; Hwang et al., 2017; Hwang et al., 2010).

Higher pH results in lower redness values for LT BF and SM FF muscles because in high pH conditions, there is an increased activity for mitochondria, and it competes with myoglobin oxidation (McKeith et al., 2016; Zhang et al., 2018).

For the sous vide cooked muscles, the L^* and a^* values indicate to a dominant red-pink pigment, a typical colour for meat sous vide at 60 °C (Bhat et al., 2018; Ismail et al., 2019b). Cooked meat from feral goats had higher L^* values ($p < 0.01$) than Boer crossbred goats, showing that feral and Boer cross meat samples differ in lightness. The colour profiles for sous vide meat from male and female Boer crosses are similar ($p > 0.05$), indicating that sex had no significant effect on colour. The PCA biplots for the colour profiles of cooked samples are also shown in **Figure 3.4** (C and D). The variability among cooked LT samples is explained primarily by PC1 (56%) and PC2 (35 %). In PC1, cooked LT muscles from feral goats can be differentiated from cooked LT muscles of BF and BM since it clusters towards L^* and hue values. For cooked SM muscles, meat from feral goats is positively correlated to b^*, while BM samples are more negatively correlated. BF samples are negatively correlated to chroma. Both PCA biplots of cooked SM and LT muscles from feral goats showed a high correlation to L^* and can be distinguished to have lighter cooked meat colour compared to BF and BM muscles. After cooking, all samples increased in L^* values, which is linked to the increased free water on the meat surface after sous vide cooking (Sánchez del Pulgar, Gázquez, & Ruiz-Carrascal, 2012). However, L^* varied between genotypes and can be explained by the differences in their composition since the light scattering property of meat is influenced by its structure (Hughes et al., 2020). The colour profile of meat is a result of a complex interplay between its meat structure and the biochemical aspect of myoglobin (Purslow et al., 2020). For our cooked samples, colour differences are mainly

attributed to the achromatic properties of meat (variations in lightness). The higher L^* from cooked feral goat muscles can be attributed to its higher collagen content. Tissues with higher collagen content have been reported to have a higher light scattering coefficient (Jacques, 2013). Moreover, varying levels of IMF between breeds might have contributed to colour differences since lipid exhibits light scattering properties (Hughes et al., 2020).

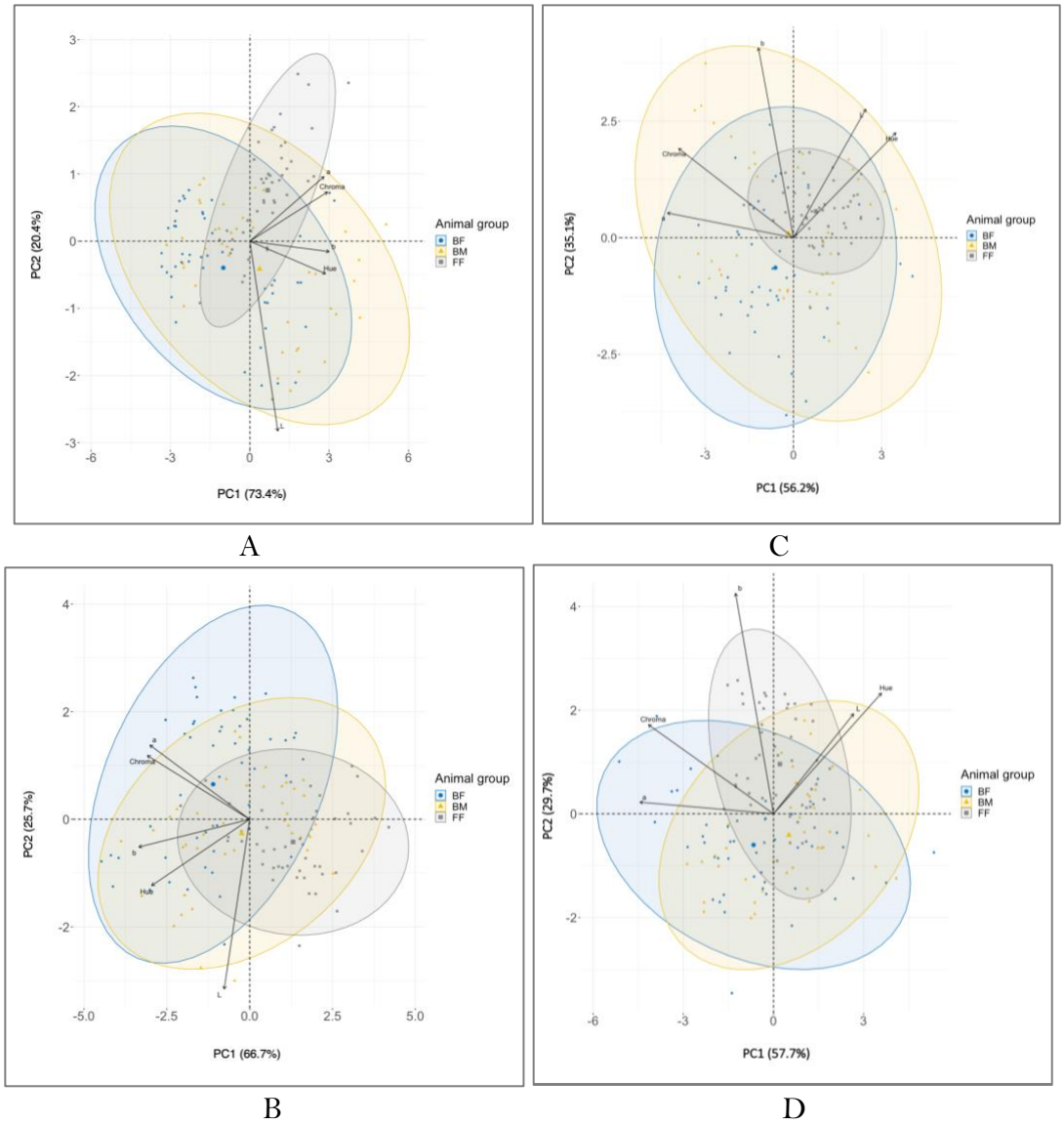


Figure 3. 4 Principal component analysis (PCA) plots showing the multivariate variation among the three animal groups (FF, BF, BM) in terms of its colour properties.

Lightness (L^*), redness (a^*), yellowness (b^*) Individual biplots are shown for (A) raw *longissimus thoracis* (LT), (B) raw *semimembranosus* (SM) muscles, (C) sous vide *longissimus thoracis* (LT) and (D) sous vide *semimembranosus* (SM) muscles. The vectors indicate the direction and strength of each trait to the overall distribution.

Table 3. 5 Colour properties of raw and sous vide cooked longissimus thoracis (LT) and semimembranosus (SM) muscles of Boer cross and feral goats.

Parameters	Muscle	Animal group			p-value[1]		
		FF	BF	BM	Animal group	Breed (FF × BF)	Sex (BF × BM)
Raw							
*L**	LT	37.26 ± 0.37^{aX}	43.03 ± 0.64^{abY}	41.36 ± 0.41^{bY}	*	ns	ns
	SM	38.19 ± 0.32^{aY}	34.70 ± 0.74^{aX}	37.94 ± 0.58^{aX}	ns	ns	ns
*a**	LT	14.08 ± 0.21^{bY}	12.98 ± 0.39^{aX}	13.20 ± 0.31^{abX}	**	**	ns
	SM	12.82 ± 0.20^{aX}	16.5 ± 0.24^{bY}	15.16 ± 0.29^{bY}	**	**	ns
*b**	LT	3.94 ± 0.22^{aX}	2.31 ± 0.27^{aX}	3.55 ± 0.31^{aX}	ns	ns	ns
	SM	3.81 ± 0.23^{aX}	5.99 ± 0.25^{aY}	5.17 ± 0.30^{aY}	ns	*	ns
Chroma	LT	14.67 ± 0.25^{aY}	11.49 ± 0.51^{aX}	13.84 ± 0.37^{aX}	*	**	ns
	SM	13.43 ± 0.24^{aX}	17.6 ± 0.29^{bY}	16.07 ± 0.38^{bY}	**	**	ns
Hue	LT	15.30 ± 0.74^{bX}	10.08 ± 0.46^{aX}	13.42 ± 1.03^{abX}	ns	ns	ns
	SM	16.10 ± 0.79^{aX}	19.69 ± 0.65^{aY}	18.31 ± 0.76^{aY}	ns	ns	ns
Sous vide							
*L**	LT	65.10 ± 0.19^{bY}	60.10 ± 0.35^{aY}	62.15 ± 0.36^{aY}	**	**	ns
	SM	62.93 ± 0.29^{aX}	57.67 ± 0.33^{aX}	59.57 ± 0.35^{aX}	**	**	ns
*a**	LT	12.95 ± 0.22^{aX}	14.37 ± 0.38^{aX}	13.99 ± 0.49^{aX}	ns	ns	ns
	SM	15.02 ± 0.19^{aY}	15.65 ± 0.42^{aY}	14.10 ± 0.37^{aX}	ns	ns	ns
*b**	LT	11.35 ± 0.07^{aX}	10.82 ± 0.21^{aX}	11.35 ± 0.31^{aX}	ns	ns	ns
	SM	12.96 ± 0.19^{bY}	11.86 ± 0.17^{aY}	11.76 ± 0.15^{aX}	*	*	ns
Chroma	LT	17.24 ± 0.20^{aX}	18.08 ± 0.34^{aX}	18.01 ± 0.52^{aX}	ns	ns	ns
	SM	19.87 ± 0.21^{aY}	19.69 ± 0.40^{aY}	18.44 ± 0.30^{aX}	ns	ns	ns
Hue	LT	41.43 ± 0.42^{aX}	37.38 ± 0.91^{aX}	39.44 ± 0.85^{aX}	ns	ns	ns
	SM	40.79 ± 0.47^{aX}	37.63 ± 0.68^{aX}	40.25 ± 0.85^{aX}	ns	ns	ns

L-lightness, a*-redness, b*-yellowness, Chroma (intensity,* $C = [a^{*2} + b^{*2}]^{1/2}$), and *Hue (discolouration,* $H^* = \tan^{-1}[b^*/a^*]$).

Animal group- **FF**- feral female, *N*=8; **BF**- Boer cross female, *N*=8; **BM**- Boer cross male, *N*=7.

FF × BF- difference between female Boer cross and female Feral (Genotype effect), **BF × BM** – difference between female and male Boer crosses (Sex effect).

[a-c] Means ± SEM within a row with the same superscript letter are not different ($p > 0.05$) between animal group.[1]ns = $p > 0.05$, *= $p < 0.05$, and ** = $p < 0.01$.

[xy]Means ± SEM within a column with the same superscript letter are not different between muscle types ($p > 0.05$).

SEM- Standard error of the mean

3.3.4 Meat ultrastructure

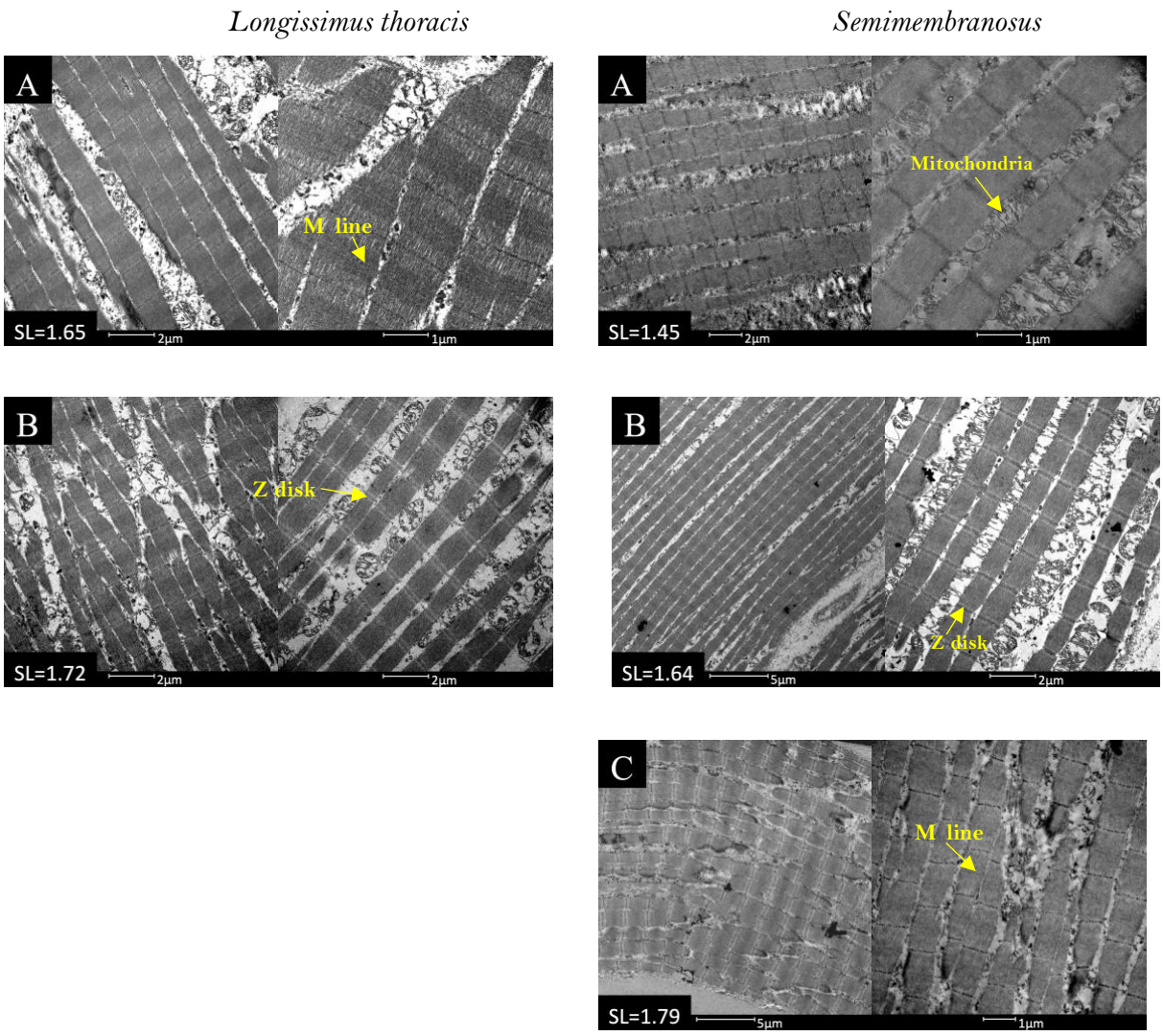


Figure 3. 5 Ultrastructural images of raw longissimus thoracis (LT) and semimembranosus (SM) muscles.

Transmission electron microscopy (TEM) images were acquired on longitudinal sections of the three animal groups: (A) FF, (B) BF, and (C) BM, after 48 h postmortem.
Images are shown in 2 magnifications.
SL: sarcomere length in µm.

Figure 3.5 shows the ultrastructure of goat meat, showing myofibrils that appeared aligned, with visible sarcomeres and intact mitochondria as observed for raw meat (Astruc et al., 2010; Supaphon et al., 2021). Additionally, a fractured z-disk along some myofibrils was

observed from BF tissues, which might be caused by some proteolytic activity since the meat was aged for 48 h. Endogenous enzymes can degrade cytoskeletal structures such as those in the Z-disk or M line (Taylor et al., 1995). The overall sarcomere length (SL) of goat meat measured from the TEM micrographs, regardless of muscle type ranged from 1.5-1.8 μm. Unfortunately, we can't present any ultrastructure of BM LT muscle because the tissues were unrecoverable due to a faulty fixative solution. Nevertheless, it can be generalized that goat meat samples from feral goats and Boer crosses had short sarcomeres (< 2 μm). A short sarcomere is a common observation for goat meat, reported being usually below 1.8 μm, associated with its susceptibility to cold shortening because of its low-fat cover (Gadiyaram et al., 2008; Hwang et al., 2019; Santos et al., 2007a). Additionally, the low SL for goat meat can be explained by its intermediate pH value. It has been reported that as pH increases from 5.5 to 6.2, sarcomere length decreases (Ertbjerg and Puolanne, 2017).

TEM micrographs revealed that FF SM and BF LT muscles had undergone cold shortening (**Figure 3.6**) based on the average SL of less than 1.4 μm (Marsh et al., 1974; Voyle, 1969). Furthermore, the TEM image of BF LT appeared super contracted, having a sarcomere length of 1.0 ± 0.02 μm, myosin filaments compressed between the Z-discs, and indistinguishable M-bands. The same observation for a super contracted beef muscle was reported by Voyle (1969). The short SL for goat meat is widely reported in the literature, and our findings proved that goat meat had undergone cold shortening and even severe muscle contraction.

Cold shortened muscles could be a result of rapid chilling of goat carcasses for the given chilling condition. Rapid chilling for Boer goat meat was reported in a study by Abhijith et al. (2021). They showed that most of the meat underwent rapid chilling and did has not

pass the cold-shortening window under common commercial processing conditions (chilling goat meat overnight at 4°C). Goat carcass is prone to a higher rate of temperature decline after slaughter because of its low-fat cover and size (Shija et al., 2013). Muscle contractions indicated by SL values are highly dependent on the postmortem conditions applied to the carcass. Different carcasses would undergo various structural changes postmortem, resulting in varying degrees of muscle contraction. Furthermore, the extent of contraction is dependent on the time-temperature profile within the muscle and the orientation of the fibres in relation to the temperature flow (Ertbjerg & Puolanne, 2017). Pophiwa et al. (2016) showed that meat from Boer and South African indigenous goats had SL values higher than 1.9 μm when electrical stimulation and delayed chilling were applied to goat carcasses. Our data show that the current practice used in handling goat carcasses leads to cold shortened goat muscles.

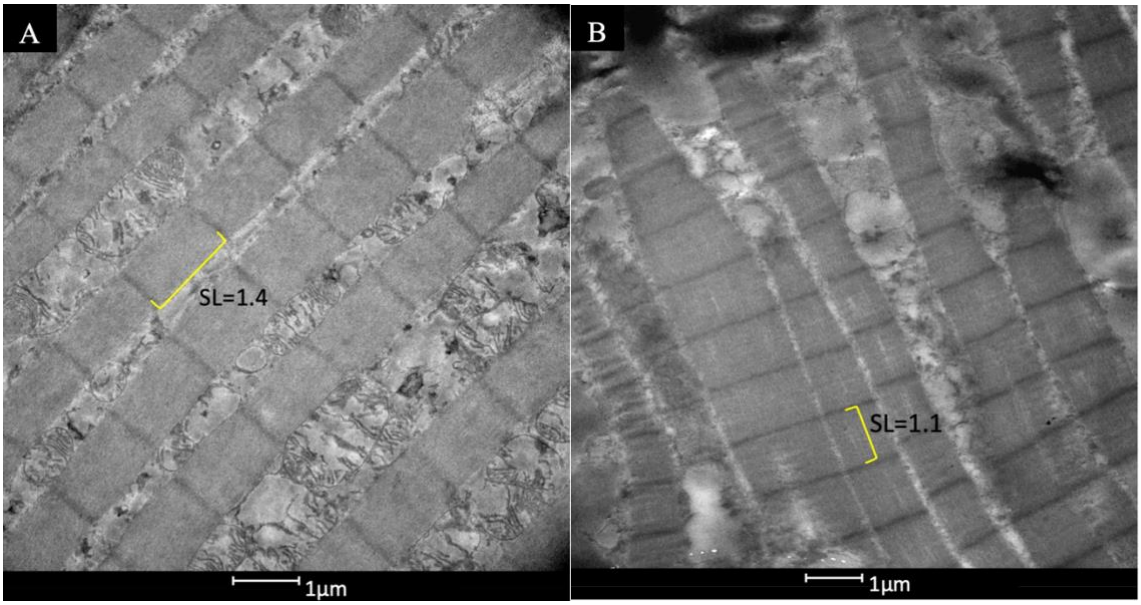


Figure 3. 6 Ultrastructural images of muscles showing hypercontracted sarcomeres. Transmission electron microscopy (TEM) images were acquired on longitudinal sections of raw semimembranosus from FF (A) and longissimus thoracis from BF (B).

TEM image of sample B was from a different semimembranosus tissue of another BF animal (See **Figure 3.5** (B) semimembranosus of BF with uncontracted sarcomere). Images show hypercontracted sarcomeres with compressed myosin filaments between the Z-discs and indistinguishable M−bands.

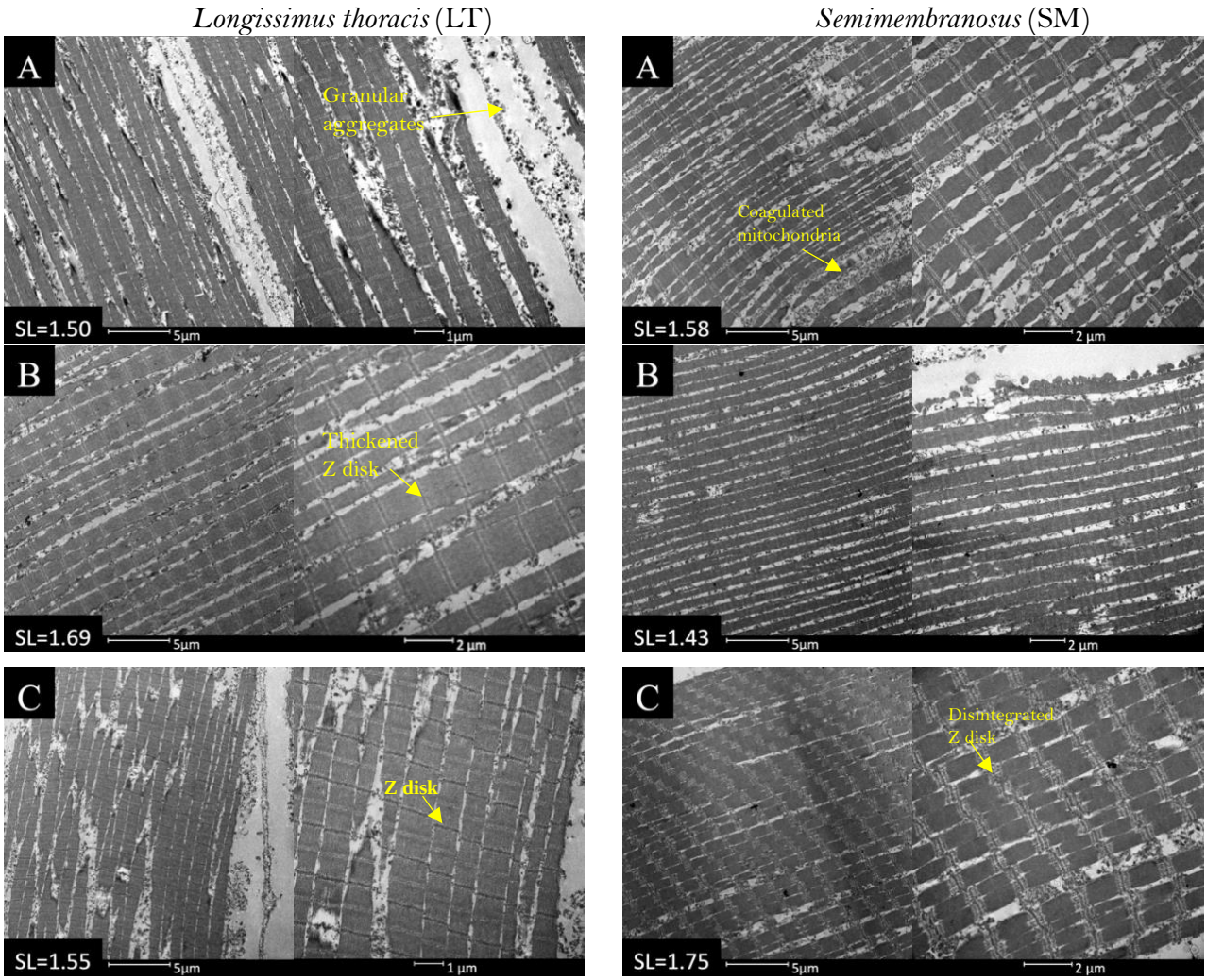


Figure 3. 7 Ultrastructural images of sous vide cooked (60 °C for 6 hrs) longissimus thoracis (LT) and semimembranosus (SM) muscles.

Transmission electron microscopy (TEM) images were acquired on longitudinal sections of the three animal groups: (A) FF, (B) BF, (C) BM). Images are shown in 2 magnifications. SL: sarcomere length in µm.

Figure 3.7 shows the representative ultrastructural images of goat meat samples after 6 h at 60 °C sous vide cooking. Average SL for the cooked muscle ranged from 1.4 -1.7 µm with no observable trends between muscle type and animal group. The sous vide cooking method used led to a general observation that the structure of the myofibrils was still clearly visible, and I bands and M lines were still detectable. Additionally, heating resulted in a

transverse and longitudinal shrinkage of meat fibres, a known behavior of muscle fibres heated at 60 °C (Astruc et al., 2010; Supaphon et al., 2021; Tornberg, 2005). However, the observed longitudinal shrinkage was minimal since the SL for cooked samples was slightly lower than for raw meat. Transverse shrinkage of myofibrils was also apparent, leading to a widened gap between fibres or increased intermyofibrillar spaces. Furthermore, the formation of granular aggregates, thickening and disintegration of Z disks, and coagulation and swollen mitochondria were visible as observed for sous vide cooked beef (Supaphon et al., 2021; Xiaojie Zhu et al., 2018).

After sous vide cooking, there were no notable differences between the ultrastructure of meat from feral goats and Boer crosses. In most cases, differences in meat structure for cooked meat are influenced mainly by processing conditions and treatments applied, as observed in various studies for beef (F. M. Chian et al., 2021; Chian et al., 2019; Supaphon et al., 2021). Between muscle types, disintegration in the Z-disk was more severe in SM muscles than in LT. This observation can partly explain why SM muscle had lower PSF value had compared to LT in our results. Many studies have shown that differences in the morphological profile of heated meat can be observed between muscle types since thermal denaturation is highly influenced by fibre type (Astruc et al., 2012; Vaskoska et al., 2021). As reported in the literature, LT and SM muscles of goat meat vary significantly in the composition of their myofibrils (Hwang et al., 2017). The recorded short SL (< 2 μm) from TEM micrographs, for all the goat meat samples and some hyper contraction for some muscles can also explain the high PSF values obtained in this study. A study by Kadim et al. (2014) attributed the toughness of LD muscle from Dhofari goats to short sarcomere length (1.4-1.6 μm). As muscles shorten, filaments tend to overlap so that, when heated, they can result in coagulation of protein and

tougher meat (Ertbjerg & Puolanne, 2017). Hence, with the known effect of cold shortening on meat toughness, the texture of goat meat can be improved by using processes that prevent cold shortening during postmortem handling. Since it has been reported that sarcomere lengths had a significant effect on meat quality characteristics (Ertbjerg & Puolanne, 2017), it is fitting to address cold shortening in goat meat.

3.4 Conclusions

Raw goat meat from New Zealand feral goats and Boer crossbreeds had low intramuscular fat values (<2%). These values show the leanness of goat meat regardless of breed and muscle type. For meat colour, the notable differences between breeds were observed in the redness (a^*) of both muscles. Even 48 h postmortem, goat meat does not readily achieve a lower pH (<5.7), and all meat samples have intermediate pH (pH, 5.7-6.3) which explains the toughness of goat meat regardless of breed. Sous vide cooking of goat meat SM and LT muscle for 6 hrs at 60 °C is not enough to obtain acceptable tenderness values (<40 N) for meat. The variability in goat meat tenderness is highly affected by meat pH and collagen content and is dependent on muscle type.

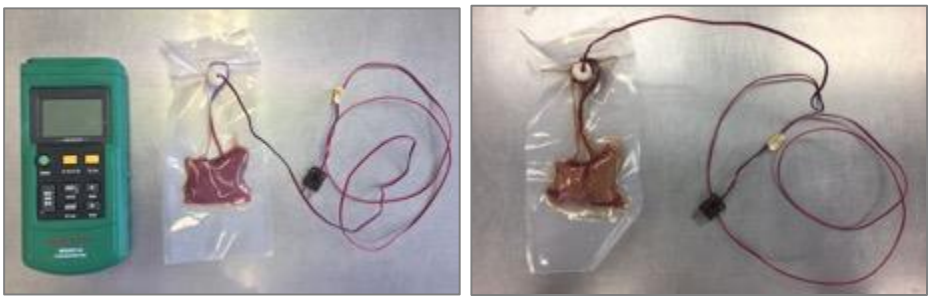
Our results highlight the high tendency of goat meat to undergo cold shortening during chilling, as revealed by the ultrastructure images obtained using TEM. We conclude that the current practice of handling goat carcasses leads to cold-shortened goat muscles regardless of breed. Moreover, muscle cold-shortening and intermediate pH values for goat meat might have significantly contributed to the known toughness of goat meat. These findings merit industrial consideration in which pre-and postmortem handling of goats should be specialized. For example, the size and fat cover of goat carcasses should be considered when

selecting chilling conditions. Furthermore, this research shows a need to conduct more research about how goats should be handled to provide the adequate process necessary to improve goat meat quality.

3.5 Supplementary material

3.5.1 Standardization of sous vide cooking

Standardization of cooking conditions was done before the actual experiment to determine the appropriate sous vide time and temperature combinations for SM and LT goat muscles. The chosen treatments consisted of 1) 60 °C for 3, 6, 12 and 14 h and 2) two-stage sous vide cooking at 45 °C for 3 h and a second temperature at 60 °C for 3 h (45+60 °C in total 6 h). The dimension of the meat used was 1.5 cm x 4 cm x 5 cm, approximately 25±5 g/slice since LT and SM muscles from goat are not large enough to have larger meat samples for cooking. With the given sample size, it only takes about 2.5 min for the coldest point to reach the set temperature; thus, during cooking, warm up time was considered negligible, and cooking was timed when the meat containing vacuum packaged bags were loaded in the water bath. Error! Reference source not found. shows the set-up used for standardizing the sous vide process.



Supplementary figure 1.1 Thermocouple and temperature meter used for monitoring meat temperature during sous vide cooking.

