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# **The Characterisation of key Processes In Sous Vide Meat Cooking**

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## Abstract

Sous vide (French for “under vacuum”) is a method of cooking under precisely controlled conditions, which employs the principles of long-time-low temperature treatment. Better control over texture, flavour, and doneness are a few of the numerous advantages that sous vide enjoys over traditional methods of cooking.

However, the requirement of a long time makes the sous vide process often uneconomical at industrial scale, particularly when applied to tougher cuts of meat, briskets for example. To improve the economics of the process, it is essential to better characterise the sous vide process, specifically understanding the cook-loss, how different conditions affect the extent of collagen dissolution and tenderisation will enable products with better sensory to be produced. The aim of the current work was, therefore, to characterise the key processes in order to facilitate the optimisation of sous vide cooking.

Samples of beef semitendinosus (‘eye of round’) were cut into blocks of approximately 60x60x100 mm and were cooked at 50—60 °C (in increments of 2 °C), 70, 80, and 90 °C for five time-points: 1.5—73.5, 1.5—49.5, 1.5—25.5, and 1.5—9.5 hours, respectively. Cook-loss (CL), Warner—Bratzler shear force (WBSF), total collagen in raw samples (TC), cook-loss-heat-soluble collagen (CLDC), and percent dissolved collagen within the cooked meat (%CMDC) were all measured (a new method was developed for determining %CMDC as no existing methods were found). Kinetic models were developed for the rate of CL and the CLDC as a function of temperature.

A rapid cook-loss (which was attributed to the denaturation of myofibrillar proteins) followed by slow phase was observed for all temperatures. The higher temperatures (70—90 °C) showed a similar equilibrium cook-loss of approximately 42%. The cook-loss of the lower temperatures did not, however, equilibrate but showed an increasing trend with increasing temperature. The WBSF measurements showed a sharp increase (from the raw measurements) then sharp decline, followed by a slow decline phase. The TC was found to be 35 mg-collagen/g-meat. The CLDC increased with both time and temperature – the

highest measured value was 3.15 mg-collagen/ml-cook-loss (80 °C, 25.5 hours). This value is very low compared to the TC and therefore CLDC is not an accurate measure of the dissolved collagen within the meat. The %CMDC increased with increasing temperature and to a lesser extent the time – the maximum %CMDC was 80% (90 °C, 9.5 hours). A two reaction, non-isothermal, first order (with fitted kinetic parameters) system was found to satisfactorily model both the CL and CLDC.

Although the mechanism of meat tenderisation is complex, the dissolution of collagen, the denaturation of myofibrillar proteins, and the level of cook-loss appear to be the key factors influencing the tenderness of the resulting meat.

The developed conceptual model integrates the key factors and shows how these undergo changes as the temperature is increased, but further research is required to elucidate these and to develop tools to rapidly identify processing conditions for different meat cuts and products.

# Acknowledgements

My sincere thanks to my supervisors, John Bronlund, Michael Parker, and Richard Edmonds, for their extraordinary guidance and support throughout the project. One of the things I really admire about Massey University is the absence of hierarchy: as a student, you and your supervisors are equals and a team, trying to wrestle the same recalcitrant, giant. This is very important because it eliminates barriers and formalities that would normally inhibit or limit a proper communication and collaboration between the student and the supervisor (s).

I would like also to thank my FIET team, especially our Dear Leader, Mike Boland, for his outstanding leadership and vision for the project. To Glenda Rosoman, Ann-Marie Jackson, John Edwards, and Steve Glasgow thank you. To John Sykes, thank you for your patience and hospitality whilst I spent countless hours in your lab for measuring collagen – you really don't get to appreciate how difficult and time-consuming collagen measurements are until you try it yourself. My gratitude must be extended to ANZCO Foods and Jonathan Cox in particular for his support of our project.

To my family and friends, thank you for your love and support.

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*In a bitterly cold November evening, Dr Hannibal Lecter walked into a small, poorly lit butchery in New York City. To the good doctor's amazement and delight, he noticed that three sous vide human brains are on display for sale: a German brain for \$300, a Japanese one for \$450, and a Somali one for \$900. He pointed to the display cabinet and asked the shopkeeper "why is the Somali brain so expensive?" To which the shopkeeper replied, "because it is hardly used!"*

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# Chapter 1 Introduction

## 1.1 Background

It is well known that tenderness is a key factor in consumers' acceptance of meat products. Research has shown that consumers are willing to pay a premium for a guaranteed tender product, providing an opportunity to significantly increase the value of middle meats by as much as \$US60 per carcass (Boleman et al., 1997, Calkins and Sullivan, 2007, Miller et al., 2001). Therefore, the meat industry is in a continual quest for methods to improve the tenderness of low to middle cuts of meat and thus upgrading them to – ideally – steak level value (Miller et al., 2001). The enormous complexity and variability of meat has, however, made this a considerable challenge.

Sous vide (French for “under vacuum”) is a relatively new method of cooking, which has not been actively studied until the 1990s and has not seen a meaningful growth until the late 2000s and early 2010s, where it was widely adopted in restaurants and homes (Baldwin, 2012). The sous vide method employs the principle of low temperature—long-time heat treatment (LTLT) and provides a better control over product texture, flavour, and doneness (Deok Jang and Sun Lee, 2005). The heat treatment and the moist environment break down the myofibrillar proteins and solubilise the connective tissue, resulting in a more tender meat (García-Segovia et al., 2007). The other main advantages of sous vide are economic (e.g. better use of labour and equipment through centralised production) and qualitative (e.g. reduced need for flavour enhancers, better preservation of vitamins, retention of juiciness, etc.) (Schellekens, 1996).

Sous vide cooking differs from traditional methods of cooking in two fundamental ways: first, the raw food is vacuum sealed in food grade, heat-stable pouches, and second, the pouches are cooked under controlled, relatively low temperatures (Myhrvold et al., 2011, Schellekens, 1996, Espinosa et al., 2015). The principal advantages of the vacuum packaged, low atmosphere environment and better heat transfer (during cooking and cooling through

elimination of air gaps between package and product), growth-inhibition of aerobic microorganisms, and space saving. The precise temperature control is more important than the vacuum packing because it allows (i) outstanding reproducibility; (ii) greater control of doneness (compared to traditional methods); (iii) the ability to pasteurise foods at lower temperatures (i.e. food does not need to be cooked to well-done to be safe); and (iv) ability to tenderise tough cuts while still maintaining medium/medium-rare doneness (Baldwin, 2012). However, the long cooking time required for this method often makes it uneconomical, which perhaps explains why it is not widely adopted in the meat industry.

## 1.2 Research aim

Predicting tenderness has been the subject of many unsuccessful attempts, and thus was not the focus of this study. Rather, the aim of this project was to measure the kinetics of the key processes occurring during sous vide cooking such as cook-loss and collagen solubilisation and their relationship to tenderness. The cook-loss and collagen solubilisation are important contributing factors to the meat tenderness and were given precedence over, for instance, myofibrillar proteins which are known to denature at comparatively low temperatures (refer to Table 2-8). The understanding of kinetics would also enable the development of a conceptual model demonstrating how sous vide conditions affect structure and composition of cooked meat and how this leads to tenderisation. A further aim was to evaluate the economic feasibility of the sous vide process and to compare it with alternative processes that may address the long cooking times that limit commercial applicability.

The conceptual model in combination with the economic feasibility will enable the industry to make wider sous vide applications.

# Chapter 2 Literature review

## 2.1 Introduction

The purpose of this chapter is to provide an overview of the sous vide cooking method and to briefly discuss the composition of meat; to highlight the different methods of tenderising meat and the underpinning mechanisms; to identify how changes in key components ultimately affect the meat tenderness; to review the kinetics of key processes and to identify opportunities for process improvement and optimisation.

## 2.2 Overview of the sous vide process

There are mainly two forms of sous vide cooking: (i) cook-hold or cook-serve and (ii) cook-chill or cook-freeze (refer to Figure 2-1) (Baldwin, 2012). The cook-hold or cook-serve method, which involves the following steps, is preferred by chefs because a large number of customers can be quickly served.

- preparing for packaging,
- vacuum packing,
- heating or pasteurising,
- finishing, and
- serving.

In contrast, the cook-chill or cook-freeze method is primarily used by the industry and involves the following steps. The first five steps are done by the industry to add value through increased tenderness, increased food safety, convenience, etc., and the last three steps are typically done by the customers or food service outlets.

- preparing for packaging,
- vacuum packing,
- pasteurising,
- rapid chilling,
- refrigerating or freezing,

- reheating or re-thermalising,
- finishing, and
- serving

**Figure 2-1.** Sous vide cooking flow diagram. The branches in red and green (the rightmost 3) are common in both restaurants and homes kitchens, while industrial food processors use only the branches in blue and green (the left most 3) (*Baldwin, 2012*).

### 2.2.1 Pre-packing treatments

To enhance the tenderness, tougher cuts of meat are often marinated with herbs and spices, brined, or mechanically tenderised before vacuum packaging. Marinades and brines can change the pH and ionic strength of the meat which may influence protein structure and denaturation during heating.

### 2.2.2 Vacuum packing

Once the meat is pre-treated (if any) it is vacuum packed. The vacuum sealing provides several advantages, including better heat transfer from the heating fluid to the food (by

removing air gaps which are a barrier to the heat transfer), better shelf life, by eliminating the risk of recontamination during storage; inhibition of off-flavours, due, for example, to oxidation; prevention of evaporative losses of volatiles flavours; and reduction of moisture losses during cooking (Church and Parsons, 2000). The absence of air also eliminates the growth of aerobic (spoilage) microorganisms, resulting in a more flavourful and nutritious product (Church, 1998, Myhrvold et al., 2011). To reduce the risk of pouch-ballooning (which is caused by steam from the water within the product), it is generally recommended that the highest possible vacuum should be used, typically 10-15 mbar for solids and 100-120 mbar for liquids (Baldwin, 2012). In some cases, a maximum vacuum may not be applied to certain products. For example, a vacuum of 10—15 mbar can significantly degrade the taste and texture of fish and poultry products (Norén and Arnold, 2009). In other words, the high vacuum mechanically damages the physical structure of these products. Myhrvold et al. (2011) suggest 30—50 mbar works best for nearly all foods.

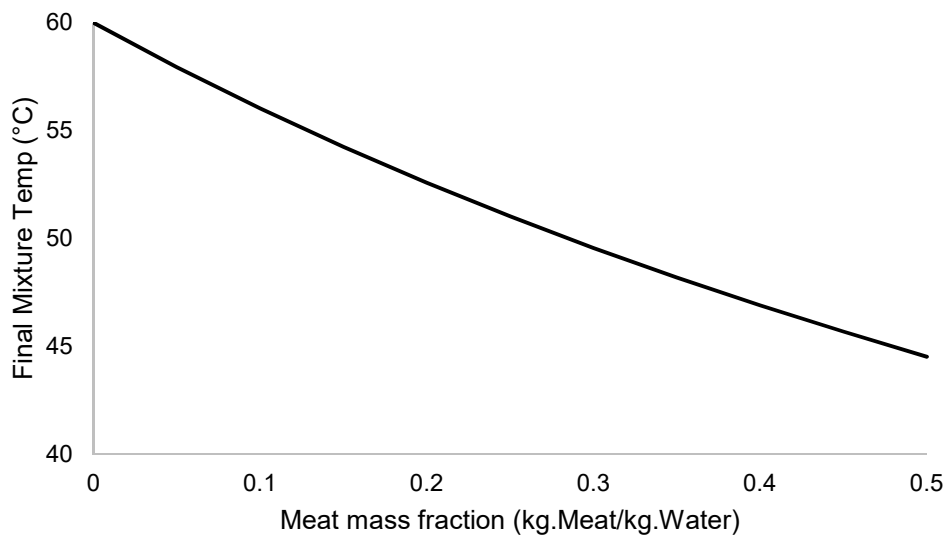
### 2.2.3 Cooking

Sous vide products are almost always cooked in water baths or steam convection ovens. However, other methods (which are not covered in this literature review) such as continuous microwave ovens are also employed.

#### 2.2.3.1 *Water baths*

Circulating water baths are popular methods of cooking because they provide uniform heating and smaller temperature fluctuations, typically less than  $\pm 0.1$  °C, which are essential for sous vide (Myhrvold et al., 2011). In an ideal system (before inserting the product) the water is preheated to the desired temperature and this is maintained by a Proportional-Integral-Derivative (PID) controller whilst at the same time the water is continuously circulated to ensure uniform temperature distribution. In reality, once the

product is inserted, the temperature of water bath drops (Figure 2-2, eq. (2.1)) and it might take some time before the desired temperature is re-established, depending on the kW capacity of the heating element (the larger the system capacity, the shorter the recovery time, Figure 2-3, eq. (2.2)). Accordingly, it is prudent to pre-heat the water to above the desired temperature (again this will depend on the quantity of product to be cooked and the kW capacity of the system).

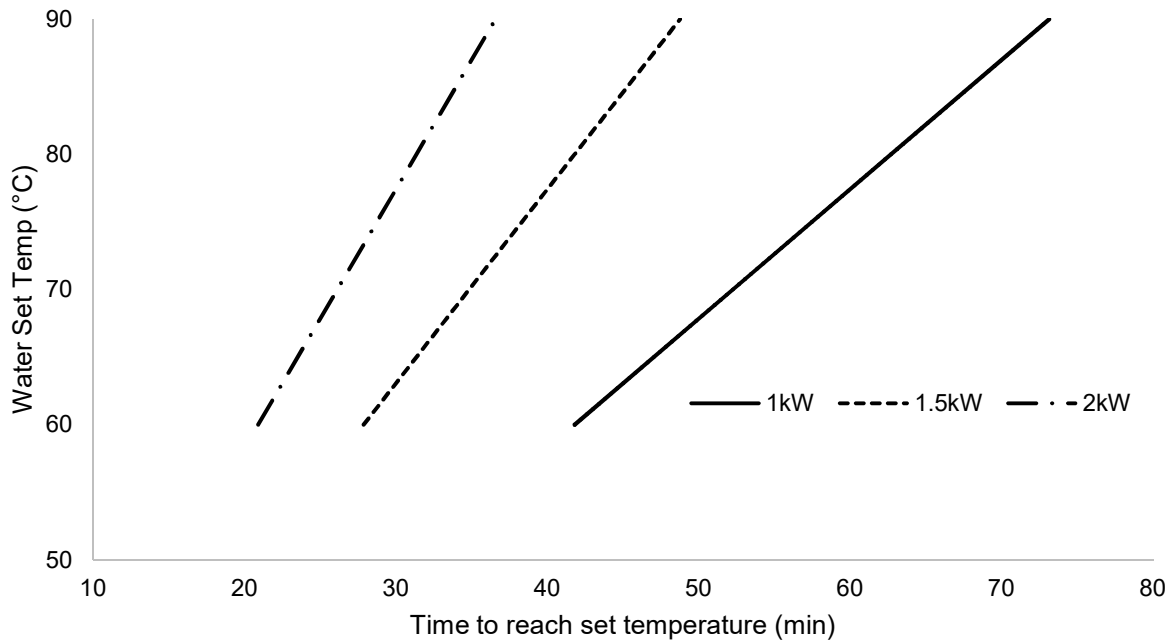


**Figure 2-2.** The temperature of the resulting mixture when fresh product is added to water bath at 60 °C, eq. (2.1) (heat loss was assumed to be negligible).

$$T_{\text{mixt}} = \frac{T_w C_{pw} + T_m C_{pm} X_m}{C_{pw} + C_{pm} X_m} \quad (2.1)$$

**Where:**

- $T_{\text{mix}}$  = mixture temperature (°C)
- $T_w, C_{pw}$  = water bath set temperature (°C) and water specif heat capacity (J kg<sup>-1</sup> °C<sup>-1</sup>)
- $T_m, C_{pm}$  = meat temperature (°C) and meat specif heat capacity (J kg<sup>-1</sup> °C<sup>-1</sup>)
- $X_m$  = meat fraction (kg.meat/kg.water)



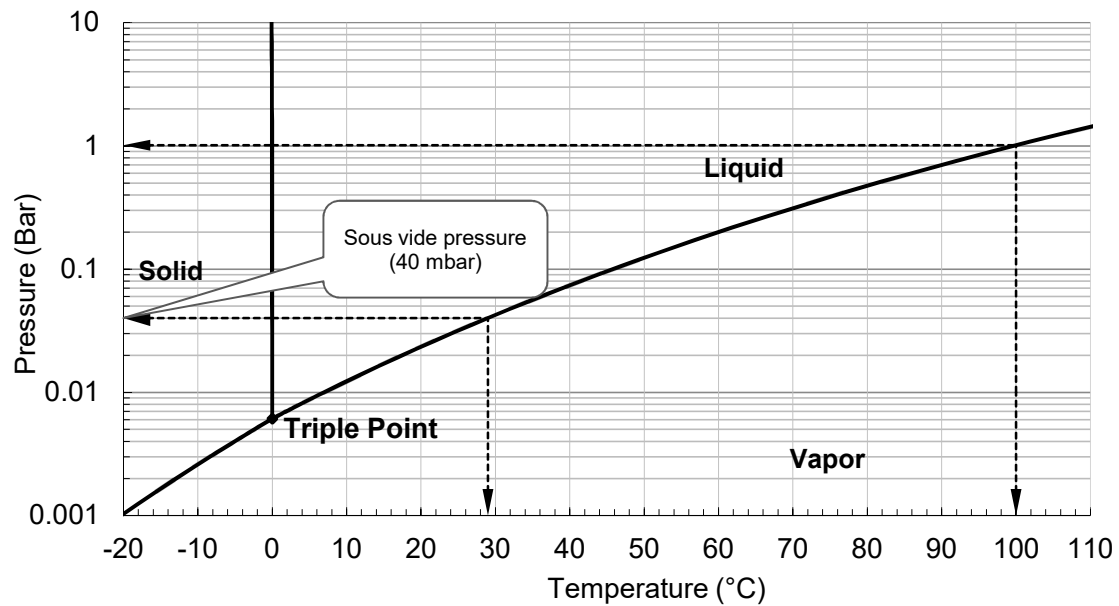
**Figure 2-3.** Time taken for 15 kg of water at 20 °C to heat up to different temperatures by different heating elements eq. (2.2).

$$t = \frac{m_w C_{pw} (T_{set} - T_{wi})}{6 \times 10^4 P}, \quad (2.2)$$

**Where:**

- t = time (mins) to reach a certain temperature (°C)
- $m_w, C_{pw}$  = mass (kg) and specific heat capacity ( $J \text{ kg}^{-1} \text{ °C}^{-1}$ ) of water
- $T_{set}$  = set temperature (°C) and meat specific heat capacity ( $J \text{ kg}^{-1} \text{ °C}^{-1}$ )
- $T_{wi}$  = initial water temperature (°C)
- P = system capacity (kW)

For the product to be cooked properly, the pouches must be completely under water and not overlapping or tightly arranged (Rybka, 1999). At low vacuum, the boiling point of water is significantly reduced (as Figure 2-4 shows) and enough vapour can evolve within the pouch which causes the pouches to balloon; thus a physical restraint, such as a wire rack, is required to keep it under water (Rybka, 1999).