3.6 Copyright information

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Chapter 4

The effects of breed and muscle type on in vitro protein digestibility of goat meat

4.1 Introduction

Meat is a very valuable source of protein. The highly ordered muscle intracellular proteins contain high levels of essential amino acids (40-45 %), making them superior to plant-derived proteins (Astruc, 2014b; Hodgkinson et al., 2018). Since protein is a bulky molecule, the body needs to convert dietary proteins to a form that it can utilise; this can be achieved through the subsequent protein hydrolysis by the action of the proteolytic systems involved in gastrointestinal digestion. However, the entire dietary protein digestion is a complex process that involves many factors.

The varying composition of food and protein structure results in a different rate of protein hydrolysis (Savoie et al., 2019). Meat is a highly digestible protein; in fact, it is categorised as a fast protein since its digestion rate is closer to what is observed with whey proteins (fast proteins) than with casein (slow proteins) (Rémond et al., 2007). Bax et al. (2013) reported a very high digestibility (about 95 %) of meat proteins in an in vivo experiment, and it was highly digestible under *in vitro* conditions (Farouk et al., 2014). Although meat is highly digestible, the rate of meat protein digestibility is not an intrinsic factor, and the effects of processing and other treatments before its final structural or molecular state can affect its digestibility (Farouk et al., 2019). Cooking temperature is a major determinant of meat protein digestibility, and meat composition also somewhat affects protein digestion (M. L. Bax, T. Sayd, et al., 2013; Kaur et al., 2014; Zou et al., 2018).

Meat protein digestibility varies between species (Wen, Zhou, Song, et al., 2015) and muscle cuts (Farouk et al., 2019; Zou et al., 2018). Since the different types of muscle contain different fibres and different amounts of connective tissues, depending on the muscle's function (Astruc, 2014b; Listrat et al., 2016b), proteins can behave differently under simulated

gastric conditions (He et al., 2018; L. Li et al., 2017; Sayd et al., 2016) and could explain the different digestibility of meat from different species or muscle types. Muscle cuts mildly affected beef's digestibility, where low-collagen *supraspinatus* muscle cuts appear more digested than more collagenous muscles (Farouk et al., 2019). For pork cuts, proteome analysis indicated that the *biceps femoris* muscle had the highest susceptibility to digestion. The protein digestibility of beef, pork, lamb and chicken has been widely studied (Mitra et al., 2022; Véronique Santé-Lhoutellier et al., 2008; Wen, Zhou, Song, et al., 2015; X. Zhu et al., 2018). To the best of our knowledge, nothing is known about the digestibility of goat meat. Hence, this chapter aims to evaluate the protein digestibility of goat meat cooked under sous vide conditions.

In our previous experiments (**Chapter 3**), the *semimembranosus* (SM) and *longissimus thoracis* (LT) showed significantly different texture values, as assessed by Warner-Bratzler peak shear force in Newton (N). Among the breeds evaluated in Chapter 3, meat samples from female feral (FF) and female Boer crossbreeds (FB) showed the same trend for the texture values. Both breeds had tougher LT muscles compared to SM. Given that toughness is related to the integrity of its myofibrils, it is hypothesised that muscles with different textures might have different digestion rates. Hence, this study aims to compare the *in vitro* digestibility of goat meat using two muscle types, LT and SM muscles, with varying levels of tenderness. This paper also aims to determine if the digestibility of meat from different goat breeds varies.

4.2 Materials and methods

4.2.1 Sample preparation and selection

The meat characteristics shown in **Table 4.1** are the data obtained for each muscle used in the previous study. The cooked meat sample was chopped into small pieces (~2 cm diameter) and ground using a stainless-steel coffee grinder (Model BCG200, Breville, AU) for five pulses. Considering the toughness of the meat, the size of the ground meat was standardised using sieving with 3 to 5 mm aperture size sieve (Jalabert-Malbos et al., 2007; Pematilleke et al., 2022). The samples were labelled as FLT and FSM for feral goat *longissimus thoracis* and *semimembranosus* samples and BLT and BSM for Boer crossbreed *longissimus thoracis* and *semimembranosus* muscles.

Table 4. 1 The characteristics of sous vide longissimus thoracis (LT) and semimembranosus (SM) muscles from feral and Boer cross.

Breed	Muscle type	Peak shear force (N)	Collagen content (%)
Feral	LT	96.07 ± 2.75^{b}	3.3 ± 0.12^{b}
	SM	65.85 ± 1.36^{a}	1.1 ± 0.02^{a}
Boer Crossbreed	LT	67.57 ± 1.47^{a}	0.9 ± 0.01^{a}
	SM	70.56 ± 2.79^{ab}	1.0 ± 0.01^{a}

N=3. Values presented are the average ± SE of the muscles selected from 3 female feral and 3 female Boer crossbreeds.

Figure 4. 1 Sample of ground goat meat for digestion.

4.2.2 In vitro protein digestibility

The simulated gastrointestinal digestion was performed in a double-jacketed glass reactor (Chian et al., 2019) according to the standardised static INFOGEST protocol described by Minekus et al. (2014) and Brodkorb et al. (2019). Digestion was carried out in triplicate for each sample. Each reactor contained 3 glass balls (3-5 mm) and 1 magnetic stirrer bar (~2.5 cm length) to facilitate stirring, mimicking the gastrointestinal motility.

The cooked meat sample was initially chopped into small pieces (~1 cm diameter). Next, a representative sample was ground using a stainless-steel coffee grinder (Model BCG200, Breville, AU) for four pulses, achieving a size of ~1.5 to 2 mm (Chian et al., 2019). The total crude protein content for raw and cooked samples was determined using the Kjeldahl method **(Chapter 3.5.1)**

4.2.2.1 Oral phase

Five grams of the prepared sample was mixed with 5.0 mL simulated salivary fluid, 1.4 mL α-amylase (10025, Sigma-Aldrich, USA) (30 U/mg), and 1.56 mL MQ water to initiate the oral phase at pH 7 ± 0.1 for 2 min.

4.2.2.2 Gastric phase

The oral phase mixture was then added with 10 mL simulated gastric fluid, 4.56 mL porcine pepsin (P7125, Sigma-Aldrich, USA) (185 U/mg) and 1.112 mL MQ water to start the gastric phase. The pH was adjusted and maintained at 3.0 ± 0.1 using 5 M HCl. Gastric digestion was carried out for 2 hrs. The digest samples were taken after 1, 10, 30, 60 and 120 min of gastric digestion, and pepsin activity was immediately stopped by adding Pepstatin A

(Abcam, UK) (200 µl/15 mL digest). The samples were immediately stored at -20 °C for further analysis.

4.2.2.3 Intestinal phase

For the small-intestinal phase, right after gastric phase, NaOH (5 M) was added to adjust and maintain the pH at 7 ± 0.1. Then, the mixture had 15.6 mL simulated intestinal fluid, 2.096 MQ water, 10 mL pancreatin (P1750, Sigma Aldrich, USA) (27 U/mg), and 4 mL bile (B8631, Sigma- Aldrich, USA). Digest samples from the small-intestinal phase were taken after 5, 10, 60, and 120 min of intestinal digestion, and the proteolytic enzyme activity was stopped using SIGMAFAST™ Protease inhibitor (Sigma Aldrich, USA) (2.5 mL/10 mL digest). All the digests were immediately stored at -20 °C for further analysis.

4.2.2.4 Pepsin activity and pancreatin Assay

The activity of pepsin (P7125, Sigma-Aldrich, USA) and pancreatin (P1750, Sigma Aldrich, USA) (27 U/mg) was determined following the INFOGEST protocol (Brodkorb et al., 2019).

4.2.3 Particle size measurement

The particle diameters and size distribution of the meat digest at time points 60, 120, 125, 130, 180 and 240 mins were measured immediately after sample collection. The analysis was carried out via static light scattering technique using the laser diffraction method, using a Mastersizer 2000 (Malvern Instruments, UK) (Mitra et al., 2017). The obscuration values in the 5% to 20% range were considered. The absorption was set as 0.001; the dispersant used was water, and the particle refractive index used was 1.45. The measurement time was every 2 min, and the temperature was 25 °C. The intensity of the scattered light was detected using

a 633 nm laser with a scattering angle of 90 °C. The values for $D_{4,3}$ represent the mean diameter in volume and were reported for the overall particle size of the digest. Other parameter such as $D_{3,2}$ represents the mean diameter in surface, $D_{0,9}$ indicates the diameter higher than 90 % of the sample particles, $D_{1,0}$ represents the size for which 10% of the sample particles have a lower size, and $D_{0,5}$ represents the size for which 50 % of the sample particles have a lower size.

4.2.4 Degree of protein hydrolysis Ninhydrin assay

The degree of protein hydrolysis was determined by quantifying free-amino nitrogen. Aliquots at the specific time-point of the gastric and intestinal phases were collected to determine the release of free amino groups (NH_2). The digest was centrifuged for 5 min at 13,000 × g and filtered using a 0.45 µm PVDF filter, Millex ® (Merck, Germany). The gastric and intestinal digests were diluted 1:7 with Milli-Q water at pH 3 and 7, respectively. The free amino nitrogen content of each sample was measured using the ninhydrin assay outlined by Moore (1968). In a tube, a 20 µL of the filtered sample was added with 980 µL of MQ water and 0.5 mL of 0.2 % ninhydrin reagent (N7285, Sigma-Aldrich, USA). The tubes were heated for 10 minutes in 100 °C. After cooling, 2.5 mL of 95 % ethanol alcohol was added, and the absorbance was read at 570 nm using a UV vis spectrophotometer (Thermo Fisher Genysis). A glycine standard was run for the calibration of free amino nitrogen concentration.

The *in vitro* degree of hydrolysis (DH) was calculated using equation 1:

$$eq\ 1. \quad DH(\%) = \frac{FAN_F - FAN_{T0}}{FAN_T - FAN_{T0}} \times 100$$

FAN_F is the final free amino nitrogen at a time point; $FAN_{(T0)}$ is the free amino nitrogen at time 0; and FAN_{TF} is the total amino nitrogen in the sample (Montoya et al., 2018).

4.2.5 Statistical analysis

Statistical analysis was performed using IBM SPSS Version 28.0.1.1(14). The determination of each parameter was done in triplicate unless otherwise stated. Before analysis, a normality test was performed using the Shapiro-Wilk test, and outliers (computed using Dixon's test) were removed when applicable. The significant differences between samples were evaluated using ANOVA. When at least one group was statistically different, multiple comparison analysis was done using the Tukey test set at a 95 % confidence interval. The simple main effects test for breed and muscle type was determined using univariate analysis with breed and muscle types as fixed factors and the animal number as the random variable.

To test the hypothesis about the relationship between variables that might influence the predictability of the digestibility values for goat meat expressed in the degree of hydrolysis, a generalised linear model (GLM) was generated. The degree of hydrolysis at the end of the gastric and intestinal phase was plotted against all the variables, with muscle type and breed as fixed factors, texture and collagen content as covariates and the individual animal as the random term. In addition, the interaction between breed × muscle type was added to the model. A stepwise backward elimination method was used to determine the best model that can explain the variation of the outcome (degree of hydrolysis). Akaike Information Criterion (AIC) was used to compare the models and the normality test for residuals for the

model validation. For the model used, the variables that were not significant were still retained in the model since their removal does not improve AIC (less power). The final model was fitted with all the fixed factors and covariates retained.

4.3 Results and discussion

4.3.1 Dynamics of meat digest particle size

The digest particle size is a physical parameter that indirectly reflects the extent of protein of hydrolysis during digestion. **Figure 4.2** shows the dynamic changes in meat particle characteristics during *in vitro* digestion starting from 60 min gastric digestion.

As expected, the meat digest particle size, that initially ranged from 5-15 mm, decreased for all samples as the digestion progressed. However, it is also clear that the characteristics of particles among the four samples vary. For FLT, there is an apparent distinction between the particles in the gastric phase and intestinal phase compared to the digest of the other samples. On the other hand, FSM shows a gradual reduction in particle size. There is no significant reduction in the average particle size from 60 to 120 min (**Table 4.2**), although smaller particles were generated.

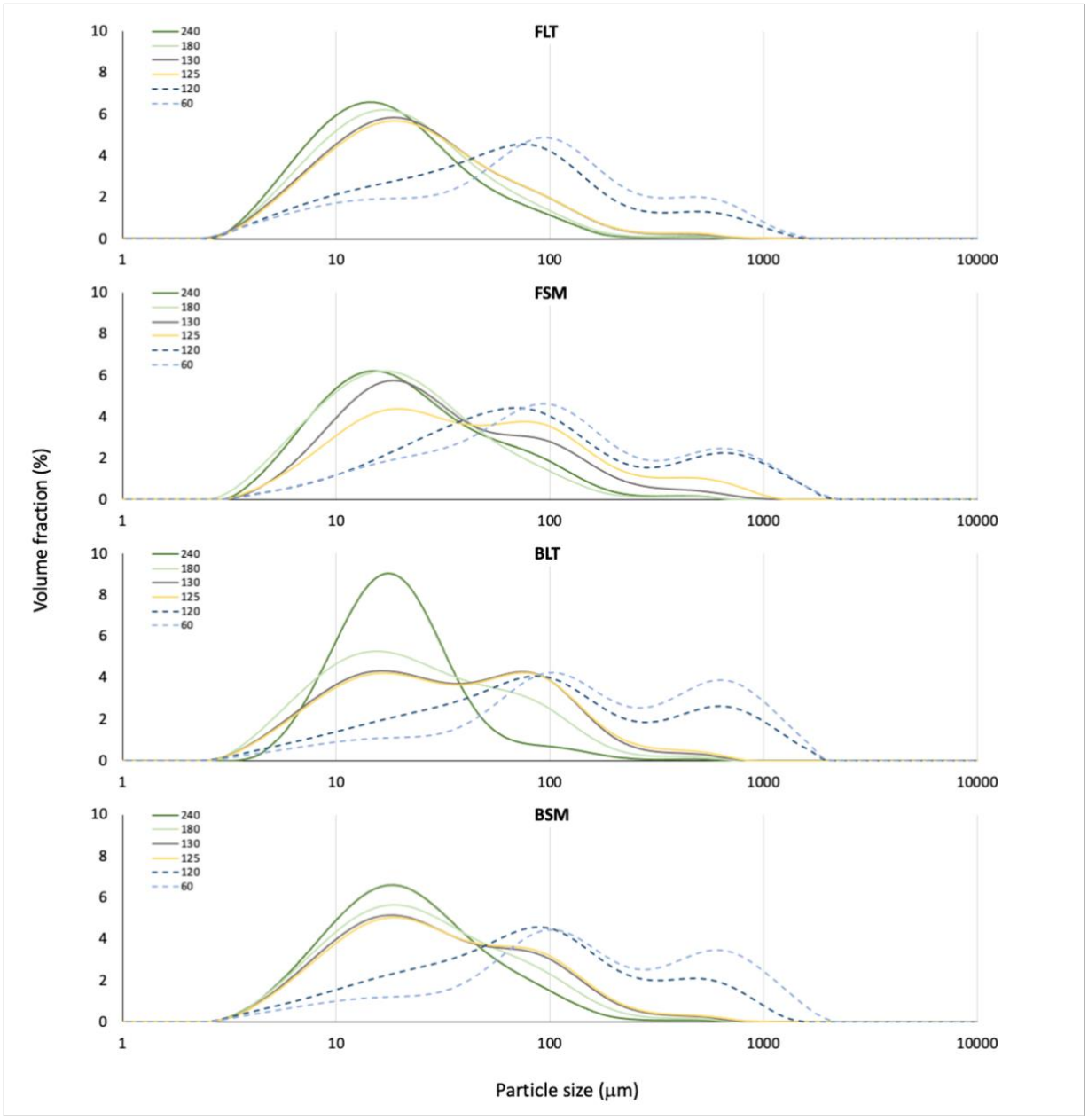


Figure 4. 2 Particle size distribution over time of the suspended solid fraction of the gastric (60 and 120 min) and intestinal digest (125, 130, 180 and 240 min).

Table 4. 2 Particle size diameter (D4,3 in μm) of goat meat digest in *in vitro* gastrointestinal digestion.

Time-point (min)		Feral		Boer cross		SD	[1]*p*-value	
		LT	SM	LT	SM		Breed	Muscle type
Gastric phase	60	156.94 ± 17.5[3a]	225.29 ± 28.45[2ab]	305.97 ± 8.34[3b]	278.72 ± 21.95[3b]	74.54	<0.001	NS
	120	117.01 ± 11.77 [2a]	205.20 ± 16.41[2b]	220.76 ± 26.79[2b]	151.60 ± 13.19[2ab]	59.14	NS	NS
Small-intestinal phase	125	42.84 ± 4.63[1a]	87.00 ± 5.51[1b]	59.93 ± 2.77[1a]	52.14 ± 4.07 [1a]	19.55	NS	NS
	130	37.33 ± 1.36[1a]	55.19 ± 9.82[1a]	54.51 ± 3.30[1a]	48.11 ± 3.24 [1a]	14.48	0.03	NS
	180	30.19 ± 1.96[1a]	43.52 ± 3.17[1b]	39.12 ± 2.23[1ab]	39.13 ± 2.08[1ab]	7.40	NS	NS
	240	26.11 ± 1.71[1ab]	34.79 ± 3.44[1b]	24.84 ± 1.35[1a]	30.78 ± 2.25[1ab]	6.67	NS	0.002

[1]NS- $p > 0.05$, and SD is the standard deviation.

[a-c] Values within a row with the same superscript letter are not significantly different at a 0.05 significance level.

[1-3] Values within a column with the same superscript number are not significantly different at 0.05 significance level.

Table 4.2 shows the tabulated particle size diameter for all the samples. The values for other particle size parameters ($D_{3,2}$, $D_{1,0}$, $D_{5,0}$, and $D_{9,0}$) are presented as supplementary data (**Supplementary Table 4.1**). At the end of the gastric phase, the particle size of the goat meat digests ranged from 151-221 μm, close to the reported particle size of pork gastric digest 142-156 μm reported by (Li et al., 2023) and beef gastric digest ~160 μm (Zhou et al. 2021). The larger values obtained for some samples in our studies compared to the other studies could be due to less homogenisation steps before digestion. In this experiment, the meat was only homogenised for five pulses before digestion, considering the reported particle size of meat after chewing (Jalabert-Malbos et al., 2007; Pematilleke et al., 2022). For the fixed effects in the gastric phase, there were significant differences between breeds for the first 60 min of digestion; meat digests from feral were significantly lower than the meat digests from Boer crosses. However, at the end of gastric digestion, there is no main effect from breed or muscle type, but significant differences exist between the four samples; this contrasts with the results of Zou et al. (2018), where no significant differences exist between the particle size of digest from different pork cuts.

In the SI phase, there was a significant reduction in particle size diameter for all the samples within just 5 minutes into the intestinal phase. At this time point, the particle size of FSM was significantly higher ($p < 0.05$) compared to FLT, BLT and BSM. The particle size continued to decrease until the end of digestion, with the end values ranging from 26-35 μm, lower than the reported value for pork at 70-74 μm (Li et al., 2023) but higher than the reported values by Zou et al. (2018) at around 7 μm. At the end of the intestinal phase, muscle type primarily affected the particle size, but no breed effect was seen; FLT and BLT had lower

values than FSM and BSM. The four samples show significant differences in the average particle size for each time-point, indicating the interaction between breed and muscle type. Hence, the particle characteristics of the goat meat digest depend not just on the muscle type but also on which breed it was sourced from.

Given that the meat samples were homogenised in the same way, it can be assumed that the starting particle size of the meat can potentially vary since the extent of myofibrillar fragmentation during homogenisation is related to tenderness (Karumendu, van de Ven, Kerr, Lanza, & Hopkins, 2009). However, there was no correlation between texture and the particle size distribution at the end of the gastric and even at the end of the intestinal phase (data not shown), indicating that myofibrillar fragmentation related to meat tenderness has little role in how meat is broken down during digestion. Hence, it can be assumed that the differences can be due to the varying composition of the meat. Collagen content was negatively correlated with the particle size in the gastric phase (R^2= -0.61 at 120 min), which means that the level of collagen can somehow explain the variability in the particle size of the meat digest in the gastric phase. The involvement of collagen is highly likely since pepsin, the main proteolytic enzyme in the gastric phase has a very high specificity for collagen, especially for low pH condition (Chang & Leung, 2014; Skierka & Sadowska, 2007). On the contrary, in the SI phase, neither the level of collagen nor texture affected the particle size for goat meat digestion.

Based on this *in vitro* digestion condition, the particle size of the meat digest from all goat meat samples after pepsin digestion was broken down to a size that is small enough to be emptied from the stomach to the intestinal phase in an in vivo gastric digestion; digestible solids empty after they are degraded to form chyme having a particle size of 2-3 mm (Meyer et al., 1988). However, this assumption cannot be demonstrated further in this study because

of the limitation of an in vivo system. Nevertheless, it is acknowledged that the particle size of meat plays a significant role during digestion since it is known that gastric emptying is influenced by the size of solids in chyme. A protein's gastric-emptying rate affects the rate of protein disappearance in the small intestine and subsequent amino acid appearance in the bloodstream (Dangin et al., 2001; Mahé et al., 1996).

4.3.2 The degree and rate of protein hydrolysis

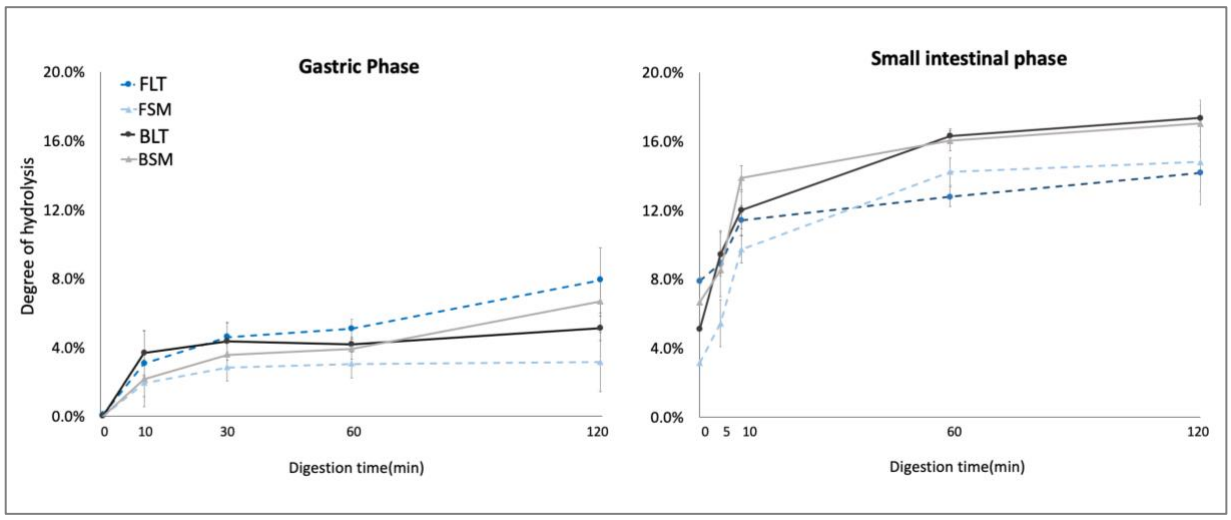


Figure 4. 3 Degree of hydrolysis based on the free amino nitrogen release during the gastric and intestinal *in vitro* digestion.

The degree of hydrolysis computed based on the amount of ninhydrin-reactive free amino nitrogen released by meat samples at different digestion time points was determined as a quantitative measure of *in vitro* protein digestibility. More hydrolysis indicates more protein hydrolysis by the digestive enzymes. The degree of hydrolysis of sous vide cooked goat meat is shown in **Figure 4.3.** After 10 min in the gastric phase, FLT and BLT were easily hydrolysed by pepsin compared to FSM and BSM, and both FLT and BLT remained more hydrolysed

until 60 min. Nevertheless, BSM became more hydrolysed at the end of the gastric phase than BLT. FLT remained the sample most hydrolysed by pepsin among the meat samples, which is also reflected in its particle size distribution in the gastric phases. Between samples, there were significant differences in the degree of hydrolysis, signifying that goat meat from different muscle types and breeds varies in the degree of hydrolysis in the gastric phase.

In the intestinal phase, there was a high increase in the degree of hydrolysis after 5 min and a continued increase in another 5 min. This increase in the degree of hydrolysis is expected due to the efficiency of proteolytic enzymes from pancreatin in hydrolysing protein. Additionally, the shift in higher pH led to a change in the structure of the folded protein, providing more cleavage sites for the enzymes. At the end of the intestinal phase, the values for LT and SM of Boer crosses were well separated from that of the feral goat meat. The average degree of hydrolysis for feral goat meat is 14.2 %, while the average degree of hydrolysis for Boer cross goat meat was significantly higher ($p < 0.05$) at 17.2 %, indicating that goat meat from Boer crossbreed goats was more digestible than meat from feral goats.

The release of free amino nitrogen was further analysed by looking into its increase over time. The rate of digestion differed ($p < 0.05$) for each digestion time point. In the gastric phase, the highest digestion rate, corresponding to the maximal increase in free amino nitrogen release over time, was recorded on the first 10 min of digestion. Among the samples, BLT had the highest rate, followed by FLT. As gastric digestion progressed, the rate of digestion decreased. For all the timepoints in the gastric phase, muscle type primarily affected the digestion rate but not breed; LT muscle from Boer and feral had a higher digestion rate than the SM muscle. Our data shows that in the gastric phase, the varying rates of digestion is dependent to the composition of meat, whether they are preferentially hydrolysed by pepsin.

This result is in agrees to the reported digestibility of meat that varies due to its composition specifically with gastric digestion by pepsin (M. L. Bax, T. Sayd, et al., 2013).

Table 4. 3 The digestion rate of goat meat in terms of changes in free amino nitrogen (ΔFAN) in terms of FAN per hour.

Digestion time-point		Feral		Boer Cross		[1]*p*-value	
		LT	SM	LT	SM	Breed	Muscle type
Gastric phase	$\Delta FAN_{g10/h}$	18.16 ± 4.70 [ab]	11.97 ± 3.32[a]	29.35 ± 3.13 [b]	13.00 ± 0.48[a]	NS	0.024
	$\Delta FAN_{g30/h}$	9.11 ± 0.58[b]	5.82 ± 0.67[a]	8.58 ± 0.88 [ab]	7.33 ± 0.69[ab]	NS	0.005
	$\Delta FAN_{g60/h}$	4.93 ± 0.19[b]	3.09 ± 0.35[a]	4.18 ± 0.18[b]	4.04 ± 0.33[ab]	NS	0.005
	$\Delta FAN_{g120/h}$	3.95 ± 0.38[c]	1.96 ± 0.16[a]	2.56 ± 0.15 [ab]	3.07 ± 0.04[bc]	NS	0.041
Small-intestinal phase	$\Delta FAN_{i5/h}$	10.17 ± 5.16 [a]	21.65 ± 5.55[a]	51.32 ± 7.04[b]	28.30 ± 2.09[a]	0.001	NS
	$\Delta FAN_{i10/h}$	18.69 ± 2.22 [a]	36.37 ± 6.88 [ab]	42.71 ± 2.19[b]	47.42 ± 5.23[b]	0.003	NS
	$\Delta FAN_{i60/h}$	4.53 ± 0.86[a]	10.30 ± 0.48[b]	11.16 ± 0.18[b]	9.87 ± 0.58[b]	0.007	NS
	$\Delta FAN_{i120/h}$	2.90 ± 0.32[a]	5.36 ± 0.23[b]	6.08 ± 0.23[b]	5.42 ± 0.14[b]	0.002	NS

ΔFAN denotes changes in free amino nitrogen: ΔFAN10g/h, ΔFAN30g/h, and ΔFAN120g/h denotes rate of digestion at 10, 30, 60 and 120 min gastric digestion, respectively. ΔFAN5i/h, ΔFAN10i/h, ΔFAN60i/h, and ΔFAN120i/h denote the rate of digestion at 5, 10, 60 and 120 min intestinal digestion, respectively.
[a-c]Means + SE within a row with the same superscript are not significantly different at a 0.05 significance level.
1ns = $p > 0.05$ and SD is the standard deviation.

In the simulated small-intestinal phase, the rate of hydrolysis was mainly affected by breed and not by muscle type. At 5 min and 10 min of intestinal digestion, there was a steep increase in the degree of hydrolysis which translates to a high rate of hydrolysis. The first 10 min intestinal time point was the largest difference in all digestion rate values. This can be attributed to the introduction of proteases from pancreatin, where the partially hydrolysed meat proteins after gastric digestion were readily hydrolysed by the combined action of the various enzymes from pancreatin (Rieder et al., 2021). In the intestinal phase, the breed had a main effect (p <0.05) on the rate of digestion but not the muscle type. The goat meat from Boer crossbreeds generally had a significantly higher rate of digestion than feral. When a correlation analysis was run between texture and digestion rate (data not shown), separate for gastric and intestinal phases, there was no significant correlation, indicating that the texture of the goat meat samples did not affect the rate of hydrolysis.

4.3.3 Variation in *in vitro* protein digestibility

The meat quality measures such as collagen content and texture and the effects of breed and muscle type as fixed effects were used to determine the predictability of the degree of hydrolysis to determine what factors affect its variability (**Table 4.4**). The interaction of muscle type and breed was also added to the model.