**Figure 2-4.** The boiling point of water at different pressures (Chemicalogic, 2017)

### 2.2.3.2 Convection steam ovens

Steam ovens use heated air, injected steam, or a combination to cook the product. Unlike water baths, in steam ovens the product is first inserted into the oven and then the system is brought up to the desired temperature. A distinct advantage of steam ovens is the ability to cook large quantities of product (i.e. a higher production throughput) but have the drawback of poor temperature distribution (Sheard and Rodger, 1995). As mentioned before, a good temperature control is essential for sous vide cooking. Sheard and Rodger (1995) evaluated the temperature distribution of ten commercial steam ovens and found that none of the them heated the product uniformly when fully loaded and set to 80 °C. In fact, it took the slowest heating pouch 70—200% longer than the fastest one to reach a core of 75 °C from 20 °C. They concluded that the degree of non-uniformity presents the possibility of overcooking/undercooking if only one pack per batch is tested.

#### 2.2.4 The set and core temperatures

An important parameter is the set temperature, irrespective of the cooking method. Before the mid-2000s, the set temperature, for both water baths and steam ovens, was usually 5 — 10 °C higher than the desired core temperature (Baldwin, 2012). To prevent overcooking the product would normally be removed from the water upon reaching the desired core temperature (Baldwin, 2012, Myhrvold et al., 2011). In this technique, however, the product is considered unpasteurised since it did not spend sufficient time in the water bath (Myhrvold et al., 2011). As such, heating the product to the desired core temperature (or one to two degrees above it) and holding at this temperature for sufficient time is preferred (Myhrvold et al., 2011).

#### 2.2.5 Chilling and Finishing

In cook-freeze or cook-chill sous vide, the product is rapidly chilled and refrigerated (0—3 °C) or frozen after pasteurising (Vaudagna et al., 2008). A number of technologies, such as air blast chillers, ice water tanks, immersion chillers, tumble chillers, and integrated systems, are used. In cook-freeze or cook-chill, before serving, the food is reheated in a water bath to 50—55 °C, which is generally considered as the optimal temperature for serving meat (Charley and Weaver, 1998). However, such a low temperature may not be safe for poultry meat. Cook-freeze or cook-chill sous vide has numerous advantages (e.g. extended shelf life) but has also several disadvantages, including the need for expensive equipment and strict transport/storage temperature control (Siddiqui and Rahman, 2015).

#### 2.2.6 Finishing and Serving

Sous vide cooked foods have generally the appearance of being poached, hence fish and poultry can be served as they are (Baldwin, 2012). Contrastingly, steaks and pork are not traditionally poached and therefore often require searing or saucing before serving (Baldwin,

2012, Myhrvold et al., 2011). The searing/saucing improves the flavour, which is attributed to the Maillard reaction (complex reactions between amino acids and reducing sugars) and the thermal, oxidative degradation of fats (Tamilmani and Pandey, 2016). The Maillard reaction can be increased in several ways including, the addition of reducing sugars (such as glucose or fructose), increasing the pH, or raising the temperature (Meynier and Mottram, 1995). The pH influences the formation of intermediates in the Maillard reaction and has been shown to have significant influence on the end flavour – even a small increase in pH, results a sweeter, nuttier and more roasted meat (Meynier and Mottram, 1995).

## 2.3 The structure and composition of meat

Meat is a complex, multicomponent, heterogeneous food product. The composition of meat is approximately 75% water, 20% proteins, 3% fat, and 2% non-protein substances (metals, vitamins, carbohydrates, etc.) (Tornberg et al., 1997). These components have different functional properties and respond differently to physical, thermal, and mechanical stresses. In the current work, only a sub-group of proteins is of interest, i.e. those which play a significant role in the tenderness of meat. These proteins are divided into three main groups: connective tissue (10—15%), myofibrillar (50—55%), and sarcoplasmic proteins, Table 2-1 (30—34%) (Tornberg, 2005). These are further discussed in the following sections.

### 2.3.1 Connective tissue

The intramuscular connective tissue (IMCT) – which consists of insoluble proteins – can be thought of as the structural beams of a building, in the sense that it connects, supports, and separates other tissues or organs. It primarily consists of collagen and elastin fibers embedded in amorphous intercellular, mostly mucopolysaccharides, substances (Baldwin, 2008, Rinehart, 1951, Talwar, 2016). There are three levels of structure in IMCT: (i) the endomysium, which surrounds individual muscle fibres; (ii) the perimysium, which surrounds

bundles of muscle fibers; and (iii) the epimysium, which surrounds a whole muscle (Purslow, 2005, Nishimura, 2010). The total collagen content of a muscle can vary from 1 to 15% (dry weight basis), Table 2-1. Elastin accounts for only a fraction of the total, typically 0.6 to 3.7% (Purslow, 2005, Bendall, 1967, Nishimura, 2010).

**Figure 2-5.** The structure of a skeletal muscle (Kraemer et al., 2011).

#### 2.3.1.1 *Collagen*

Collagen is a fibrous, structural protein comprising of a right-handed bundle of three parallel, left-handed polyproline type II helices, Figure 2-6 (Shoulders and Raines, 2009). Collagen is the most abundant protein in animals (primarily due to skin) and is known for its outstanding mechanical properties (Fratzl, 2008). The IMCT collagen consists of collagen types I, II, III, IV, V, VI, VII, and XIV (Purslow, 2005, Bailey and Light, 1989, Nishimura, 2010). “Types I and III are the major fibre-forming types in endomysium, perimysium and epimysium, and type IV collagen is the principal non-fibre-forming component of the basement membrane linking the fibrous (reticular) layer of the endomysium to the muscle cell membrane (sarcolemma)” (Purslow, 2005).

**Figure 2-6.** the triple helix structure of collagen (*Shoulders and Raines, 2009*).

The toughness of a cooked meat is often ranked based on the collagen content; however, precise correlations between textural measurements (for example using the Warner-Bratzler shear-force WBSF), and the collagen content have been reported to be poor (Purslow, 2005, Nishimura, 2010, Purslow, 1999). Several studies have shown that the separation of the perimysium from the endomysium (Figure 2-7) in cooked meat is relatively easy, but the individual perimysial layers are very strong and difficult to fracture and are as such considered a major contributor to meat toughness.

**Figure 2-7.** Bovine *semitendinosus* muscle cooked to 80 °C and pulled perpendicular to the muscle fibre direction (Purslow, 2005).

Figure 2-7 shows the separation of a cooked muscle at 80 °C. The left-hand side of the figure shows the first phase of separation, where the endomysial and the perimysial layers separate, leaving the perimysium intact in the gaps between them (Purslow, 2005). The right-hand side of the figure shows that the perimysial layers have to be broken down to separate the muscle into pieces – this process mimics what happens during mastication (Purslow, 2005). The perimysium collagen content has been reported to vary much more in between muscles than the endomysium. Purslow (1999) reported that, in a study involving fourteen beef muscles, the perimysial collagen varied from 0.45 to 4.76% (dry weight basis), whilst the endomysial collagen varied from 0.47 to 1.2%. Light et al. (1985) also reported similar findings (1.4-7% perimysium, 0.1-0.5% endomysium). Although the techniques of separating these two structures are less than perfect, it is evident that it is the amount of perimysium that predominantly varies between functionally different muscles (Purslow, 2005).

Brooks and Savell (2004) studied the perimysium thickness of eight bovine animals and found that the perimysial thickness in a *semitendinosus* muscle was on average 2.4 that of psoas major from the same animal. Purslow (1999) conducted a similar study involving

fourteen beef muscles and found that the average relative difference (between the thickest and the thinnest) was 2.2 (although the primary perimysium had greater variability compared to the secondary perimysium, 2.53 vs 1.9). That being said, the findings of several studies (including Swatland et al. (1995)) indicate that the perimysium thickness does not significantly influence the meat tenderness. Purslow (1999) found that there was little correlation between the shear-force and the perimysium thickness of a meat cooked to 80 °C. He also showed that, though there were similarities in ranking toughness and perimysial content, the correlation between the WBS force and the perimysial content or perimysial thickness was poor.

#### 2.3.1.2 *Elastin*

Elastin is an insoluble, extracellular protein and the second most abundant in connective tissue (Wise and Weiss, 2009). It provides the flexibility of elastic tissue and can withstand decades of repetitive forces (Baldock et al., 2011). From tenderisation point view, elastin is difficult to fracture, but fortunately its content in meat is relatively low and is not a major factor in meat tenderness (Brown, 2014).

#### 2.3.2 Myofibrillar proteins

Myofibrillar proteins are the major components of the basic unit of the muscle fibre, the sarcomere (Figure 2-8). As shown on Figure 2-8, myosin forms the thick filaments while actin, tropomyosin, and troponin principally form the thin filaments of the sarcomere complex (Nishimura, 2010). Although there about 20 different myofibrillar proteins, as much as 65—70% are myosin or actin (Baldwin, 2012). According to Zarkadas et al. (1988), myosin and actin respectively account for 43 and 22% of the total myofibrillar proteins in skeletal muscles, Table 2-1. Troponin and tropomyosin have both a molar ratio of 1:7 with actin (Marston et al. (1998), Sousa and Farah (2002), Yates and Greaser (1983)). Assuming that

different types of muscles have approximately the same myosin to actin ratio of ~2:1 (43:22%), the percentages of troponin and tropomyosin can be estimated as follows:

$$\text{Tropomyosin} = \frac{1}{7} \text{Actin (on molar ratio)}$$

$$\text{Molar mass of actin} = 42 \text{ kDa}$$

$$\text{Molar mass of tropomyosin} = 70 \text{ kDa}$$

$$\text{Troponin (Troponin C, Troponin Tnl, Troponin TnT)} = 77.5 \text{ kDa (Januzzi (2010))}$$

$$\text{mass ratio} = \frac{65}{42} \left[ \frac{\text{kDa Tropomyosin}}{\text{kDa Actin}} \right] \times \frac{7}{1} = 0.238 \left( \frac{\text{Tropomyosin}}{\text{Actin}} \right)$$

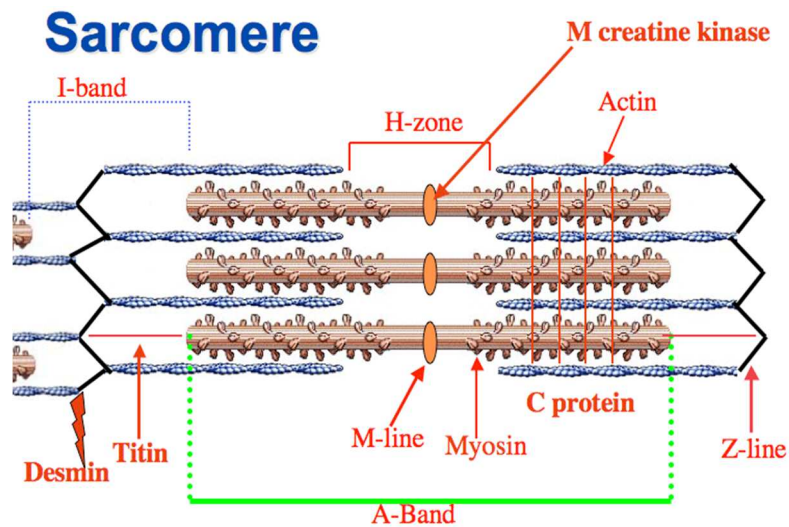
$$\begin{aligned} \therefore \text{Tropomyosin} &= 22\% \left( \frac{\text{Actin}}{\text{Myofibrillar}} \right) \times 0.238 \left( \frac{\text{Tropomyosin}}{\text{Actin}} \right) \\ &= 5.2\% \left( \frac{\text{Tropomyosin}}{\text{Myofibrillar}} \right) \end{aligned}$$

The same calculation was performed for troponin and was found to be 5.8%. Table 2-1 summarises the composition of meat.

**Table 2-1.** The composition of meat.

Component	Sub-components	Composition	Source
Meat	Water	75%	Tornberg et al. (1997)
	Proteins	20%	
	Fats	3%	
	Other	2%	
Main Proteins	Myofibrillar	50—55%	Tornberg (2005)
	Connective tissue	10—15%	
	Sarcoplasmic	30—34%	
Myofibrillar Proteins	Myosin	43%	Zarkadas et al. (1988)
	Actin	22%	
	Tropomyosin	5.2%	Calculated
	Troponin	5.8%	Calculated
Connective Tissue	Collagen	1—15%*	Nishimura (2010), Purslow (2005)
	Elastin	0.6—3.7% *	

\* Total quantity in muscle (on dry basis)



**Figure 2-8.** The structure of sarcomere (UNM, 2016).

Myofibrillar proteins play a key role in the contraction and relaxation of muscles and thus have an influence on the toughness of meat. When a carcass goes into rigor mortis, these proteins form a rigid complex which causes the muscle to toughen. Therefore, it is important to understand how this occurs.

We begin by first highlighting the key steps in the 'sliding filament theory' (Figure 2-9). When a muscle action is initiated, a number of things happen, which are summarised as follows (Marieb and Hoehn, 2013):

**Figure 2-9.** The sliding filament theory in muscle contraction (Marieb and Hoehn, 2013)

- An action potential is generated from the brain (voluntary activity) or from the spinal cord (reflex activity).
- The sarcoplasmic reticulum releases calcium ions ( $\text{Ca}^{2+}$ )
- The  $\text{Ca}^{2+}$  ions, bind to troponin, which then changes its conformation
- In the resting state of the muscle, the myosin active sites are blocked by tropomyosin, however, the conformation change in troponin forces tropomyosin to vacate the actin active sites and expose them for myosin head to bind.
- The myosin head (already in the cocked position and attached to ADP plus inorganic phosphate) binds to the actin active site.
- The ADP and inorganic phosphate are detached and the myosin head pivots, pulling the actin filaments towards the M-line and contracting the sarcomere.
- A new ATP binds to the myosin head and breaks the actin-myosin bridge
- Finally, the ATP is hydrolysed into ADP plus inorganic phosphate, the energy released is used to cock the myosin head, and the cycle starts all over again

When an aerobic animal dies, the carcass's oxygen (which is essential for the synthesis of ATP), is depleted. However, the body can still produce some ATP via anaerobic glycolysis. Eventually, the body's glycogen is depleted (or the pH drops to a point where anaerobic glycolysis stops), the ATP concentration drops, and the body enters rigor mortis – a state characterised by stiff limbs which is caused by the formation of actin-myosin cross bridge and sarcomere contraction. The absence of ATP means that this bridge cannot be broken down and continues to exist until endogenous enzymes (or bacteria) are activated to break down the rigid complex, rendering the muscles relaxed and tender once more.

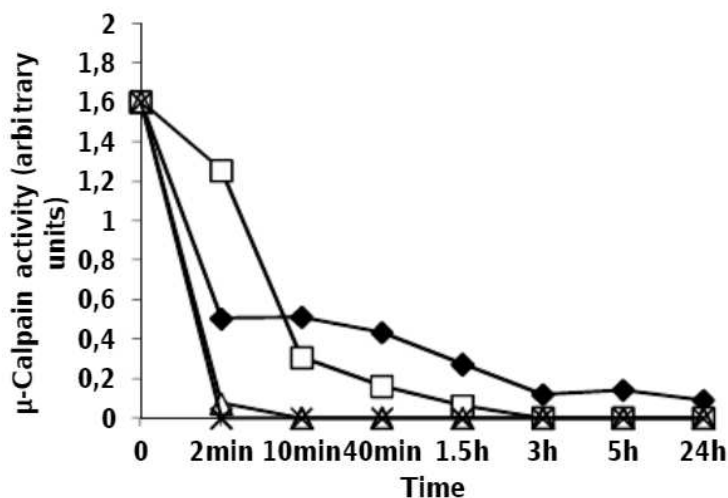
### 2.3.3 Sarcoplasmic Proteins

Sarcoplasmic proteins consist of about 50 components, mostly enzymes and myoglobin – the protein responsible for the colour of meat; specifically, the ratio of metmyoglobin (MMb<sup>+</sup>), myoglobin (MB), and oxymyoglobin (MbO<sub>2</sub>) determines the meat colour (Charley and Weaver, 1998, García-Segovia et al., 2007). The sarcoplasmic proteins are soluble in both water and low-strength ionic solutions (Tornberg, 2005). The calpain and lysosomal cathepsins are very important for the aging of meat (Tornberg, 2005). The calpain proteases consist of at least three enzymes:  $\mu$ -calpain, m-calpain, p94 or calpain 3, and an inhibitor of the  $\mu$  and m calpains (Brevie, 2007).

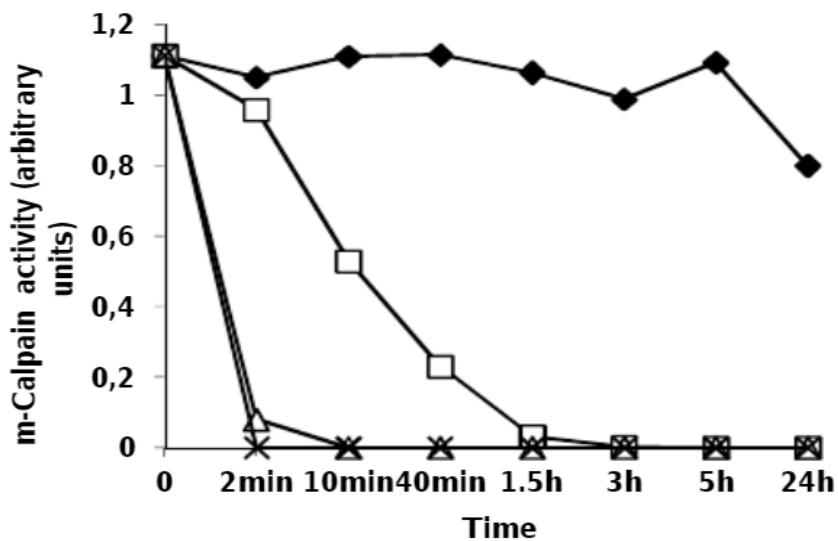
The calpains and cathepsins catalyse the hydrolysis of myofibrillar and connective tissue proteins: the calpains catalyse the hydrolysis of the Z-line proteins (Figure 2-8), while the cathepsins catalyse the hydrolysis of myosin, actin, troponin, and collagen (Baldwin, 2012, Kemp et al., 2010). However, the changes induced by the cathepsin enzymes are not well correlated with the tenderisation of meat in post-mortem aging (Taylor et al., 1995). For a proteolytic system to be considered as a contributor to meat aging (and hence tenderness), it must have access to the substrate and must produce the same patterns observed after post-mortem storage (Brevie, 2007). According to Brevie (2007), when cathepsins were incubated with myofibrillar proteins, they produced different patterns than those observed in post-mortem storage, and therefore it can be concluded that cathepsins have a limited role in post-mortem meat aging. The conclusions of Brevie (2007) are echoed by Du and McCormick (2009) who asserted that previous studies (e.g. Matsukura et al. (1981), Mikami et al. (1987)), which suggested cathepsins contribute to meat tenderness, are flawed because they were conducted under conditions of high temperature and low pH, which are atypical to a myofibrillar post-mortem environment. Another hurdle for the proponents of cathepsin-contributions to tenderness is the fact that actin and myosin are generally not degraded in post-mortem aging (Du and McCormick, 2009).

The muscle cellular environment undergoes a significant pH and ionic strength shift during the initial period of rigor mortis, from approximately neutral pH to about pH 5.6 and from approximately 165 mM NaCl in living muscle to approximately 295mM in meat (Maddock et al., 2005). Maddock et al. (2005) measured the  $\mu$  and m-calpains activities at pH of 7.5, 6.5, and 6.0 with either 165 or 295 mM NaCl ionic solutions. Overall, the  $\mu$ -calpain activity was highest at pH 6.5 at each ionic strength but decreased with increasing ionic strength (Maddock et al., 2005). The m-calpain activity was highest at pH 7.5 and was undetectable at pH 6.0. The m-calpain activity also decreased with increasing ionic strength (Maddock et al., 2005). Fidalgo et al. (2014) suggest 6.0 is the optimal pH for cathepsin B, which is approximately the pH of post-mortem meat. However, as discussed above, the role of cathepsins in post-mortem aging is disputed among the scholars.

Ertbjerg et al. (2012) studied the effect of time and temperature on the activity of extractable  $\mu$  and m-calpain and cathepsin B+L. Samples of porcine *longissimus* muscles at 24 hours post-mortem were incubated at 25, 40, 55, and 70 °C for 24 hours (Ertbjerg et al., 2012). As shown in Figure 2-10, Figure 2-11, at 25 °C  $\mu$ -calpain lost most of its activity within the first three hours, whereas the m-calpain was more stable, although there was a significant activity drop between 5 and 24 hours (Ertbjerg et al., 2012). At 40 °C, both the calpains were inactivated within three hours and at 55 and 70 °C within two minutes.

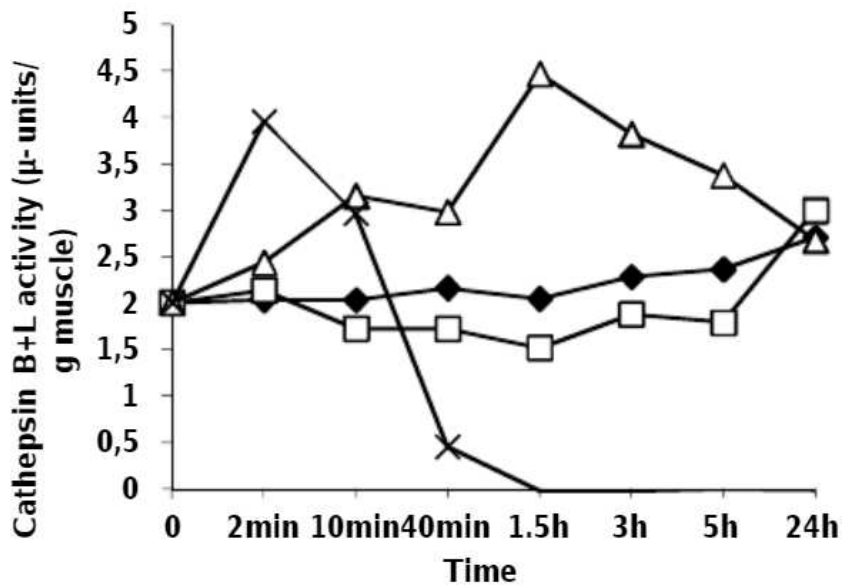


**Figure 2-10.** Extractable  $\mu$ -calpain activity from porcine *longissimus* muscle treated at ◆ 25, □ 40, △ 55, and ×70 °C for up to 24 hours (Ertbjerg et al., 2012).



**Figure 2-11.** Extractable m-calpain activity from porcine *longissimus* muscle treat at ◆ 25, □ 40, Δ 55, and ×70 °C for up to 24 hours (Ertbjerg et al., 2012).

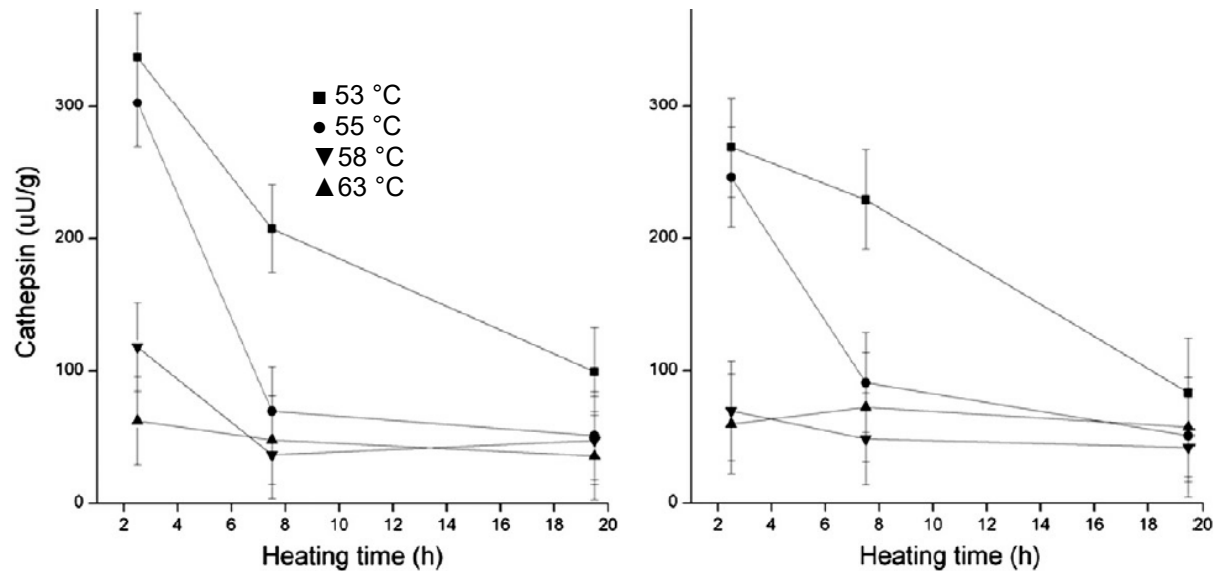
The tested cathepsin enzymes were significantly more heat-stable (than the calpains) and remained active throughout the experiments at 25, 40, and 55 °C, Figure 2-12 (Ertbjerg et al., 2012). In fact, at 55 °C, the enzymic activity increased with time, peaking at 1.5 hours, which suggests that part of the cathepsin exists as a pro-enzyme and is activated by heat (Ertbjerg et al., 2012). At 70 °C, the activity spiked at 2 minutes but declined rapidly thereafter and was completely lost at 1.5 hours (Ertbjerg et al., 2012). These results suggest that proteolysis is accelerated during the initial stage of the cooking process (Ertbjerg et al., 2012).



**Figure 2-12.** Extractable cathepsin B+L activity from porcine *longissimus* muscle treat at ◆ 25, □ 40, △ 55, and ×70 °C for up to 24 hours (Ertbjerg et al., 2012).

Christensen et al. (2013) studied the activity of cathepsins B+L in the cook-loss of *semitendinosus* samples from cows and young bulls, heated at 53, 55, 58 and 63°C for 2.5, 7.5, and 19.5 hours (Figure 2-13). The cathepsins activity was significantly influenced by the heating temperature, but there was no significant difference between the different animals, which suggests an equal proteolytic potential in long-time-low-temperature (LTLT) cooking (Christensen et al., 2013). The enzymatic activity was highest at 53 °C and decreased with increasing temperature and time. Unlike the 53 °C treated samples, the activity difference between 2.5 and 19.5 hours in the 63 °C samples, was almost zero. The presence of enzymatic activity even after 19.5 hours of cooking at 63 °C suggests that these enzymes have the potential to contribute to the mechanical weakening of muscle proteins, including collagen (Christensen et al., 2013). Heat induces unfolding of the triple helix structure of collagen which is thought to make the peptide chain more susceptible to enzymatic degradation, thereby increasing the collagen solubility (Christensen et al., 2013). Additionally, cathepsin L+B are endopeptidases that catalyse the hydrolysis of internal peptide bonds of proteins and have been previously reported to be involved in the

weakening of collagen, leading to greater collagen solubility (Christensen et al., 2011, Christensen et al., 2013, Ertbjerg et al., 2012). The temperature stability and tenderisation effects of the cathepsins make them important in the sous vide process.



**Figure 2-13.** Cathepsins B+L activity of *semitendinosus* from young bulls (left) and cows (right) heated at 53, 55, 58 and 63°C for 2.5, 7.5, and 19.5 hours (pH 5.4—5.8).

## 2.4 Meat Tenderisation and the effects of cooking on key components

The tenderness of meat is determined by three factors: the background toughness, the toughening phase, and the biochemical tenderisation phase (the latter two occur in the post-mortem storage period) (Brevie, 2007). The background toughness, defined as the resistance to shearing of the unshortened muscle, exists at the time of the slaughter, and does not (significantly) change with storage (Brevie, 2007)(Brevie, 2007) This toughness is caused by the connective tissue of the muscle. Specifically, the organisation of the perimysium appears to have a greater effect on this toughness, since positive correlation have been reported for both beef and chicken (Brevie, 2007).

(Brevie, 2007). When a carcass enters the rigor mortis phase, the sarcomere shortens, and the muscle toughens. According to Brevie (2007), studies have shown that there is a strong

relationship between sarcomere length and meat toughness – the shorter the sarcomere, the tougher the meat. A sarcomere length less than 2  $\mu\text{m}$  results in a tougher meat (Brevie, 2007).

Meat is normally heat treated before being eaten; accordingly, it is important to understand the changes that take place during cooking. The cooking temperature and time have a significant influence on the physical properties and the eating quality of meat (García-Segovia et al., 2007). The most important factors which control meat toughness are the myofibrillar and connective tissue proteins (García-Segovia et al., 2007). Heating induces significant changes, such as the destruction of cell membrane, shrinkage of meat fibers, the aggregation and gelation of myofibrillar and sarcoplasmic proteins, and the shrinkage and solubilisation of connective tissue proteins (García-Segovia et al., 2007, Tornberg, 2005).

The toughness (or tenderness) of meat is quantified by how easily it can be chewed or cut. Tenderness, juiciness, and flavour are considered the major components of food sensory quality (Vaudagna et al., 2008, Nishimura, 2010). When meat is chewed, it is deformed and fractured; the forces involved are shear, compressive, and tensile (Baldwin, 2012, Martens et al., 1982). To quantify tenderness, most studies use a Warner–Bratzler (WB) shear test, which involves cutting a cylindrical or rectangular sample perpendicular to the fibre direction with a flat blade. The WB measurements seems to correlate well with taste tests (Møller, 1981). The W—B shear decreases after cooking from 50°C to about 65°C and then increases in meat cooked up to 80°C (Baldwin, 2012).

The increase in tenderness is due to the weakening of the connective tissue. Although both the connective tissue and the myofibrillar proteins contribute to toughness, in many cuts, the connective tissue is the major source of toughness, and the collagen content and its solubility are considered the essential factors (Baldwin, 2012). Collagen from older animals or those which have endured extensive exercise is tougher than that of young animals or muscle of little exercise (Laakkonen et al., 1970). This toughness is caused by a higher degree of cross-linking, which intern elevates the denaturation temperature and reduces the

collagen solubility (Hill, 1966, Kim et al., 1967, Laakkonen et al., 1970, Schaub, 1963, Hopkins and Przybylski, 2015). Nishimura (2010) elucidated this point by stating as the animal grows, the collagen cross-linking attains a greater stability and the mechanical strength of the intramuscular connective tissue (IMCT) increases, contributing to the toughening of meat.

When myofibrillar proteins are the dominant source of toughness, it is termed actomyosin toughness (Nishimura, 2010) and can be a major factor in young animals or muscles of little exercise.

#### 2.4.1 Enzymatic Tenderisation

The calpain and cathepsin sarcoplasmic enzymes are essential for tenderising actomyosin toughness as they catalyse the breakdown of the myofibrillar proteins. As afore explained, when a carcass enters rigor mortis, the actin-myosin cross-bridge is locked in place (due to the absence of ATP) and the meat toughens. After some time, the calpain and cathepsin proteases are activated, the myofibrillar proteins are degraded and the meat regains tenderness (Brevie, 2007). The use of exogenous enzymes (traditionally derived from plants) is also possible.

#### 2.4.2 Artificial Tenderisation

##### 2.4.2.1 *Brining and marinating*

Brining and curing are popular methods for tenderising meat, especially for pork and poultry products (Baldwin, 2012, Shahidi et al., 2014). There are two methods of brining: traditional brining and equilibrium brining. In the former, the meat is placed in a 3—10% salt solution for about two hours (Myhrvold et al., 2011). Equilibrium brining is done as follows (Myhrvold et al., 2011, Baldwin, 2012):

- Meat and water are mixed and weighed
- 0.75—1.25% of the mixture-weight of salt is added for a brining or 2—3% for curing
- The meat is held in the solution until the salt concentration in the meat and the water have equalised (which can take hours or even days)
- Finally, the meat is rinsed and cooked as usual

Brining dissolves some of the support structure of the muscle fibres, impeding the formation of dense fibre aggregates (McGee, 1984). But it also allows the meat to absorb 10 to 25% of its weight in water, which may include aromatics from herbs and spices (McGee, 1984).

According to Offer et al. (1989), the myofibrils swell laterally and take up water – probably by an entropic mechanism. Studies have shown that seasoning with salt prior to cooking enhances juiciness, tenderness and browning (Juárez et al., 2011). The injection of sodium chloride solutions (especially those containing phosphates) have been reported to be highly effective in improving meat tenderness and juiciness (Juárez et al., 2011). Injecting the muscle with calcium-based salts stimulates the calpain system but can lead to loss of flavour (Juárez et al., 2011). An alternative option might be aqueous solutions containing ammonia, which have been reported to have similar results as calcium chloride and phosphate but without the negative effects on flavour (Juárez et al., 2011).

Marination is another method of pre-tenderising tough meat. “Most marinades are acidic as they contain either vinegar, wine, fruit juice, buttermilk or yoghurt. Acids disrupt the hydrogen bonds of the collagen fibrils and cause the connective tissue to swell” (Juárez et al., 2011). Introducing alcohol to acid marinated meat enhances the diffusion of the marinades, since alcohol can dissolve in fats (Juárez et al., 2011). It is, however, recommended that the use of alcohol be minimised as the lower vapor pressure of alcohol will cause the vacuumed pouches to balloon during cooking (Baldwin, 2012). The use of alcohol has also commercial implications. For instance, a meat treated with alcohol becomes non-Halal and is unsellable in the Halal market.

#### 2.4.2.2 *Mechanical tenderisation*

Mechanical tenderisation, also known as blade tenderisation or pinning, involves the insertion of a large number of closely spaced, very thin blades into the meat, which disrupt the internal fibres of the meat (Kerry and Ledward, 2009). A number of studies have shown that consumers were able to differentiate the tenderness of mechanically tenderised meat from that of natural tenderness (Suriaatmaja, 2013). The drawback of mechanical tenderising is the potential to push surface pathogens into the interior of the meat (a normally sterile environment); as such, mechanical tenderisation requires post pasteurisation to be safe (Baldwin, 2012, Juárez et al., 2011). Mechanical tenderisation may also detrimentally affect the shelf life, colour, drip loss (or cook-loss), and the yield of fresh beef (Kerry and Ledward, 2009).

#### 2.4.3 Heat tenderisation

Heat treatment of meat has three fundamental purposes: to render the meat safe, to tenderise it, and to develop its flavours. As discussed above, biochemical and artificial methods are important for tenderising meat; however, they are comparatively less effective than heat tenderisation. On the other hand, heat tenderisation is, of course, more severe than the other methods and results in a reduction in the functional properties of the meat (via denaturation, leaching, colour change, etc.). The essence of *sous vide* is finding the balance between food safety, nutrient retainment, and tenderness. The heat treatment has various effects on the many different components of meat. In the following sections, the key effects are highlighted.

During cooking, connective tissue, sarcoplasmic and myofibrillar proteins denature, shrink and their solubilities changes, leading to changes in mechanical properties, colour, and cooking losses of the meat (García-Segovia et al., 2007, Liu et al., 2013, Tornberg, 2005). The denaturation of these proteins is determined by the heating temperature and, to a lesser

extent, the time (Baldwin, 2012). Both the collagen and the myofibrillar proteins contract when heated, whilst the sarcoplasmic proteins expand (Davey and Gilbert, 1974). On the other hand, elastin fibers, the other major component of connective tissue, do not denature when heated and display rubber-like properties (Baldwin, 2012). However, as the elastin content is relatively minor, it does not play a major role in meat tenderness, except in muscles involved pulling the legs backwards or cuts from the neck and shoulders (Brown, 2014, Weston et al., 2002, Zayas, 1997). These claims are supported by the works of Lowe and Kastelic (1961) and Cross et al. (1973).

#### *2.4.3.1 Connective Tissue and gelatin formation*

Even though collagen represents only about 2% of the total protein in muscles, it is responsible for a large number of textural changes in cooked meat (Powell et al., 2000). When collagen is heated, it denatures, shrinks and begins to dissolve, as summarised below (Covington and Covington, 2009). Notice, only the first step is reversible.

Intact  $\rightleftharpoons$  Shrinking  $\rightarrow$  Shrunk  $\rightarrow$  Gelatinisation  $\rightarrow$  Solubilisation

The rate and the extent at which collagen changes occur depend on a number of factors, such as the maturity of the collagen, the heating rate, and relative humidity (Powell et al., 2000). According to Christensen et al. (2011), collagen denatures between 53 and 63 °C, which is postulated to involve the breakage of the hydrogen bonds, which loosens the fibrillar structure followed by the contraction of the collagen fibres. The effect of heat on collagen can be reversible or irreversible (Wright and Humphrey, 2002). Reversible changes, involving localised unfolding, are caused by moderate temperatures and can be reversed upon the restoration to normal temperatures (Wright and Humphrey, 2002). Severe heating, on the other hand, results in time-dependent, irreversible transformation of the native structure into water-soluble, random coils called gelatin (Wright and Humphrey, 2002). This transformation is caused by the breakage of longer sequences of hydrogen bonds that

normally stabilised the collagen triple helix (Wright and Humphrey, 2002). Powell et al. (2000) suggests at 64 °C, the triple helix begins to break down (starting at the ends of the molecule and at the period crimp), and, as the heating is continued, the molecule eventually shrinks to about ¼ of its original length, resulting in a toughening of the muscle (Powell et al., 2000, Purslow, 2005). Continuing the heating to 70—75 °C causes partial solubilisation of collagen and the formation of gelatin (Powell et al., 2000). Although there is no one specific temperature at which collagen is said to be completely denatured, the rate of denaturation does increase exponentially with higher temperatures, and – for safety reasons – 55°C is considered as the lowest practical temperature which collagen denatures (Baldwin, 2012).

When cooking tough cuts of meat, a long cooking time or a high temperature is necessary for the conversion of collagen to gelatin and the reduction of inter-fiber adhesion. Prolonged cooking has been reported to double tenderness by dissolving all the collagen into gelatin and almost completely destroying the inter-fiber adhesion of muscles (Baldwin, 2012).

Christensen et al. (2011), measured the meat toughness (WBS force), the cook-loss, the colour change ( $L^*a^*b$ ), the total collagen, and the heat-soluble collagen in *Longissimus dorsi* and *Semitendinosus* muscles from slaughter pigs and sows cooked to 48, 53, 58 and 63 °C for Tc (time to reach a core temperature equal to the water bath), Tc+5 hours, and Tc+17 hours.

The averaged results are shown in Table 2-2. As can be seen from the table, the sow muscles had higher collagen content but was more resistant to heat. The heat soluble collagen (measured in the cook-loss) in pigs increased both with increasing temperature and heating, Figure 2-14 (A, B), but no significant changes were observed for the sow muscles, Figure 2-14 (C) (Christensen et al., 2011).