For the gastric digestion phase, collagen content and breed type significantly affected the degree of hydrolysis of the samples. Although, food texture has been recognised as a factor influencing gastric emptying (Santangelo et al., 1998), tenderness measured in terms of WBSF was not a significant predictor for the degree of hydrolysis of meat *in vitro* (**Table 4.4**). Regarding the composition of meat, the amount of collagen in it can affect its digestibility,

specifically in the gastric phase. The role of collagen content in meat digestion can be linked to the specificity of pepsin for collagen. All the goat meat samples were cooked using sous vide at 60 °C for 6 hrs. This step denatured the collagen. Pepsin can efficiently hydrolyse a broad range of peptide bonds of a denatured protein structure at low pH at elevated temperatures (Z. Fu et al., 2021). In the case of gastric conditions, pepsin activity is generally favoured—the varying protein structure of food results in a different rate of pepsin hydrolysis. While the collagen structure is typically known for its stability owing to its intermolecular crosslinks, the collagen present in the sample may not exhibit a high level of intermolecular crosslinks. This is because the goat meat samples were sourced from goats aged around 1.5-2 years old. Animals tend to have a higher level of cross-linked connective tissue as they age (Purslow, 2014). Moreover, even with higher crosslinks pepsin can still efficiently break down collagen since it removes non-helical ends (telopeptides) of the collagen, and the removal of telopeptide region consequently removes the intermolecular crosslinks (Hickman et al., 2000).

Table 4. 4 Muscle traits that significantly affected the degree of hydrolysis.

[1]Parameters	**Gastric phase**			**Intestinal phase**		
	Coefficient	Std error	p-value	Coefficient	Std error	*p* -value
Covariates						
Collagen content	0.031	0.005	<0.001	0.005	0.003	*0.060
Texture	-0.001	0.001	*0.285	-0.001	0.000	*0.183
Fixed factors						
Breed	0.489	0.085	<0.001	0.157	0.00	<0.001
Muscle type	0.035	0.107	*0.736	0.00	0.00	*0.051
Breed × muscle type	-0.208	0.117	*0.076	0.016	0.31	0.019

[1]Parameters are all the variables used for the generalised linear model.
[*]*p-value*, which is not significant at a 95 % confidence interval.

When all the selected factors were also used as predictors for the resulting degree of hydrolysis at the end of the *in vitro* gastrointestinal digestion, neither meat texture nor collagen content affected the variability of the results ($p > 0.05$). Our findings suggest that the digestibility of goat meat is not dependent on its texture; a lower shear force value does not equate to higher digestibility. The same result can be seen in literature where sous vide beef semitendinosus muscle having significantly different textures resulted in the same digestibility (Bhat et al., 2020). The significant predictors for the overall digestibility of meat are the breed and a significant interaction effect of breed and muscle type; this means that the digestibility of the goat LT and SM depends on which breed they were obtained from. Additionally, the effect of the breed is significant, indicating that the digestibility between breeds varies; the LT and SM muscles of the Boer cross had higher overall digestibility compared to feral meat in terms of degree of hydrolysis.

As reported in the literature, the digestibility of meat from different pork and beef cuts varies (Farouk et al., 2019; Zou et al., 2018). Although for beef, muscle cuts only mildly affected meat digestibility, and the collagenous cuts appeared more undigested at the end of digestion (Farouk et al., 2019). In our findings, although there is a positive correlation between collagen content and digestibility in the gastric phase, the meat with high collagen might not be efficiently further hydrolysed in the intestinal phase since it contains high proline. A proline-containing peptide was reported to mainly resist pancreatic enzyme hydrolysis primarily because of their structure (Shan et al., 2002). Hence, the overall digestibility of higher collagen meat might be lower. In this experiment, however, there is no relevant evidence that collagen affects the overall digestibility. Moreover, the level of collagen in the meat sample was not very high. It is possible that some other meat proteins played a

significant role in the differences in the degree of hydrolysis of the meat samples from Boer and feral goats. The differences in hydrolysis among samples cannot be further explained with any other variable since our data is limited only to the variables presented.

The composition of the muscle can affect the digestibility and rate of hydrolysis of meat proteins during digestion. As shown in our results, the difference in digestibility between muscle cuts is possible since different cuts could vary in composition with varying levels of muscle proteins with different thermal properties and susceptibility to various proteolytic enzymes (Liu et al., 2019; Montoya et al., 2018; Zhang et al., 2013). Our findings agree with the reported results in the literature, where different muscle proteins were found to behave differently under simulated gastric conditions (He et al., 2018; L. Li et al., 2017; Sayd et al., 2016). Regarding collagen content alone, its amount, spatial distribution, composition, and degree of crosslinking vary considerably between muscles (Lepetit, 2007; Purslow, 2014). Moreover, the types and amounts of specific amino acids in meat also determine the extent of protein digestion in the gastrointestinal tract. For example, phenylalanine, tyrosine, tryptophan, lysine and arginine can easily be hydrolysed since these amino acids are the target cleavage sites of pepsin or trypsin or chymotrypsin (Savoie et al., 2019).

4.4 Conclusion

The overall digestibility of sous vide goat meat muscles from feral, and Boer cross in terms of the degree of hydrolysis were different. Meat from Boer crossbreed goats had higher digestibility than meat from feral goats in terms of the degree of hydrolysis *in vitro*. Moreover, the digestibility varies between LT and SM but the values depend on which breed they were sourced from. Additionally, our results show that the level of collagen increased the invitro

digestion rate, specifically in the gastric phase. This would have important implications in the consumption of meat since the digestion rate in the stomach dictates the rate of gastric emptying and consequently the postprandial appearance of amino acids. On the other hand, meat texture measured in terms of peak shear force (N) did not affect the digestibility of goat meat. These results confirm that knowledge of meat composition can explain a significant portion of the variation in meat digestibility.

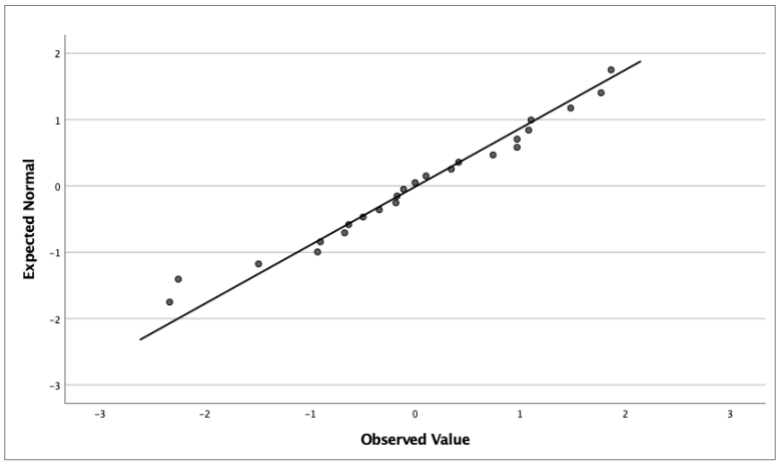
4.5 Supplementary data

Supplementary Table 4.1. Muscle traits that significantly affected the particle size predictability during *in vitro* gastric digestion.

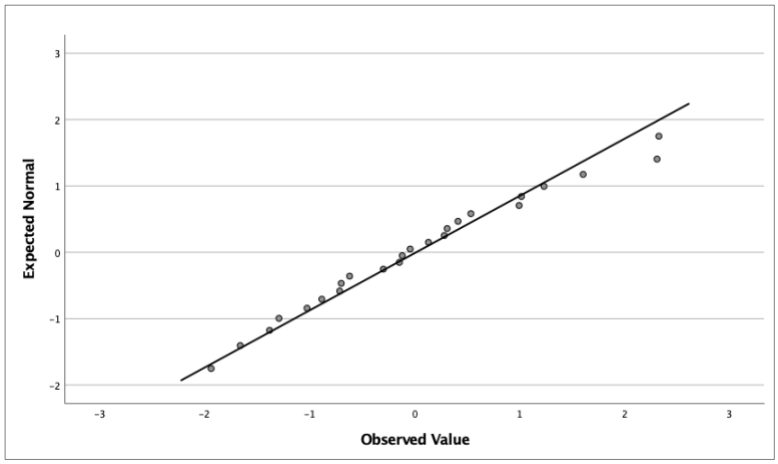
Parameters	Gastric		
	Coefficient	Std error	*p* – value
Covariates			
Collagen content	0.031	1.56	<0.001
Texture	-0.001	0.63	NS
Fixed factors			
Breed	0.489	-	NS
Muscle type	0.035	22.6	0.04
Breed × muscle type	-0.208	-	NS

Supplementary Table 4.3 The degree of hydrolysis for gastric and intestinal digestion.

Digestion time-point	FF		BF		SD	p-value		
	LT	SM	LT	SM		Breed	Muscle type	Breed× Muscle type
10	3.06ab	1.99a	4.89b	2.17a	1.62	0.132	NS	0.002
30	4.55b	2.91a	4.29ab	3.66ab	1.04	NS	0.005	0.018
60	4.93b	3.09a	4.18b	4.04ab	.92	NS	0.005	0.001
120	7.91c	3.92a	5.12ab	6.15b	1.95	<0.001	0.038	NS
125	8.49b	5.72a	9.40b	8.50b	1.62	.003	0.003	<0.001
130	11.02a	9.98a	12.24ab	14.05b	2.18	0.001	NS	0.002
180	12.43a	14.22b	16.28c	16.02c	2.94	<0.001	NS	<0.001
240	13.70a	14.64a	17.52b	16.99b	1.98	<0.001	NS	<0.001



Supplementary figure 4.1. Plot showing the Normal Q-Q Plot of the residual for the degree of hydrolysis at 120 min gastric digestion (p=1.0 using Shapiro-Wilk).



Supplementary figure 4.2. Plot showing the Normal Q-Q Plot of the residual for degree of hydrolysis after 240 min gastrointestinal *in vitro* digestion (p=1.0 using Shapiro-Wilk).

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Chapter 5

Effect of microwave processing in comparison to sous-vide cooking on goat and lamb meat quality and muscle structure

5.1 Introduction

Thermal treatment is widely used to make meat appealing and safe for consumption. Thermal processes can be carried out in different methods and modes of heating, such as steaming, boiling, braising, pan frying, deep-frying, roasting, and drying. Consequently, the severity of the process in terms of the temperature and length of cooking time can affect the qualities of the cooked meat, such as texture, colour, and cooking loss. Furthermore, the type of thermal process also modifies meat structure, affecting its technological properties (Astruc, 2014b).

Meat quality is often associated with tenderness, where tender meat means better quality for consumers (Huffman et al., 1996). Based on the literature and our previous findings, goat meat is tough (Kannan et al., 2014; Shija et al., 2013). Hence, using technologies that significantly improve its tenderness would gain consumer satisfaction. The effect of various thermal processing conditions has been examined for cooking goat meat. Steaming, boiling, and braising were proven to be suitable methods that resulted in better nutritional values for goat meat (Jiao et al., 2020). Additionally, moist heat cooking (boiling and steaming) is a better option than roasting in improving the texture of goat *longissimus* muscle (Jia et al., 2022). Among the processing conditions reported for goat meat, the use of low-temperature long-time cooking, such as sous vide, was found to achieve an acceptable tenderness significantly (Ismail et al., 2019a, 2019b).

Sous vide (SV) is a low-temperature process for a prolonged duration and is a popular method of cooking vacuum-packed food in controlled heating (Baldwin, 2012). It is also described as a novel process that produces superior quality cooked meat, even for low-value beef (Silva et al., 2016; Uttaro et al., 2019). The long cooking time during SV makes this

process popular for tough meat cuts (Karki et al., 2022; Uttaro et al., 2019). For goat meat applications, the tenderizing effect of low-temperature processing can be attributed to some types of collagen that solubilize in prolonged cooking (Purslow, 2014). Additionally, at low temperatures, collagen fibres experience decreased breaking strength due to partial denaturation and shrinkage (Christensen et al., 2000; Tornberg, 2005), and endogenous enzymes such as cathepsins B and L and serine proteases have an effect in degrading muscle proteins (L. Christensen et al., 2011; Purslow, 2014; Zielbauer et al., 2016).

In our previous experiment, SV cooking was carried out for 6 hr at 60 °C. Even though the suggested optimum cooking temperature is at 50-60 °C (Ismail et al., 2019b), both *longissimus* and *semimembranosus* muscles still had high (>60 N) peak shear force values (PSF). Hence, the cooking condition was not enough to achieve tender goat meat, and for tough goat muscle, tenderization can be achieved with prolonged cooking, more than 6 h. It has been reported in the literature that beef chuck and pork shoulder become tender after 10–12 h at 80 °C and 24–48 h at 55–60 °C, respectively (Baldwin, 2012). Getting the precise time and proper temperature for different meat cuts is challenging for SV. Although SV processing has shown a favourable impact on meat, its main downside is the length of time for the cooking process, which is energy-intensive, not ideal, and practical for industry application.

In contrast to SV cooking, which involves a slow process with long-time low-temperature heat treatment and precise temperature control, microwave (MW) heating is a fast-cooking method at elevated temperatures. Hence, this method significantly reduces food preparation time (Tang et al., 2018). The mechanism of MW heating uses the polarization effect of electromagnetic radiation that transforms electromagnetic energy into thermal energy. It relies heavily on the food dielectric property that affects the degree of heat

generated within the system (Soni et al., 2020). In recent years, MW processing has gained much attention because of the development of microwave-assisted thermal processing (MATS), which aims to produce ready-to-eat products on an industrial scale via a fast-heating process (Soni et al., 2020; Tang, 2015). This process is thought to be a green technology that can be used to prolong the shelf-life of minimally processed food.

The effects of microwave heating on goat meat have been studied. Yarmand & Homayouni (2009) compared microwave cooking to conventional oven heating, where MW processing resulted in high cook loss with fat migration. Yarmand & Homayouni (2010) also evaluated the effect of microwave and conventional cooking on goat meat muscle structure. Electron micrographs revealed that microwave heating caused more structural damage (shrinkage and breakdown) in the connective tissue and the myofibrillar elements in semimembranosus muscle. However, the result was only limited to the structural changes seen from the surface of the meat, and the effect of MW on the texture of the meat was not evaluated.

Although studies have been published on SV and MW that contribute to our understanding of the effects of SV and MW processing on goat meat, gaps still need to be filled. In this experiment, we aim to understand the impact of MW compared to SV cooking on the quality of cooked goat meat, in which both processes can achieve acceptable tenderness values. Furthermore, the meat ultrastructural examination of goat meat undergoing SV and MW processes was determined considering the disruption of major muscle structures and the shrinkage of muscle fibres. This study also compared goat meat to lamb to understand how the selected processes affect different kinds of meat.

5.2 Materials and methods

5.2.1 Meat samples

The *biceps femoris* (BF) muscles of lamb and goat meat were used in this study. The BF is a large muscle on the lateral surface of the mammalian hindlimb and is considered a less tender meat that requires more extended cooking methods (Calkins & Sullivan, 2007). For this experiment, BF muscle was taken only from Boer crossbreed goats since it has the size and shape suitable for the conditions for MW processing.

A total of 6 animals of each species were used in the study. The lamb samples were purchased as a bone-in lamb leg cut, supplied by Wilson Hellaby (Auckland, New Zealand), and purchased from The Mad Butcher, Palmerston North, New Zealand. The lamb was wet-aged for 10 days at 4 °C after slaughter, and its average pH was 5.34, measured by an insertion electrode (Mettler-Toledo Inlab 427). The goat meat samples were dissected whole BF from female Boer crossbreed goats supplied by Shingle Creek Chevon (Central Otago, New Zealand) with 15 kg, an average empty body weight and a fat score of 3 to 4 girth rib (GR). The goat meat samples were wet-aged for four days at 4 °C and had a pH of 5.18.

The muscles were dissected from the whole leg cut, and the epimysial sheath was removed. From both ends of each BF muscle, approximately 50 g was taken where a portion was immediately used for colour analysis, while the remaining samples were minced and stored at -80 °C for proximate and protein structure analysis. The remaining large portion of the meat sample, with an average weight of 176 ± 5 g and 165 ± 5 g for lamb and goat meat samples respectively, were prepared for processing. **Figure 5.1** shows the sampling procedure on the *biceps femoris* muscle.

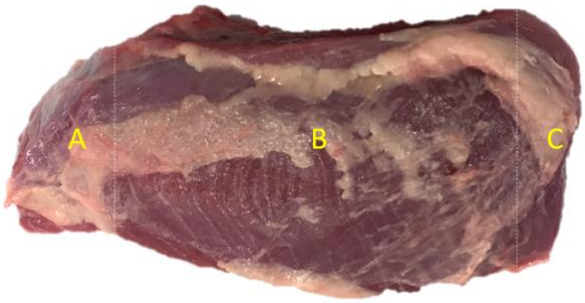


Figure 5. 1 Sampling procedure on biceps femoris muscle.
A and C are for proximate composition and raw meat structure, and B is for microwave and sous vide processing/ultrastructure/ shear force.

5.2.2 Sample Packaging

For each sample, a cylindrical PicoVacQ temperature sensor and MI-Orion probe (TMI-USA, Inc., VA, USA) were inserted in the BF muscle for temperature monitoring. Then, the meat was vacuum-packed in BNB1 vacuum pouches (Cryovac, Hamilton, New Zealand) and sealed (0.023 MPa) using a Multivac C200 vacuum sealer (Multivac NZ Ltd, Auckland, New Zealand). These vacuum pouches are the designated packaging for the MW equipment. Packed samples are shown in **Figure 5.2**.

Figure 5. 2 The *biceps femoris* samples packed in BNB1 pouches.

5.2.3 Sample processing

5.2.3.1 Microwave cooking

The MW processing was carried out using coaxially induced microwave pasteurization and sterilization (CiMPAS) equipment manufactured by Meyer Burger Germany GmbH (Hohenstein-Ernstthal, Germany) with the industrial microwave parts manufactured by MUEGGE GmbH (Reichelsheim, Germany) (**Figure 5.3**). This equipment uses industrial MW heating at 915 MHz and operates at 30 kW. The MW process was chosen after a series of preliminary experiments.

Figure 5. 3 Coaxially induced microwave pasteurization and sterilization (CiMPAS) equipment.

The vacuum-packed samples were loaded in a tray, placed inside the heating chamber under pressure at 0.25 MPa, and flushed with water. The meat was exposed to microwave for four consecutive passes at 100 cm/min speed. The CiMPAS enables efficient heat penetration since MW energy is emitted above and below the food. After MW exposure, 60 °C water was

flushed to reduce the temperature, and the process was stopped after 20 min holding time. The internal temperature profile of the meat samples was measured using the TMI probes inserted in the middle of the meat samples. After the calibration of the TMI probes using fibre optics, the values of for temperature was based on the corrected temperature using fibre optics. Overall, MW exposure led to an approximately 10°C/min increase in temperature throughout the sample. By the end of the process, the maximum average temperature of the core reached 104.7 °C for goat meat and 117.8 °C for lamb. A sample temperature profile of the whole MW process is shown in **Figure 5.4**. After the entire process, the pouches were immediately immersed in an ice bath. Although the MW equipment involved elevated pressure, the main physicochemical changes undergone by the MW processed meat are attributed to the effects of high temperature induced by MW heating. The pressure of the chamber was only 0.25 MPa compared to the industrial HPP equipment that uses 100-900 MPa.

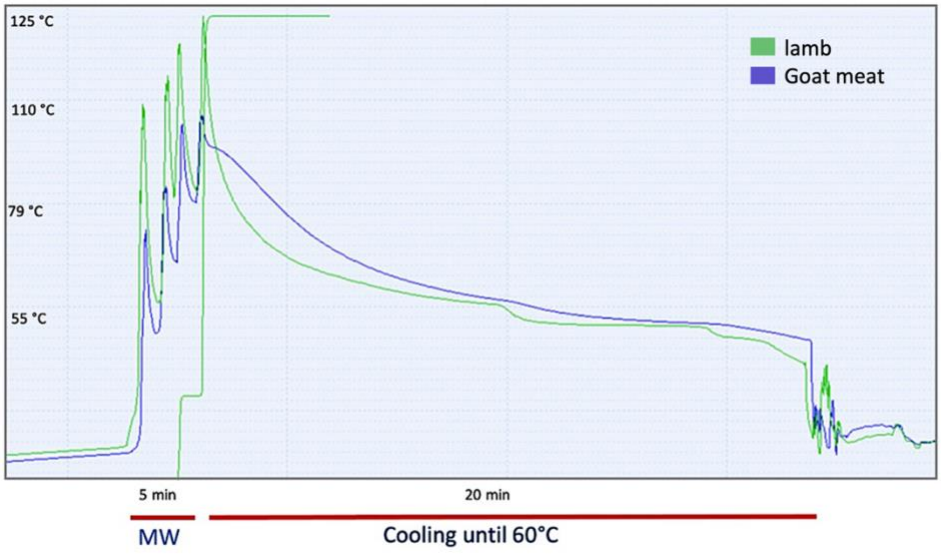


Figure 5. 4 Sample temperature profile of a MW process.

5.2.3.2 Sous vide cooking

For SV cooking, BF muscle was prepared and vacuum-packed the same way as samples used for MW processing. Preliminary experiments were conducted to determine the SV cooking time that can provide the same level of tenderness as that of the MW meat. Packed meat was subjected to SV cooking at 60 °C using various cooking times (6, 8, 10, and 12 hours). A temperature of 60 °C was selected since it is the water temperature in the MW chamber, a widely used temperature for SV, and an approved temperature for cooking meat in New Zealand according to Ministry for Primary Industry guidelines (MPI, 2015).

5.2.4 Meat quality evaluation

5.2.4.1 Texture

The instrumental texture tenderness of the samples was analysed using the Warner-Bratzler shear force (WBSF) method. A TA.XT plus texture analyser (Stable Microsystems, Godalming, UK) was fitted with a Warner-Bratzler Shear device (V-notched cutting blade, with a blade thickness of 1.1684 mm and a V-notched angle at 60°). The cooked meat was chilled overnight at 4 °C before the sample preparation and analysis. For each sample, six cuboids (1 cm^2 shear area) with a fibre direction parallel to the length of the sample were taken and sheared perpendicularly. The peak force was obtained using a 50 N load cell with a crosshead speed of 250 mm/min (Ismail et al., 2019b)

5.2.4.2 Cooking loss

After cooling, the cooked SV and MW meat samples were taken from the vacuum pouch, blot-dried with filter paper, and weighed. The cooking loss (%) was computed using Eq. 1.

$$\textit{Eq. 1.} \qquad Cooking\ loss\ (\%) = \frac{raw\ weight - cooked\ weight}{raw\ weight} \times 100$$

5.2.4.3 Colour

The mid-section of the whole BF muscle was cut, and three sections with a thickness of 12–15 mm were taken and allowed to bloom on a tray at 4 °C for 30 min. After blooming, two locations for each cut section were randomly chosen for the analysis; six measurement points were taken for each location. Instrument settings and colour parameters were done using the details described in **Chapter 3.4.2**.

5.2.5 Meat ultrastructure

The meat ultrastructure was evaluated using Transmission Electron Microscopy (TEM) using the FEI Tecnai G² Spirit BioTWIN transmission electron microscope (FEI Corp., Brno-Cernovice, Czech Republic). The sample collection, preparation, and staining were done following the method in **Chapter 3.6.** Ultrastructural examination for all the treatments was carried out using one representative animal sample each for lamb and goat. A total of 10 images were examined for one sample, and 10 muscle fibres per image were randomly chosen for sarcomere length measurement.

5.2.6 Statistical analysis

Statistical analysis was performed using Minitab Version 19.2020.2.0 (Minitab Inc., State College, PA, USA). The determinations of each parameter were done in triplicate unless otherwise stated. Before analysis, a normality test was performed using the Shapiro-Wilk test, and outliers (computed using Dixon's test) were removed where applicable. The analysis of variance was determined using the Minitab General Mixed Model, with processing condition as a fixed factor and the animal number as the random variable. When at least one group was statistically different, multiple comparison analysis was done using the Tukey test set at 95 % confidence interval.

5.3 Results and discussion

5.3.1 Preliminary experiment

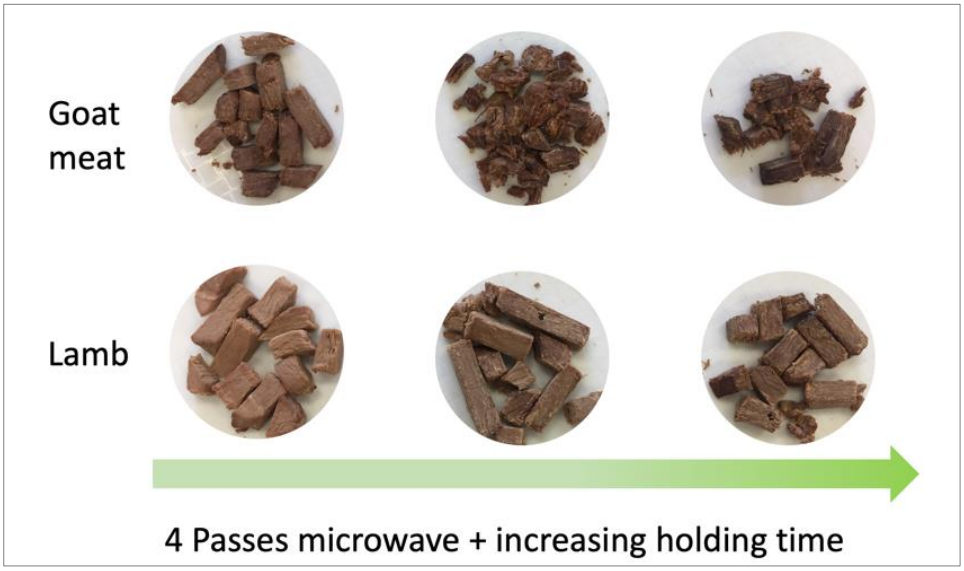


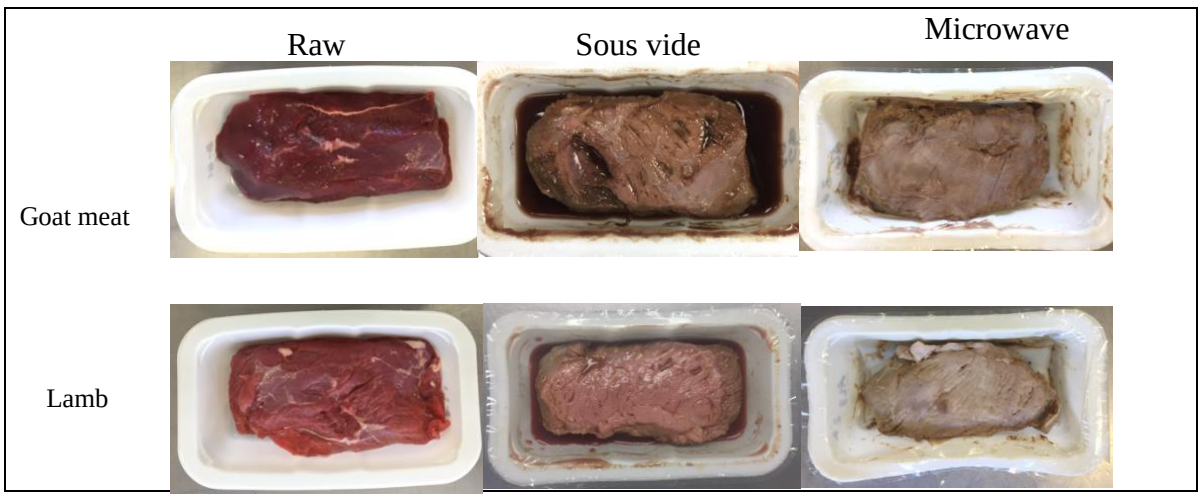
Figure 5. 5 Meat samples cooked with prolonged microwave passes.

Before the final process was chosen for SV and MW, the BF muscle was first subjected to various processing conditions to determine the condition that could achieve acceptable meat texture values in terms of WBSF. Here, we use the scale for the acceptable tenderness values for beef, where WBSF values > 52.68 N and < 42.87 N are perceived by most consumers as "tough" and "tender", respectively (Destefanis et al., 2008; Shackelford et al., 1991).

Four passes were used as the standard number for the MW process to reach an acceptable temperature based on the CIMPAS application. After four passes, the samples were held in various durations before flushing with water at 60 °C. The prolonged MW process led to overcooked meat (very dry and brittle), darker cooked meat, and very high cook loss (>

40 %) for lamb and goat meat (**Figure 5.5**). The cook loss values obtained are usually the values obtained for high-temperature processing (>100 C) due to excessive muscle fibre shrinkage (Palka & Daun, 1999). For tenderness, a longer MW process resulted in low WBSF values, less than 20 N. Hence, a prolonged MW process will result in more tender meat but with high cooking losses and darker meat. On the other hand, shorter MW processes and fewer passes led to low internal meat temperature (<80 °C) and higher WBSF values. The final process for MW used four passes, with the maximum average temperature of the core reaching 104.7 °C for goat meat and 117.8 °C for lamb), then immediate flushing of 60 °C water for 20 minutes.

To determine the counterpart SV process, the BF muscle vacuum packed in the same way as the MW process set-up was subjected to 60 °C SV cooking for using 6, 8, 9, 10 and 12 hours. As the cooking duration decreased, the tenderness values of the meat also decreased. Among the SV time tests, the 9 h SV was used as a reference to carry out the whole study since this condition resulted in cooked meat having the same tenderness values as the MW processed meat**. Figure 5.6** shows the samples before and after the chosen SV and MW processing conditions.



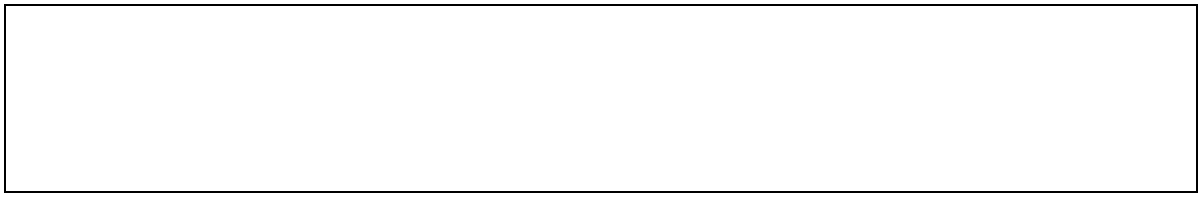


Figure 5. 6 Raw, SV, and MW lamb and goat meat.