**Table 2-2.** Mean values for animal weight, pH (24h post slaughter), total collagen and heat soluble collagen of *Longissimus dorsi* (LD) and *Semitendinosus* muscles (ST) from slaughter pigs and sows (Christensen et al., 2011).

		Pigs	n	Sows	n
Slaughter weight (kg)		79 ( $\pm$ 2)	12	172 ( $\pm$ 40)	12
pH24	LD	5.62 ( $\pm$ 0.05)	24	5.56 ( $\pm$ 0.07)	24
	ST	5.94 ( $\pm$ 0.19)	72	5.84 ( $\pm$ 0.23)	21
Total collagen (mg/g meat)	LD	4.2	a	5.3 ( $\pm$ 0.7)	12
	ST	7.7	a	8.0	a
Heat soluble collagen (% of total collagen)	LD	34.4%	a	12.5% ( $\pm$ 3.4)	12
	ST	39.5%	a	16.5%	a

<sup>a</sup> All raw materials within each muscle and type of animal were pooled.

**Figure 2-14.** Mean heat soluble collagen. A) *Longissimus dorsi* from slaughter pigs, B) *Semitendinosus* from slaughter pigs, and C) *Longissimus dorsi* from slaughter sows (Christensen et al., 2011).

Liu et al. (2013) and Li et al. (2010) also evaluated collagen solubility using goat *semimembranosus* and beef *semitendinosus* muscles, respectively. Their data has been used to produce Figure 2-15. As shows, the collagen solubility increased significantly between 50 and 60 °C and remained practically constant from 65 to 80 °C, (Liu et al., 2013)

– the solubility of the raw samples was only  $3.69 \pm 0.31$ . This suggests that collagen denatures between 50 and 60 °C and agrees with what Martens et al. (1982) reported (i.e. collagen denaturation occurs between 53 and 60 °C) (Liu et al., 2013). However, the results of Li et al. (2010) had somewhat erratic and unaccountable variations with temperature (except in the middle range, 60—80 °C) which they could not explain, Figure 2-15. Nonetheless, there is an obvious peak at 55 °C, which lends some support to the theory that collagen denatures between 50 and 60 °C.

**Figure 2-15.** Collagen solubility of goat *semimembranosus* muscle, *solid line* (note: the solubility of the raw samples was  $3.69 \pm 0.31$ ) and beef *semitendinosus* muscle, *dotted line*. The data used to plot graph is from Liu et al. (2013), (Li et al., 2010).

#### 2.4.3.2 Myofibrillar and sarcoplasmic proteins

The myofibrillar proteins start to shrink around 35—40°C and this continues almost linearly up to 70°C (Baldwin, 2012). The solubility of myofibrillar proteins increases with increasing temperatures as Figure 2-16 shows (Liu et al., 2013). Unlike connective tissue and myofibrillar proteins, which shrink when heated, sarcoplasmic proteins expand when heated (Baldwin, 2012). These proteins begin to aggregate and gel around 40 °C and finish around

65 °C. However, Davey and Gilbert (1974) reported the aggregation of sarcoplasmic proteins from a bull neck muscle could extend up to 90 °C. In any case, before the sarcoplasmic enzymes denature, they can significantly increase the tenderness of the meat (Baldwin, 2012). The aggregation of the sarcoplasmic proteins also contributes to the consistency of cooked meat by forming a gel between the different structural elements of the meat, thereby linking them together (Davey et al., 1976, Tornberg, 2005, Tornberg et al., 1997).

In sous vide, the temperatures are typically 55—65 °C; at these temperatures, many of the sarcoplasmic enzymes would have been denatured, however, some of the collagenases (enzymes attacking native collagen at near physiological pH) are still active and can significantly increase the tenderness after about six hours (Tornberg, 2005, Laakkonen et al., 1970).

The solubility of myofibrillar proteins does not significantly change with increasing end point temperatures, but significant solubility decrease (from raw) is observed for the sarcoplasmic proteins, Figure 2-16 (Liu et al., 2013, Tornberg et al., 1997)

**Figure 2-16.** The sarcoplasmic and myofibrillar protein solubilities as a function of end point temperatures (Liu et al., 2013).

### 2.4.3.3 *Cooking losses and meat tenderness*

Cooking causes the shrinkage of myofibrillar and sarcoplasmic protein as well as the shrinkage and solubilisation of collagen, which reduces the water holding capacity of the meat (García-Segovia et al., 2007, Liu et al., 2013, Tornberg, 2005). “The water-holding capacity of whole muscle meat is governed by the shrinking and swelling of myofibrils. Around 80% of the water in muscle meat is held within the myofibrils between the thick (myosin) and thin (actin) filaments” (Baldwin, 2012). A change of water content and/or its distribution is primarily due to changes in these voids (Offer et al., 1989, Liu et al., 2013). At lower cooking temperatures, between 40 and 60°C, the muscle fibres shrink transversely, widening the gap between the individual muscles fibres (Baldwin, 2012); at higher temperatures, 60-65 °C, the fibres shrink longitudinally, which increases with increasing temperature, causing significant water loss and toughening of the muscle (Baldwin, 2012). The shrinkage which causes the water loss is not limited to heat denaturation: it can also be caused by a fall in pH or the sarcomere contraction during rigor mortis (Tornberg, 2005). The key changes are summarised as below (Tornberg, 2005, Liu et al., 2013):

- A transverse shrinkage (to the axis of the fibres) occurs at 40—60 °C
- This shrinkage widens the gap already induced by rigor mortis between the fibres and their surrounding endomysium
- At 60-70 °C, the connective tissue and the muscle fibres cooperatively shrink longitudinally, increasing with temperature – this shrinkage causes the greatest water loss observed in cooking
- It is thought, then, that the water is squeezed out by pressure exerted by shrinking connective tissue. To be clear, water loss will inevitably occur even below 60°C, but the rate and level of water loss is comparatively less.

These statements are consistent with what Liu et al. (2013) experimentally found (Figure 2-17). That is, the cooking losses increased with increasing cooking temperature,

primarily in two distant phases. The first increase occurred at 60 to 65 °C and the second occurred at 70 to 75 °C (Liu et al., 2013). Although there are some differences, which may be due to the different muscle types, experimental conditions, etc., the work of Palka and Daun (1999) (Figure 2-18) showed similar results. There is also inverse relationship between the sarcomere length and cooking losses (Figure 2-18). According to Palka and Daun (1999), the sarcomere length appears to be a good indicator of changes in meat during cooking, particularly the cooking losses.

**Figure 2-17.** Cook-losses of goat *semimembranosus* muscles heated to different end point temperatures. The data used to plot graph is from Liu et al. (2013).

**Figure 2-18.** Cook-losses and sarcomere length of *semitendinosus* bovine muscle heated to different end point temperatures (Palka and Daun, 1999).

The overall water loss of the muscle is the evaporation from the surface and the exudate when the muscle is cut (Tornberg, 2005). The exudate (also known as drip loss), is drained from the cut surface of the muscle by gravity if its viscosity is low and the capillary forces are not strong enough to retain it (Tornberg, 2005). Offer et al. (1989) has shown that the drip loss is from the longitudinal channels between the fibre bundles. Allowing the meat to cool down before cutting, reduces its temperature which in turn increases its viscosity, facilitating gel formation and thus drip loss reduction.

Meat tenderness is inextricably linked to cooking losses since it is well established that water losses result in a tougher meat. Liu et al. (2013) found meat toughness (measured as the shear-force) occurred in four phases: (i) a rise from raw to 55 °C, (ii) a fall from 55 to 60 °C, (iii) a significant rise from 70 to 75 °C, and (iv) a fall from 75 to a stable value at 80 °C (Figure 2-19). The increase in shear-force below 60 °C is attributed to myofibrillar protein denaturation, whilst the second rise (below 80 °C) is attributed to the connective tissue denaturation. The cooking losses are believed to have contributed to this toughening as well (especially since it increased between 70 and 75 °C, see Figure 2-17) (Liu et al., 2013).

Bouton and Harris (1981) evaluated the tenderness of tough cuts of meat (from animals of one to four years old), cooked to 50—65 °C for 1 or 24 hours, Table 2-3. They found that meat cooked at 60 °C was significantly more tender than one cooked at 50 °C for the same amount of time. In fact, there was no significant difference in tenderness when the meat was cooked at 50 °C for 1 and 24 hours. However, there was a significant difference (26—76%) when the meat was cooked at 60 °C for 1 or 24 hours. Davies et al. (1976) reported at 80 °C, the tenderisation of beef *sternomandibularis* muscle occurs with 12—24 hours, and only a slight increase is achieved when the meat was cooked for 50-100 hours at 80 °C

**Figure 2-19.** Influence of end point cooking temperature on shear-force of goat *semimembranosus* muscles (Liu et al., 2013).

**Table 2-3.** effects of cooking at 50/60 °C for 1 or 24 hours (Bouton and Harris, 1981).

Parameter measured	Age group	Cooking procedure <sup>b</sup>	Cooking temp (°C)/time (hr)				LSD <sup>c</sup>
			50/1	60/1	50//24	60/24	
WB peak shear-force (kg)	Veal	N	17.69	6.60	15.32	2.38	1.31
		R	5.17	5.17	4.58	1.76	
	1 yr old	N	10.45	5.83	11.26	3.07	1.33
		R	7.12	6.26	5.30	2.70	
4-8 yr old	N	12.83	9.64	13.51	6.09	0.95	
	R	9.62	10.08	10.70	4.49		
Sarcomere of cooked meat (µm)	Veal	N	3.17	2.91	3.03	2.60	0.09
		R	2.07	2.14	2.19	2.45	
Cooking loss (%)	4-8 yr. old	N	3.30	6.60	12.70	30.6	0.5
		R	34.7	35.6	39.2	39.1	

<sup>a</sup> sampled both normally (N) and after a further cook at 80 °C for 1 hr (R) on the shear properties of DP muscle from veal, 1 yr and 4-8 yr old animals plus sarcomere lengths of the cooked veal muscles and cooking losses from the old animals

<sup>b</sup> N = Normally cooking procedure; R = recooked at 80 °C/1 hr

<sup>c</sup> Least significant difference at  $p < 0.05$

## 2.5 Thermal kinetics of key proteins and microbial inactivation

Determining the cooking kinetics allows one to model the behaviour of the key components under different cooking conditions, thereby facilitating process optimisation without a great deal of experimental work (i.e. a trial and error approach). In the literature, the heating

kinetics of meat proteins have been studied in two forms: (i) the isolated form, where specific proteins have been extracted, and (ii) the intact form, where the components of interest were studied while intact with the whole muscle. However, caution should be exercised when comparing the kinetics obtained from the two different methods because it is thought that the meat components act as a cooperative unit when intact, and therefore, the thermal properties of isolated components might be somewhat different to those of the intact form (Findlay et al., 1986).

Differential scanning calorimetry (DSC) is a popular method for studying meat protein denaturation kinetics\*. According to Miles et al. (1995), while there several methods (such as polarimetry, spectroscopy circular dichroism, etc.) for studying the physical state of certain proteins, most of these are not applicable to the measurement of fibrous tissue. DSC provides a potential method for examining changes in fibrous proteins and has been used extensively to study changes in collagen both in solution and in solid form (Graiver et al., 2006). However, calculating the activation enthalpy or entropy from DSC thermograms can be difficult – especially for collagen (Wright and Humphrey, 2002).

To extract kinetic parameters from the DSC thermogram, three methods have been developed over the years: (i) the D and z formulation, (ii) the Arrhenius equation, and (iii) the absolute rate theory. The details of these theories are discussed by Miles et al. (1995) and are not be covered here.

### 2.5.1 Connective tissue and sarcoplasmic proteins

Because of its small contribution to the overall composition of connective tissue, the denaturation and kinetics of elastin is seldom studied in the literature. Using a differential scanning calorimetry, Samouillan et al. (2011) evaluated the first and second transitions of elastin in hydrated and dehydrated state (the second transition is concerned with the glass transition temperature and occurs at very temperatures thus bores no relevance in the

current context). Figure 2-20 shows the thermogram of pre-frozen, hydrated elastin. Unlike collagen and pericardium, only one peak occurs around 0 °C which is attributed to the melting of ice (Samouillan et al., 2011). There is, however, a very small peak around 45 °C, but this is most likely an experimental anomaly – as opposed a true transition. The absence of transition peaks strongly supports the widely held believe that elastin does not denature when heated. Bailey and Light (1989) suggest that elastin denatures at 121 °C, which is well above the normal range of sous vide operating temperature. The small fraction of elastin present in meat and its high denaturation temperatures, makes unworthy of pursuing further.

**Figure 2-20.** DSC thermograms of hydrated elastin (faint-grey line) alone with collagen and pericardium (Samouillan et al., 2011).

The denaturation kinetics of collagen has been extensively studied (Liu and Li, 2010, Miles, 1993, Miles et al., 1995, Samouillan et al., 2011). Using differential scanning calorimetry, Miles (1993) studied the kinetics of collagen denaturation in intact mammalian lens capsules, isothermally stored in a water bath at different temperatures (45.7, 46.8, 47.9 and 49 °C). The results showed that the inactivation of native collagen followed approximately first-order kinetics (Figure 2-21), and the rate constant was highly temperature dependent – increasing by a factor of ten for every 2.3 °C rise in temperature, (Miles, 1993). The activation energy

and the enthalpy of activation were found to be  $860 \pm 44$  and  $858 \pm 45$  kJ/mol, respectively (Miles, 1993). The entropy was found to be positive and large,  $2.38 \pm 0.41$  kJ/mol.K, indicating significant structural change (Miles, 1993).

**Figure 2-21.** Enthalpy of denaturation (at 47.9 °C) as a function of time. Figures are expressed on basis of lens capsule dry matter (Miles, 1993).

Miles et al. (1995) did a similar study using collagen derived from rat tail. To investigate the length of the cooperative unit, the samples were immersed either in distilled water or 0.5 M (molar) acetic acid. The collagen fibrils swollen in acetic acid acted independently and their enthalpy of denaturation indicated a cooperative unit of  $65 \pm 5$  residues (Miles et al., 1995). Contrastingly, the water-swollen fibrils were intact and had a cooperative unit of only  $26 \pm 1$  residues long (Miles et al., 1995). The reduction in the size of the cooperative unit is compensated by the fact that the surrounding molecules stabilise the whole molecule (Miles et al., 1995). For the samples immersed in water, the activation enthalpy and entropy were  $518 \pm 16$  kJ/mol and  $1.485 \pm 0.049$  kJ/mol.K; the corresponding acetic acid figures were  $1306 \pm 0.099$  kJ/mol and  $4.142 \pm 0.323$  kJ/mol.K (Miles et al., 1995). Liu and Li (2010) studied the kinetics of the thermal denaturation of extracted type I collagen from bovine hide. The kinetics were evaluated using a DSC with different heating rates. The minor peaks in Figure 2-22 are due to the breakdown of the hydrogen bonds, whilst the major peaks are attributed to the transformation of the collagen helical structure (Liu and Li, 2010). Also from Figure 2-22, the basic shape of the graphs remains the same, except the peak temperature

( $T_{max}$ ) increases and shifts to the right with increasing heating rate (Liu and Li, 2010).

However, the change in enthalpy (H) was not significant ( $p>0.05$ ) with increasing heating rate and agrees with what Miles et al. (1995) observed (Liu and Li, 2010).

**Figure 2-22.** DSC curves of the 16 mg/ml collagen solution at different heating rates (Liu and Li, 2010).

Liu and Li (2010) evaluated the kinetic parameters of collagen using a *Multivar-NLR* kinetic analysis (essentially a model fitting approach). Four models (t:r,f, d:f,  $F_n$ , and  $F_1$ ) were fitted to the experimental data, and the obtained kinetics parameters are shown in Table 2-4. The four models were ranked based on the minimum sum of least squares (LSQ), the mean value of deviations (MD), the correlation coefficient, and visual comparison of predictions. The t:r,f *Lumry-Eyring* model (Eq. (2.3) was found to be the most suitable, Figure 2-23 (Liu and Li, 2010).



**Where:**

$N$	=	<i>the native triple helix state</i>
$U$	=	<i>the partially unfolded state</i>
$D$	=	<i>the denatured state</i>
$k_1, k_2, k_3,$	=	<i>the rate constant (1/s) for the three reactions</i>

**Table 2-4.** Kinetics parameters and reaction order for 8, 16 mg/ml collagen solution (Liu and Li, 2010)

Model	Kinetic parameters	Collagen	
		16 mg/ml	8 mg/ml
t:r,f	$\lg A_1/s^{-1}$	240.827	294.581
	$E_1/kJ mol^{-1}$	1444.115	1763.860
	$n_1$	1.910	2.058
	$\lg A_1/s^{-1}$	75.276	37.147
	$E_2/kJ mol^{-1}$	450.824	218.743
	$n_2$	2.019	1.937
	$\lg A_3/s^{-1}$	43.338	17.149
	$E_3/kJ mol^{-1}$	263.474	106.896
	$n_3$	1.417	1.159
d:f	$\lg A_1/s^{-1}$	205.952	214.938
	$E_1/kJ mol^{-1}$	1237.194	1292.312
	$n_1$	1.851	1.8711
	$\lg A_1/s^{-1}$	79.713	24.081
	$E_2/kJ mol^{-1}$	484.318	150.597
	$n_2$	1.259	0.773
Fn	$\lg A_1/s^{-1}$	182.894	173.633
	$E/kJ mol^{-1}$	1101.228	1046.774
	$n$	1.895	1.637
F1	$\lg A_1/s^{-1}$	139.770	141.912
	$E_1/kJ mol^{-1}$	844.63	857.836

**Figure 2-23.** The experimental data (solid lines) versus the predicted figures (dotted lines) (Liu and Li, 2010).

Kajitani et al. (2011) studied the kinetics of cured pork containing different amounts of sodium chloride. The DSC thermograms showed three distinct peaks, attributed to (i) myosin, (ii) sarcoplasmic protein and collagen, and (iii) actin (Kajitani et al., 2011). As the salt concentration was increased, the rate constant increased for all proteins but to different extents (Kajitani et al., 2011). The average activation energy for the sarcoplasmic proteins and collagen was  $326 \pm 61$  kJ/mol and the Arrhenius frequency factor ranged from  $2.3 \times 10^{40}$  to  $4.63 \times 10^{48} \text{ s}^{-1}$  (Kajitani et al., 2011). Bertola et al. (1994) studied the kinetics of *semitendinosus* muscle from 26 steers cooked to 66, 67.5 and 68°C. Their DSC scans also showed three distinct peaks (Figure 2-24) of which the second peak was thought to be mostly collagen (Bertola et al., 1994). The activation energy was found to be 604.6 kJ/mol (Bertola et al., 1994). Machlik and Draudt (1963) reported a similar figure of 589.4 kJ/mol for *semitendinosus* beef muscle.

**Figure 2-24.** DSC scans of semitendinosus beef muscle proteins after different heat treatments (Bertola et al., 1994).

Considerable controversy surrounds the various methods developed over the years for determining the kinetics parameters (i.e., the activation energy,  $E_a$ , and the frequency factor,  $k_0$ ). Traditionally, it was assumed that the activation energy is independent of temperature and that the frequency factor carries similar importance as the activation energy. However, Vyazovkin (2006) has put forth a convincing argument, challenging these assumptions. His argument hinges on three key points: (a) contrary what was traditionally assumed, most reactions cannot be approximated by a simple first order reaction; rather, multiple reactions are involved; (b) the activation energy varies with temperature but is *constant* for a given fractional conversion ( $\alpha$ ); and (c) the frequency factor ( $k_0$ ) has no physical meaning, therefore there is no point in determining it. Apart from the interpretability issue, the frequency factor tends to be stupendously large and thus masks changes arising from a change in the activation energy. However, it is possible to circumvent this problem by employing a reparameterised form of the Arrhenius expression, Eq. (2.4).

$$k = k_r \cdot \exp \left[ -\frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T_r} \right) \right] \quad (2.4)$$

**Where:**

$k$	=	rate constant (1/sec or 1/hr)
$k_r$	=	rate constant (1/sec or 1/hr) corresponding to a reference temperature
$T_r$	=	Reference temperature (K)
$t$	=	Time
$E_a$	=	Activation energy (J/mol)
$R$	=	universal gas constant (J/mol/K)

Vyazovkin (2006) has proposed an alternative method: the *model-free kinetics* (sometimes referred to as the *advanced Vyazovkin isoconversional method*), which is essentially a minimisation function, Eq. (2.5) – for full details, please refer to Vyazovkin (2006), Vyazovkin et al. (2007). Dubaj et al. (2015) proposed a somewhat similar method: the isoconversional, incremental method. The kinetic model is directly fitted to the experimental data using orthogonal non-linear regression least square fitting (Dubaj et al., 2015). To preserve the experimental error distribution, the data is processed without any transformation (Dubaj et al., 2015). Moreover, unlike the Vyazovkin approach, the frequency

factor ( $k_0$ ) and the activation energy ( $E_a$ ) are treated as equally important and are determined simultaneously because – they claim, in solid state kinetics – the activation energy should not be reported and interpreted in isolation from the value of the frequency factor. They also assumed that both parameters depend on the fractional conversion and there exists a linear relationship between the parameters as described by Eq. (2.6).

$$\Phi(E_\alpha) = \sum_{i=1}^n \sum_{j \neq i}^n \frac{J[E_\alpha, T_i(t_\alpha)]}{J[E_\alpha, T_j(t_\alpha)]} \quad (2.5)$$

*Where:*

$$J[E_\alpha, T_i(t_\alpha)] \equiv \int_{t_\alpha - \Delta\alpha}^{t_\alpha} \exp\left[\frac{-E_\alpha}{RT_i(t)}\right] dt$$

$$\ln(k_0(\alpha)) = b + eE_a(\alpha) \quad (2.6)$$

*Where  $b$  &  $e$  are fitting parameters*

Using the model-free kinetics and the Kissinger method (as a comparison), Vyazovkin et al. (2007) evaluated the thermal denaturation of collagen from re-hydrated bovine Achilles tendon. From Figure 2-25, the Kissinger method predicted an activation energy of 319 kJ/mol (A), but the model-free yielded a decreasing activation energy with increasing reaction fractional conversion. The observed decreased is explained by the Lumry-Eyring model for collagen denaturation, see Eq. (2.3) (Vyazovkin et al., 2007). The decreasing activation energy pattern is in agreement with what Liu and Li (2010) reported, Table 2-4 – except the values of the Liu and Li (2010) are higher, which might be due to the fact that their samples were in solution, suggesting that the kinetic parameters obtained from solutions are not comparable to those obtained from solid samples.

**Figure 2-25.** The activation energy of re-hydrated bovine Achilles collagen as predicted by the Kissinger method (**A**) and by the model-free method (**B**) (Vyazovkin et al., 2007).

Dubaj et al. (2015) evaluated their model by applying it to the non-isothermal cold crystallization of polyethylene terephthalate (PET). The activation energy was found to be dependent on the conversion and decreased with increasing conversion until eventually plateauing (Figure 2-26), which is similar to what Vyazovkin et al. (2007) reported (Dubaj et al., 2015). Figure 2-27 also shows that the kinetic parameters ( $E_a$  and  $k_o$ ) are strongly correlated ( $r = -0.992$ ), and therefore no conclusion can be drawn from only one parameter (Dubaj et al., 2015). Although both methods appear to work rather well, they both require multiple heating rates and are computationally demanding – especially the Vyazovkin method even though it provides only one kinetic parameter,  $E_a$ .

**Figure 2-28.** The activation energy and the natural logarithm of the frequency factor ( $A=k_0$ ) as a function of conversion (Dubaj et al., 2015).

## 2.5.2 Myofibrillar Proteins

### 2.5.2.1 Myosin

Using differential scanning calorimetry at different heating rates, Beas et al. (1991) studied the thermal denaturation and the kinetics of myofibrillar proteins from pre and post-spawning hake fish. All the post-spawning proteins had higher enthalpies compared to the pre-spawning— in other words, the post-spawning proteins were more temperature sensitive as demonstrated by the values of the following kinetic parameters. The activation energy ( $E_a$ ), the reaction rate constant ( $k$ ), and the frequency factor ( $k_0$ ) were found to be 218 versus 283 kJ/mol, 5.09 versus 21 min<sup>-1</sup>, and  $9.33 \times 10^{35}$  versus  $4.94 \times 10^{48}$  for pre-and post-spawning hake fish, respectively (Beas et al., 1991). Kajitani et al. (2011) reported a similar activation energy value of  $241 \pm 45$  kJ/mol for cured pork samples.

### 2.5.2.2 Actin

Kajitani et al. (2011) found the thermal inactivation energy of actin to be  $250 \pm 100$  kJ/mol. Bertola et al. (1994) reported somewhat higher value of 362.4 kJ/mol for actin derived from bovine, which agrees with what was reported in earlier studies: 350 kJ/mol (Martens et al.,

1982) and 378.7 kJ/mol (Wagner and Anon, 1985). However, the findings of Findlay et al. (1986) seem rather different than what is reported by the other authors. According to Figure 2-24, the  $T_{\max}$  for peaks I, II, and III are (approximately) 53, 64 and 76 °C, whilst the corresponding figures reported by Findlay et al. (1986) (Figure 2-29) are (approximately) 56, 67 and 82 °C. According to the work of Martens et al. (1982),

**Table 2-5**, no active actin should remain at 82 °C. Furthermore, the actin activation energy (654 kJ/mol at  $T_{\max}$  of 82 °C) reported by Findlay et al. (1986) is significantly higher than those reported by the other authors. Findlay et al. (1986) have, however, stated that the thermal properties of proteins are quite different when they are studied in isolation and in situ. Findlay et al. (1986) found that the rate constant for actin was  $0.045 \text{ min}^{-1}$  and the reaction orders were 0.6 to 2.1, 3.5 to 5.1 and 1.5 to 3.1 for peaks I, II, and III, respectively.

**Figure 2-29.** DSC curves of beef *sternomandibularis* muscle with sarcomere lengths of 1.4 and 2.4  $\mu\text{m}$  at 5 °C for 4 days post-mortem (Findlay et al., 1986).

**Table 2-5.** The denaturation of various proteins in bovine *m. semimembranosus* muscle after 5 minutes of holding time at different temperatures (Martens et al., 1982).

End Temperature (°C)	45	53	63	67	73	79	85
STRUCTURAL PROTEIN:							
Myosin (LMM)	80	20	0	0	0	0	0
Myosin (HMM)	100	100	0	0	0	0	0
Collagen	100	100	0	0	0	0	0
Actin	100	100	100	80	10	0	0
SOLUBLE PROTEINS:							
Sarcoplasmic proteins	100	90	30	10	1	0	0
Heme-proteins (myoglobin, haemoglobin)	100	100	100	100	70	10	0

Partial denaturation fractions are approximate  
LMM = Light Meromyosin, HMM = Heavy Meromyosin

### 2.5.2.3 Tropomyosin

Chen et al. (2006), using an enzyme-linked immunosorbent assay (ELISA), studied the thermal denaturation of tropomyosin (a myofibrillar protein). The kinetic parameters were derived from isothermal heating of beef muscle extracts at 54.4, 57.2, 60, and 62.8 °C (Chen et al., 2006). As Figure 2-30 shows, as the temperature was increased, the rate of reaction accelerated (Chen et al., 2006). This is demonstrated by the high-temperature dependency of the reaction rate constants (0.0016, 0.0139, 0.0507 and 0.14 s<sup>-1</sup> at 54.4, 57.2, 60, and 62.8 °C) (Chen et al., 2006). The denaturation reaction was shown to follow first order kinetics (between 54 and 70 °C) with an activation energy of 484 kJ/mol (Chen et al., 2006). A predictive model was developed and was evaluated under different conditions (e.g. by

holding the temperature constant but varying the heating time) (Chen et al., 2006). The model was in close agreement with the experimental data – the percent error between the predicted and the measured ranged from -5.1 to +5.3% (Chen et al., 2006).

**Figure 2-30.** Effects of temperature and time on residual tropomyosin in muscle extracts under isothermal conditions (Chen et al., 2006).

### 2.5.3 Myoglobin

Myoglobin is responsible for the red colour of meat; hence the doneness of a steak can be related to the denaturation of myoglobin. A redder meat (rare to medium rare) has less myoglobin denaturation, whereas a lighter meat (well done) has most (if not all) of its myoglobin denatured. Myoglobin and haemoglobin are substantially less stable in meat than when in solution at the same pH and ionic strength (Geileskey et al., 1998). Ledward (1978) suggests that at temperatures well below its denaturation temperature, myoglobin undergoes a conformational change, partially exposing the haem (also spelt heme) and allowing it to interact with the sarcoplasmic proteins, which themselves have been already denatured at this temperature. Other mechanisms have also been suggested; but irrespective of the exact mechanism, it is obvious the thermally induced changes of myoglobin lead to colour changes in meat, which has implications for the quality and the safety of the product (Geileskey et al., 1998).

Geileskey et al. (1998) studied the rate of cooked meat hemoprotein formations (measured as the rate of loss of myoglobin solubility) and found that it initially obeys first order kinetics. However, at 70 °C and above, the expected rate is much slower than what was experimentally observed, Figure 2-31. This suggests that at a higher temperature, there is a different mechanism operating, i.e. the reaction no longer obeys first order kinetics (Geileskey et al., 1998). Nonetheless, the critical denaturation temperature for myoglobin is 55 – 70 °C (Geileskey et al., 1998). Sen et al. (2014) found that the percent myoglobin denaturation in mutton cooked to end temperatures of 51, 65, 71 and 79 °C were 25.7, 45.7 62.6 and 77.3%, respectively.

**Figure 2-31.** Arrhenius plots for myoglobin precipitation in different cuts of meat (Geileskey et al., 1998). The calculated activation energy values are shown in Table 2-6.

**Table 2-6.** Activation energies for myoglobin denaturation in meat, aqueous muscle extracts and BSA - myoglobin mixture (Geileskey et al., 1998).

System	Ea <sup>a</sup> (kJ mol <sup>-1</sup> )	r <sup>a</sup>	Ea <sup>a</sup> (kJ mol <sup>-1</sup> )	r <sup>b</sup>
Beef shin	237	0.980	284	0.998
Beef chuck	230	0.989	290	0.999

Beef <i>m. l. dorsi</i>	216	0.972	309	0.999
Lamb <i>m. l. dorsi</i>	189	0.986	218	0.992
Aqueous extract	194	0.976	250	–
BSA/Mb solution	–	–	344	0.999

r=linear correlation coefficient for the Arrhenius plots over the above temperature ranges.

<sup>a</sup> including rate constants found at 75 and 80 °C.

<sup>b</sup> Over the range 55 to 70 °C.

As Table 2-7 shows, the denaturation of myoglobin in beef *m. l. dorsi* was much faster than in chuck or shin beef at all temperature, while overall, at 60 and 65 °C, lamb *m. l. dorsi* was the fastest. All muscles yielded similar activation energies of about 250 kJmol<sup>-1</sup> (Geileskey et al., 1998).

**Table 2-7.** Rate constant for the different muscles at different temperatures (Geileskey et al., 1998).

Muscle	Temp (°C)	Rate (min <sup>-1</sup> )	pH	No. of samples
Beef shin	60	0.027 ± 0.003	5.79 ± 0.03	2
Beef chuck	60	0.035 ± 0.013	5.56 ± 0.06	7
Beef <i>m. l. dorsi</i>	60	0.065 ± 0.020	5.58 ± 0.08	9
Lamb <i>m. l. dorsi</i>	60	0.125 ± 0.005	5.38 ± 0.02	2
Beef shin	65	0.186 ± 0.003	5.47 ± 0.06	2
Beef chuck	65	0.150	5.57	1
Beef <i>m. l. dorsi</i>	65	0.330	5.57	1
Lamb <i>m. l. dorsi</i>	65	0.635 ± 0.025	5.38 ± 0.02	2
Beef shin	70	0.61 ± 0.03	5.76 ± 0.05	2
Beef chuck	70	0.74 ± 0.014	5.56 ± 0.08	20
Beef <i>m. l. dorsi</i>	70	1.68 ± 0.07	5.56 ± 0.07	4
Lamb <i>m. l. dorsi</i>	70	1.46 ± 0.05	5.38 ± 0.02	2
Beef shin	80	3.70 ± 0.10	5.73 ± 0.05	2
Beef chuck	80	3.84 ± 0.12	5.73 ± 0.05	4
Beef <i>m. l. dorsi</i>	80	5.58 ± 0.12	5.58 ± 0.07	4
Lamb <i>m. l. dorsi</i>	80	5.57 ± 0.04	5.38 ± 0.02	2

## 2.5.4 SUMMARY – Denaturation temperatures and kinetics parameters

### 2.5.4.1 Denaturation temperatures of key proteins

It is rather difficult to pin-point the starting and ending denaturation points for proteins, therefore the given range might not hold for all proteins in all situations.

**Table 2-8.** Denaturation temperatures of key proteins.

PROTEIN	Denaturation Temperature [°C]	Source
Collagen	54 – 75	(Baldwin, 2012)
	50 – 75	(Powell et al., 2000)
	53 – 63	(Christensen et al., 2011, Martens et al., 1982)
Sarcoplasmic Proteins	40 – 60	(Tornberg, 2005)
Myosin	35 – 70	(Tornberg, 2005)
	54 – 58	(Graiver et al., 2006)
Actin	71 – 83	(Findlay et al., 1986, Gómez-Guillén et al., 2011)
	66-73	(Liu et al., 2013)
Myoglobin	45-80	(Geileskey et al., 1998)
	51-79	(Sen et al., 2014)

### 2.5.4.2 Summary of kinetic parameters

In Table 2-9, the kinetic parameters obtained in the literature review are summarised.

**Table 2-9.** Summary of kinetics parameters.

Protein	E <sub>a</sub> [kJ mol <sup>-1</sup> ]	k [min <sup>-1</sup> ]	k <sub>o</sub> [min <sup>-1</sup> ]	n [--]	Sample Source	Reference	
Actin	250 ± 100	0.1 (at 70 °C with 2 mg/g NaCl)	1.47x10 <sup>37</sup>	n=1	Pork, sirloin	(Kajitani et al., 2011)	
	362.4	0.002 (at 66 °C)	6.4x10 <sup>35**</sup>	n=1	Bovine, <i>semitendinosus</i> muscle	(Bertola et al., 1994)	
	350 (at pH 5.4), 381 (at pH 6.2)	--	1.3x10 <sup>52</sup> , 9.4 x10 <sup>61</sup>	n=1	Bovine, <i>m. semimembranosus</i> muscle	(Martens et al., 1982)	
	378.7 ± 28.5	--	3.9x10 <sup>57</sup>	n=1	Bovine, <i>semitendinosus</i> muscle	(Wagner and Anon, 1985)	
	654 (at T <sub>max</sub> 82 °C)	0.045	7.8x10 <sup>56</sup>	n= 1.5-3.1	Beef, <i>sternomandibularis</i> muscle	(Findlay et al., 1986)	
Collagen (and sarcoplasmic proteins)	860 ± 44	--	--	n≈1	Intact mammalian lens capsules	(Miles, 1993)	
	E <sub>a1</sub> : 1444, 1763.9 E <sub>a2</sub> : 450.8, 218.7 E <sub>a3</sub> : 263.5, 106.9	--	k <sub>o1</sub> : 3.9x10 <sup>104</sup> , 8.6 x10 <sup>127</sup> k <sub>o2</sub> : 4.9x10 <sup>32</sup> , 1.4 x10 <sup>16</sup> k <sub>o3</sub> : 6.6x10 <sup>18</sup> , 2.8 x10 <sup>7</sup>	n <sub>1</sub> : 1.9, 2 n <sub>2</sub> : 2, 1.9 n <sub>3</sub> : 1.4, 1.16	bovine hide (solution, 16, 8 mg/ml)	(Liu and Li, 2010) (t:r,f model)	
	326 ± 61	--	2.3x10 <sup>40</sup> to 4.63x10 <sup>48</sup>	--	Pork, sirloin	(Kajitani et al., 2011)	
	604.6 kJ/mol	0.013 (at 66 °C)	1.65x10 <sup>90**</sup>	Assumed n=1	Bovine, <i>semitendinosus</i> muscle	(Bertola et al., 1994)	
	589.4	--	--	--	Beef, <i>semitendinosus</i> beef	(Machlik and Draudt, 1963)	
Myoglobin	E <sub>a1</sub> : 284 E <sub>a2</sub> : 290 E <sub>a3</sub> : 309 E <sub>a4</sub> : 218	k <sub>1</sub> : 0.027 ± 0.003 k <sub>2</sub> : 0.035 ± 0.013 k <sub>3</sub> : 0.065 ± 0.020 k <sub>4</sub> : 0.125 ± 0.005 (at 60 °C)	-- --	n=1 (for the first part of the reaction)	Beef shin, beef chuck, beef <i>m. l. dorsi</i> and lamb <i>m. l. dorsi</i>	(Geileskey et al., 1998)	
	Myosin	218, 283 (pre-&-post spawning)	5.09, 21	9.33x10 <sup>35</sup> 4.94x10 <sup>48</sup>	--	Hake fish tissue	(Beas et al., 1991)
		241 ± 45	--	--	--	Pork, sirloin	(Kajitani et al., 2011)
	Tropomyosin	484	3.042, 8.616 (at 60, 62.8 °C)	2.36x10 <sup>76**</sup> , 1.56x10 <sup>76**</sup>	n=1	Bovine muscle extract	(Chen et al., 2006)

Note: E<sub>a</sub> = activation energy, k= rate constant, k<sub>o</sub> = Arrhenius frequency factor, n = reaction order. \*\* has been calculated using the Arrhenius equation.

### 2.5.4.3 Overall graph

The kinetics of the various proteins and three pathogenic microorganisms (*listeria*, *E. coli* and *salmonella*) are shown in Figure 2-32. The figure was generated using data obtained from the literature search (Table 2-10) and the reparametrized Arrhenius expression, Eq. (2.4). A reference temperature of 60 °C was used, except in actin where slightly higher temperature of 66 °C was used. As the figure shows, under the current sous vide processing conditions, the examined pathogens are inactivated and all the protein, save collagen and actin, are denatured. The denaturation of collagen is obviously desirable as it reduces toughness, but its shrinkage is not desirable as it squeezes water out of the meat. However, the complete denaturation of myoglobin might not be desirable as this will result in a significant colour change in the meat. Moreover, the denaturation of myoglobin and the subsequent drip loss is also detrimental because beneficial trace elements, such as iron, will also be lost – unless the drip loss is controlled by other means.