5.3.2 Meat quality

Table 5.1 presents the quality parameters for the MW and SV samples. Using the same processing conditions for both MW and SV, lamb and goat meat had different tenderness values but both were within the acceptable tenderness value range (~< 43 N) (Destefanis et al., 2008). The MW and SV processed lamb resulted in low WBSF values from 21.45 to 23 N, which can be considered very tender (WBSF < 32.96 N). Hence, for SV, 9 hrs 60 °C processing for lamb would be too much since it resulted in very tender cooked meat. However, for goat meat, the chosen SV and MW processes resulted in WBSF values from 42.75 to 43.78 N (intermediate tenderness:42 N < WBSF < 52 N) (Destefanis et al., 2008; Shackelford et al., 1991).

Table 5. 1 Meat colour profile and texture and cooking loss of sous vide and microwave-processed lamb and goat meat.

Parameters	Raw	MW	SV	*p*-value
Goat meat				SV×MW
Texture (N)		42.75 ± 2.00 [a]	43.78 ± 5.6 [a]	NS
Cooking loss (%)		32.40 ± 2.01 [b]	29.00 ± 1.02 [a]	0.04
L*	41.9 ± 0.32 [a]	54.2 ± 0.40 [c]	58.8 ± 0.32 [c]	<0.001
a*	14.5 ± 0.21 [c]	6.25 ± 0.10 [a]	12.00 ± 0.42 [b]	<0.001
b*	6.80 ± 0.22 [b]	8.84 ± 0.21 [a]	9.59 ± 0.20 [a]	0.003
Chroma	16.09 ± 0.34 [b]	10.83 ± 0.23 [a]	15.40 ± 0.70 [a]	<0.001
Hue	24.78 ± 0.52 [a]	54.72 ± 0.42 [c]	39.04 ± 0.41 [b]	<0.001
Lamb				
Texture (N)		23.14 ± 0.61 [a]	21.45 ± 1.30 [a]	NS
Cooking loss (%)		33.5 ± 1.02 [b]	23.00 ± 2.03 [a]	<0.001

L*	46.9 ± 0.52 [a]	58.26 ± 0.70 [b]	60.20 ± 0.45 [b]	0.017
a*	18.51 ± 0.32	6.27 ± 0.24 [a]	13.60 ± 0.66 [b]	<0.001
b*	10.57 ± 0.31 [a]	9.58 ± 0.36 [b]	10.75 ± 0.21 [a]	<0.001
Chroma	21.4 ± 0.60 [c]	11.4 ± 0.38 [a]	17.5 ± 0.44 [b]	<0.001
Hue	29.6 ± 0.43 [a]	56.8 ± 0.33 [c]	39.2 ± 1.60 [b]	<0.001

All values are reported as the mean ± SE, where *N*=6 (6 replicates with 3 measurements).
Values within a row not having common superscripts differ significantly ($p < 0.05$). [1]NS = $p > 0.05$.
L-lightness, a*-redness, b*-yellowness, Chroma (intensity,* $C = [a^{*2} + b^{*2}]^{1/2}$) and *Hue (discolouration,* $H^* = \tan^{-1}[b^*/a^*]$).

The more tender cooked lamb than goat meat in this study can be explained by its longer aging time, which was ten days, compared to goat meat, which was just four days. Although a direct comparison between the lamb and goat meat samples cannot be done, it is clear that the effect of the MW and SV process would differ depending on the meat sample. In this case, goat BF muscle, aged for four days, would require a more prolonged SV or MW cooking if more tender meat is sought.

In this experiment, the shear force values obtained from Boer crossbreed goat meat are lower than those reported for the BF muscle of Korean native black goat, which was subjected to SV at 60 °C for 12 hrs (Ismail et al., 2019b). Additionally, their study showed shear force values for elevated temperature and more prolonged duration were high at WBSF >50 N. The high toughness of the Korean black goat compared to our samples in this study is probably because the native Korean goat samples were from the uncastrated males, while our samples were from female Boer crosses. It has been reported that, generally, female goat are more tender than males (Hogg et al., 1992; A. Kirton, 1970).

The cooking loss for MW samples was significantly higher than the SV samples for goat meat and lamb. These results agree with the literature observation that MW results in higher cooking loss than water bath processing (Wang et al., 2019) and conventional cooking (Yarmand & Homayouni, 2009). However, MW treatment offers a fast method of

cooking, resulting in higher cooking loss, which is a typical observation for meat cooked at high temperatures (Bax et al., 2012; Dominguez-Hernandez et al., 2018; Roldán et al., 2013). Hence, SV would be a better processing option for tough goat meat to achieve juicier meat.

Generally, the capacity of meat to retain moisture is governed by the shrinking and swelling of myofibrils. Nevertheless, various factors can explain the varying cooking loss between treatments. First, the different degrees of muscle fibre and connective tissue shrinkage are influenced by the temperature, where a high temperature (>100 °C) can induce a higher degree of longitudinal shrinkage, resulting in more significant water loss compared to the SV (Roldán et al., 2013). In this study, MW resulted in high-temperature processing (>100 °C), which causes shrinkage of myofibrils and the connective tissue network, releasing moisture, solubilized proteins, and fats. Conversely, SV process with low temperature and slow heating can cause limited longitudinal shrinkage compared to higher temperature cooking (Dominguez-Hernandez et al., 2018). Another factor is the difference in heating rate between the SV and MW process. A fast-heating rate was shown to cause a significantly higher cooking loss than slow heating in pork (Wu et al., 2007). It has been demonstrated that a higher heating rate can induce significantly higher cooking loss than slow heating because it results in a higher mobility of bound water (Mortensen et al., 2006). Lastly, the varying cooking loss can be explained by the length of cooking time. The SV processed meat had a lower cooking loss because longer cooking duration results in a higher level of solubilization and gelation of collagen, which enhances the water-holding capacity of the meat matrix (Tornberg, 2005; Zielbauer et al., 2016). With short heating duration in MW processing, collagen is not gelatinized and cannot aid in retaining moisture inside the meat (Purslow, 2014).

For meat colour, both SV and MW caused a significant reduction of a^* (redness) for both lamb and goat meat, but MW samples had a significantly lower ($p < 0.05$) redness compared to SV samples (**Table 5.1**). The MW process led to a distinct cooked appearance, suggesting a higher degree of doneness than the SV samples. Relative to the raw sample, MW led to a higher ($p < 0.05$) pigment discolouration (hue) with significantly lower ($p < 0.05$) intensity (chroma) compared to SV. The temperature difference between SV and MW can explain the colour difference in the cooked meat since the colour of cooked meat is highly dependent on the endpoint temperature (Lawrie, 1998). Even for a short duration, the high-temperature cooking for MW resulted in a significant reduction in a^* and increased L^*. On the other hand, the SV-cooked at 60 °C had less myoglobin denaturation. This finding agrees with observations for SV processing, where meat cooked by SV displays a more intense reddish and less brown colour (García-Segovia et al., 2007). The redness value for MW samples (< 7) is close to the redness of meat typically processed with temperatures above 80 °C (Liu et al., 2013; Wattanachant et al., 2008). The high endpoint temperature of MW meat was sufficient to denature myoglobin, which is unstable at temperatures > 60°C (García-Segovia et al., 2007; Hugas et al., 2002). For high temperatures, the mechanisms of the redox chemistry of myoglobin involve a reduction of the amount of deoxymyoglobin and oxymyoglobin and an increase in metmyoglobin.

For all the colour parameters, the goat meat sample had lower values than the lamb, which is reflected by the chroma (intensity) of the cooked meat. Moreover, goat meat samples had lower lightness values than lamb, and with the same redness level, they would appear darker than lamb. These values agree with what has been reported in the literature that goat meat tends to be darker red compared to other red meat (Casey et al., 2003; Kadim et al., 2010;

Schönfeldt, Naude, Bok, Van Heerden, Smit, et al., 1993; Schönfeldt, Naude, Bok, Van Heerden, Sowden, et al., 1993; R. Sheridan et al., 2003). For the reported values of SV cooked goat meat, cooked BF from Boer crossbreed had a higher redness value (39) compared to the reported values for Korean native black goat, which is under 30 but with the same level of intensity, that which is around 21 (Ismail et al., 2019b).

5.3.3 Meat ultrastructure

MW and SV processes led to changes in meat structure (**Figure 5.7**). Regardless of the type of meat, both processes led to an increase in Z-disk thickness, swollen coagulated mitochondrial matrix, weakening of the Z-disk and I-band junctions, and detachment of the sarcolemma from the myofibrils. Furthermore, granular aggregates in gaps between the endomysium and myofibrillar mass were seen. These observations are expected changes in red meat that undergoes thermal processing (Chian et al., 2019; Tornberg, 2005). However, the resulting ultrastructural damage level varied between the two processes.

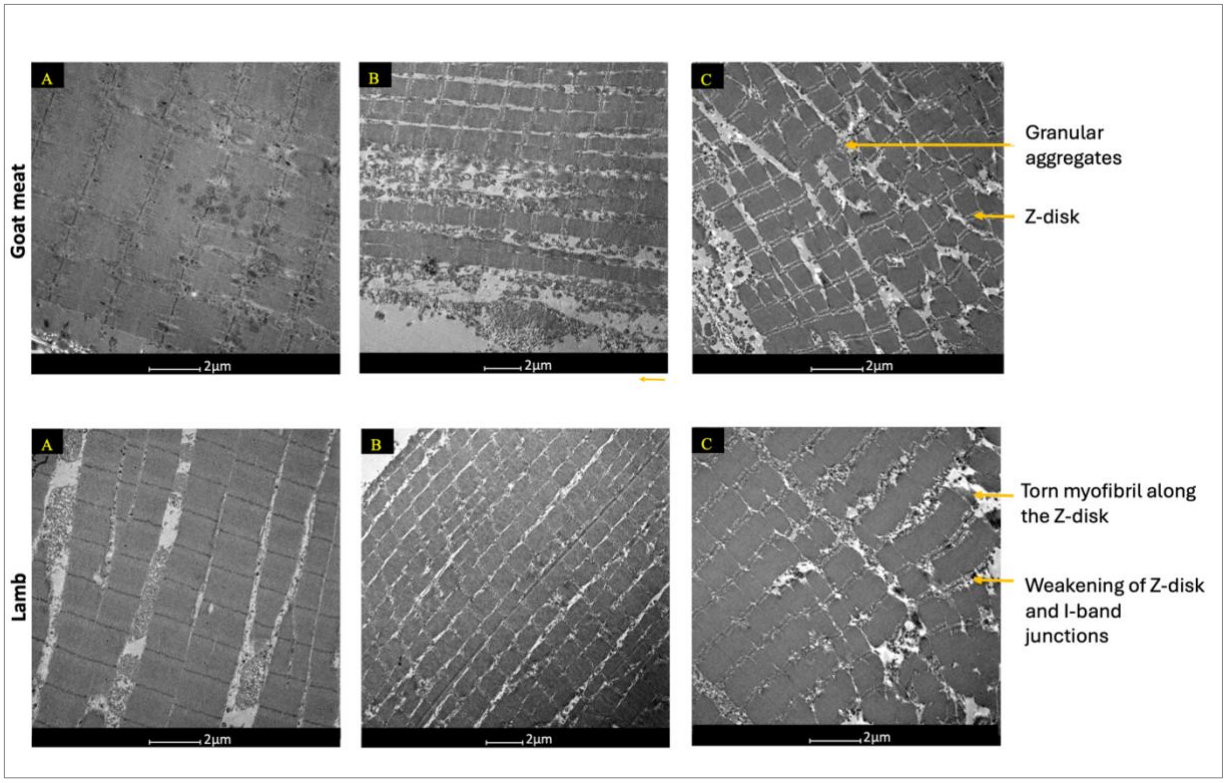


Figure 5. 7 Meat ultrastructure.

Transmission electron microscopy (TEM) images were taken from the longitudinal sections of raw (A), sou vide (B), and microwave (C) processed goat meat and lamb *biceps femoris*.

Muscle fibre disruption was present in SV samples but to a very low degree. The goat meat sample's ultrastructure closely resembles the SV LT and SM muscle from our previous experiments (**Chapter 3**). Collectively, for the SV processed goat meat sample, including the LT and SM for 6 h at 60 C, SV processing resulted in cooked goat meat with myofibrils that were easily identifiable, still parallel, and mostly without breaks. The same observation for SV beef at 60 C was also reported by Supaphon et al. (2021).

Rapid MW heating resulted in significantly torn myofibrils, which occurred randomly between muscle fibres and along the Z-line and breakage of the sarcomere. Furthermore, the myofibrils were not parallel anymore, mostly disoriented with a significant number of z-disks disrupted. The observation for the MW images can be likened to the structure of meat

processed using high temperature (> 90 °C). An obvious breakage of the sarcomere was reported for high-temperature processing at 90 °C (Liu et al., 2013; Wattanachant et al., 2005).

Table 5. 2 Longitudinal and horizontal shrinkage of goat meat and lamb biceps femoris after sous vide (SV) (60 °C for 9 hrs) and microwave (MW) process measured from TEM image.

		Raw	SV	MW
Goat meat				
	Muscle fibre diameter (μm)	0.99 ± 0.03	0.96 ± 0.03	0.82 ± 0.03
	Sarcomere length (μm)	2.38 ± 0.03	1.78 ± 0.03	1.46 ± 0.03
	Longitudinal shrinkage		25 %[a]	39%[b]
	Transverse shrinkage		3% [a]	17%[b]
Lamb				
	Muscle fibre diameter	0.91 ± 0.04	0.82 ± 0.03	0.61 ± 0.03
	Sarcomere length	1.53 ± 0.02	1.47 ± 0.02	1.41 ± 0.05
	Longitudinal shrinkage		4% [a]	8%[b]
	Transverse shrinkage		10% [a]	34%[b]

All values are reported as the mean ± SE, where N=1 (1 animal with 10 images with 10 measurements from each image).
Values within a row not having common superscripts differ significantly within a row ($p < 0.05$).
MW-microwave process and SV-sous vide process.

General changes in muscle ultrastructure after SV and MW also involved lateral and longitudinal shrinkage for all samples (**Table 5.2**). When meat is processed at a temperature above 60 °C, muscle fibre shrinkage proceeds, in both the transverse and longitudinal axes (Dominguez-Hernandez et al., 2018; Hughes et al., 2014; Palka & Daun, 1999). Between processes, it was evident that MW processing resulted in a higher degree of longitudinal and transverse shrinkage. This higher shrinkage for MW can further explain the higher cook losses of the MW samples. The decreased muscle fibre dimension for the MW sample is due to the high temperature generated during MW compared to SV at 60 °C. As the temperature

increases, muscle volume decreases due to the shrinkage of individual muscle fibres and myofibrils (Purslow et al., 2016; Supaphon et al., 2021).

After SV and MW processing, goat meat responded differently compared to lamb. For goat meat samples, longitudinal shrinkage was higher than 25%, while the transverse shrinkage was just below 20%. Notably, transverse shrinkage in SV samples was significantly less than the MW samples. On the contrary, the lamb had higher transverse shrinkage than longitudinal shrinkage for both MW and SV processes. The higher transverse shrinkage for lamb meat is probably related to the effect of aging; transverse shrinkage increases the longer the meat is aged (Purslow et al., 2016). Moreover, the difference in pattern for muscle shrinkage can be explained by the difference in MW temperature generated between lamb and goat meat muscles. Lamb reached a higher peak temperature (117 °C) than goat meat (107 °C) on average, and the temperature dependence of muscle shrinkage is known (Palka & Daun, 1999). Furthermore, the differences in muscle composition between lamb and goat meat can also affect the resulting quality of a cooked meat from different species. The response of muscle fibres to temperature depends on the muscle fibre types composed of various proteins with different thermal stability (Astruc et al., 2012; Purslow et al., 2016).

The images of the meat ultrastructure clearly showed the significant differences between processes. The significant disruption of the meat structure, such as obvious breakage of sarcomere and excessive thermal shrinkage of myofibrillar proteins by MW, resulted in the tenderness of the meat having the same level as the SV sample cooked for a long time. Furthermore, MW processing operates at higher temperatures that can easily solubilize some collagen, reducing muscle integrity.

5.4 Conclusions

MW cooking can cook lamb and goat meat *biceps femoris* with the same tenderness as meat SV processed at 60 °C for 9 h. However, there was a significant difference in the quality of the cooked samples. The MW process resulted in a higher cooking loss, which should be addressed when designing pre-packed meals using MW technology. The significant disruption of meat ultrastructure can explain the texture of MW meat that was the same as the SV processed for a longer time. Additionally, goat meat responded differently compared to lamb, indicating that the MW and SV processes depend on the muscle condition; in our study, the process applied resulted in very tender lamb but resulted in goat meat with intermediate tenderness.

5.5 Acknowledgements

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5.6 Copyright information

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Chapter 6

The modification of goat meat protein structure by microwave and sous vide and its impact on in vitro gastrointestinal protein digestion

6.1 Introduction

Various thermal processes can induce a series of transformations in meat, affecting its physical, chemical, and structural properties (Christensen et al., 2000; Veronique Santé-Lhoutellier et al., 2008; Tornberg, 2005). In our previous experiment (**Chapter 5**), the effect of sous vide (SV) and microwave (MW) processing was mainly evaluated in terms of meat qualities such as texture, colour, cooking loss, and muscle structure. In this chapter, the effect of processing on meat protein digestibility will be examined since one of the relevant effects of processing is how it modifies the molecular structure of meat, which affects its nutritional value.

The impact of processing on meat protein quality has been widely studied by evaluating protein digestibility *in vitro* (Mitra et al., 2022; Orlien et al., 2021; Yin, Zhou, et al., 2020). In the literature, when different heating conditions are involved, heating temperature is often the key determinant of the meat protein *in vitro* digestibility (Bax et al., 2012; Kaur et al., 2014). Heating can either improve or decrease the digestibility of meat proteins. Heat treatments can enhance the accessibility of digestive enzymes to cleavage sites by denaturing proteins and breaking structures (Astruc, 2014b; Bax et al., 2012). However, heating can also reduce protein digestibility by causing significant protein aggregation that hinders efficient enzymatic hydrolysis (Aurélie Promeyrat et al., 2010; Sante-Lhoutellier et al., 2007). Thus, various thermal processing methods can affect the digestibility of meat in different ways.

The effect of MW heating and its nonthermal mechanism on protein secondary structure has been linked to lower nutritive quality of meat proteins (Calabrò & Magazù, 2014, 2020). Cai et al. (2018) reported that microwave radiation may increase aggregation rates and

denature sarcoplasm and myofibrils, leading to a higher probability of irreversible aggregation. Although structural examinations of proteins suggested compromised protein structure after MW processing, the implications of MW on meat protein digestibility have not been extensively studied, and a call for further investigation was raised (Cai et al., 2018). Recent digestibility studies involving MW were carried out for gluten (Xiang et al., 2020) pea (Sun et al., 2020), and shrimp (Dong et al., 2021). The effect of MW on the digestibility of beef, chicken, pork, and kangaroo meat has been evaluated (Ferreira et al., 2018; Luo et al., 2018; Menezes et al., 2018), but this study did not examine the effect of protein structural changes on protein digestibility. Based on our knowledge, the direct impact of the MW process on red meat structural changes and its influence on protein digestibility has not been demonstrated, specifically for goat meat.

Unlike MW, SV processing is described as a novel process that produces superior quality cooked meat. The nutritive value of SV-processed meat in terms of protein digestibility has been widely studied (Bhat et al., 2020; Chian et al., 2019; Xiaojie Zhu et al., 2018) and SV processing enhanced the digestibility of meat (Bhat et al., 2020). Despite the positive effects of sous vide (low-temperature processing) for meat protein digestibility, it can also potentially result in lower digestibility for meat. Some SV processes use a very long processing time, and it has been reported that prolonged heating can result in further aggregation of proteins (Cai et al., 2018; Mitra et al., 2017; Tornberg, 2005). More aggregated protein structures rebury exposed structures, limiting enzyme activity or leading to longer digestion time. Ultimately, the effect of thermal processing conditions on the digestibility of meat proteins is related to the resulting meat protein molecular structure, dependent on time and temperature conditions.

In this chapter, the focus is on the effect of thermal processing on the protein digestibility of goat meat. We aim to understand the impact of different processes on meat molecular structure and how these modifications influence the *in vitro* digestibility of proteins. Moreover, the meat samples from two different species, goat meat and lamb, were compared to understand further how the selected processes can affect different kinds of meat. Therefore, this study was designed to determine the effect of MW, which involves high-temperature intensive processing, versus the SV process, which requires a long-time and low-temperature process on meat protein digestibility. We hypothesised that MW processing would lead to intense structural modification of meat proteins, resulting in reduced *in vitro* protein digestibility compared to the milder effects of SV cooking at 60 °C for 9 hours.

6.2 Materials and methods

6.2.1 Extraction of myofibrils

The degree of protein aggregation was measured by analysing the particle size distribution of the myofibrillar protein. The myofibrillar protein was extracted following the methods (Martinaud et al., 1997) with modifications. A 2.5 g aliquot of thawed tissue was minced and homogenised for 1 min in an Ultra-Turrax T homogeniser (Ultra-Turrax T8 Ika Labortechnik, Germany) with 25 mL of cold isolation buffer containing 150 mM NaCl, 25 mM KCl, 3 mM MgCl2, 0.4 mM Pefabloc and 4 mM EDTA at pH 6.5. The collagen was eliminated by filtration on gauze. After 30 min of stirring in ice, the extract was centrifuged in 2000 × g for 15 min at 4 °C. Next, the pellet was collected and washed twice with 25 mL of a 50 mM KCl and 5 mM mercaptoethanol solution at pH 6.4 and once with 25 ml of 20 mM

phosphate buffer at pH 6.0. After washing, the pellet was resuspended in the phosphate buffer at pH 5.8. The protein concentration of the extracted myofibril was measured using the Bradford microplate standard assay with bovine serum albumin (BSA) as a standard; absorbance was read at 280 nm using Synergy 2 Multi-Detection microplate reader (BioTek, USA). The myofibrillar protein isolate (MPI) was stored in a 4 °C cold room and analysed within 24 h.

6.2.2 Protein degree of aggregation

The particle diameters and size distribution were measured via static light scattering technique using the laser diffraction method, Mastersizer 2000 (Malvern Instruments, UK) (Mitra et al., 2017). The volume-weighted ($D_{4,3}$) and surface-weighted ($D_{3,2}$) mean particle diameters were computed from the particle size distributions. The absorption was set as 0.001; the dispersant used was water, and the particle refractive index used was 1.45. The measurement time was every 2 min, and the temperature was 25°C. The intensity of the scattered light was detected using a 633 nm laser with a scattering angle of 90°.

6.2.3 Myofibrillar protein surface hydrophobicity

The surface hydrophobicity of extracted myofibrils (as mentioned in section 6.2.1) was determined using bromophenol blue (BPB) sodium salt binding (Chelh et al., 2006), with modifications. A 1 mL aliquot from a 5mg/mL myofibrillar fraction was added to 200 µl of BPB solution (1 mg BPB/ml Milli-Q water) and mixed well. A control sample was prepared by adding 200 µl of BPB solution to 1 ml of 20 mM phosphate buffer at pH 6. Samples and

control were kept under agitation at room temperature for 10 min and then centrifuged for 15 min at 2000 × g at 4 °C. The supernatant was diluted to 1/10 with phosphate buffer, and the absorbance of the resulting solution was measured at 595 nm. The myofibrillar surface hydrophobicity in terms of bound BPB was computed using Eq 6.1:

$$Eq\ 6.1 \qquad BPB\ bound(mg) = 200mg \times \frac{Abs\ control - Abs\ sample}{Abs\ control}$$

$$Abs = absorbance\ at\ 595\ nm$$

6.2.4 Fourier transform infrared (FTIR) spectroscopy

After processing, the qualitative changes in meat structure were evaluated using Fourier transform infrared (FTIR) spectroscopy. The samples were first lyophilised and had a final moisture content of 6.0-6.9 %. Then, the dried samples were ground, powdered, and sieved using a 316 stainless steel mesh with a 65 μm aperture. The analysis was carried out using a Nicolet™ iS™ 5 FTIR Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) with an iD7 ATR accessory (Thermo Fisher Scientific Inc., Waltham, MA, USA). The scan was conducted between 4000 and 450 cm^{-1} with a resolution of 2 cm^{-1}. The background spectrum was obtained initially and automatically subtracted from every acquisition; it was reset after scanning for 1 h. For each sample spectrum, 32 scans were accumulated and averaged. Finally, each spectrum was baseline corrected, normalised, and averaged for qualitative interpretation of spectra.

6.2.5 Deconvolution of the amide I region

The deconvolution of the amide I region (1600 and 1700 cm^{-1}) was done for further quantitative analysis using Peakfit 4.12 software (Systat Software, Inc., CA, USA). First, each spectrum was processed for baseline correction and smoothing using a Fast Fourier Transform (FFT) filter with a smoothing width set at 10 %. Next, peak fitting was done using the second derivative, and subsequent peak fitting was done using the assumption of the Voigt curve (Fellows et al., 2020). Several iterations were done to reach a fitting curve with a corrected $R^2 \geq 0.99$. The resulting peaks after deconvolution were identified based on known IR spectral data for protein secondary structures. Finally, the area under each peak was evaluated against the total area and expressed as a percentage. The reported values for β-sheet and β-turns are the accumulated values of more than one peak.

6.2.6 In vitro protein digestibility

The *in vitro* digestion system used in this experiment followed the same method outlined in **Chapter 4.2.2.** In this study, the sample used was 8 g per reactor instead of 5 g.

6.2.6.1 Oral phase

Eight grams of cooked meat sample, including the cooking loss, was mixed with 5.0 mL simulated salivary fluid, 1.4 mL α-amylase (10025, Sigma-Aldrich, USA) (30 U/mg), and 1.56 mL MQ water to initiate the oral phase at pH 7 ± 0.1 for 2 min.

6.2.6.2 Gastric phase

After oral phase, the gastric phase mixture had 10 mL simulated gastric fluid, 4.56 mL porcine pepsin (P7125, Sigma-Aldrich, USA) (185 U/mg) and 1.112 mL MQ water added to start the gastric phase. The pH was adjusted and maintained at 3.0 ± 0.1 using 0.5 M HCl. Gastric digestion was carried out for 2 h. The digest samples were taken after 1, 10, and 120 min of gastric digestion and pepsin activity was immediately stopped by adding Pepstatin A (Abcam, UK), (200 µL/15 mL digest).

6.2.6.3 Intestinal phase

For the intestinal phase, the mixture had 15.6 mL simulated intestinal fluid, 2.096 MQ water, 10 mL pancreatin (P1750, Sigma Aldrich, USA) (27 U/mg), and 4 mL bile (B8631, Sigma-Aldrich, USA). The pH was adjusted and maintained at 7 ± 0.1 by adding NaOH (0.1 M). Digest samples from the small-intestinal phase were taken after 10, 60, and 120 min of small-intestinal digestion, and the proteolytic enzyme activity was stopped using SIGMAFAST™ Protease inhibitor (Sigma Aldrich, USA) (2.5 mL/10 mL digest). All the digests were immediately stored at -20 °C for further analysis.

6.2.7 SDS-PAGE analysis of digests

The protein profile of each digest was evaluated using reduced-tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Kaur et al. (2010), with modifications. The digest was thawed and centrifuged at 13000 $\times g$ for 2 min, and the supernatant was diluted with Milli-Q water, adjusting the volume to the desired protein concentration. The diluted digests were mixed in a 1:1 ratio with premixed tricine sample buffer (1610739, Bio-Rad Laboratories, USA) with 2 % β-mercaptoethanol (M6250, Sigma-

Aldrich, USA) and then heated at 100 °C for 5 min. The electrophoresis was run using 16.5% Criterion™ Tris-Tricine Gel (Bio-Rad Laboratories Pty Ltd., New Zealand) at 125 V for 2 h, followed by fixing in fixing solution (40% methanol and 10% acetic acid) for 1 hr and staining with premixed staining solution Bio-Safe™ Coomassie Stain (Bio-Rad Laboratories Pty Ltd., New Zealand) for another hour. Afterwards, the gels were rinsed and soaked with MQ water overnight, with agitation. The gel image was obtained using Gel Doc XR+ scanning densitometer (Bio-Rad Laboratories Pty Ltd., New Zealand), and bands were analysed using Image Lab™ software version 6.0.0 (Bio-Rad Laboratories, Inc., CA, USA).

6.2.8 Protein hydrolysis (%)

The degree of protein hydrolysis was determined by quantifying free-amino nitrogen. The digest was centrifuged for 5 min at 13,000 x g and filtered using a 0.45 µm PVDF filter, Millex ® (Merck, Germany). The gastric and small intestinal digests were diluted with Milli-Q water at pH 3 and 7, respectively. The free amino nitrogen content of each sample was measured using the ninhydrin assay outlined by Moore (1968) using 0.2 % ninhydrin reagent (N7285, Sigma-Aldrich, USA).

6.2.9 Statistical analysis

Statistical analysis was performed using Minitab Version 19.2020.2.0 (Minitab Inc., State College, PA, USA). The determinations of each parameter were done in triplicate unless otherwise stated. Before analysis, a normality test was performed using the Shapiro-Wilk test, and outliers (computed using Dixon's test) were removed where applicable. T-Test was used to determine the difference between the two processes. The analysis of variance for the

hydrolysis values over time was determined using the Minitab General Mixed Model (GMM), with time as a fixed factor and the animal number as the random variable. When at least one group was statistically different, multiple comparison analysis was done using the Tukey test set at 95 % confidence interval. General Mixed Model was also used to determine the contribution of the individual molecular structure (as covariates) to the predictability of the hydrolysis values.

6.3 Results and discussion

6.3.1 Myofibrillar protein surface hydrophobicity

Myofibrillar protein surface hydrophobicity results from protein unfolding as the protein structure changes and is related to the tertiary protein structure. Our values show that both treatments significantly induced protein unfolding, exposing the hydrophobic side chains of amino acids that bind with BPB (**Figure 6.1**). Between SV and MW, for both lamb and goat meat, MW induced a higher degree of protein unfolding compared to SV. The MW process resulted in 185 and 180 μg hydrophobicity values for lamb and goat meat, which are significantly higher ($p < 0.05$) compared to the hydrophobicity values of SV samples. The results suggest that hydrophobicity is higher with high processing temperatures, consistent with the trend reported in the literature (Chelh et al., 2006; A. Promeyrat et al., 2010).