**Table 2-10.** Kinetics parameters of key proteins.

Protein	$k_{ref}$ ( $\text{min}^{-1}$ )	$E_a$ ( $\text{kJ mol}^{-1}$ )	$T_{ref}$ (°C)	Reference
Actin	0.002	362.4	66	Bertola et a. (1994)
Collagen	0.00031	604.9	60	Bertola et a. (1994)
Myoglobin	0.125	218	60	Geileskey et al (1998)
Myosin	5.09	218	60	Beas et al. (1991)
Tropomyosin	3.042	484	60	Chen et a. (2006)

$k_{ref}$ =reference rate constant,  $E_a$ =activation energy,  $T_{ref}$ =reference temperature.

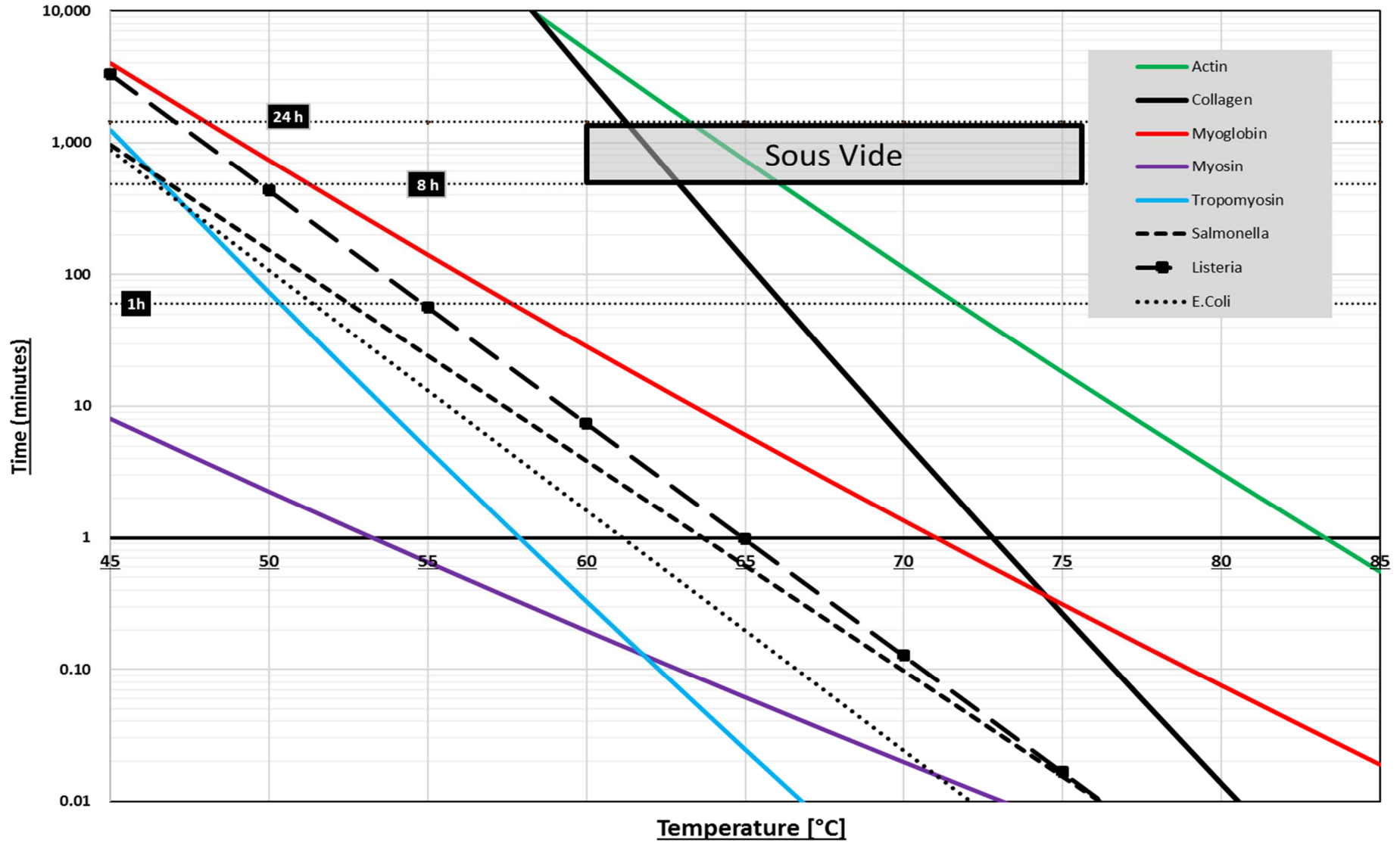


Figure 2-32. Time (mins) for 95% of kinetic changes in meat components and microbial inactivation.

### 2.5.5 Microbial inactivation

As in with all meat processes, there are microbiological concerns associated with sous vide. *Clostridium botulinum* is of particular concern in sous vide, due to the preparation, distribution and storage methods of sous vide products (Hyytiä-Trees et al., 2000). The combination of low heat and vacuum packaging may create favourable conditions for *C. botulinum* and increase the potential for botulism (Hyytiä-Trees et al., 2000). Furthermore, sous vide products are often prepared with little or no inhibitory barriers, such as high/low pH, low water activity ( $a_w$ ) or salts, which would normally inhibit or slow microbial growth (Hyytiä-Trees et al., 2000). As such, strict adherence to refrigeration temperature of 3.3 °C is essential to ensure that sous vide products are safe (Hyytiä-Trees et al., 2000). Certain sous vide recipes (especially in fish dishes) merely warm up the food, and any pathogens or parasites are likely to survive; such foods should only be served to informed, healthy individuals and never to immunodeficient individuals (Baldwin, 2012). These issues are not usually encountered in cook-hold or cook-serve sous vide, as the food is served hot or held around 54 °C. However, the obvious downside of this process is that the product continues to cook, which may not be desirable – especially if the food is to be held at this temperature for an extended amount of time.

Based on the slowest heating point, it is possible to compute pasteurisation tables (time and temperature combinations) for different product thicknesses, Table 2-11.

**Table 2-11.** Approximate time (hours : minutes) required to heat and cook refrigerated beef (NSW, 2012).

Thickness (mm)	Water bath temperature °C											
	55	56	57	58	59	60	61	62	63	64	65	66
5	1:16	0:54	0:38	0:28	0:21	0:17	0:14	0:12	0:09	0:08	0:07	0:06
10	1:24	1:02	0:47	0:38	0:31	0:27	0:23	0:21	0:19	0:18	0:16	0:15
15	1:37	1:15	1:00	0:51	0:44	0:39	0:35	0:32	0:30	0:28	0:26	0:25
20	1:54	1:32	1:17	1:06	0:59	0:53	0:49	0:45	0:42	0:39	0:37	0:35
25	2:08	1:46	1:31	1:20	1:11	1:05	1:00	0:56	0:52	0:49	0:47	0:44
30	2:23	2:00	1:44	1:33	1:24	1:17	1:11	1:07	1:03	0:59	0:56	0:54
35	2:38	2:15	1:58	1:46	1:36	1:29	1:23	1:18	1:13	1:09	1:06	1:03
40	2:53	2:29	2:12	1:59	1:49	1:41	1:34	1:29	1:24	1:20	1:16	1:13
45	3:15	2:51	2:32	2:18	2:07	1:58	1:51	1:45	1:39	1:34	1:30	1:27
50	3:39	3:13	2:54	2:39	2:27	2:17	2:09	2:02	1:56	1:50	1:46	1:41
55	4:04	3:37	3:17	3:01	2:48	2:37	2:28	2:20	2:13	2:07	2:02	1:57
60	4:31	4:03	3:42	3:24	3:10	2:58	2:48	2:40	2:32	2:26	2:20	2:14
65	4:59	4:30	4:07	3:49	3:34	3:21	3:10	3:00	2:52	2:45	2:38	2:32
70	5:30	4:59	4:34	4:14	3:58	3:44	3:32	3:22	3:13	3:05	2:58	2:51

Hyytiä-Trees et al. (2000) evaluated the safety with respect to *clostridium botulinum* of sixteen different sous vide processed products. Two predictive models, Food MicroModel (FMM) and Pathogen Modelling Programme (PMP), which are based on the classical microbial thermal inactivation, Eq. (2.7), were evaluated against experimental data. After cooking, the samples were stored at 4 and 8 °C and examined for the presence of botulinal spores and neurotoxin on sell-by-date and seven days post sell-by-date (Hyytiä-Trees et al., 2000). The majority of the thermal processes were found to be inadequate for eliminating spores, and both predictive models were found to be inconsistent with the experimental observations (Hyytiä-Trees et al., 2000). Hyytiä-Trees et al. (200) attributed the model inaccuracies to a limited number and range of the controlling factors in the model and concluded that the safety of sous vide products need to be evaluated product-by-product, i.e. the results of the predictive models are not transposable to all products. Vijay et al. (2008) evaluated the inactivation of *E. Coli* (0157:H7) in sous vide meat cooked at 55, 58, 60, or 62 °C for one hour, then held at 55 °C for 240 minutes or 10 minutes at 62.5 °C. They found that the inactivation kinetics deviated from first-order kinetics (Juneja et al., 2009). The experimental D value ranged from 67.79 minutes at 55 °C to 2.01 minutes at 62.5 °C (Juneja

et al., 2009). The logistic model, Eq. (2.8), on the other hand, predicted 36.22 and 112.79 minutes at 55 °C and 1.39 and 3 minutes at 62.5 °C for D1 (the survival of the major population) and D2 (the minor population), respectively (Juneja et al., 2009).

$$LR = \frac{1}{D_{ref}} \int_0^t 10^{\frac{T(t')-T_{ref}}{z}} dt' \quad (2.7)$$

**Where:**

*LR* = Log reduction  
*D<sub>ref</sub>* = Time required for one decimal reduction at a reference temperature,  
*T<sub>ref</sub>* = Reference temperature  
*t* = Time  
*Z* = The temperature increase needed for a 10-fold decrease in *D*

$$\log \frac{N(t)}{N_0} = -\frac{t}{D} \quad (2.8)$$

**Where:**

*N(t)* = Number of active organisms at time *t*  
*N<sub>0</sub>* = Initial number of active microorganisms  
*t* = Time  
*D* = Time required for one decimal reduction at a reference temperature

## 2.6 Literature Review Conclusions

Although factors such as colour and flavour are important, the focus of this review was the factors controlling tenderness. Myofibrillar protein denaturation and connective tissue breakdown are the primary factors contributing to meat tenderness. There is a consensus in the literature that the required temperature should be high enough to trigger collagen breakdown and myofibrillar denaturation but low enough to minimise water and nutritional losses. There is no known optimal temperature for sous vide, but there appears to be an emphasis on the temperature range 55-65 °C; there are cases, however, where sous vide has been processed at much higher temperatures (around 80 °C) – the rationale here is to speed up the process to improve product profitability. Heating, enzymatic treatments, brining, and marination are the main methods of tenderising meat. These are briefly summarised as below:

### **HEAT TREATMENT**

*Heating induces the thermal denaturation of the various proteins which cause toughness.*

*But temperature control is important: too low of a temperature and the meat is not pasteurised, too high of a temperature and undesirable changes are induced including water losses, leading to toughening.*

### **ENDOGENOUS ENZYMES**

*Endogenous enzymes are important for reversing the rigor mortis toughness by degrading the myofibrillar proteins. However, they comparatively denature at lower temperatures and are more sensitive to pH change; nonetheless, they can cause significant tenderisation prior to denaturing.*

### **BRINES**

*Brines (generally 3—10% salt solutions) contribute to tenderness by dissolving the support structure of muscle fibres and prevent the formation of dense fibre aggregates.*

## **MARINADES**

*Marinades (which usually contain acidic ingredients such as vinegar) disrupt the hydrogen bonds of the collagen fibrils and cause connective tissue swelling. The addition of alcohol can enhance the diffusion of marinades, but its use should be minimised to prevent pouch ballooning.*

The sous vide process appears to be more of an art than a concise science. Therefore, a better scientific understanding is first necessary for the optimisation of the sous vide process. That is, (i) improving product profitability, by reducing the cooking time, and (ii) maximising product tenderness, by maximising connective tissue solubilisation, optimising myofibrillar protein denaturation, minimizing drip loss, etc. The first step to materialise these goals is to measure the rates and understand underlying mechanism of the key processes in sous vide cooking.

## Chapter 3 Sous vide financial feasibility

### 3.1 Introduction

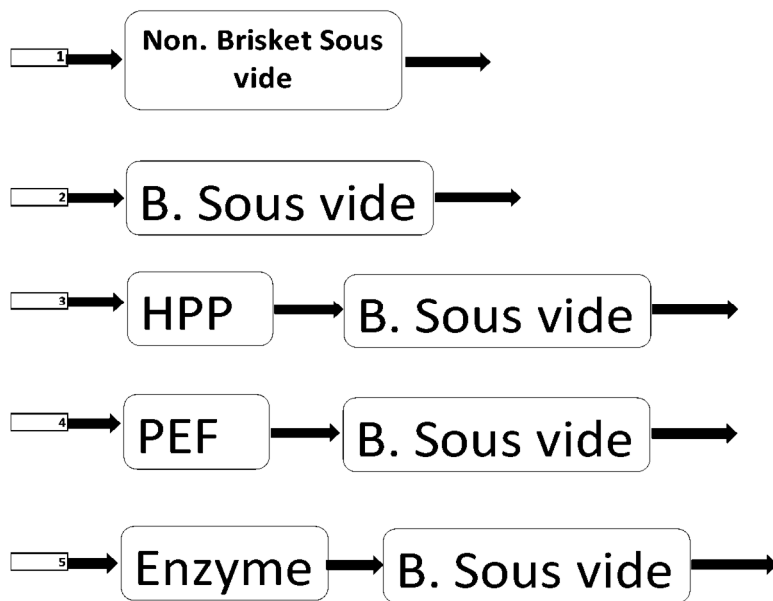
Sous vide is an excellent method for producing a consistent product, but it is not applicable to all products on an industrial scale due to increased processing costs. Relatively tender cuts of meats, such as lamb shanks, are currently sous vide industrially because appreciable tenderness can be achieved within reasonable times (approximately 8—10 hours). In contrast, tougher cuts such as briskets are less economical because they require cooking times of 20—36 hours at 80 °C (Baldwin, 2008).

By better characterising and understanding the sous vide process, ways and means of reducing the cooking time can be identified and the economics of the process can be improved. Also, the employment of supplementary technologies has the potential to address the problem of long cooking times by sufficiently pre-tenderising the meat, thereby reducing the required sous vide time and improving the economics of the process. Moreover, it is possible that these technologies deliver additional benefits in the form of improved product quality, through reduced cook-loss, modified nutritional functionality, and colour retainment.

In addition to sous vide, three other potential technologies were considered: high-pressure processing (HPP), addition of processing enzymes, and pulsed electric field (PEF). The use of HPP and enzymes is not new, but the application of PEF to meat is relatively recent. PEF works by inducing microstructural changes (which alter the meat's functional properties and quality) through electroporation and subsequent action of proteolysis; additionally, PEF has been reported to fragment the myofibrillar structure, which enhances the tenderisation process (Alahakoon et al., 2017).

Using data from the literature, machinery suppliers and food processors, an interactive spreadsheet model was developed. Any data which could not be found was estimated. As Figure 3-1 shows, the considered cases were as follows: case (1) is for relatively less tough

cuts of meat with a cooking time of 10 hours at 80 °C (industry practice); case (2) is a brisket-like sous vide and requires 20—36 hours at 80 °C (industry practice); cases (3) and (4) respectively employ HPP and PEF as pre-tenderisers to reduce the sous vide cook time; and case (5) is similar but uses enzymes for the pre-tenderisation step – thus its cost was considered as an additional cost variable.



**Figure 3-1.** Considered sous vide cases. B = brisket-like.

### 3.2 Process integration

The HPP, PEF, and the enzyme technologies would need to be appropriately integrated with the sous vide cooker to achieve a process that is efficient and is able to produce the desired end-product. The HPP and PEF would be implemented in similar fashion: the product would be inserted (or conveyed) into the treatment chamber; and, once the desired level of tenderisation is achieved (based on pre-set processing conditions: voltage, pressure, residence time, etc.), the product would be conveyed into an accumulator (at low temperatures for food safety reasons), where, once enough product is accumulated based on the size of the oven, it would be transferred into the sous vide cooker. The HPP and PEF would need to be continuously operated to ensure that enough product is accumulated while the previous batch cooks in the sous vide oven. According to the unpublished work of

Alahakoon et al. (2017), no tenderness differences were observed in PEF treated brisket samples that were aged at 4 °C for different times. Meaning, enough product (for an oven batch) can be accumulated without seeing (significant) tenderness variations in the end-product.

The enzyme injector holds an enzyme solution (of pre-determined enzyme concentration levels which can be tailored to specific products) and injects the product with a certain amount of solution as it is conveyed across the injection bed. Then, the product is (usually) tumbled to improve the distribution of enzymes (Zhu, 2017). Finally, the product is transferred to the sous vide cooker, where it is cooked for significantly less time (compared to the HPP and PEF treated samples, see next section). It is important that the cooker temperature is high enough to inactivate the enzymes, or they will over tenderise the meat and transform it into structureless, mushy substance – the inactivation time and temperature combination would either have to be determined experimentally or obtained from the literature. Also, unlike the HPP and PEF set ups, it is important that the enzyme injector is large enough to process enough product for ‘an oven batch’ within a short time to ensure tenderness variations arising from different enzyme exposure times can be minimised, to produce a uniform end-product. If large enough injectors cannot be sourced, multiple injectors may be used, or the batch size may be reduced. To minimise over handling and labour costs, (ideally) all processes should be continuous, however, this may not be always possible, especially for the HPP system.

### 3.3 Methodology

Although there were some assumptions specific to some of the cases (as outlined below), most of the assumptions were common to all five cases. This is advantageous since bias is minimised.

### 1. Processing conditions

Product temperature	=	5 °C
Cooking Temperature	=	80 °C
Cook-time	=	10 hours ( <b>case 1</b> ) or 20 hours ( <b>cases 2—5</b> )

### 2. Base equipment (used in all five cases)

Number of ovens	=	1 oven
Oven dimensions	=	1.7m (length) x 2.1m (width) x 5.5m (depth)
Oven capacity	=	1,000 kg of meat

### 3. Power requirements

Electricity Cost	=	0.15 \$/kWh
Meat heat capacity ( $c_p$ )	=	3,190 J kg <sup>-1</sup> °C <sup>-1</sup>
Heating + chilling energy	=	$2*mc_p\Delta T = 2*1000*3190*(80-5) =$ 479 MJ
Energy efficiency	=	80%

### 4. Working period (personnel communication with industry partner)

Number of work days	=	5 days
Work hours	=	20 hours
Available hours	=	5,000 hours/year

### 5. Throughput (personnel communication with industry partner, November 2016)

500,000 kg of meat per annum (or 100 kg/h) for all processes

### 6. Labour (personnel communication with industry partner)

Number of workers	=	cases (1) — (2): <b>1 unit each</b> and cases (3) — (5): <b>3 units each</b>
Labour rate	=	22 \$/h
Shift duration	=	20 hours

### 7. Capital Installation Factors (source: Bouman et al. (2005))

Combined Lang factor	=	1.3
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### 8. Capital amortisation and running cost

Machine life	=	10 years (or 120 months)
Machine salvage value (after 10 years)	=	\$0.0
Capital interest rate	=	8.0 % per annum (0.67% per month)
Depreciation	=	straight line method
Payment frequency	=	monthly ( $\therefore$ number of payments is 120)
Machine annual running cost	=	8.0% of capital cost*

\*Based on the figure given by Muth et al. (2013)

### 9. Reduction in cooking time by HPP, PEF & Actinidin Enzymes

It was assumed that the HPP and PEF processes would reduce the cooking time from 20 hours to 10 hours whilst the enzyme process (based on studies done by Zhu (2017)) will reduce the cooking time to 0.5 hours. However, allowances were made for loading, unloading, change overs, etc., and a total 'cooking time' of 1.5 hours was assumed.