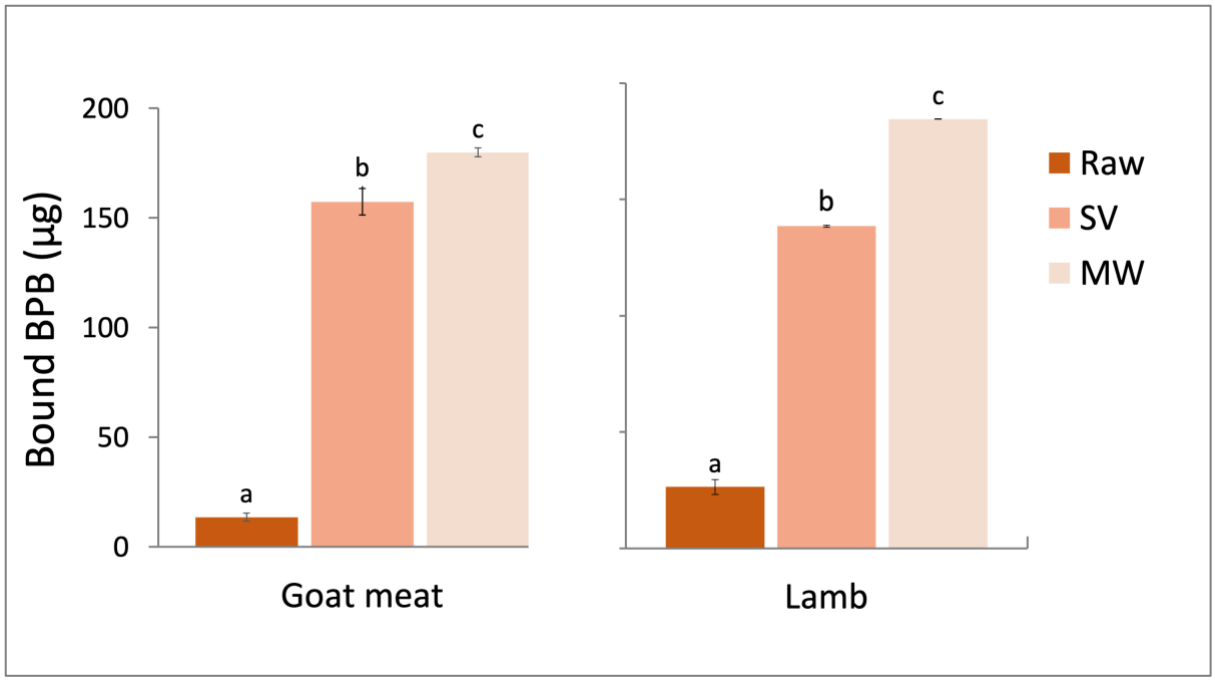

Figure 6.1 Myofibrillar protein surface hydrophobicity of Raw, sous vide (SV) and Microwave (MW) processed meat.

The high myofibrillar protein hydrophobicity in MW samples can also be linked to how heat is generated in meat through dielectric heating by MW. In meat proteins, the rupture of hydrogen bonds is cited as the leading cause of increased hydrophobicity (Sante-Lhoutellier et al., 2007). Therefore, the MW process could have resulted in a higher degree of hydrogen bond disruption since the alternating electromagnetic field of a microwave propagates through the polar water molecules within the muscle fibre. Furthermore, the MW electric field could also directly affect myofibrils due to the tearing effect on the protein structure (Cai et al., 2018; Cao et al., 2019)

On the other hand, the lower hydrophobicity values for SV meat can be caused by lower temperatures and prolonged heating conditions (Cao et al., 2019). Protein surface hydrophobicity increases as heating duration increases (Chelh et al., 2006; A. Promeyrat et al., 2010). However, increased surface hydrophobicity can also cause more extensive bonding

and aggregation, and the hydrophobic side chains of denatured proteins tend to be embedded in clusters when proteins aggregate further, thus reducing the observable surface hydrophobicity.

6.3.2 Protein degree of aggregation

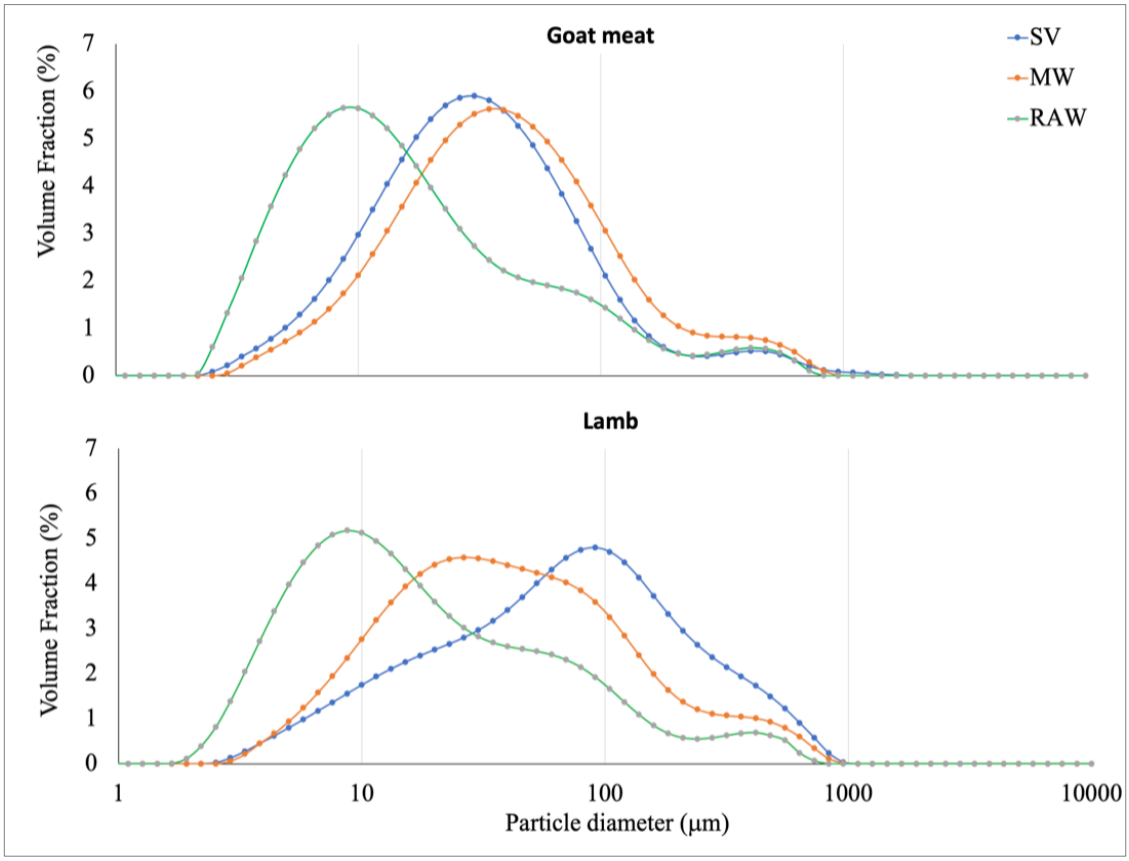

Figure 6. 2 Particle size distribution of the myofibrillar isolates from raw, SV and MW meat.

Changes in the number, size and form of myofibrillar particles due to heat can describe protein aggregation process (Traore et al., 2012). The volume moment value (D4,3) indicates the size of the coarse particulates that make up the bulk of the isolated myofibrils; this value can provide the degree of myofibrillar aggregation and dissolution. **Figure 6.2** shows the distribution of the myofibrillar protein particle size. The graph shows that goat meat myofibrillar proteins behave differently compared to lamb samples. The goat meat SV and

MW samples had very close distribution, indicating that the aggregation of the myofibrillar protein did not differ significantly. On the other hand, the SV and MW lamb samples show a clear difference in myofibrillar aggregation.

Table 6. 1 Myofibrillar protein particle size (D4,3 and D3,2), specific surface area, and span of raw, microwave (MW), and sous vide (SV) processed lamb and goat meat.

Parameters	Raw	SV	MW	[1]*p*-Value SV × MW
Goat meat				
$D_{4,3}$ (μm)	39.03 ± 3.67[a]	61.35 ± 5.11[b]	67.83 ± 4.75[b]	NS
$D_{3,2}$ (μm)	9.66 ± 0.13[a]	21.92 ± 1.11[b]	24.71 ± 1.97[b]	NS
Specific surface area (m^2/g)	0.62 ± 0.01[a]	0.28 ± 0.01[b]	0.26 ± 0.02[b]	NS
Span	6.72 ± 0.36[a]	3.33 ± 0.39[b]	3.59 ± 0.10[b]	NS
Lamb				
$D_{4,3}$ (μm)	42.73 ± 2.77[a]	110.84 ± 5.30[c]	74.07 ± 4.70[b]	<0.001
$D_{3,2}$ (μm)	9.99 ± 0.45[a]	28.60 ± 0.97[c]	22.32 ± 1.45[b]	0.002
Specific surface area (m^2/g)	0.61 ± 0.03[a]	0.21 ± 0.00[b]	0.28 ± 0.02[b]	0.007
Span	6.82 ± 0.30[a]	4.09 ± 0.08[b]	4.68 ± 0.16[b]	0.005

All values are reported as the mean ± SE, where $N=3$ (3 replicates with 3 measurements from each replicate). Values within a row not having common superscripts differ significantly ($p < 0.05$).
[1]NS = $p > 0.05$.

MW and SV treatment for both meat samples promoted myofibrillar aggregations based on their significantly higher ($p < 0.05$) $D_{4,3}$ values than the raw meat samples (**Table 6.1**). Additionally, $D_{3,2}$ was higher ($p < 0.05$) for MW and SV samples compared to raw, signifying a larger size for the fine particulates for MW and SV samples. It was evident that the temperatures during SV and MW processing were enough to induce significant aggregate formation as the intra or intermolecular cross-links between unfolded protein molecules formed, starting at 30 °C (Tornberg, 2005).

Values show that the aggregation mechanisms between the two processing conditions are different. The SV process induced greater aggregation based on higher $D_{4,3}$ values and

lower $D_{3,2}$ values than MW. Although high temperature has been reported to cause greater unfolding of myosin and rapid formation of larger aggregates (Cao et al., 2019), the larger aggregates in SV treated samples can be linked to the effect of heating time. Prolonged heating increases protein aggregation since protein aggregates cluster over time (Cao et al., 2019). This observation was also reported by Mitra et al. (2017), where a high-temperature short-time process had lower $D_{4,3}$, and protein aggregation required a longer cooking time for larger aggregates to form. On the other hand, for the goat meat sample, MW and SV showed no significant differences ($p > 0.05$) for $D_{4,3}$ (61 - 68 μm) and $D_{3,2}$ (21-25 μm); SV and MW led to the same degree of aggregation. The characteristics of protein aggregation after processing could also depend on the sample type. Other parameters, such as specific surface area and span (width of distribution) of the particulates, were the same for both treatments for both lamb and goat meat samples. The significantly higher ($p < 0.05$) span values for raw meat indicate a more uniform distribution of myofibrils compared to lower span values for SV and MW samples.

6.3.3 FTIR spectroscopy

FTIR spectra can show qualitative information about protein conformational changes after processing. The FTIR spectra for raw, SV, and MW samples are shown in **Figure 6.3**, highlighting the major amide bands (amide I, II, III, A, and B); these are characteristic bands present for protein samples. The figure shows that the spectra for the SV and MW goat meat seemed superimposed compared to those of lamb. The details of the location and assignment of each peak are in **Table 6.2**

Compared to SV and MW, the absorption intensity of overall FTIR spectra of raw samples was higher for both lamb and goat meat. However, after processing, the SV and MW caused a significant shift in peak location for most amide bands. Interestingly, the effect of SV and MW processes on peak location was observed in the lamb sample only; amide A and B for SV lamb had low wavenumbers. The lower frequency for the amide A and B region for the lamb can be explained by the significant differences in protein aggregation and hydrophobicity between SV and MW lamb, which is related to hydrogen bond formation.

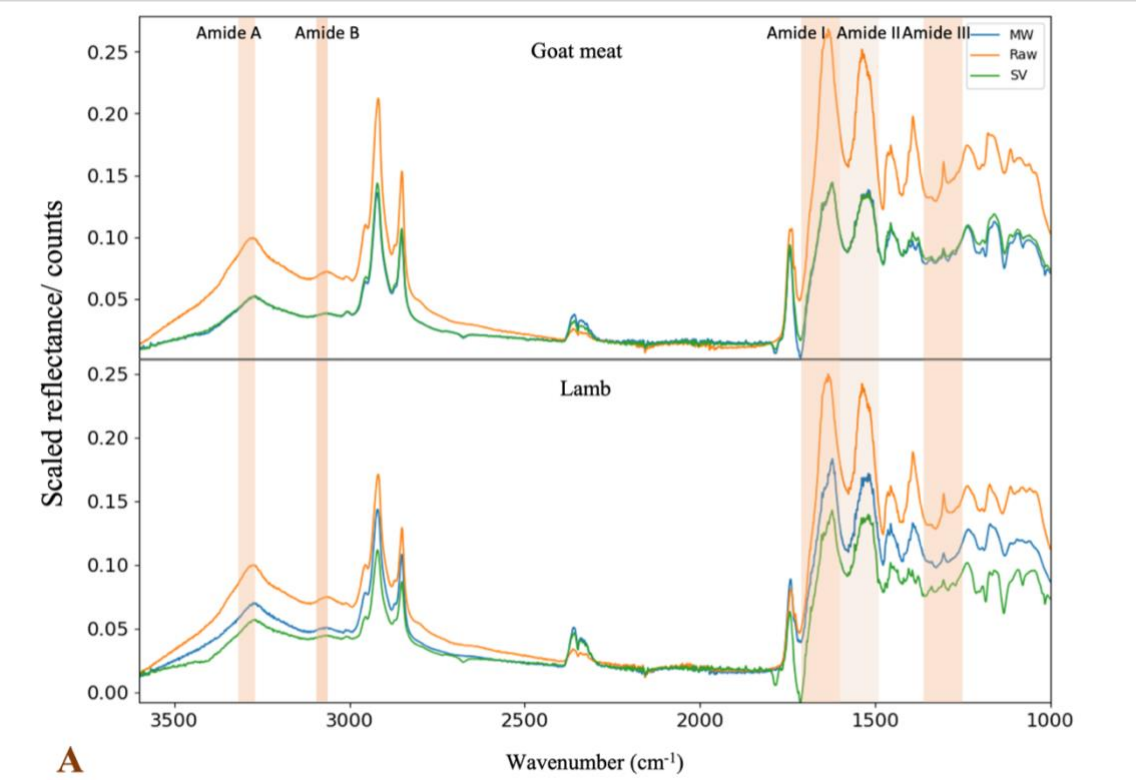

Figure 6. 3 FTIR spectra of raw, sous vide (SV), and microwave (MW) goat meat and lamb biceps femoris.

Referenced location of secondary structures: Aggregated strands (1610-1620 cm^{-1}), β-sheet (1620-1640 cm^{-1}), Random coil (1643-1650 cm^{-1}), α-helix (1650-1660 cm^{-1}) and β-turn (1660-1695 cm^{-1}) (Barth, 2007; Ngarize et al., 2004).

The peak location for the amide I (the most intense absorption band in proteins) shifted from 1634-1635 cm^{-1} to 1622-1623 cm^{-1} after SV and MW processing. The lower

wavenumber for the processed samples is a characteristic peak of a denatured protein after processing (Ngarize et al., 2004). The exact position of the amide I band results from the backbone conformation and hydrogen bonding characteristics of proteins, which is primarily exhibited by the stretching vibrations of C=O (70-85%) and C-N (10-20%) (Barth, 2007). Amide I is sensitive to the strength and the number of hydrogen bonds, which downshifts the natural frequency of the amide (Lorenz-Fonfria, 2020). The effect of hydrogen bonding can also be clearly observed for amide II vibration, which is very sensitive to hydrogen bonding. SV and MW resulted in the same frequency downshift in the amide I and II regions for lamb and goat meat.

Table 6. 2 Location and assignment of amide peaks in FTIR spectra.

Region	Peak wavenumber (cm^{-1})						Band Assignment
	Goat meat			Lamb			
	Raw	SV	MW	Raw	SV	MW	
Amide A	3279 ± 0^{b}	3272 ± 0^{a}	3271 ± 0^{a}	3277 ± 0^{c}	3272 ± 0^{a}	3273 ± 1^{b}	3310 -3270 cm^{-1} N– H and O– H stretching vibration
Amide B	3065 ± 0^{a}	3068 ± 1^{b}	3069 ± 0^{b}	3065 ± 0^{a}	3066 ± 1^{a}	3071 ± 0^{b}	3060-3090 cm^{-1} overtone of NH-bending
Amide I	1635 ± 0^{b}	1622 ± 0^{a}	1622 ± 0^{a}	1634 ± 0^{b}	1623 ± 0^{a}	1623 ± 0^{a}	1600–1700 cm^{-1} C=O stretching vibration and minor C– N stretching
Amide II	1538 ± 0^{c}	1522 ± 2^{b}	1520 ± 0^{a}	1538 ± 0^{b}	1523 ± 0^{a}	1522 ± 1^{a}	1500–1600 cm^{-1} C– N stretching and N– H bending
Amide III	1305 ± 0^{a}	1305 ± 0^{a}	1305 ± 0^{a}	1305 ± 0^{a}	1305 ± 0^{a}	1305 ± 0^{a}	1250-1350 in-phase combination of NH bending and CN stretching vibration

The location of the identified peaks is from the reported peaks in the literature (Barth, 2007; Ngarize et al., 2004).
All values are reported as the mean ± SEM, where $N=3$ (3 replicates with 3 measurements from each replicate).
Values within a row not having common superscripts differ significantly ($p < 0.05$).
MW-microwave process and SV-Sous vide process.

6.3.4 Secondary structure of proteins in amide I region

The amide I region (1600-1700 cm^{-1}) was further examined (**Figure 6.4**), its deconvolution provides secondary structure data that describes how protein molecules are coiled and folded in a specific direction relative to the secondary structure of the backbone (Barth, 2007).

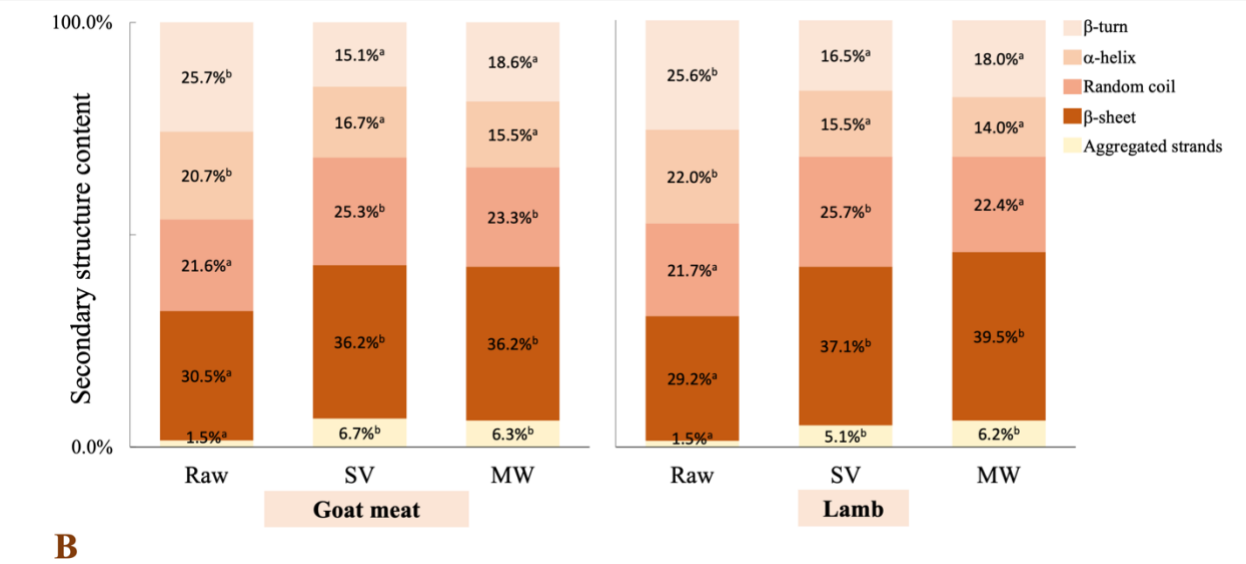

Figure 6. 4 Secondary structure content (B) of raw, sous vide (SV), and microwave (MW) goat meat and lamb biceps femoris.

Referenced location of secondary structures: Aggregated strands (1610-1620 cm^{-1}), β-sheet (1620-1640 cm^{-1}), Random coil (1643-1650 cm^{-1}), α-helix (1650-1660 cm^{-1}) and β-turn (1660-1695 cm^{-1}) (Barth, 2007; Ngarize et al., 2004). All values are reported as the mean ± SEM, where $N=3$ (3 replicates with 3 measurements).
Values within a row not having common superscripts differ significantly ($p < 0.05$).

Surprisingly, although SV and MW processing differ in their heating mechanisms, both processes showed the same trend in the increase or decrease of the identified secondary structures. After SV and MW processing, the secondary structure of lamb and goat meat samples showed a marked increase in β-sheet, random coil, and aggregated strand structure, with a significant reduction in α-helix and β-turns, a common observation for heat-induced protein denaturation in meat (Beattie et al., 2008; Berhe et al., 2014; Ngarize et al., 2004). The decrease in α-helix after MW and SV processing indicates a shift from the tighter and very

compact α-helix structure to more spread out β-sheet and random coil. MW increases non-organised structure and β-sheet that agrees with the reported trend in the literature (Cai et al., 2018; Calabrò & Magazù, 2014). The increase in the bands of aggregated proteins (1610-1620 cm^{-1}) and random coils (1643-1650 cm^{-1}) after processing occurs because many proteins preferentially adopt a more random coil conformation, a state with a maximum degree of disorder, in the presence of denaturants or upon heating. The increased β-sheet formation can be associated with a more stabilised secondary structure driven by the increase in protein surface hydrophobicity after the collapse of the α-helix structure.

There were no significant differences ($p < 0.05$) between the secondary structure contents of SV and MW goat meat samples,. This observation may be related to the same tenderness values after the treatments. Some researchers have reported a correlation between protein structure and tenderness, specifically for α-helix and β-sheet (Beattie et al., 2008; Beattie et al., 2004; Liu et al., 2019). On the other hand, the secondary structure contents of SV and MW treated lamb resulted in a significantly higher ($p < 0.05$) amount of random coil after the SV process. The trend toward the more significant formation of random coils in SV-treated lamb can be directly related to the myofibrillar surface hydrophobicity. Hydrophobic interactions play a dominant role in stabilising β-sheet conformations (Narayanan & Dias, 2013), and between SV and MW, MW induced significantly higher surface hydrophobicity as discussed (Section 6.3.1), driving more β -sheet structure for the MW sample.

The significant difference in secondary structure changes was only observed for the lamb sample. Although both lamb and goat meat samples are mainly composed of myofibrillar and sarcoplasmic protein, the differences in protein composition and the initial states of the proteins (e.g., more hydrolysed proteins) will have contributed to the resulting different levels

of secondary structure formation when exposed to different thermal processes. The lamb meat proteins, aged longer, have undergone more proteolysis from the endogenous enzyme. Hence, its protein structure is more susceptible to denaturation and subsequent structural changes, supported by its myofibrillar hydrophobicity and aggregation behaviour.

6.3.5 Protein *in vitro* digestibility

6.3.5.1 Protein profile of meat digests

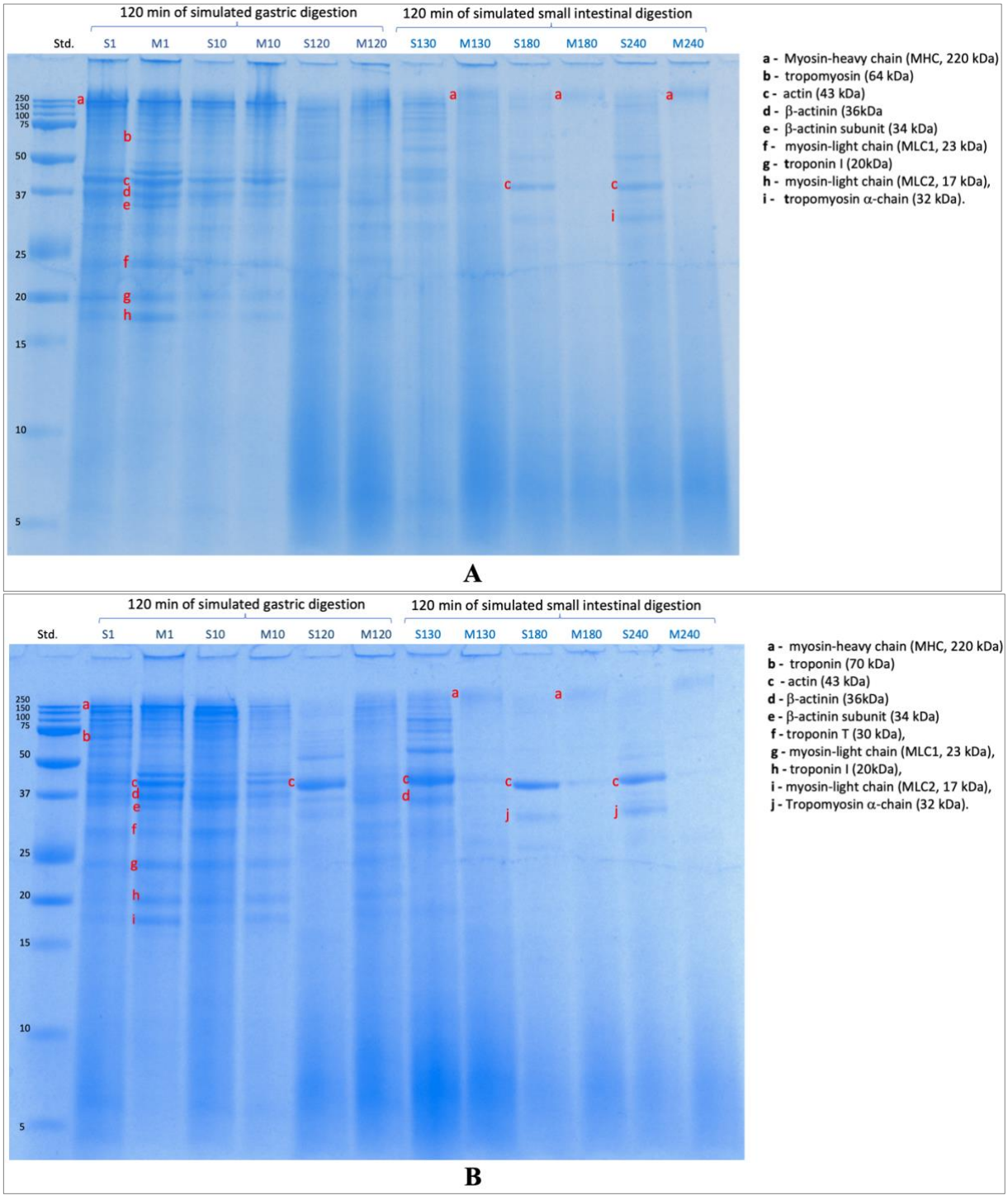

Figure 6. 5 Tricine SDS-PAGE electrophoretograms of sous vide and microwave goat meat (A) and lamb (B) meat digests after 1, 10, and 120 min of simulated gastric digestion and 130, 180, and 240 min of simulated gastro-small intestinal digestion.

S-sous vide and M- microwave for each well in the gel label.

The SV and MW process led to a similar pattern of protein breakdown during digestion for both species based on the qualitative data using SDS-PAGE (**Figure 6.5**). After 1 min gastric digestion, MW meat appeared to have a higher degree of protein breakdown; low molecular weight protein (20-23 kDa) already appeared in high intensity compared to the SV sample. These low molecular weight proteins include myosin light chains (20-23 kDa) that are known as the enzymatic digestion products of heavy myosin (220 kDa) (Chian et al., 2019). Additionally, the significant components of the Z-disk were efficiently hydrolysed based on the disappearance of β-actinin (130 kDa) and α-actinin (95 kDa).

As the digestion progressed, bands for actin (43 kDa) and tropomyosin α-chain (32 kDa) were still intense for SV, even at the end of the small intestinal digestion phase. These bands indicate that these proteins are still in high amounts in the SV meat digest compared to the MW meat sample. On the other hand, the actin band (43 kDa) in MW meat samples was already very faint at the end of digestion. Since actin is an abundant muscle protein that acts as a structural element of the sarcomere (Obinata et al., 1981), the intense disruption of the Z-disk shown in TEM images of MW can explain its efficient breakdown. Higher Z-disk disruption allowed pepsin to easily attack the proteins because of their accessibility (Astruc, 2014b).

The high temperature MW heating probably resulted in a heterogenous aggregate formation, some of which might be more resistant to digestion, as seen in our gels. It has been reported that MW induced the formation of irregular aggregate (A. Promeyrat et al., 2010) affecting the digestibility of cooked meat. An interesting observation for MW digest is the detectable bands for myosin-heavy chain (MHC, 220 kDa) even at the end of small-intestinal

digestion, consistent for both meat samples. This high molecular weight protein possibly resisted digestion even after the small intestinal phase.

The protein digestion pattern for SV-cooked sample showed slow and progressive hydrolysis of peptides as the digestion progresses. SV processing might have led to more extensive but more uniform aggregated myofibrillar proteins that require a longer time to be fully digested. As explained by Bax et al. (2012), more compact protein aggregates have fewer cleavage sites, thus reducing the rate of proteolysis. Only small peptides were visible at the end of the digestion of SV treated samples.

6.3.5.2 Degree of protein hydrolysis

The results in **Table 6.3** show the degree of protein digestibility measured by free amino N release for both lamb and goat meat. In the gastric phase, free amino nitrogen values were not significantly different at $p = 0.05$ for each time point. As the digestion progressed to the small intestinal phase, the values started to reach the same level, and the efficient hydrolysis by pancreatic enzymes was apparent. The free amino N significantly increased after the addition of pancreatin, attributable to pancreatic enzymes being more efficient in hydrolysing proteins and possibly better solubility of proteins at a neutral pH.

Table 6. 3 Percent in vitro protein digestibility of sous vide (SV) and microwave (MW) samples after 0, 10, and 120 min of simulated gastric digestion and 10, 60, and 120 min of simulated small intestinal digestion.

	Goat meat		Lamb	
Digestion Time (min)	SV	MW	SV	MW
0	1.52 ± 0.00^{a1}	1.56 ± 0.00 ^{a1}	2.63 ± 0.00 ^{a1}	1.80 ± 0.01 ^{a1}
1	3.09 ± 0.17^{a1}	2.35 ± 0.21^{a1}	3.80 ± 0.13^{a1}	2.56 ± 0.10^{a1}
10	3.19 ± 0.09^{a1}	3.47 ± 0.83^{ab1}	5.19 ± 0.73^{a1}	3.77 ± 0.54^{a1}

gastric digestion	120	3.90 ± 0.38^{a1}	5.08 ± 0.42^{b1}	6.75 ± 0.27^{ab1}	4.44 ± 0.77^{a1}
small-intestinal digestion	130	8.82 ± 0.44^{b1}	9.12 ± 0.65^{c1}	9.83 ± 0.86^{bc1}	9.15 ± 1.07^{b1}
	180	9.87 ± 0.49^{b1}	10.87 ± 0.12^{c1}	10.88 ± 1.09^{bc1}	10.93 ± 1.05^{b1}
	240	10.33 ± 1.77^{b1}	10.93 ± 0.22^{c1}	13.61 ± 1.65^{c1}	12.48 ± 1.28^{b1}

All values are reported as the mean ± SE, where $N=3$ (3 replicates with 3 measurements from each replicate).
[a-c] Values within a column not having common alpha superscripts differ significantly within species ($p < 0.05$).
[1]Values within a row having common superscripts do not differ significantly within species ($p < 0.05$).

At the end of intestinal digestion, our data shows that the varying conditions between SV and MW processing resulted in a non-significant difference ($p < 0.05$) in the overall goat meat and lamb protein digestibility, even though SV and MW resulted in a different pattern of how the meat proteins were hydrolysed (section 3.6.1). This result contrasts with the common reports in the literature that meat protein digestibility depends on both temperature and time of cooking (M. L. Bax, T. Sayd, et al., 2013; Farouk et al., 2019).

A univariate analysis was carried out to determine the effect of all the determined parameters, and this was done for each meat source (**Supplementary Table 6.1**). All the aggregation, hydrophobicity and secondary structure values were fitted as covariates. Although the generated model did not achieve a very high R^2 value, the data were presented to understand the contribution of the parameters in the obtained digestibility values. For the gastric phase, the myofibrillar hydrophobicity negatively correlates to the digestibility value of goat meat ($p = 0.024$). However, no factor can be identified for the lamb sample to affect the digestibility value. For the intestinal phase, only the myofibrillar aggregation which is positively correlated ($p < 0.001$) to the overall digestibility values, affects the level of intestinal digestion, but for goat meat only. On the other hand, no significant predictor can be identified for the lamb sample.

The protein hydrophobicity and aggregation affect the digestibility, but its effect is probably limited only to the digestion rate related to the efficiency of enzymes that can attack cleavage sites through time but not on the overall digestibility of protein. SV resulted in a larger myofibrillar aggregates, but the aggregates formed may have a less compact structure that was reported for low temperature processing (A. Promeyrat et al., 2010). These large aggregates have more random coils or unordered structures that the digestive enzymes can easily attack. On the other hand, MW resulted in smaller aggregates than the SV, but these aggregates are formed by a more stable β-sheet) and compact structure. The smaller aggregates with more surface area exposed were attacked quickly by the digestive enzymes. The effect of secondary structure on meat protein digestibility was not seen and was not a significant predictor for the gastric and intestinal digestibility values. However, others have reported that the nutritive value of a protein is also influenced by the secondary structure (Calabrò & Magazù, 2014; Yu, 2005). In this study, there is only a minimal difference in secondary structure between the SV and MW samples (only random coil content for the lamb sample).

While it is likely that the *in vitro* digestibility of MW and SV-cooked lamb and goat meat may have been affected by the differences in the time and temperature of processing or the mechanism of heating, we could not provide any evidence of differences in the measures of total digestibility *in vitro* in terms of the release of free amino nitrogen. The SV and MW processes resulted in proteins with varying structural features that affect how digestive enzymes can attack the cleavage sites but can still result in the same digestibility in terms of the release of free amino nitrogen. In this case, the mild SV cooking appears not superior to MW that uses high temperature processing. The advantage of MW processing could be its

ability to cleave thiol bonds (Ferreira et al., 2018) that could facilitate the enzyme action. However, it should be noted that the protein profile of the digest showed a noticeable difference. Moreover, the use of free amino N as the basis for protein digestibility has limitations, such that it only estimates the level of free amino N but not how polypeptides are degraded to their constituent amino acids. This is a limitation of this study that needs to be clarified using a more extensive method, such as peptidomics.

Meat digestibility also depends on the types of meat due to the varying composition, as was demonstrated in our previous experiment (**Chapter 4**). In this chapter, goat and lamb meat samples resulted in varying levels of free amino N at the end of the digestion. The SV and MW processed goat meat had digestibility values of 10.3-10.9 %, while lamb had 12.5-13.6 %. This difference is probably due to the varying postmortem conditions of the raw materials before processing. Lamb was aged longer than goat meat, and this could have resulted in greater proteolysis due to endogenous enzyme activity. Consequently, this condition led to a more tender lamb than goat meat. The lesser muscle structure integrity facilitates digestive enzyme bioaccessibility, resulting in higher digestibility values for lamb (Astruc, 2014b). Other studies also have reported protein digestibility values for processed meat in terms of ninhydrin free amino nitrogen released. For example, 9.8 % and 12.6 % for SV and pulsed electric field treated SV beef (Feng Ming Chian et al., 2021), around 10.5-14 % for beef cooked at 100 °C (Kaur et al., 2014) and 17-20 % for beef under high pressure processing (HPP) (Kaur et al., 2016). In comparison to these digestibility values for processed beef, what we got for SV and MW goat meat was closer to most of the values that uses heat or involves cooking such as SV or high temperature cooking. Although it should be noted that all the processes are not directly comparable since the process differs in terms of the level of

heat or length of cooking. Furthermore, the post-mortem conditions of beef and goat meat were different.

6.4 Conclusions

The MW and SV can change the protein structure of meat differently, and its effect varies with meat types. Generally, SV induced higher myofibrillar protein aggregation, while MW induced higher hydrophobicity than SV. Additionally, more random coils were formed for SV than MW. In terms of *in vitro* protein digestibility, even though MW is an intense process (high-temperature process), it does not result in lower protein digestibility of meat compared to the milder effects of SV cooking at 60 °C in terms of the free amino nitrogen release. Two extremely different techniques for cooking meat can result in the same digestibility values based on protein hydrolysis. Moreover, goat meat had lower digestibility values than lamb meat, considering the conditions of the meat used in this study.

6.5 Acknowledgement

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their butchering facility; and Mr. Simon Wishnowsky of Venison Packers Feilding Ltd. for the use of their slaughtering facility.

6.6 Supplementary data

Supplementary Table 6.1 The contribution of various protein molecular structures as predictors for the release of free amino nitrogen at the end of gastric and intestinal *in vitro* protein digestion.

Parameters	End of gastric		End of intestinal	
	Goat meat R^2=0.57	Lamb R^2=0.69	Goat meat (R^2=0.80)	Lamb (R^2=0.53)
Covariates				
Myofibrillar aggregation	0.116	0.283	**<0.001**	0.420
Myofibrillar hydrophobicity	**0.024**	0.126	0.168	0.520
α-helix	0.643	0.844	0.373	0.337
β-sheet	0.394	0.818	0.222	0.329
Random coil	0.301	0.893	0.201	0.344
Aggregated strands	0.386	0.850	0.234	0.322
β-turn	0.391	0.868	0.222	0.321

Values greater than **0.05** means the parameter is not a significant predictor.
Fitted values are the covariates only to determine the individual contribution of the studied parameters on the level of free amino nitrogen released.

6.7 Copyright information

This part has been published:

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Chapter 7

Identification and quantification of peptides released during in vitro digestion of microwaved and sous vide goat meat

7.1 Introduction

In *in vitro* protein digestion studies, the effects of processing on meat protein digestibility can be demonstrated by evaluating the degree of protein hydrolysis during *in vitro* digestion (Bhat et al., 2020; Farouk et al., 2019; Kaur et al., 2023a). These studies have investigated the processing effects on protein by evaluating the release of free amino acids and the protein profile on polyacrylamide gel electropherograms (PAGE). Both methods can directly give an overview of the protein hydrolysis at a particular point in time. In our previous experiment (**Chapter 6**), the effect of processing on the digestibility of meat proteins was evaluated by monitoring protein hydrolysis based on the level of free amino nitrogen. Our result (**Chapter 6**) has shown that sous vide (SV) and microwave (MW) processed goat meat samples had the same level of digestibility regarding free-amino nitrogen release. Examining SDS-PAGE gels, the protein profile of the digest can describe and indicate how sizeable molecular weight proteins are degraded during digestion by measuring the band intensity on SDS gels. Based on the SDS-PAGE gels, the protein profile of the meat digest between processes showed that major proteins were broken down differently between processes. Some bands were clearly still visible for SV processed meat after a complete gastrointestinal digestion phase compared to the MW sample. We further sought to investigate the fate of goat meat protein during digestion between the two processes using a peptidomics approach.

Proteomics, specifically peptidomics technique based on mass spectrometry (MS), has been extensively used in protein digestion studies (Dupont, 2017; Liu et al., 2021; Martini et al., 2019). Currently, for dietary protein digestion, peptidomics is employed to monitor the proteolysis of major proteins from food in the gastrointestinal tract during digestion (He et al., 2018; L. Li et al., 2017). Its efficiency is best suited for tracking the evolution of digestive

processes in both in vivo and *in vitro* systems (Bauchart et al., 2007; Nyemb et al., 2016). With peptidomics, it is possible to characterise peptides and to track if they are resistant to hydrolysis (Dupont, 2017). Furthermore, peptidomics can also be used to identify oxidation of specific amino acid residues in hydrolysed peptides (Mitra, Lametsch, Akcan, et al., 2018).

The peptidomics evaluation of digests from thermally treated meat shows that varying temperatures can affect the hydrolysis of major muscle proteins (He et al., 2018; L. Li et al., 2017; Sayd et al., 2016). The peptide profiling of meat *in vitro* digests from beef (Yin, Pereira, et al., 2020; Zhao et al., 2020), pork (L. Li et al., 2017; Zou et al., 2018), chicken and fish (Martini et al., 2019; Wen, Zhou, Song, et al., 2015) have been investigated. To the best of our knowledge, the characterisation of peptides released from goat meat digestion has not been investigated. Furthermore, a comparative study about the pattern of specific muscle protein hydrolysis as affected by SV, or MW, needs to be done to understand the impact of processing on meat digestibility and better understand the mechanisms of food disintegration in the gastrointestinal tract. This information can help us strengthen our understanding of the effect of food on human health.

The main objective of this chapter is to determine the digestion pattern of the significant muscle proteins and to characterise the peptides released from SV and MW goat meat *biceps femoris* muscle during simulated *in vitro* digestion. With the vast number of proteins that can be identified using LC-MS, our *in vitro* digestion study only focused on the dominant structural proteins, the major proteins in meat.

7.2 Materials and methods

7.2.1 Sample preparation for LC-MS analysis

7.2.1.1 Gel electrophoresis

The digests from the MW and SV processed meat (**Chapter 6**) were thawed and centrifuged at 13000 × *g* for 2 min, and the supernatants were diluted with Milli-Q water, adjusting the volume to 1 mg/ml protein concentration. Protein concentration was determined using Kjeldahl method **(Chapter 3.5.1)**. The diluted digests were mixed in a 1:1 ratio with premixed tricine sample buffer (1610739, Bio-Rad Laboratories, USA) with 2 % β-mercaptoethanol (M6250, Sigma-Aldrich, USA) and then heated at 50 °C for 15 min in a shaking incubator. The electrophoresis was run using 4-15% MP TGX Gel (Bio-Rad Laboratories Pty Ltd., New Zealand) at 120 V for 1.5 h. The gel was fixed in a fixing solution (40% v/v ethanol and 10% v/v acetic acid), stained using a colloidal Coomassie blue stain (0.1 % Coomassie Brilliant Blue G-250; 2% w/v ortho-phosphoric acid and 10% ammonium sulphate) and destained using 1% aqueous acetic acid solution.

7.2.1.2 Destaining

The colloidal stained gel was prepared where each lane contains one time point. The separated proteins in each lane were divided into five sections to have manageable amounts of gel pieces for the succeeding steps. Each section was cut into small pieces (approximately 1 × 1 mm^2). Then, the gel pieces were destained with 1 Part 100 mM ammonium bicarbonate (ABC) and 50% methanol and dehydrated using 80% acetonitrile (ACN).

7.2.1.3 Reduction, alkylation and digestion

The proteins were then reduced, alkylated, and digested. Protein reduction was carried out using dithiothreitol (DTT), and alkylation was performed using 20 mM iodoacetamide in

an aqueous solution. Subsequent dehydration was done using 80% ACN. Afterwards, tryptic using trypsin (20 ng/ µL in 50 mM ABC) digestion was carried out overnight at 37 °C. ABC was added to make sure gels won't dry up.

7.2.1.4 Extraction

After digestion, the peptides were extracted from gel pieces using 50% ACN/5% formic acid (FA). Moreover, subsequent extraction using 0.1% FA/80 % ACN until the gel becomes opaque, for a complete extraction. The pooled volume was reduced using Speedvac, and the peptide extract was stored in a -80 °C freezer for further analysis.

7.2.2 Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis

7.2.2.1 LC-MS/MS analysis

Four microliters of each sample (comprising 3 biological replicates) were injected into a 1.0mm × 5 mm PepMap 100 C18 trap column with a particle size of 5µm, operating at a flow rate of 15 µl/min. Subsequently, they were transferred to a 75 µm × 50 cm PepMap C18 column with a particle size of 3µm, flowing at a rate of 300nl/min, utilizing a Dionex UltimateTM 3000 RSL nano system (Thermo Fisher Scientific, Massachusetts, USA). The mobile phase consisted of 3% acetonitrile and 0.1% formic acid in MS-grade H2O. Peptides were eluted using a linear gradient ranging from 3% to 30% acetonitrile, with 0.1% formic acid over a span of 55 minutes. The eluted peptides were analysed employing a Q Exactive Plus mass spectrometer with a Nano Flex ionization source, operated using Xcalibur acquisition software (Thermo Fisher Scientific, Massachusetts, USA). The mass spectrometer was externally calibrated and operated in data-dependent mode. Full MS1 scans were conducted across a mass range of 370-1,600 m/z with a resolution setting of 70,000, while fragment ion spectra

were acquired at a resolution of 17,500. For data-dependent acquisition of HCD spectra, the top ten most intense ions were chosen for fragmentation in each scan cycle, and full MS and fragment ion spectra were detected using the Orbitrap mass analyser. Exclusion conditions were optimized based on observed peak width, around 10 secs.

7.2.2.2 Protein identification and peptide characterisation

The raw data generated from LC-MS/MS analysis was processed using Proteome Discoverer version 2.4 SP1 (Thermo Fisher Scientific; Massachusetts, USA). The following search parameters were used for non-specific protein identification: peptide mass tolerance 10 ppm, MS/MS mass tolerance 0.02 Da, up to two missed cleavages allowed, and minimum peptide length, and six amino acids. Carbamidomethylation of cysteine was set as a fixed modification, and oxidation of methionine, lysine and proline, acetylation of the N-terminal residue, and galactosyl, glucosyl modifications of lysine were set as variable modifications. The false discovery rate (FDR) was set at 1%. Data were searched NCBI database (taxonomy: *Capra hircus*, released 22-06-2023). In addition, a separate database search was done to the determine the oxidation of methionine, phenylalanine, tryptophan, and tyrosine, as these amino acids are good oxidation indicator for the major muscle proteins.

In screening the identified proteins, the minimal number of unique peptides for each protein was set to two, peptide spectral matches (PSM) were at least two, and protein FRD confidence was set to high. A non-targeted approach was applied to identify peptides. In peptide analysis, peptide IDs were accepted if the peptides were detected in at least two experimental replicates. The data originating from pepsin and trypsin were removed.

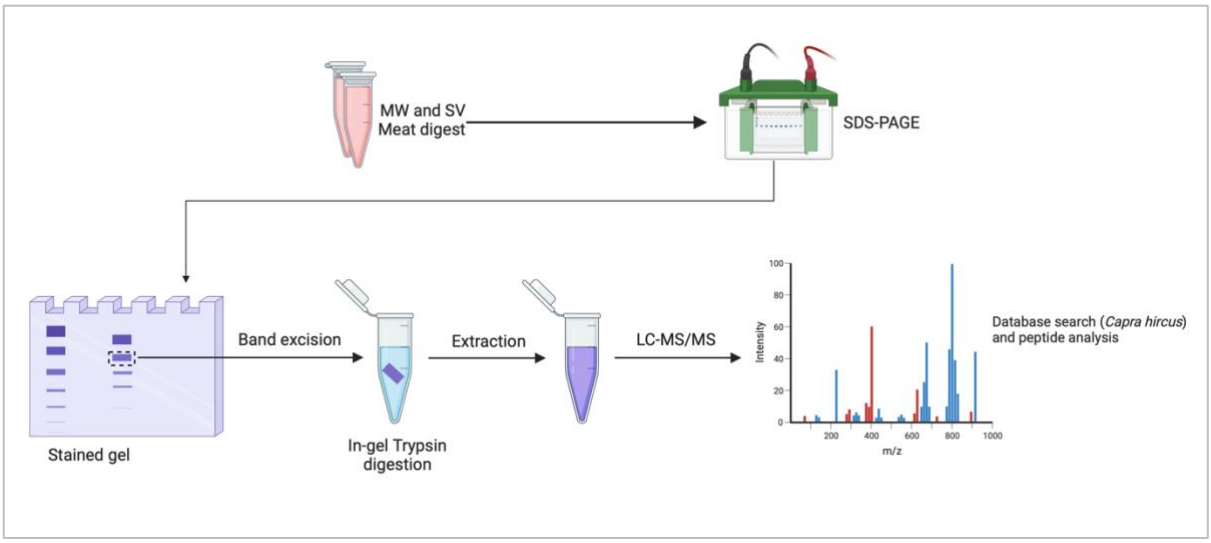

Figure 7. 1 The workflow of the LC-MS analysis.
Biorender.com

7.2.3 Statistical analysis and graphical representation

7.2.3.1 Boxplot of peptide size distribution.

Peptide size distribution was visualised with a boxplot generated by R programming (version 3.5.3) using the ggplot2 package (Wickham, 2016).

7.2.3.2 Heatmap of protein abundance and peptide patterns

The protein abundance patterns for each sample were generated by the identified protein abundance and abundance count ratio and normalised using min-max normalisation. The data were then log-transformed (base 10) and scaled using mean centering to correct for heteroscedasticity, reduce skewness, and reduce mask effects. The Hierarchical cluster analysis (HCA) was clustered by Euclidean distance and Ward's minimum variance method. The PCA and the heatmap analysis were produced using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/MetaboAnalyst/). The chosen colour code ranged from red,

representing the maximal abundance within the corresponding protein and digestion phase, to blue, representing medium to low.

7.2.3.3 Venn diagram of resistant sequences.

The identical peptides between processes were represented with Venn diagrams using the http://bioinformatics.psb.ugent.be/webtools/Venn/. The duplicated peptides were removed for the total count.

7.2.3.4 Replication of experiments and statistical analysis

The digests were taken from three independent *in vitro* digestion experiments. The Statistical analysis between the processes was performed using SPSS 18.0 (SPSS Inc., Chicago, IL). Significant differences were considered between groups when $p < 0.05$.

7.3 Results and discussion

7.3.1 Protein composition of gastric and small-intestinal compartments

Peptidomics revealed the specific protein profile of the digest. On average, the number of proteins identified after gastric digestion was 195 for SV and 301 for MW. After the whole gastro-small intestinal phase, the number of proteins identified was down to 187 for SV and 197 for MW. The values show that between processes, more muscle proteins were identified in MW than in SV. The number of proteins that can be identified was expected to decrease due to the reduced number of peptides that can be unambiguously identified by mass spectrometric analysis (Dupont, 2017). Furthermore, further hydrolysis at the end of digestion would result in shorter peptides that can lead to a lower peptide-spectrum match.

Muscle proteins can be classified by their functions or location in the cell. In the present study, most proteins can be classified as myofibrillar and sarcoplasmic. Most of the myofibrillar proteins are structural proteins, which play a significant role in physicochemical properties in meat. For our data, the huge number of proteins was narrowed down to the top 30 most abundant proteins. The details of the top 30 most abundant proteins in the sample, which show the relative abundance and sequence coverage, are shown in **Supplementary Table 7.1**.

Figure 7.2 shows the hierarchical cluster analysis (HCA) for the abundance of the top 30 most abundant proteins in the goat meat digest. These proteins composed approximately 50 % of the muscle proteins identified in the samples. The abundance of proteins generally decreased as the digestion progressed from 1 min gastric digestion (G1) to the end of gastrointestinal digestion (I120). However, it is notable that there is a distinct pattern of protein abundance between processes. The abundance of the proteins in the microwave digest

is still high compared to SV. Cluster analysis shows that there are two major clusters of proteins. For MW, the cluster from fructose bisphosphate aldolase down to Troponin C (**Figure 7.2**) were proteins highly abundant in G1 and were found in fewer amounts after gastric and intestinal digestion. In contrast, the other clusters SI (Myosin-2 down to Troponin I) were still abundant even at the end of SI. In contrast to MW, overall, the SV-treated digest was detected in lower abundance than the MW sample, and in fact, all of the proteins were found in very small amounts at the end of I120.

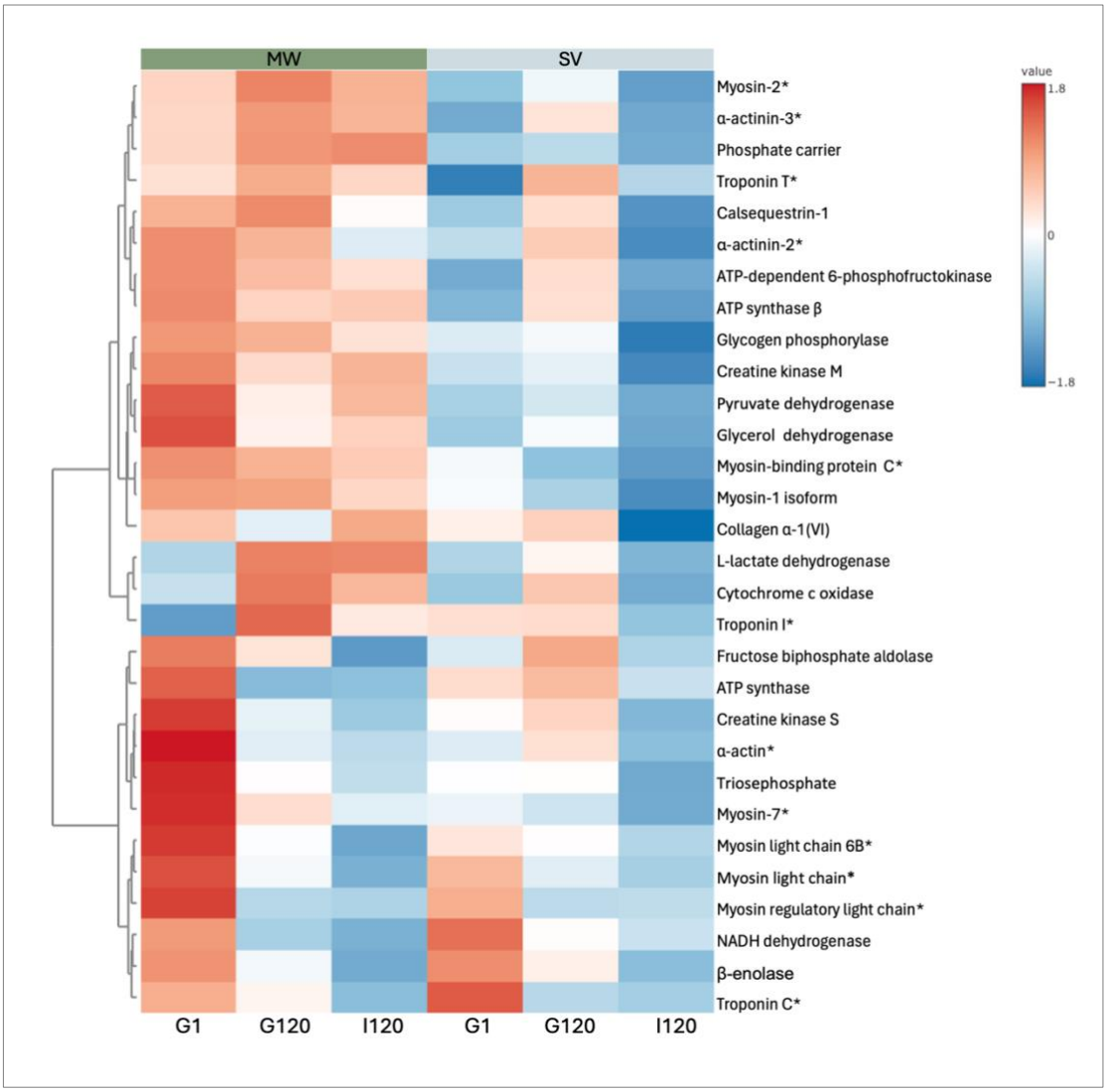

Figure 7. 2 Hierarchical cluster analysis (HCA) of the top 30 most abundant proteins in goat meat digest after 1 min (G1), 120 min (G12) gastric digestion and 120 min intestinal digestion (I120).

The heatmap colours reflect the abundance of goat meat proteins. The protein IDs followed with * represents the structural proteins.

The difference between the abundance of proteins between processes, at G1 specifically, could possibly be due to the breakdown of the major muscle proteins by endogenous enzymes (cathepsins) during SV processing that facilitated peptide hydrolysis at the early stage of gastric digestion. Although cathepsin activity was not measured in our experiment, cathepsins B and L were reported to be stable at 60 °C (Christensen et al., 2013; Kaur et al., 2020). The SV process that lasted for 9 hrs would have allowed the endogenous enzymes to hydrolyse peptide bonds and made them more susceptible to pepsin digestion.

For the specific muscle proteins identified, our results are in agreement with what has been reported that myofibrillar proteins, particularly actin and myosin, were the primary sources of peptides in all the *in vitro* digested pork, beef, chicken and turkey meat (Martini et al., 2019) and pork (Jiang et al., 2021). Furthermore, sarcoplasmic proteins such as creatine kinase and β-enolase were also found in high abundance in goat meat digest, the same major proteins reported in beef (Sayd et al., 2016). In tracking the digestibility of the individual muscle protein, it is vital to consider the abundance, sequence coverage, and peptide counts (Portmann et al., 2023). However, sequence coverage does not correlate with the abundance data.

7.3.2 Peptidome of goat meat digests

To evaluate the processing effects on meat protein digestion, we focused the identified peptide sequences on the parent protein, and HCA was used to visualise the effects of

processing. The identified peptides were fragments of abundant muscle proteins in the digest. Among the top 30 muscle proteins, the meat structural protein myosin is the most important precursor of peptides in the gastrointestinal tract, in agreement with the commonly reported data for meat peptidomics (Escudero et al., 2010; Martini et al., 2019; Mitra, Lametsch, Akcan, et al., 2018).

Cluster analysis showed that muscle proteins treated with SV and MW show a different hydrolysis pattern. SV and MW proteins were clustered into two major clusters. In the gastric phase (G120), more peptides were released from the MW sample compared to the SV sample, and the cluster was more evident after the complete gastrointestinal phase (I120). It was apparent that more peptides were released from myosin 2, myosin regulatory light chain, and ATP synthase for the SV sample. However, it appears that more peptides were released from the major muscle proteins from the MW sample. This data contradicts what has been reported that high-temperature processing, such as roasting and boiling, could lead to fewer peptides released during digestion than SV processing due to protein aggregation (Yin, Zhou, et al., 2020).

Since myosin was the protein identified as the most sensitive to oxidation (Morzel et al., 2006), its main chemical modifications during oxidation, such as the formation of carbonyl groups, thiol oxidation, and aromatic hydroxylation can explain the different patterns of how peptides were released from the major muscle proteins (Lund et al., 2011; Morzel et al., 2006). In the structural examination of the meat samples in our previous chapter (**Chapter *5***), MW samples had higher hydrophobicity and lower aggregation than SV. The oxidative modifications in MW increased the susceptibility of oxidised proteins towards proteolysis (Grune et al., 2004). The unfolding process resulting from oxidation can lead to higher peptide

release for the MW sample. However, it should be noted that the HCA data were only monitored for the most abundant proteins.