### 3.3.1 Case analysis

#### 3.3.1.1 Cases (1) and two – *Sous Vide & Brisket like Sous Vide*

Case (2) is very similar to case (1), however the cook-time is doubled (see assumption 1). Consequently, the annual production is halved. As such, the lost output had to be recovered though the procurement of a second oven.

#### 3.3.1.2 Case (3) – *Sous Vide + HPP*

This process consists of an oven and an HPP machine. The necessary additional information and assumptions are shown below:

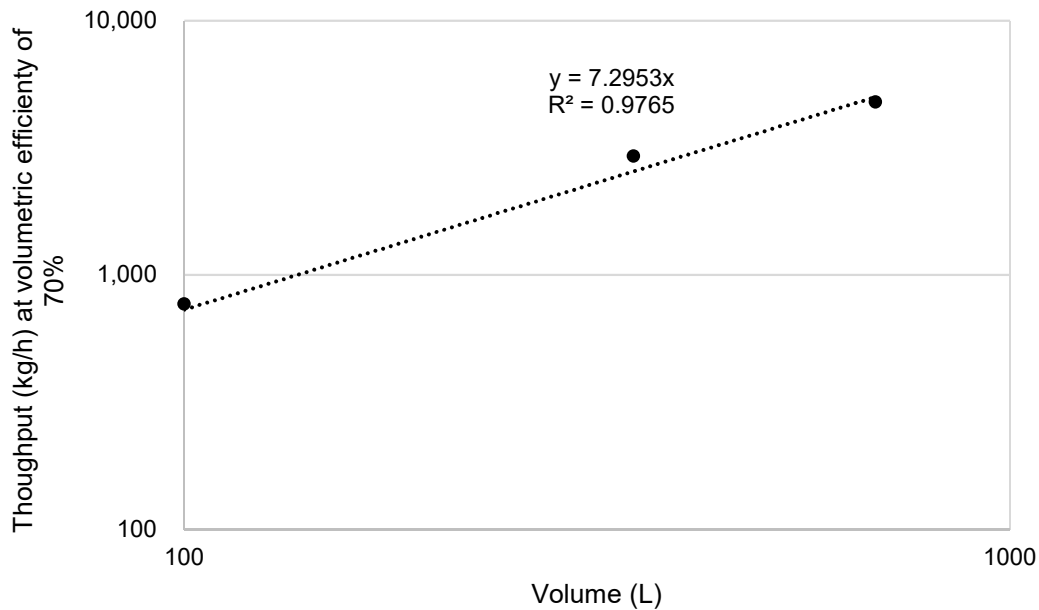
<b><u>Additional info</u></b> (Muth et al., 2013)	
Number of HPPs	= 1 machine
Pressure	= 600 MPa
Volume	= 100 L
Capacity	= 70 kg
Cycle rate	= 11 cycles/h
Throughput	= 770 kg/h

To find a relationship between the cost, the throughput (in kg-meat/h), and the volume of the HPP machine, the data provided by Muth et al. (2013) was used. Finding the relationship between these variables facilitates objective comparison between all cases. Figure 3-2 shows the relationship between the volume (at a given volumetric efficiency) and the expected throughput. As Figure 3-2 shows, the linear model (Eq. (3.1)) does not perfectly fit the data but is sufficient for the purpose of this exercise.

$$N = \frac{7.2953 \times V \times \beta}{70} \quad (3.1)$$

**Where:**

- $N$  = throughput (kg-meat/h)
- $V$  = HPP chamber volume (L)
- $\beta$  = expected volumetric efficiency (typically 55-75%)



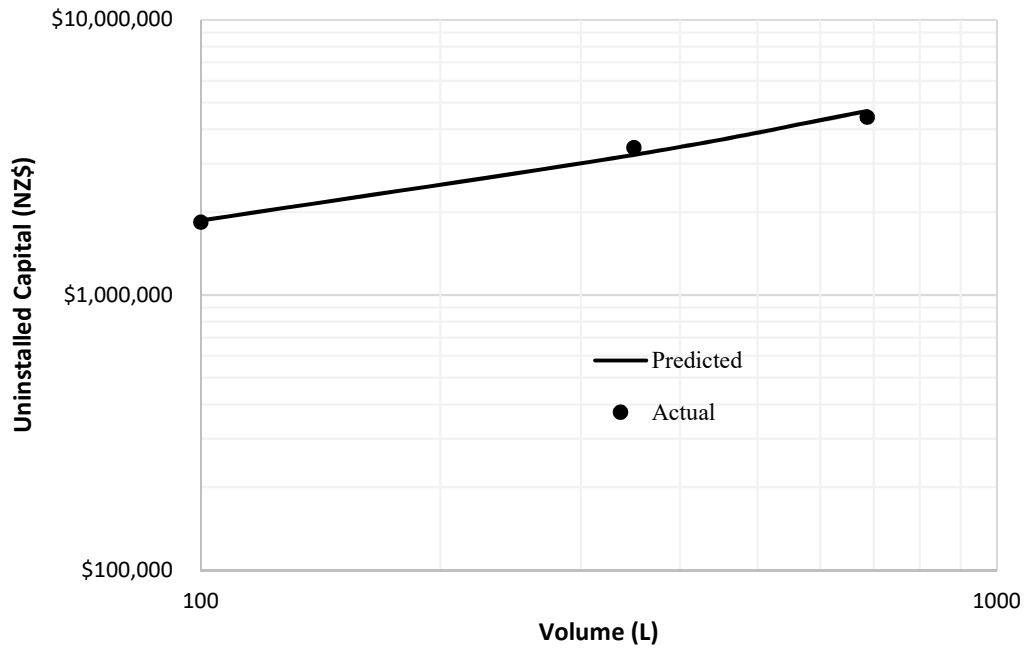
**Figure 3-2.** Throughput as a function of volume with  $\beta=70\%$ , Eq. (1) (original data is from Muth et al. (2013)).

Using Eq. (3.2), a relationship was sought between the initial capital and the HPP chamber volume. Based on the data obtained from Muth et al. (2013), the scaling factor,  $k$ , was determined to be 0.45. Then, by using Eq. (3.2) with  $k = 0.45$ , Figure 3-3 was obtained. As shown, there is a good agreement between the actual data and the predicted (max error  $\pm 6\%$  within the shown interval).

$$C = C_i \left( \frac{V}{V_i} \right)^k \quad (3.2)$$

**Where:**

- $C$  = the unknown cost (\$) (i.e. the sought cost)
- $V$  = the volume (L) corresponding to the unknown cost,  $C$
- $C_i$  = a known cost (\$)
- $V_i$  = a known volume (L) corresponding to  $C_i$
- $k$  = a dimensionless scaling factor



**Figure 3-3.** The initial capital cost as a function of chamber volume (actual data is from Muth et al. (2013)). Exchange rate: 1 USD = 1.42 NZD.

Assuming a throughput of 100 kg/h and a volumetric efficiency of 65%, the required HPP volume and corresponding cost were determined using equations (3.1) and (3.2). The data is summarised as below:

Number of HPPs	=	1 machine
Pressure	=	600 MPa
Volume	≈	15 L
Volumetric efficiency	=	65%
Capacity	≈	9.5 kg
Cycle rate	=	11 cycles/h
Throughput	=	100 kg/h

### 3.3.1.3 Case (3) – Sous Vide + PEF

**Assumptions:**

Number of PEFs	=	1 units
Throughput capacity	=	100 kg/h (same as case (3))
Additional labour	=	2 units

The cost of the PEF machine was provided by Prof. E. Oey of Otago University (personal communication, November 2016).

#### 3.3.1.4 Case (3) – *Sous Vide* + Enzyme Process

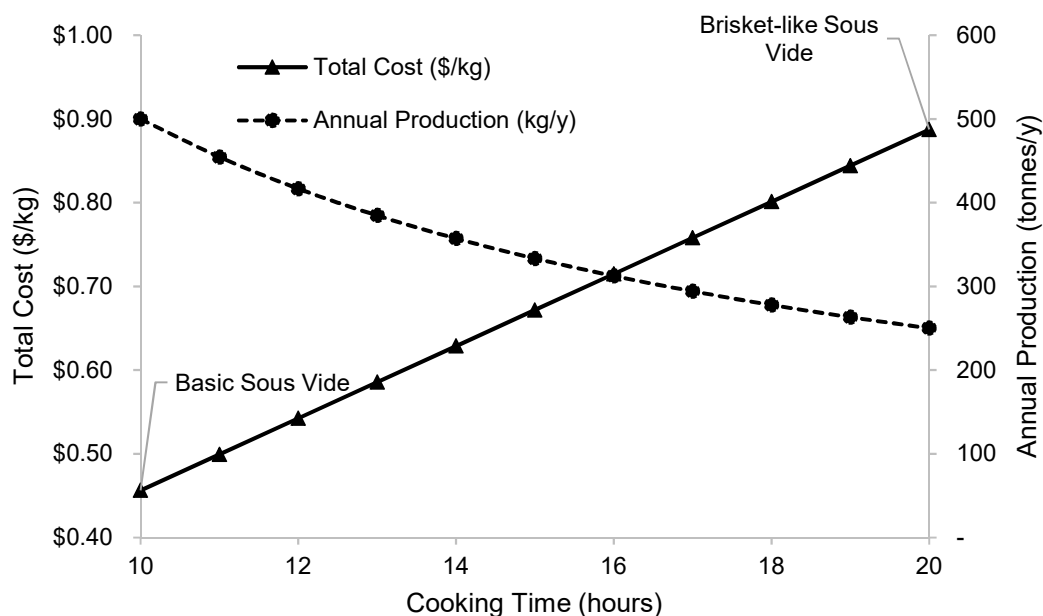
**Assumptions:**

Number of injectors	=	1 units
Throughput capacity	=	100 kg/h
Additional labour Units	=	2 units
Enzyme concentration	=	0.02% (kg-enzyme/kg meat) ( <i>Massey University, July 2017</i> )
Enzyme cost	=	\$129.10 \$/kg ( <i>Massey University, July 2017</i> )

The cost of an injector with a throughput capacity of 450 kg/h is \$210,000 (Marel Meat Processing Inc., November 2016). This was adjusted for a capacity of 100 kg/h using Eq. (3.2) by assuming k value of 0.6.

### 3.4 Results and discussions

Increasing the cooking time of a sous vide process (e.g. lamb vs brisket), reduces the annual production of the process, which in turn leads to an increased production costs (Figure 3-4). However, the obvious potential is that products which would normally be sold as 'low' grade meat (at a low price) can now be sold as premium, tender products; thus, improving the economics of the process. The gained benefits may not always offset the additional costs incurred via an increased cooking time. Figure 3-4 can be used to assess the feasibility for a given required cook time that is found to transform a product.



**Figure 3-4.** The cost profile and production rate (for 1000 kg oven) of the basic Sous Vide process as a function of cooking time.

Table 3-1 shows the various values calculated for cases (1) — (5). To improve the interpretability of the data, all the values were reduced to dollars per kg of annual production, Table 3-2. As previously stated, the basic sous vide (i.e. case (1)), which is for tender cuts of meat such as lamb shanks, is economical; but when dealing with significantly tougher cuts of meat, it is not reasonable to use case (1) as a base case for determining the viability of the other options. Case (2) was therefore chosen as the base case, which had an overall cost of \$0.66 per kg of meat (Table 3-2).

As shown, the cost of the HPP supplemented system is more than twice that of the base case. This is not surprising since HPP has a reputation for being expensive; and, given how long the technology has been in use, it is unlikely to see a significant price reduction in the foreseeable future. The PEF system is also significantly higher than the base case (~ 62%). However, this is a new technology and is likely to see a significant capital price reduction; therefore, it should not be prematurely dismissed. Additionally, it may be possible that these technologies bring additional benefits such, as an improved product quality, that add significant value to the products. Finally, as shown in Table 3-2, the Enzyme system (\$0.58 per kg) is cheaper than the base case and is therefore the process of choice. The Enzyme process may also offer additional benefits that are unaccounted in the model. For example, the reduced cooking time of the meat, may result in a superior product than achieved by a 20-hour sous vide.

**Table 3-1.** Cost of the different processes.

Component	Sous vide	Brisket like sous vide	Sous Vide + HPP	Sous Vide + PEFF	Sous Vide + Enzyme Process
<i>No. of Units</i>	<i>1 oven</i>	<i>2 ovens</i>	<i>1 oven +1 HPP</i>	<i>1 oven +1 PEF</i>	<i>1 oven +1 Injector</i>
Uninstalled Cost	\$ 380,000	\$ 760,000	\$ 1,163,000	\$ 1,135,000	\$ 462,300
Fixed Capital Cost <sup>a</sup>	\$ 494,000	\$ 988,000	\$ 1,512,000	\$ 1,475,500	\$ 601,870
Amortised Capital	\$ 51,079	\$ 102,157	\$ 156,337	\$ 152,569	\$ 62,230
Running Cost	\$39,520	\$ 79,040	\$ 120,960	\$ 118,040	\$ 48,150
Labour Cost	\$ 125,000	\$ 125,000	\$ 375,000	\$ 250,000	\$ 156,250
Power Cost <sup>b</sup>	\$ 12,461	\$ 24,920	\$ 24,920	\$ 24,920	\$ 24,920

<sup>a</sup> After accounting for the Lang factor

<sup>b</sup>The 'power cost' is the energy of cooking and cooling the product. The machinery power consumption is accounted in the 'running cost'

**Table 3-2.** Summary of the main results. All figures are in \$/kg.

<u>Overall Cost</u>	Basic Sous vide	Brisket-like Sous vide	SV <sup>a</sup> +HPP	SV+PEF	SV+EZ <sup>b</sup>
Labour	\$0.25	\$0.25	\$0.75	\$0.50	\$0.31
Power	\$0.02	\$0.05	\$0.02	\$0.02	\$0.02
Capital (oven)	\$0.10	\$0.20	\$0.10	\$0.10	\$0.10
Running Cost (oven)	\$0.08	\$0.16	\$0.08	\$0.08	\$0.08
Capital <sup>c</sup> (HPP/PEF or Injector)	--	--	\$0.21	\$0.20	\$0.02
Running Cost (HPP/PEF/Injector)	--	--	\$0.16	\$0.16	\$0.02
Enzyme Cost	--	--	--	--	\$0.02
<b>TOTAL (\$/kg)</b>	<b>\$0.46</b>	<b>\$0.66</b>	<b>\$1.33</b>	<b>\$1.07</b>	<b>\$0.58</b>

<sup>a</sup> SV = brisket like sous vide, <sup>b</sup> EZ = enzyme process, <sup>c</sup> Amortised capital

**Table 3-1** shows the individual component cost contributions for cases (1) to (5). As shown, the labour component is the biggest contributor to the overall cost – this is especially true for the HPP, PEF, and the Enzyme processes. Of course, this cost can be reduced by introducing greater automation – provided that the additional investment is justifiable.

### 3.5 Chapter conclusions

Several assumptions and extrapolations were necessary for performing the calculations carried out in this feasibility study, which could potentially introduce significant errors.

Nonetheless, based on this model, the HPP system was found to be not viable. Similarly, the PEF system is unviable according to the current standing of the model; but it should not be eliminated since it is likely that a meaningful capital price reduction may occur in the near future and the technology may offer superior product. According to the model, the Enzyme process is economically viable and should be the process of choice to supplement the sous vide process.

If the sous vide process is considered as a standalone process, reducing the cooking time has an obvious economic advantage. However, for this advantage to be realised, the sous vide process must be first characterised. For example, by understanding the kinetics of the

key reactions, the potential reduction in the cooking time can be modelled and then can be translated into economic returns. Characterising the process has also additional benefits, for example, how the cook-loss can be minimised and what cooking conditions produce the best product in terms of tenderness, juiciness, flavour, etc. For this reason, the current work was carried out on sous vide to achieve these goals without processing technologies.

## Chapter 4 Cook loss and tenderness during sous vide cooking

### 4.1 Introduction

Cook-loss, defined as the water that is expelled during cooking, is principally caused by protein denaturation and shrinkage due to thermal and mechanical stresses occurring during cooking. Understanding the kinetics of cook-loss is important as cook-loss impacts the sous vide process in several ways. First, any dispelled water negatively impacts the consumer's acceptability of the product. Second, once water is expelled, the meat fibre density increases and therefore the toughness is likely to increase. Third, the expelled water is likely to carry with it certain nutrients that would normally be retained within the meat, thus detrimentally impacting the nutritional value of the meat. It is also important to study the shear-force in conjunction with the cook-loss and to investigate the correlation (if any) between them.

The key objectives of this chapter were to measure the cook-loss at different temperature-time combinations, to determine the cook-loss kinetics, and to measure the Warner-Bratzler shear-force of raw and cooked samples.

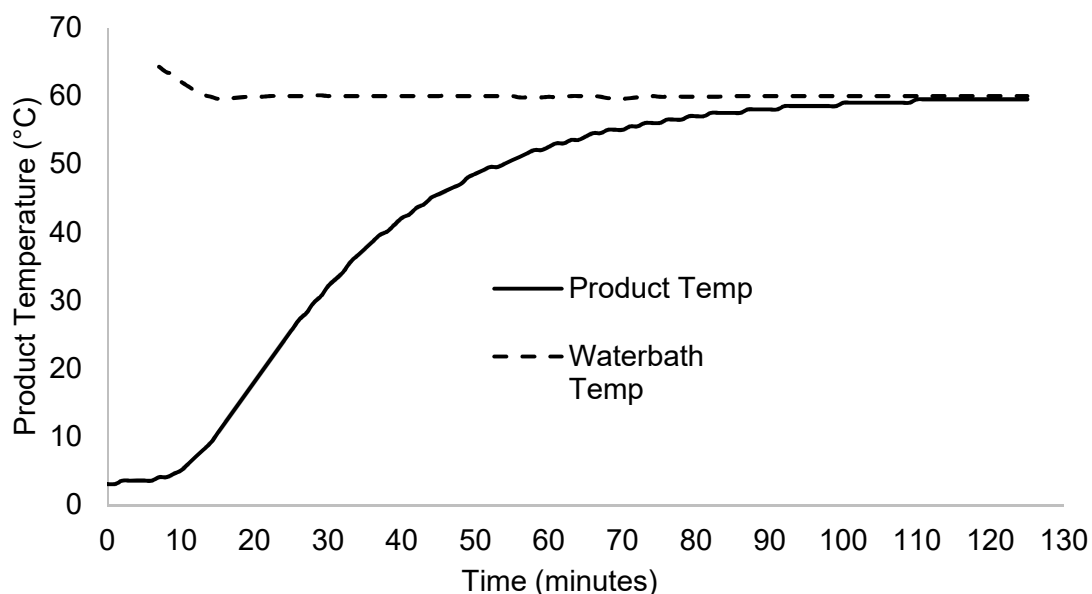
### 4.2 Sample preparation

Samples of *semitendinosus* ('eye of round') from 18—20 months heifer animals were purchased from a local butcher, 5—7 days post-slaughter. The *semitendinosus* muscle was chosen because it is widely studied and the obtained results could be compared with the literature values. The samples were cut into (approximately) 60x60x100 mm pieces, vacuum packed, accurately weighed and transported in an ice-cooled chiller (the pH was not measured). It was initially planned to obtain all the samples at once; however, it became evident that as the meat aged, it was losing blood that would have introduced significant differences in the cook-loss between samples cooked on the day of purchase and those cooked at a later date. Hence, only enough samples for one temperature treatment (i.e. a batch) was bought at a time (all the samples in each batch were from the same animal).