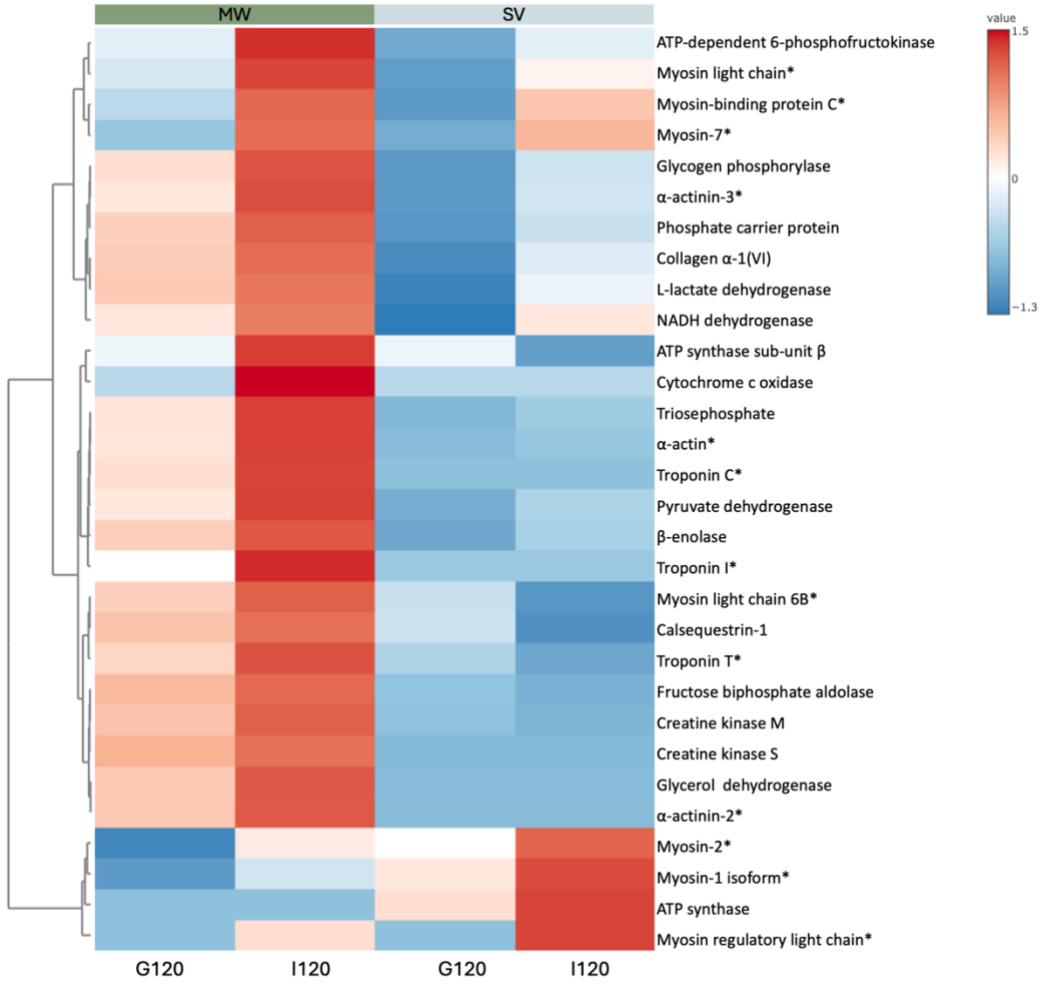

Figure 7. *3* Hierarchical cluster analysis (HCA) for the number of peptides released from the mutual parental proteins after 120 min gastric (G120) and 120 min intestinal digestion.
The protein IDs followed with * represents the structural proteins.

7.3.3 Peptide molecular weight distribution

The proteolytic enzymes hydrolyse the peptide bonds between amino acids of proteins, resulting in a mixture of peptides of different lengths and free amino acids. The peptide molecular weight profile can be used to determine the level of hydrolysis and aggregation of

meat peptides (Deb-Choudhury et al., 2014; Portmann et al., 2023). **Figure 7.4** shows the peptide size distribution of the digests, and the number of data points is equal to the number of peptides identified in the sample. In general, the generated peptide during digestion resulted in a mixture of low molecular weight peptides ranging from 800 to 5000 Da and most of the peptides are distributed around 1500 to 2500 Da, where the median molecular weight is at 2000 Da. After the gastrointestinal digestion, 3397 and 3137 peptides were identified in the SV and MW digests, respectively.

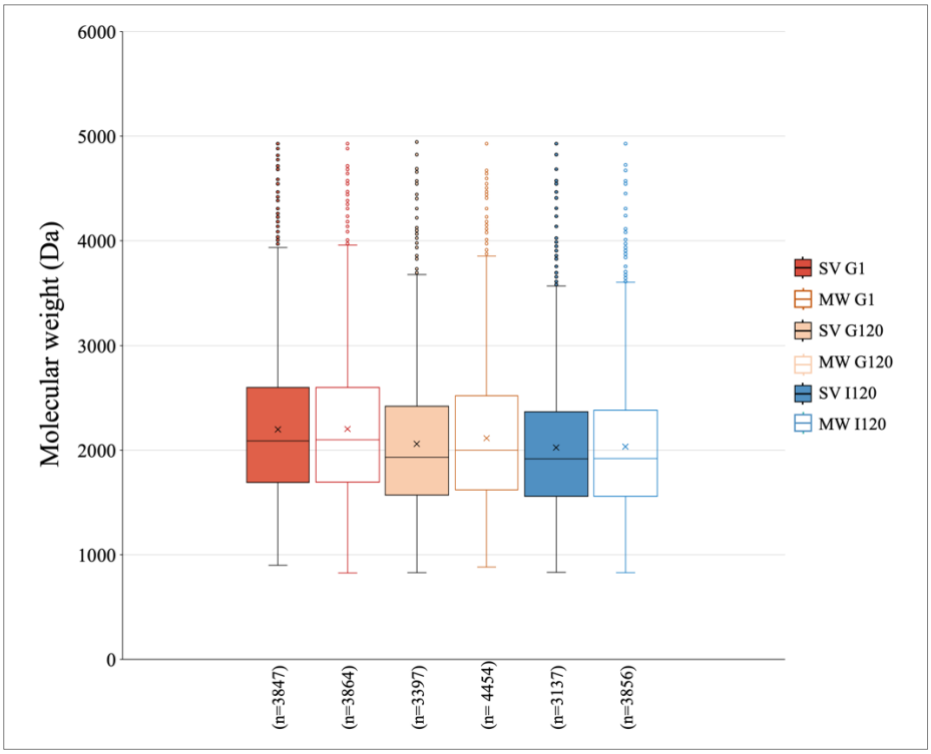

Figure 7. 4 Boxplot peptide size distribution.

The number of data points (n) represents the peptides identified in each digest.

As expected, the peptide size becomes smaller as the digestion progresses from gastric until the end of the intestinal digestion. Despite the expected significant increase in the degree of hydrolysis in the intestinal phase (**Chapter 6**), this drastic decrease in molecular weight

was not very evident when it came to peptide fragmentation. There was only a slight shift towards lower molecular weight fragments from the gastric to the intestinal phase. The median molecular weight after the intestinal phase was still around 1920 Da compared to the peptides at the end of the gastric phase, which was at 1960 Da. In the gastric phase, the peptide molecular weight distribution for MW peptides was higher, with a median value of 1998 Da, while for SV, it was around 1930 Da.

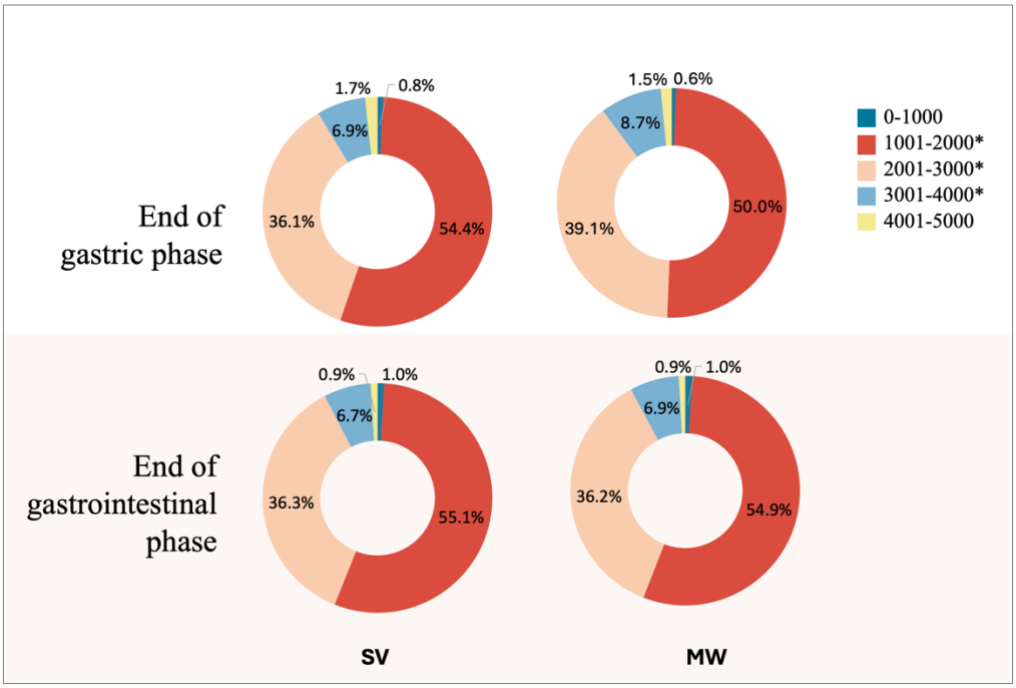

Figure 7. 5 Characteristics of peptides released after in-vitro digestion of goat meat, clustered based on molecular weight (Da).

Molecular weight range with * means values differ significantly ($p < 0.05$) between SV and MW for the gastric phase.

When the peptides were further grouped according to their molecular weights, the composition of the digests could be further examined (**Figure 7.5**). The gastric digests were mainly composed of 1001-2000 Da peptides. This value is similar to the reported values for beef and pork, where proteins from this red meat were mainly degraded by pepsin to

fragments of around 1500 Da (Wen, Zhou, Li, et al., 2015). In the gastric phase, the peptide profile between samples can be differentiated. The MW-treated samples had digests with higher proportions for larger peptides. The MW digest had a higher number of peptides with 2001-3000 Da and 3001-4000 Da, while the digest from the SV had more peptides in the 1001-2000 Da range.

The obtained values show that the goat meat proteins treated with MW were cleaved by pepsin into larger peptide fragments than SV. This pattern implies that different cooking methods affected goat protein digestibility. It is established that pepsin is more efficient at the cleavage sites of aromatic amino acids such as tryptophan and tyrosine (Z. Fu et al., 2021). However, these amino acids are very susceptible to oxidation during cooking (Veronique Santé-Lhoutellier et al., 2008). When myosin specific modification was examined for methionine, tyrosine and tryptophan, the number of oxidations differ between the SV and MW samples (**Table 7.1**). MW resulted in a higher oxidation of methionine and tryptophan residues while SV had more oxidation of tyrosine residues. This data, which is limited only to myosin, show that high-temperature processing by MW leads to a greater extent of oxidation resulting into the decreased protease hydrolysis due to altered cleavage sites of the affected amino acid residue. Therefore, the higher protein modification in samples treated with MW resulted in a more significant proportion of longer peptides (1200−2000 Da) than the SV meat.

The further digestion of peptides from gastric to intestinal phases can also be monitored based on the changes in the peptide molecular weight. For SV samples, most of the change in peptide size was from a large drop in 4001-5000 Da and a corresponding increase in 0-1000 Da. On the other hand, for the MW sample, a considerable decrease in larger

peptides (3001-4000 Da, 2001-3000 Da) corresponds to a significant increase in smaller peptides (1001-2000 Da). At the end of the intestinal phase, most of the digest corresponds to peptides around 1001-2000 Da for both treatments. These values were consistent with the reported *in vitro* digestion peptide profiles for intact meat such as pork (L. Li et al., 2017; Zou et al., 2018) and beef (Yin, Zhou, et al., 2020), but were higher than the reported peptides released from *in vitro* experiments for ham, which corresponds to peptides around 800–200 Da and 1700 Da (Paolella et al., 2015). The lower values for ham could be due to its comminuted structure.

Table 7. 1 Myosin peptide sequences identified with amino acid residue level oxidation from MW and SV processed meat samples.

Annotated Sequence (MW processed meat)	Amino acid residue and position	Annotated Sequence (SV processed meat)	Amino acid residue and position
[K].EGIEWEFIDFGMDLAAC.[I]	[M12]	[-].MVDAEMAAFGEAAPYLR.[K]	[M6]
[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y10; Y16; W21]	[H].FSLIHYAGTVDYNIIGWLQK.[N]	[Y12]
[R].RDSLLIIQWN.[I]	[W9]	[H].FSLIHYAGTVDYNITGWLDK.[N]	[W17]
[R].RDSLLIIQW.[N]	[W9]	[K].AAYLQGLNSADLLK.[A]	[Y3]
[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y10; Y/W]	[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[W/Y]
[K].GKPEAHFSLIHYAGTVDYNIIGWLQK.[N]	[Y12; Y18; W23]	[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y]
[K].AEAHFSLIHYAGVVDYNITGWLEK.[N]	[Y10; Y16]	[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y10; Y/W]
[K].MLSSLFANYAGFDTPIEK.[G]	[M1; Y9]	[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y10; Y16]
[E].LIEKPMGIFSILEEECMFPK.[A]	[M6]	[K].GKAEAHFSLIHYAGTVDYNITGWLDK.[N]	[Y12]
[K].IEDMAMLTFLHEPAVLYNLK.[E]	[M4; M6; Y17]	[K].GKPEAHFSLIHYAGTVDYNIIGWLQK.[N]	[Y12]
[R].DSLLIIQWNIR.[A]	[W8]	[K].GQTVEQVYNAVGALAK.[A]	[Y8]
[R].SYHIFYQIMSNK.[K]	[M/Y]	[K].GQTVQQVYNAVGALAK.[A]	[Y8]
[K].GKPEAHFSLIHYAGTVDYNIIGWLQK.[N]	[Y12]	[K].IEDMAMLTFLHEPAVLYNLK.[E]	[M4; M6; Y17]
[ML].GIFSILEEECMFPK.[A]	[M11]	[K].KMEADLSQLQTEVEEAVQECR.[N]	[M2]
[K].SMEWFTVIEHYHR.[T]	[M2; W4]	[K].KPELIEMLLITTNPYDYAFVS.[Q]	[M7; Y15]
[A].ACIELIEKPMGIFSILEEECMFPK.[A]	[M10]	[K].KPELLDMLLITNNPYDYAFIS.[Q]	[M7; Y15]
[K].AEAHFSLIHYAGVVDYNITGWLEK.[N]	[Y/W]	[K].LASADIETYLLEK.[S]	[Y9]
[K].EGIEWEFIDFGMDLQAC.[I]	[M12]	[K].LQQFFNHHMFVLEQEEYKR.[E]	[M9; Y17]
[H].FSLIHYAGTVDYNITGWLDK.[N]	[W17]	[K].LVVEISNPDLPLKWFK.[N]	[W14]
[K].LVVEISNPDLPLKWFK.[N]	[W14]	[K].MEADLSQLQTEVEEAVQECR.[N]	[M1]
[C].IDLIEKPMGIMSILEEECMFPK.[A]	[M8; M11; M19]	[K].MFLWMVTR.[I]	[M/W]
[R].DYHIFYQILSNK.[K]	[Y2]	[K].MFLWMVTR.[I]	[M1; W4; M5]
[R].RDSLLIIQWNIR.[A]	[W9]	[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1]
[R].NYHIFYQILSNK.[K]	[Y2]	[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M]
[H].FSLIHYAGTVDYNIIGWLQK.[N]	[W17]	[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1; M10]
[E].LIEKPMGIFSILEEECMFPK.[A]	[M6; M17]	[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1; M10]
[K].PMGIFSILEEECMFPK.[A]	[M2; M13]	[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1; M10]
[Q].ACIDLIEKPMGIMSILEEECMFPK.[A]	[M10; M13; M21]	[K].MLSSLFANYAGFDTPIEK.[G]	[M/Y]
[M].TWEPPVYDGGSPILGYFIER.[K]	[W2]	[K].MLSSLFANYAGFDTPIEK.[G]	[M1; Y9]
[K].EGIEWEFIDFGMDLQACIDLIEKPMGIM.[S]	[M12; M25; M28]	[K].PMGIFSILEEECMFPK.[A]	[M2; M13]
[-].MVDAEMAAFGEAAPYLR.[K]	[M6]	[K].PMGIFSILEEECMFPK.[A]	[M]
[K].PMGIFSILEEECMFPK.[A]	[M]	[K].RSEAPPHIFSISDNAYQYMLTDR.[E]	[Y/M]
[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[W/Y]	[K].SMEWFTVIEHYHR.[T]	[M/Y]
[K].AEAHFSLIHYAGTVDYNITGWLDK.[N]	[W/Y]	[K].SMEWFTVIEHYHR.[T]	[W4; Y11]

[A].ACIELIEKPMGIFSILEEECMFPK.[A]	[M10; M21]	[K].VAIYKLTGGVMHYGNLK.[F]	[Y4]
[K].EGIEWEFIDFGMDLAACIELIEKPMGIF.[S]	[M12; M25]	[ML].GIFSILEEECMFPK.[A]	[M11]
[K].EGIEWEFIDFGMDLAACIELIEK.[P]	[M12]	[R].DSLLIIQWNIR.[A]	[W8]
[R].HTSCTVSDLIMGNEYYFR.[V]	[M11]	[R].DYHIFYQILSNK.[K]	[Y2]
[K].KKMEADLSQLQTEVEEAVQECR.[N]	[M3]	[R].HTSCTVSDLIMGNEYYFR.[V]	[M11]
[R].SEAPPHIFSISDNAYQYMLTDR.[E]	[M/Y]	[R].NMWAAFPPDVGGNVDYK.[N]	[M2; W3]
[K].KMEADLSQLQTEVEEAVQECR.[N]	[M2]	[R].NYHIFYQILSNK.[K]	[Y2]
[K].MEADLSQLQTEVEEAVQECR.[N]	[M1]	[R].RDSLLIIQWNIR.[A]	[W9]
[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1; M10]	[R].SYHIFYQIMSNK.[K]	[M/Y]
[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M1; M10]	[R].SYHIFYQIMSNK.[K]	[Y2; M9]
[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M]	[R].VIQYFATIAVTGEK.[K]	[Y4]
[K].MKEEEVEALMAGQEDSNGCINYEAFVK.[H]	[M]	[R].VIQYFAVIAAIGDR.[S]	[Y4]
[R].MTEEEVESVLAGHEDSSGCINYEAFLK.[H]	[M1]	[R].YKVLNASAIPEGQFIDSK.[K]	[Y1]

Myosin specific oxidation for methionine [M], tyrosine [Y] and tryptophan [W].
For **MW** processed meat, the average number of oxidized amino acid residues was: 45 for (M), 19 for (Y), and 14 for (W), and for **SV** processed meat was 36 for (M), 31 for (Y), and 8 for (W).

Unlike the gastric phase, the composition of the digest from the intestinal phase in terms of molecular weight distribution is the same between treatments. This result can explain the same level of digestibility between samples in terms of the degree of hydrolysis reported in **Chapter 6**. Based on the molecular weight distribution of the digest, most peptides are expected to have an amino acid length of around 15-20 AA residues. The sizes of the peptides at the end of *in vitro* digestion show that the peptide lengths are still long compared to the actual peptide size in the in vivo system. Larger peptides are expected since the *in vitro* model lacks the brush border enzymes necessary to hydrolyse peptides further down to di- and tri-peptides.

Overall, goat meat processed using SV and MW resulted in the same peptide characteristics at the end of the *in vitro* gastrointestinal digestion. The similarity of the digest profile, in terms of the molecular weight composition, agrees with the reported efficiency of the proteolytic enzyme in digesting proteins from meat (Bauchart et al., 2007). These findings also support our initial results from the free amino nitrogen assay (**Chapter 6**), which indicated that SV and MW resulted in similar levels of hydrolysis. In this case, it can be inferred that the effect of processing on peptide fragmentation was mainly observed during

the gastric phase, but the pancreatic enzymes were so efficient that at the end of the gastrointestinal phase, the impact of processing became negligible. Our data support the conclusion that the processing of meat affects the kinetics of digestion, but its effect on the overall proteolysis of meat is insignificant (M. L. Bax, T. Sayd, et al., 2013; Dupont, 2017).

7.3.4 Resistant and unique peptides

A Venn diagram was used to visualise the number of unique peptides present at a given phase of digestion (**Figure 7.6**). The proportions of undigested peptides from G1 to G120 were higher in MW than in SV, with 54.6 % and 71.2 %, respectively. These data supported the discussion in the previous section about the effect of high-temperature processing by MW that leads to a lesser digestibility of goat meat proteins during the gastric phase. At the end of the gastrointestinal phase, some peptides from gastric-digested samples could also be detected, indicating that these peptides resisted further digestion by pancreatic enzymes. When the proportion of the peptides was computed, 55.6% and 56.9% of peptides at G1 were still present in the SV and MW digest from the intestinal phase, respectively. The same observation was also reported for a pork digest (Y. J. Li et al., 2017), where there was still a high proportion of peptide fragments left intact at the end of *in vitro* digestion.

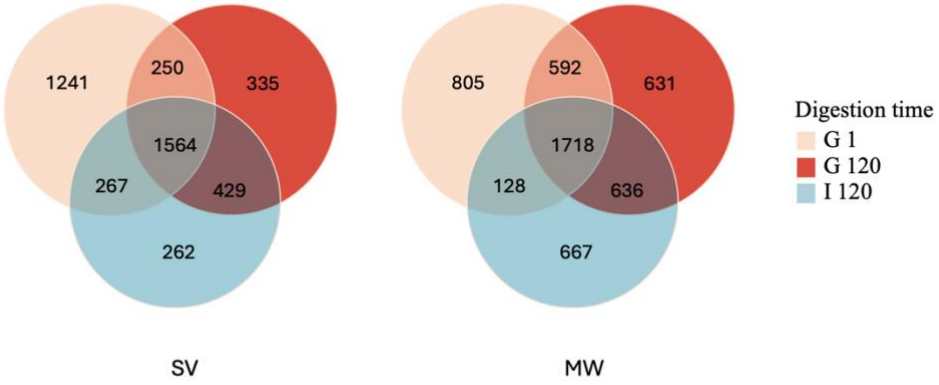

Figure 7. 6 Venn diagram showing the number of common and specific peptides.
Peptides were the digested samples from 1 min gastric (G1) at 120 min gastric (G120) and the end of the gastrointestinal phase (I120).
http://bioinformatics.psb.ugent.be/webtools/Venn/

Although more unique peptides were expected to be generated after the gastrointestinal phase than during the gastric phase, this was not evident in our results. The number of peptides detected after the gastrointestinal digestion was less than those found in the gastric-digested samples. A lesser number of peptides detected after intestinal digestion has been widely reported in the literature for studies using peptidomics (L. Li et al., 2017; Portmann et al., 2023; Zhao et al., 2020), and this appears to be a major drawback for the LC-MS technique for studying digestibility. After the gastrointestinal phase, the peptide sequence was possibly reduced to very low molecular weight peptides (<7 AA residues) that were not detected by LC-MS. There is a high tendency to underestimate the detectable peptides, since digestion products can be reduced to peptide sequences less than 4 AA residues (De Cicco et al., 2019; Dupont, 2017). Moreover, protein digestion is not just a peptide-releasing process; amino acids are also generated for absorption. However, LC-MS has limitations when detecting low molecular peptides (MW< 700). Several signals from very low molecular weight

peptides (MW< 700) detected as mono-charged ions can be unassigned due to the lower limit range of analysis (Dupont, 2017; Portmann et al., 2023). Hence, small peptides (<6–7 residues), especially the very short peptides (2–5 residues), are often underrepresented. Hence, although the number of peptides generated can describe the level of hydrolysis, the digestibility of proteins cannot be directly inferred using these values.

7.4 Conclusion

The current research revealed that different cooking methods, such as SV and MW, notably affected goat meat protein hydrolysis regarding the peptide profile of gastrointestinal digest. Major proteins were hydrolysed differently between the two treatments, and this can be explained by the different levels of oxidation of amino acid residues under different heating conditions. The overall peptide profile of the digest showed that the effect of processing was mainly observed in the gastric phase; MW treatment led to longer peptides than SV-treated meat in the gastric phase. However, the overall hydrolysis regarding the peptide characteristic did not vary after the end of *in vitro* digestion.

7.5 Acknowledgement

This LC-MS analysis would not have been possible without the valuable support of Trevor Loo from the School of Natural Sciences; his expertise in this area is very much acknowledged.

7.6 Supplementary Data

Supplementary table 7.1 The top 30 most abundant proteins where most of the peptides from digest were generated.

	Protein	Accession	Molecular weight (Da)	Relative abundance (%)				Coverage (%)			
				SV-G	SV-I	MW-C	MW-I	SV-G	SV-I	MW-G	MW
Myofibrillar	Phosphorylatable fast skeletal muscle myosin light chain	AEK21248.1	19	1.6	1.2	1.7%	1.7	66	70	43	72
	myosin light chain 6B	XP_005680424.1	23.4	1.0	0.8	0.5%	0.9	26	17	33	39
	Myosin regulatory light chain 2	XP_005691504.1	18.9	0.9	0.1	1.5%	0.8	50	47	45	46
	Actin, alpha skeletal muscle	XP_017897926.1	42	1.4	1.3	2.7%	1.3	17	67	75	75
	Alpha-actinin-2	XP_017897988.1	103.7	0.5	0.6	1.9%	0.5	38	44	41	45
	Alpha-actinin-3 isoform X1	XP_017898846.1	106.3	0.8	0.2	1.3%	0.9	26	40	34	42
	Troponin I, fast skeletal muscle	XP_017898958.1	21.3	0.4	0.2	0.9%	0.5	47	16	24	24
	Myosin-7	XP_017910093.1	223	1.5	1.8	2.1%	0.5	17	35	29	39
	Myosin-binding protein C, fast-type	XP_017918332.1	127.6	1.1	1.3	0.5%	0.7	39	33	26	34
	Troponin T, slow skeletal muscle isoform X1	XP_017918598.1	31.3	0.2	0.4	1.9%	0.3	14	14	14	20
	Myosin-1 isoform X1	XP_017920146.1	222.9	1.2	1.4	4.2%	1.5	16	41	44	44
	Low quality protein: myosin-2	XP_017920148.1	222.8	4.9	6.3	0.5%	4.6	55	40	31	41
	Troponin C, skeletal muscle	NP_001272597.1	18.1	1.2	1.0	0.9%	1.5	64	56	56	58
Mitochondrial	Cytochrome c oxidase subunit NDUFA4	XP_005679030.1	9.3	0.3	0.4	1.3%	0.2	37	44	44	45
	ATP synthase subunit beta, mitochondrial	XP_005680388.1	56.1	1.0	0.6	0.9%	1.5	30	47	43	52
	ATP synthase subunit gamma, mitochondrial isoform X1	XP_017912454.1	33.1	1.5	1.5	0.2%	1.4	20	30	30	30
	NADH dehydrogenase [ubiquinone] 1 α-subcomplex subunit 8	XP_005687095.1	20.1	1.8	2.0	0.3%	2.7	7	22	33	35
	Phosphate carrier protein, mitochondrial isoform X1	XP_005680534.1	40.1	1.7	1.5	0.5%	1.9	44	24	22	34
Sarcoplasmic	Calsequestrin-1	XP_005677287.1	45.2	1.6	1.5	1.5%	1.3	40	55	48	56
	Glycerol-3-phosphate dehydrogenase [NAD(+)]	XP_005680034.1	48.3	1.1	0.8	0.5%	0.9	52	25	31	40
	L-lactate dehydrogenase B chain	XP_005680842.2	36.5	1.1	0.1	0.7%	1.0	51	20	29	26
	Creatine kinase M-type	XP_005692693.1	43	1.1	0.1	4.6%	1.3	34	52	51	54
	Beta-enolase isoform X1	XP_005693511.1	47.8	0.9	0.9	1.5%	1.2	44	44	43	51
	Pyruvate dehydrogenase E1 component subunit beta	XP_005695840.1	39.1	0.2	0.3	0.2%	0.2	41	30	16	46
	Fructose-bisphosphate aldolase A isoform X1	XP_005697728.1	45.1	2.6	2.4	0.8%	2.1	24	42	42	46
	Glycogen phosphorylase	XP_005699921.2	97.2	0.1	0.1	1.4%	0.2	39	42	34	47
	ATP-dependent 6-phosphofructokinase, muscle type	XP_017903352.1	85.5	1.0	1.1	1.0%	0.5	39	27	24	33
	Triosephosphate isomerase	XP_017904037.1	30.6	1.8	1.5	1.3%	1.9	34	39	47	47
	Creatine kinase S-type, mitochondrial	XP_017905548.1	47.3	6.6	6.5	0.9%	4.2	31	33	38	42
Stromal	Collagen alpha-1(VI) chain	XP_017907809.1	108.5	0.9	9.4	0.2%	0.5	19	13	10	19

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Chapter 8

General discussion and recommendation

8.1 Overall discussions

This thesis explored the quality of goat meat and its nutritional properties in terms of in vitro protein digestibility as influenced by processing methods. In New Zealand (NZ), the vital sources of goat meat are feral goats, Boer crosses and dairy goats (Scholtens et al., 2017). In this study, the quality characteristics of feral goats were compared to those of Boer crossbreeds. The goat meat quality characteristics were evaluated using important meat parameterssuchascookingloss,colourandtenderness.Anemphasiswasgiventothequality of meat in terms of texture since it is the most significant quality parameter for consumers, and goats have been reported to produce tough meat (Kannan et al., 2014; Pophiwa et al., 2020). The effects of sous vide and microwave cooking methods on goat meat quality, molecular structure and ultrastructure were examined considering the level of texture achieved during processing. This thesis further explored the impact of sous vide and microwave processing on goat meat in vitro protein digestibility. Advances in scientific techniques were used to elucidate the mechanism of muscle protein structural modification and its implications for the technological and nutritional quality of goat meat. This research takes a multidisciplinary approach to link processing methods to the nutritional quality of meat proteins, particularly in terms of protein digestibility.

8.1.1 The quality of New Zealand goat meat

The first main objective of the study was to evaluate the quality characteristics of the significant goat breeds in New Zealand. Feral goat is the country's primary source of goat meat (Chapter 2). Hence, the quality characteristics of NZ feral goat meat was investigated and compared with a known premium goat meat source which is the Boer crossbreed. Due to

the unavailability of some raw materials such as the dairy breeds and male feral goats, the samples were limited to female feral goats and female and male Boer crossbreeds.

The meat quality evaluation between breeds was presented in Chapter 3. Only selected muscle cuts from feral and Boer crossbreeds were used based on their tenderness profiles; the semimembranosus (SM) from the tough cuts and the longissimus thoracis (LT) for the tender cuts. The results showed that goat meat from both NZ feral goats and Boer crossbreeds had low intramuscular fat values (<2%) showing the leanness of goat meat regardless of breed and muscle type. The notable differences between breeds were observed in colour; the redness (a*) of feral goats was higher than Boer crosses. For the raw meat pH, even at 48 h postmortem, goat meat did not readily achieve a lower pH (<5.7) regardless of breed, all the meat samples had an intermediate pH range (5.7–6.3). These values agree with what has been reported for goat meat from other breeds (Kadim et al., 2004; Tshabalala et al., 2003).

The quality of cooked goat meat samples was examined by cooking goat meat using sous vide (SV) at 60 °C for 6 hrs. After SV processing, the tenderness values, measured in terms of peak shear force (PSF), remained high. Hence, the SV conditions used were insufficient to achieve an average tenderness with PSF value of less than 40 N, which is considered tender (Warner et al., 2017; Webb et al., 2005). Therefore, regardless of breed, the SM and LT muscles would require a longer SV time or an additional processing intervention to achieve lower shear force values. Between the breeds, meat from feral goats was tougher than meat from Boer crosses. Interestingly, looking into the differences between muscle types, meat from the LT was tougher than meat from the SM muscle. A similar finding was also obtained in a study with Australian feral goat, with LT muscle had high instrumental tenderness values than other cuts (N. M. Werdi Pratiwi et al., 2007).

Since the toughness of meat is a complex trait dependent on many factors (Tornberg, 2005), its variability among the goat meat samples was further understood by modelling the available parameters (collagen content, pH, collagen solubility, muscle type and breed) in a Mixed-effects model. The model revealed that toughness was highly correlated with meat pH and collagen content, which depended on muscle type. It can be concluded that the pH of goat meat (intermediate pH, ~5.7–6.3) plays a significant role in the postmortem biochemical processes affecting goat meat toughness. As goat meat reaches intermediate pH range, its PSF value increases. Meat toughness in the intermediate pH range can be explained by less proteolytic activity of lysosomal enzymes (cathepsins) and less degradation of myofibrillar proteins as exhibited by some intermediate pH meats (Lomiwes, Farouk, et al., 2014). The collagen content was positively correlated to texture values ($p < 0.05$). The toughness of the LT muscle can then be explained by the level of its collagen that was significantly higher than the SM muscle. On the other hand, collagen solubility had less or no role in tenderising goat meat. Furthermore, muscle type had a significant ($p < 0.01$) main effect on the texture variability, in agreement with the reports that muscle type significantly affects texture due to the variability of muscle fibre types and degree of collagen crosslinking for each muscle (Listrat et al., 2016b).

To further understand the mechanisms behind the quality of goat meat, muscle ultrastructure from representative tissues was excised and analysed using transmission electron microscopy (TEM). For the raw meat tissue, for the first time, it was evident that goat muscle had undergone cold shortening even at 48 hrs postmortem. The tissue samples from feral SM and Boer crossbreed LT muscles exhibited very short sarcomere lengths and unrecognisable Z-disks. These findings highlight the high tendency of goat meat to undergo

cold shortening during chilling as revealed by the ultrastructure images. These results agree with the commonly reported reasons why goat meat can be tough (Abhijith et al., 2021; Smith et al., 1978). The current practice of handling goat carcasses may lead to cold-shortened goat muscles regardless of breed. This phenomenon needs be further investigated to establish the required postmortem conditions for goat meat carcass.

The ultrastructure of goat meat tissues before and after SV processing was also evaluated. The TEM images of the raw and cooked meat samples revealed no drastic muscle fibre disintegration or disruption for all the samples. This observation implies lesser damage to muscle fibres, that can further explain why the SV cooked muscles were still tough. Additionally, after SV cooking at 60 °C for 6 hrs, there were no notable differences between the ultrastructure of meat from feral goats and meat from Boer crosses. The average sarcomere length for the cooked muscle ranged from 1.4 −1.7 μm with no observable trends between muscle type or animal group. These results indicate that muscle structure between feral goats and Boer crossbreeds does not significantly differ.

8.1.2 The impact of different processing technologies on goat meat quality

The impact of processing on goat meat quality was initially explored using SV process to evaluate the cooked characteristics of SM and LT muscles from various breeds (Chapter 3). The instrumental tenderness values of cooked goat meat after SV cooking for 6 hrs at 60 °C still ranges from 40 to 80 N. Hence, the initial process used could not achieve acceptable instrumental tenderness values. (Destefanis et al., 2008). To further investigate the effect of processing on goat meat, goat meat was subjected to SV for low-temperature processing and microwave(MW) for high-temperature processing using goat *biceps femoris* (BF) muscle

(Chapter 5). The BF muscle was subjected to various SV conditions to achieve an acceptable level of tenderness. An SV process at 60 °C for 9 hrs resulted in texture values ~40 N. Our SV trials revealed that goat meat should be cooked for 9 hrs or longer if 60 °C is used to achieve texture values ≤ 40 N. For the MW process, a short time high temperature MW processing (5 min) also resulted in cooked goat meat that reached ~40 N. Then the SV and MW treated meats with the same level of tenderness were compared, noting that the length of cooking time and end point temperature were different. Also, in this experiment, the quality of goat meat was compared with lamb, processed using the same SV and MW conditions.

The MW process resulted in a higher cooking loss compared to SV samples for all meat samples. Although the level of texture for the MW sample was acceptable, the cooked meat was easily disintegrated and very dry. Furthermore, the redness values for the MW sample were very low, indicating a higher level of myoglobin denaturation. The high moisture loss for the MW-treated meat agreed with the reports in the literature that MW cooking results in higher cooking loss than water bath processing (Wang et al., 2019) and conventional cooking methods (Yarmand & Homayouni, 2009). High moisture loss for the MW treated sample can be due to a fast-heating rate (Wu et al., 2007) since a higher heating rate can result in higher mobility of bound water (Mortensen et al., 2006). Furthermore, alternating electromagnetic field of a microwave propagates through the polar water molecules within the muscle fibre, disrupting muscle fibre resulting in higher expulsion of water molecules (Cai et al., 2018; Cao et al., 2019). Varying cooking loss can also be explained by the length of cooking time. Longer cooking times in SV resulted in a higher level of solubilisation and gelation of some collagen, which enhances the water-holding capacity of the meat matrix (Purslow, 2014; Tornberg, 2005; Zielbauer et al., 2016). The high levels of collagen solubilisation and

gelatinisation could not take place with the short heating duration in MW processing. Hence, SV cooking that involves longer cooking time would be ideal for goat meat with high collagen content. On the other hand, the high moisture loss during MW would be a challenge for meat processing employing MW technology.

When the ultrastructure of goat meat was examined before and after processing, goat meat muscle responded differently between the two processes. The high-temperature MW processing (>100 °C) induced a higher degree of longitudinal shrinkage and transverse shrinkage that can further explain more significant water loss in MW compared to the SV (Roldán et al., 2013). Conversely, SV process with low temperature and slow heating caused limited longitudinal shrinkage. For meat ultrastructure, in comparison to lamb, after SV and MW processing, goat meat responded differently compared to lamb. For goat meat samples, longitudinal shrinkage was higher than 25%, while the transverse shrinkage was just below 20%. Notably, transverse shrinkage in SV samples was significantly less than the MW samples. On the contrary, the lamb had higher transverse shrinkage than longitudinal shrinkage for both MW and SV processes. The higher transverse shrinkage for lamb meat is probably related to the effect of aging where transverse shrinkage increases the longer the meat is aged (Purslow et al., 2016). Moreover, the difference in pattern for muscle shrinkage can be explainedbythedifferenceinMWtemperaturegeneratedbetweenlambandgoatmeat muscles. Lamb reached a higher peak temperature (117 °C) than goat meat (107 °C) on average, and the temperature dependence of muscle shrinkage is known (Palka & Daun, 1999). Furthermore, the differences in muscle composition between lamb and goat meat can also affect the resulting quality of a cooked meat from different species. The response of muscle

fibres to temperature depends on the muscle fibre types composed of various proteins with different thermal stability (Astruc et al., 2012; Purslow et al., 2016).

Compared to the SV process discussed in Chapter 3, the changes in muscle structure for the BF muscle under longer SV processing and microwave (MW) conditions were more pronounced than those observed in the SM and LT muscles processed at 60°C for 6 hours. These findings indicate that temperature significantly affects the degree of muscle fibre and connective tissue shrinkage, which is directly related to the quality of cooked goat meat. Although high structural damage seen from the surface of the meat caused by MW was initially reported (Liu et al., 2013; Yarmand & Homayouni, 2010), the TEM images in this study clearly showed that level of disruption on meat structure includes obvious breakage of sarcomere, high number of torn myofibrils along the Z-line, and excessive thermal shrinkage of myofibrillar proteins by MW. These damages resulted in myofibrils with less structural integrity that can explain why the texture of goat meat cooked in MW condition for shorter time was comparable to SV samples cooked for a long time. The structural damage caused by MW is comparable to a structural damage caused by high-temperature processing.

Although MW is a very short process, its end point temperature play a crucial role in its effect on muscle proteins and meat quality. Furthermore, the effect of dielectric heating involving the electromagnetic field of a microwave propagating through the polar water molecules of muscle fibre induces thermal effect on muscle protein that directly affects protein molecular structure.

8.1.3 The impact of processing on goat meat protein digestibility

The effect of SV and MW on the molecular structure of goat meat proteins was investigated in Chapter 6. Between processes, for both lamb and goat meat, MW induced a higher degree of protein unfolding compared to SV, and this can be attributed to the higher temperature processing during MW, consistent with the trend reported in the literature (Chelh et al., 2006; A. Promeyrat et al., 2010). Furthermore, the effect of electromagnetic heating on protein structure could have also exacerbated protein unfolding, as reported in the literature (Cai et al., 2018; George et al., 2008).

When the myofibrillar protein aggregation was examined, the aggregation behaviour of goat meat proteins after processing was almost the same between SV and MW. On the other hand, lamb proteins had different levels of aggregation between the processes, indicating that goat meat myofibrillar proteins behave differently compared to lamb samples. Hence, this suggests that the effect of processing on meat proteins is also dependent on the muscle type or condition. The data also indicates that even though SV was operating at a lower temperature, it could induce higher protein aggregation than MW due to the length of heating. Prolonged heating increases protein aggregation since protein tend to cluster and form aggregates over time (Cao et al., 2019; He et al., 2018; Mitra et al., 2017).

The protein secondary structure was also evaluated after SV and MW processing. Surprisingly, although SV and MW processes differ in their heating mechanisms, both processes showed the same trend in increasing or decreasing the identified secondary structures. For both lamb and goat meat samples, there was a marked increase in β-sheet, random coil, and aggregated strand structure, with a significant reduction in α-helix and β-turns, a common observation for heat-induced protein denaturation in meat (Beattie et al.,

2008; Berhe et al., 2014; Ngarize et al., 2004). The decrease in α-helix after MW and SV processing indicates a shift from a very compact structure to more spread out β-sheet and random coil structures. MW processing increases the amount of non-organised structure and β-sheet that agrees with the reported trend in the literature (Cai et al., 2018; Calabrò & Magazù, 2014). The significant observation between the two processes is the higher amount of random coil for SV- treated meat, which was substantial for the lamb sample; this can be directly related to the myofibrillar surface hydrophobicity. Hydrophobic interactions play a dominant role in stabilisingβ-sheet conformations (Narayanan & Dias, 2013), and between SV and MW, MW induced significantlyhighersurfacehydrophobicityasdiscussed(Section6.4.1), driving more β-sheet structure for the MW sample.

Generally, the protein molecular structure analysis shows that both SV and MW processing induced molecular changes in goat meat protein structure. However, the level of difference was not very high compared to the data observed from the lamb samples. This again highlights that the effect of processing conditions can vary between different types of meat.

8.1.4 The impact of processing on goat meat protein digestibility

The protein digestibility of goat meat was determined using an in vitro digestion system. The first in vitro digestion experiment was conducted to determine the overall digestibility of goat meat muscles from feral and Boer crossbreeds cooked using sous vide (6 hrs at 60 °C) and these samples had different textures (Chapter 4). Results show that meat from LT and SM muscles of Boer crossbreed goats had higher digestibility than meat from feral goats in terms of the degree of hydrolysis in vitro. Statistical modelling was used to examine the effect of texture, collagen content, breed and muscle type on the degree of

hydrolysis. Texture was found to be not a significant predictor for the degree of hydrolysis of meat in vitro or even the digestion kinetics although food texture has been recognised as a factor influencing gastric emptying (Santangelo et al., 1998). On the other hand, the level of collagen significantly affected the variability in the meat digestion rate, specifically in the gastric phase. Collagen content is positively correlated with the rate of digestion. The role of collagen content in meat digestion can be linked to the specificity of pepsin for collagen. Pepsin can efficiently hydrolyse a broad range of peptide bonds of a denatured protein structure at low pH at 37 °C (Z. Fu et al., 2021). In the case of gastric conditions, pepsin activity is generally favoured and the varying protein structure of food results in a different rate of pepsin hydrolysis. Although collagen is known for its rigid structure due to higher crosslinks (Purslow, 2014), pepsin can still efficiently break down collagen since it removes non-helical ends (telopeptides) of the collagen. The removal telopeptides consequently removes the intermolecular crosslinks (Hickman et al., 2000).

The protein digestibility of goat meat was further investigated by determining the influence of different thermal processing on goat meat (Chapter 6). Studies on meat protein digestibility have demonstrated that various processing conditions can affect meat in vitro protein digestibility (Mitra et al., 2022; Orlien et al., 2021; Yin, Zhou, et al., 2020). When goat meatBFmusclewassubjectedtoSVandMWprocessing,theoveralldigestibilityofthemeat did not vary between SV and MW when measured in terms of the degree of hydrolysis. This can probably be explained by a minimal difference on the degree of protein hydrophobicity, myofibrillar aggregation and secondary structure as discussed earlier, although these features could affect protein digestibility (Zhang et al., 2020; Zhang et al., 2013). However, SDS-PAGE

data show that different processes resulted in different digestion profiles of the muscle proteins.

Goat meat digest was subjected to LC-MS analysis (peptidomics) to further examine the fate of the major muscle proteins during digestion (Chapter 7). In agreement with our initial SDS-PAGE gel findings, peptidomics revealed that major muscle proteins were hydrolysed differently between processing methods as shown by the cluster analysis. For example, the peptides originating from myosin 2, myosin regulatory light chain and ATP synthase were released mainly at the end of gastrointestinal digestion for the SV-treated meat, but this was not the case for the MW-treated sample. Based on the release of peptides using cluster analysis, MW proteins appeared more hydrolysed than SV; this data contradicts whathasbeenreportedthathigh-temperatureprocessing,suchasroastingandboiling,could lead to fewer peptides released during digestion than SV processing (Yin, Zhou, et al., 2020).

While the peptide released revealed heightened hydrolysis in MW-treated meat, it is important to acknowledge that the cluster analysis was restricted to monitoring the most prevalent proteins, constituting approximately 50% of the total protein abundance. Furthermore, the possibility of an underestimated number of peptides detected in SV digest should also be considered, since digestion products can be reduced to peptide fragments that are less detectable by LC-MS (De Cicco et al., 2019; Dupont, 2017).

To understand the whole picture of the peptides as digestion products, all the peptides were further characterised, revealing that the peptide profiles of the meat digests between the two processes differed. The MW digest had a higher number of peptides with 2001-3000 Da and 3001-4000 Da ranges, while the digest from the SV had more peptides in the 1001-2000 Da range. This is possibly due to the different extent of oxidation during processing. Further

examination of myosin specific modification for methionine, tyrosine and tryptophan revealed that MW resulted in a more oxidized methionine and tryptophan residues while SV had more oxidized tyrosine residues. This data, though limited only to myosin, showed that high-temperature processing by MW led to greater extent of oxidation resulting in the decreased protease hydrolysis due to altered cleavage sites of the affected amino acid residues. However, the difference in peptide molecular weight distribution was mainly observed in the gastric phase. But after the complete gastrointestinal digestion, the peptide profile of the digest was the same between samples. This result explains the same level of digestibility between the samples in terms of the degree of hydrolysis as reported in Chapter 6.

In our study, all our protein digestibility findings show that when comparing the meat protein source, the meat's composition dictates the protein's fate during digestion. The lamb sample had a higher degree of hydrolysis than goat meat. On the other hand, when processing conditions vary, the release of peptides from major muscle proteins can be affected. Overall, the peptide profiles of the digests revealed that the effect of processing can significantly impact meat protein digestion in the gastric phase; this can be relevant to metabolic processes that require a faster rate of digestion and absorption. However, at the end ofinvitrodigestion,theoverallhydrolysisintermsofthedegreeofhydrolysisandeven peptide characteristics did not vary. This can be explained by the efficiency of the proteolytic enzymes in hydrolysing meat proteins. Furthermore, after the entire gastrointestinal digestion, the values reinforce previous reports that meat is a highly digestible protein.

8.2 Overall conclusion

This research makes a significant original contribution to the knowledge of goat meat quality, muscle structure, and protein digestibility as affected by processing conditions. With the emerging global markets for goat meat, its leanness positions it as a healthier protein source. In this study, regardless of breed, goat meat from feral goats and Boer crossbreed was lean and tough. The combined effects of muscle cold-shortening, intermediate pH, less intramuscular fat, and collagen content can explain the goat meat toughness. These findings merit industrial consideration, suggesting that pre- and postmortem handling practices should be tailored to the unique characteristics of goat meat.

Processing goat meat using single-stage SV cooking at 60 °C requires a longer processing time that could reach up to 9 hrs to achieve an acceptable tenderness level. On the other hand, the MW process can be a fast-cooking option, but it would result in dry meat due to very high cooking loss. This data would have significant implications when processing pre-pack meals using microwave technology. Between SV and MW processing, SV would be a better processing option for tough goat meat to achieve juicier meat.

In general, the composition of meat such as the level of collagen dictates the fate of protein during digestion, which can affect the digestion kinetics and the overall degree of hydrolysis. The protein digestibility of goat meat can vary between breeds and muscle types; meat from Boer crossbreed goats had higher digestibility than meat from feral goats. When goat meat was subjected to different processing conditions, the severity of a process in terms of the temperature and length of the processing time can affect protein molecular structure and have implications on meat protein digestibility. Based on our findings, SV does not appear to be a better cooking method than MW in terms of overall protein digestibility. However,

overall, the effect of processing on goat meat protein digestibility is highly compartmentalised. Processing can affect the fate of muscle proteins during the gastric digestion phase but not at the end of gastrointestinal digestion. Different gastric digestion kinetics would have important implications for meat consumption since the digestion rate in the stomach dictates the gastric emptying rate and, consequently, the postprandial appearance of amino acids. Our data support the conclusion of other authors that meat is a highly digestible protein, and the impact of processing can be more on the kinetics of digestion in the gastric phase but would be negligible in the small-intestinal phase.

8.3 Recommendations and future work

Although this PhD project has produced new information about NZ goat meat, some questions need to be answered in future studies. The recommendations and future work related to this research are outlined as follows:

8.3.1 Improving NZ goat meat quality

- The findings of our study emphasize the need for further research on the goat meat supply chain in NZ. There is a need to conduct more research on how goats should be handled to provide the adequate process necessary to improve goat meat quality and optimize its potential. For example, adequate nutrition, electrical stimulation, and delayed chilling might improve the quality of goat meat. These methods can be studied together with the investigation of meat pH and the activity of the endogenous proteolytic systems to have a better knowledge of mitigating goat meat toughness. The impact of intermediate pH on the myofibrillar integrity of goat meat should be examined further.

- Although our research evaluated the quality characteristics of feral goat meat, we didn't present any data regarding its body or carcass quality which would give a very important information regarding the saleability of the current domesticated feral goat meat. The last comprehensive data on NZ feral goat meat was conducted by Kirton et al. (1970) and their data, such as carcass weight and dressing percentages seemed far from the records of our goat meat carcass profiles. There is a need to examine the carcass and body characteristics of NZ feral goat meat compared to other breeds.

Furthermore, NZ developed new breeding stock for goat meat production, the Kiko and Kikonui, but there are no current data regarding their carcass and meat quality. This is good area to explore knowing the growing demand for goat meat, although there would be challenge getting enough sample when conducting the research.

8.3.2 Exploration of various processing technology

- The equipment used to evaluate the effect of microwave processing on goat meat was CiMPAS, a green technology machine designed for microwave-assisted thermal sterilisation (MATS) to produce sterilised prepack meals as ready-to-eat food. In our study, we only explored the effect of MW on goat meat quality and structure without the complete sterilisation process. Nevertheless, our previous trials using a complete sterilisation regime resulted in cooked meat losing a lot of moisture and muscle fibres quickly disintegrating, a significant drawback when microwaving meat. Hence, for further evaluation of MATS for meat processing, it is recommended that the meat be prepared in curry form or any process that can mask significant moisture loss or intense structural damage. Product development involving MATS can be optimised to benefit from MATS.

- The single-stage cooking was used in this study for SV processing. However, multi-stage (double or triple) SV processing can also be explored in the future. This concept has produced improved texture for tough meat (Ismail et al., 2019a; Mitra, Lametsch, Greco, et al., 2018) and would probably be suitable for goat meat.

- In addition to the processing techniques studied in this thesis, emerging technologies in meat processing can be explored to produce more tender meat. The use of pulsed electric field (PEF), high-pressure processing (HPP) and ultrasonic processing can be

investigated. Furthermore, the use of pre-treatment such as application of enzymes can also be considered. In addition to examining whole muscle goat meat, the impact of different processing methods on comminuted goat meat products is another area that can be studied.

8.3.3 Evaluation of the effects of processing on protein structure

- Food peptidomics are now encouraged to be integrated with health biomarker science in nutrition, and this is specifically important for meat and the implications of processing on meat quality. For example, oxidation in meat processing has always been the explanation for the lesser protein digestibility of processed products and this can be monitored through peptidomics. In our study, the oxidation of the samples was evaluated on the raw, undigested sample, but we did not compare the modifications of peptides after processing between the untreated (fresh meat) and the treated samples. Hence, for further study, this area can be specifically examined to directly confirm modifications on a peptide level after processing and this can be linked to how specific protein oxidation can directly affect meat protein digestibility.

- A deeper knowledge of how thermal treatments of goat meat can affect the proteolytic susceptibility of oxidised myofibrillar proteins can be further investigated using different time-temperature combinations. Experiments investigating the relationship between protein modifications such as carbonyl formation, loss of free sulfhydryl groups, Maillard product formation, and aggregation can be carried out.

8.3.4 Goat meat protein digestibility

- One limitation of using goat meat samples is their small size, limiting the amount of samples that can be collected for further analysis or using an alternative setup for digestion studies. For example, the *in vitro* experiment could have been conducted using the dynamic models since they are available in the lab; however, the meat samples were not large enough to perform a complete run for a dynamic *in vitro* digestion setup. Dynamic models can give a better gastric motility effect since the effect of meat texture is mainly observed in the gastric compartment, and digestion kinetics can be closer to the ideal system. Moreover, a dynamic model is relevant to gastric emptying, and it should be used in the future to simulate the dynamic and transient nature of the human digestive system, such as the movement of the stomach wall and gastric emptying.

- Although the *in vitro* system has advantages over in vivo models, it is recommended that in vivo models should be used in the future. Based on our findings, the effect of meat composition or processing on meat was mainly observed in the gastric phase; its impact on the overall digestibility of meat proteins will be more on the digestion kinetics that relies on the particle size of peptides/food to be transported to the small intestine that can be ideally studied under in vivo system. Even with a dynamic *in vitro* system, it is still a challenge for *in vitro* gastric models to simulate realistic gastric emptying. Additionally, since this study uses the *in vitro* system, one of the limitations of our results is the protein digestibility caused only by the pepsin and then by the pancreatic enzymes. This setup could not completely mimic the efficiency of the human intestinal tract that has enterocyte peptidases. Hence, the data on peptidomics would

underrepresent more digested meat proteins in human gastrointestinal digestion. In vivo systems should be used to achieve more ideal results.

- Current advancements in digestion studies involve exploring digestion beyond the small intestine. The involvement of the gut microbiota in nutrition and health is gaining more relevance. Hence, the effect of meat processing on digestion can be further explored for future studies, this time involving the gut microbiome.

- It is recommended that in future work, meat protein digestibility should include the evaluation of amino acid bioaccessibility. Oxidised proteins can lead to an impaired digestion, caused by the loss of essential amino acids due to oxidative degradations and formation of resistant peptides (Soladoye et al., 2015). Amino acid bioaccessibility values can provide information regarding the loss of nutritional value of a protein. However, we did not evaluate amino acid bioaccessibility. Additionally, determination of *in vitro* digestible indispensable amino acid score (DIAAS) which was recently reported (Sousa et al., 2023) can also be carried out following the analytical workflow based from INFOGEST *in vitro* digestion protocol. This method is a practical tool in identifying the protein quality of meat and would be very important in determining the nutritional impact of processing.

- Although our findings show that meat texture is not a significant predictor for the degree of hydrolysis of goat meat, understanding the relationship between meat texture, moisture content, and bolus formation would be significant. Various meat textures require different chewing patterns (Mioche et al., 2003); the harder the food, the longer the chewing, which could lead to a smaller bolus size that can affect the starting physical structure of meat for protein digestion. Hence, it is recommended

that for future studies, the digestibility of goat meat should also be evaluated with bolus formation. This can be done by having individual perform the mastication of the sample before commencing *in vitro* gastrointestinal digestion.

- Studies have shown that the consumption of red meat sometimes declines because of its texture, firmness and taste profile, and older consumers (e.g. aged 65 years or more) may miss out on this nutrient-dense food that provides protein, essential amino acids, lipids, iron, zinc, and B vitamins (Biesalski & Nohr, 2009; McAfee et al., 2010). For further studies, with mastication, the adjustment of food textural properties of meat for elderly patients can be further investigated. Moreover, developing strategies to produce meat products with improved ingestion and protein digestibility for the elderly would be relevant. An enzymatically softened goat meat or textured modified meat for the elderly can be developed instead of focusing on heat treatment or minimal treatment to have better meat texture.

- The *in vitro* model in this study is only for adults, but understanding the impact of tough meat on the elderly or people with impaired dental health is also an excellent area to explore. Hence, the *in vitro* digestion conditions can be altered, this time using the conditions for the elderly, and the effect of meat texture and processing conditions can be monitored under this condition.

- Meat is often consumed with a carbohydrate source, and it would be very worthwhile to conduct a study by looking into the protein digestibility of meat in combination with other food sources. This study can be specifically designed to examine protein digestion kinetics and evaluate the consequent postprandial release of amino acids.

- LC-MS is a perfect method to understand how a specific muscle protein can be hydrolysed during digestion. However, this method is not a stand-alone technique to measure the digestibility of the food based on the generated peptide. It is possible that in doing LC-MS, there will be an incomplete characterisation of the digests since the LC-MS method has the aa cut-off limit, where smaller peptides cannot be detected, it is difficult to establish whether the missed identification of peptides from specific protein domains is a consequence of their complete breakdown to amino acids or their survival as large peptides.
- In using LC-MS for the characterisation of meat digests, the protocol used for the LC-MS can be improved. Instead of in-gel digestion, peptides can be directly separated using a peptide filtration technique with a specific cut-off.
- Much work has been done regarding the bioactive peptides from meat, but less has been done when it comes to evaluating the processing of meat. For further studies, it is recommended that complete characterisation of complex digestomes can be done to bridge the gaps between composition, structural properties, and biological effects of peptides originating from dietary proteins.

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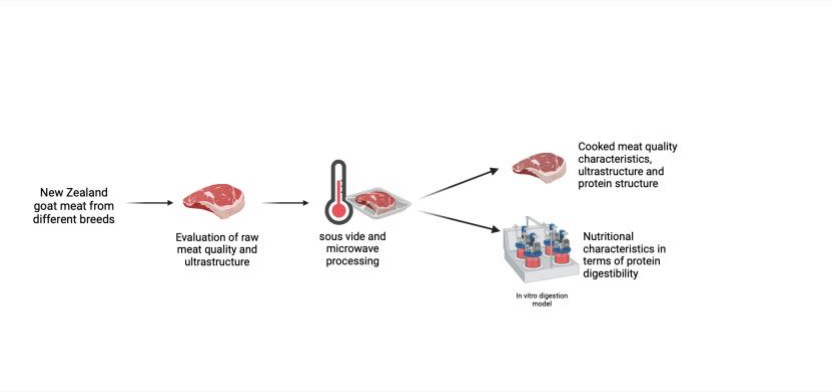

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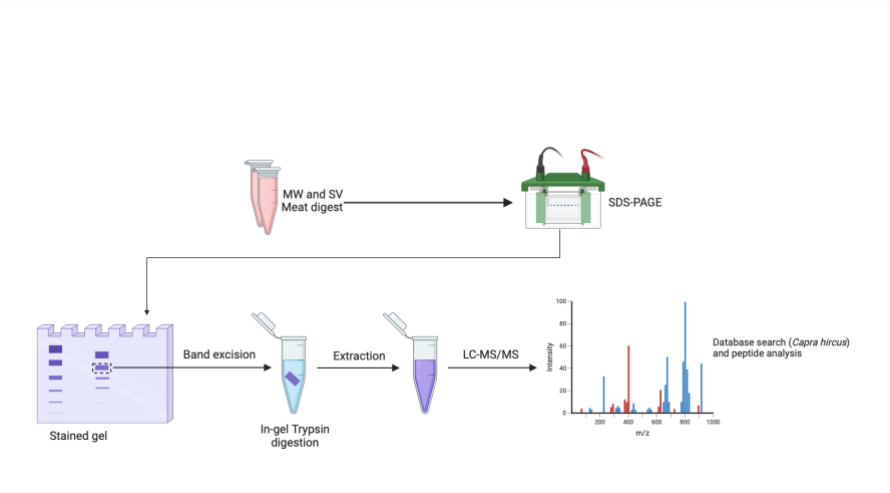

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