Furthermore, to eliminate any sample temperature variations, each batch was bought one day earlier, stored at 0°C for overnight and cooked immediately after taking out of the chiller. Each batch consisted of 13 samples: 10 samples were assigned to five temperature treatment points and the remaining three samples were for the background cook-loss, the Warner-Bratzler shear-force, and the total collagen, respectively.

### 4.3 Cooking treatments

The samples were cooked in a water bath (600x300x180 mm) with an integrated stirrer and a temperature controller (Labortechnik GmbH, model ED (v2), Germany) with a control accuracy of  $\pm 1^\circ\text{C}$ . To ensure that the water bath did not go below the set temperature, before introducing the samples, the water was pre-heated to 4°C above the desired temperature (Figure 4-1) but immediately after introducing the samples, the set temperature was dropped back to the desired temperature. For example, if the desired temperature was 60 °C, the water bath was set to 64 °C, product was added after 64°C was achieved and the set temperature was immediately reduced to 60 °C.



**Figure 4-1.** The centre-temperature profile of samples cooked at 60°C. The product temperature was recorded with an iButtons® (as explained below). It is worth noting that the mean average temperature will be changing faster than the slowest heating point.

To produce sufficient data for extracting the cook-loss kinetics, the samples were cooked at five different temperature-time combinations. The samples were first cooked for a long time (slow cook sous vide) (Table 4-1). However, the cook-loss data showed that the equilibrium cook-loss for all temperatures – except 60°C – was reached at much earlier point in time. In other words, the dynamic phase of the cook-loss – which is used for determining the kinetics – occurs in the very early part of the cooking process. Accordingly, the temperature-time combinations were modified in accordance with Table 4-2. In the later phase of the project, additional ‘slow’ sous vide experiments (between 50 and 58 °C) were conducted to test the validity of formulated hypothesises,

**Table 4-3.**

**Table 4-1.** Temperature-time combination for the *slow* sous vide cooking (Tc=1.5 hours).

EXP.	*50, 52, 54, 56, 58, 60°C	70°C	80°C	90°C
1	Tc	Tc	Tc	Tc
2	Tc+7h	Tc+8h	Tc+5h	Tc+2h
3	Tc+24h	Tc+21h	Tc+8h	Tc+4h
4	Tc+48h	Tc+35h	Tc+13h	Tc+6h
5	Tc+72h	Tc+48h	Tc+24h	Tc+8h

\*50, 52, 54, 56, and 58 °C were not part of the initial experiment. Each temperature-point represents a batch.

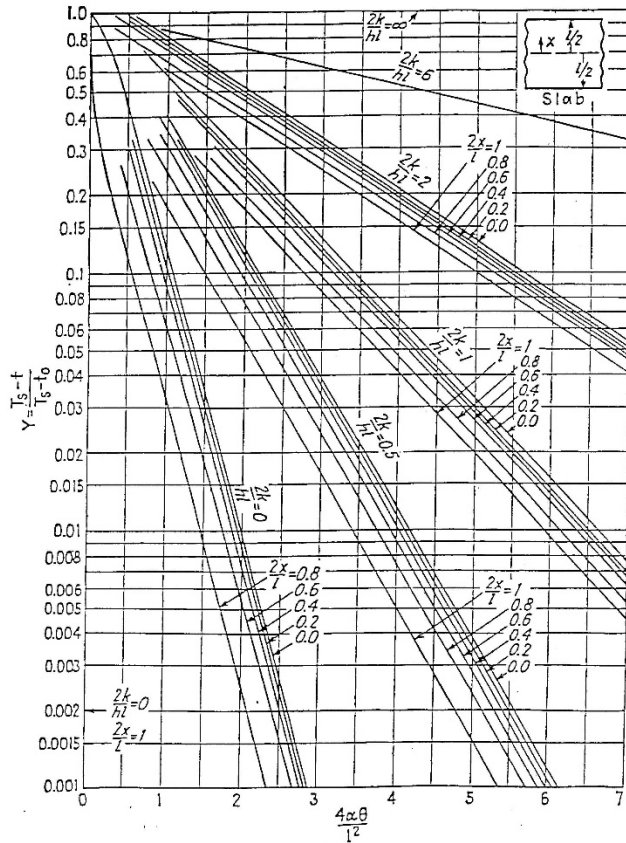
**Table 4-2.** Temperature-time combination for the *fast* sous vide cooking (Tc=1.5 hours).

EXP.	60°C	70°C	80°C	90°C
1	Tc	1h	1h	0.5h
2	Tc+7h	2h	2h	1h
3	Tc+24h	3h	3h	1.5h
4	Tc+48h	4.5h	4h	2h
5	Tc+72h	7h	5h	3h

**Table 4-3.** Temperature-time combination for slow sous vide ( $T_c=1.5$  hours).

EXP.	50, 52, 54, 56, 58 °C
1	$T_c$
2	$T_c+7h$
3	$T_c+24h$
4	$T_c+48h$
5	$T_c+72h$

In Table 4-1,  $T_c$  refers to the time at which the product temperature reaches the water bath temperature. This is normally determined by inserting thermocouples into the centre of the meat and monitoring the temperature evolution. However, such method presents several issues. First, since the probe conducts heat better than the meat, it can lead to localised heating and resulting in inaccurate temperature readings. Second, it is difficult to know the exact centre point, which will vary from sample-to-sample. To resolve the first issue, Maxim's iButtons® temperature sensors were inserted into the centre of the samples; this, however, exacerbated the second aforesaid issue and created an additional problem by making a hole for the water to diffuse out – this was proven by the fact that samples containing the iButtons had comparatively higher cook-loss than those without them. Due to these problems, it was decided to fix  $T_c$  at 1.5 hours for all treatments, which, based on the initial experiments, was estimated as the time it takes for the centre temperature to reach 60 °C. This is justified by recognising that the fractional unaccomplished temperature change ( $Y$ ), for a given time and constant sample dimensions, is the same regardless of temperature (Figure 4-2).



**Figure 4-2.** The Gurney-Lurie chart for a slab (Kern, 1950).

#### 4.4 Cook-loss and moisture content measurements

The cook-loss was determined per the method of Liu et al. (2013). Briefly, upon cooking the samples, the bag was opened, the cook-loss was drained (and stored at  $< -18$  °C for subsequent soluble collagen analysis), the meat was blot-dried with a paper towel, and the samples were immediately reweighed. The cook-loss was calculated from Eq. (4.1).

$$CL = \left( \frac{M_i - M_c}{M_i} \right) \times 100\% \quad (4.1)$$

**Where:**

- CL = cook-loss (w/w%)
- $M_i$  = sample initial weight (g)
- $M_c$  = cooked sample weight (g)

The moisture content was determined by cutting raw samples into thin slices and spreading them over an oven tray. The slices were then placed in an oven (Contherm, Digital Series) at 105°C for 24 hours. Finally, the moisture content was calculated from Eq. (4.2).

$$MC_i = \frac{M_i}{DM} - 1 \quad (4.2)$$

**Where:**

*MC<sub>i</sub>* = initial meat moisture content on dry basis (g-Water/g-Solids)

*M<sub>i</sub>* = sample initial weight (g)

*DM* = dry matter weight (g)

The moisture content of the cooked samples, MC (which is a function of time) can be calculated from the cook-loss using Eq. (4.3)

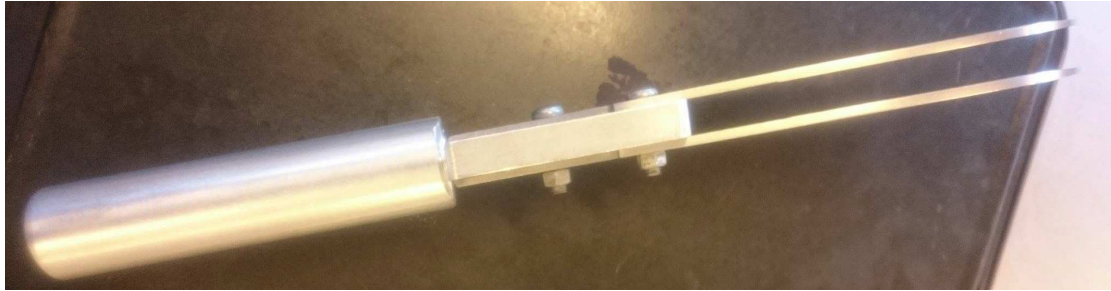
$$MC = MC_i - \frac{CL}{100} (1 + MC_i) \quad (4.3)$$

#### 4.5 Warner-Bratzler measurements

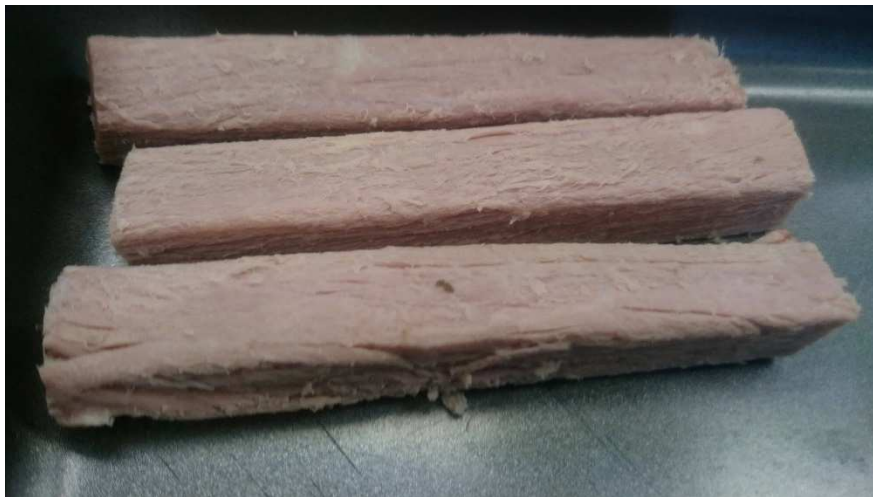
The Warner-Bratzler shear-force (WBSF) was determined according to the method of Christensen et al. (2011) and Liu et al. (2013) with some modifications. After blot-drying the cooked samples, they were sealed in a bag and stored at 3°C to retard further cooking until all the samples (including the raw samples) have reached an equal temperature of 3°C. At first, the cooked samples were vacuum packed (upon blot-drying) but the vacuum dried the samples further by removing as much as 7 w/w% of additional water, which was deemed significant enough to influence the texture measurements, hence the vacuum packing was abandoned.

Using a two-bladed knife (Figure 4-3), four rectangular samples (~10x10x50mm) were cut from the raw and cooked samples (Figure 4-4). Each sample was then sheared twice perpendicular to the fibre direction using a TA XT Plus Texture Analyser (Stable Micro

Systems, UK), equipped with a triangular, flathead Warner-Bratzler blade (10 mm/min). The average and standard deviation peak force (i.e. the firmness/hardness) was calculated.



**Figure 4-3.** knife used to cut the meat.



**Figure 4-4.** Meat specimens for measuring the Warner-Bratzler shear-force.

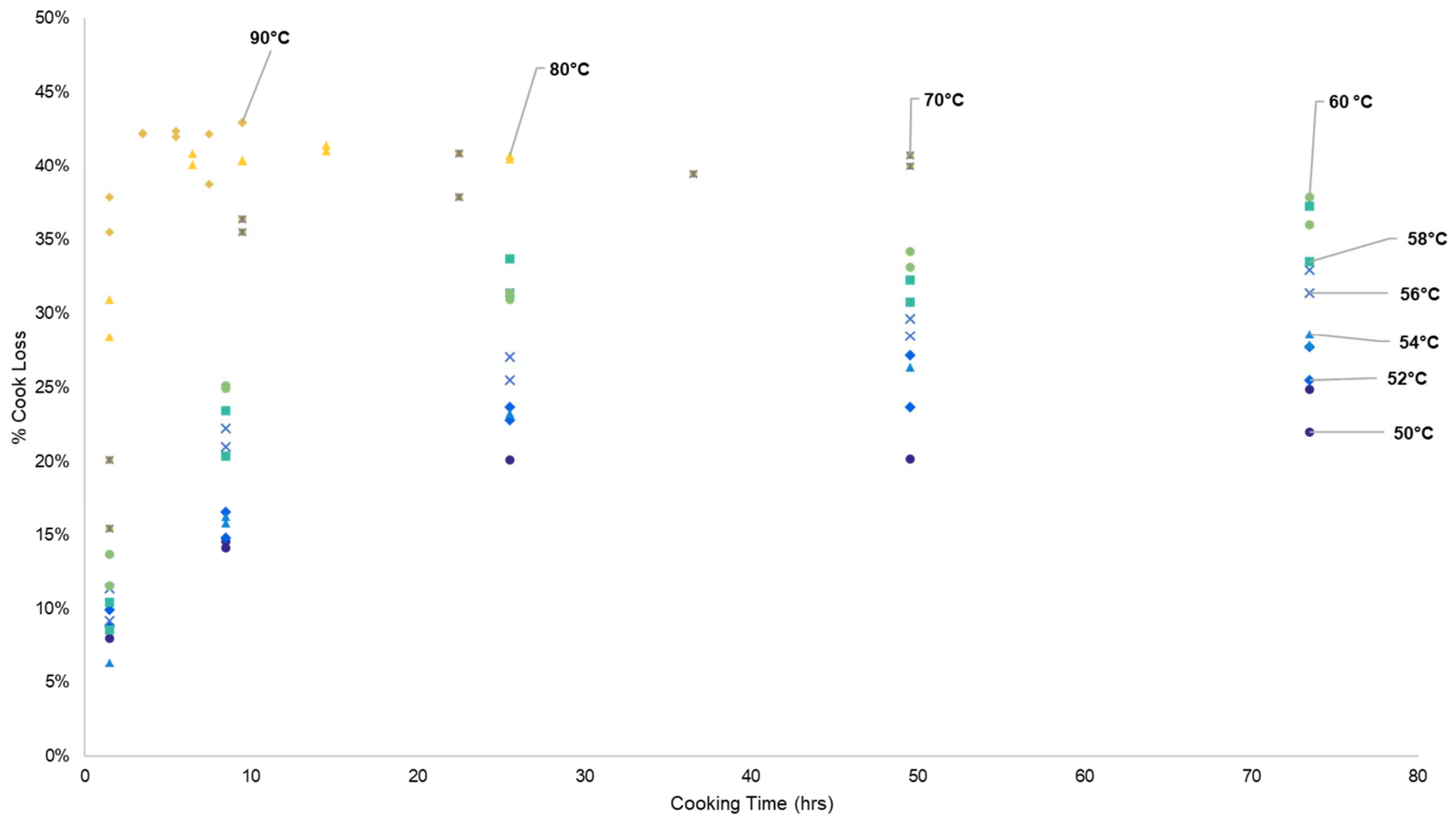
## 4.6 Results and discussion

### 4.6.1 Cook-loss

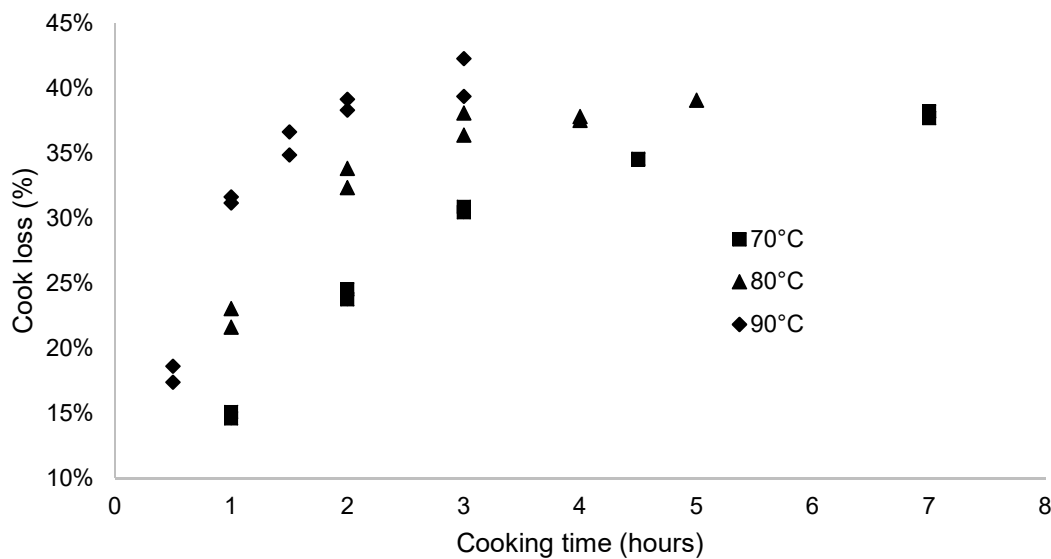
Cook-loss is a result of meat fibres contracting as the meat is heated and the proteins denature, aided by the contraction of the connective tissue. Figure 4-5 shows the measured cook-loss of samples of the slow cook sous vide. Several observations can be drawn: (1) initially, there is a steep gradient for all treatments, although the degree of steepness does significantly increase with an increasing temperature; (2) at lower temperatures (i.e. 50—60), the difference between the cook loss of consecutive treatments is negligible (it is also

probable the same trend exists at the higher temperatures); (3) at high temperatures (70—90 °C), a similar equilibrium cook-loss exists across the different treatments; and (4), in general, the higher the treatment temperature, the faster the equilibrium point is reached. Figure 4-6 shows the cook-loss of samples cooked at 70, 80 and 90 °C but for shorter times, which was intended for the characterisation of the cook-loss kinetics. As shown, comparable results were obtained. Meaning that at higher temperatures, most of the cook-loss takes places within a short period of time.

For the high temperature experiments, no equilibrium cook-loss was reached for the samples treated between 50 and 60, even after 73.5 hours of cooking. It does, however, stand to reason that, if the cooking is continued, eventually, an equilibrium cook-loss would be established; which, based on the results of other treatments, might well be around 40—43%. Oillic et al. (2011) have reported the cook-loss of beef *semitendinosus* samples cooked for five hours at 50, 70 and 90 °C to be 19, 31, and 42%, respectively. Liu and Li (2010) also reported a similar equilibrium cook-loss of 43% (Figure 2-17, Figure 2-18). From the results obtained in this study and what is reported in the literature, it appears that the equilibrium cook-loss is around 40-43%, irrespective of cooking temperature; which suggests that there is, perhaps, something intrinsic to the meat that is creating this limitation as opposed to the cooking conditions.



**Figure 4-5.** Cook-loss of samples cooked at 50 — 60, 70, 80 and 90°C for extended period (most of the samples points were in duplicate).



**Figure 4-6.** Cook-loss of samples cooked at 70, 80 and 90°C for a comparatively brief period.

The moisture content of the raw *semitendinosus* samples was found to be 76 w/w% (or 3.17 on dry basis). This agrees well with what is reported in the literature: 78, 75, 73.4 w/w% (Raiymbek et al. (2013), Li et al. (2008) and Cho et al. (2016)).

#### 4.6.2 Cook-loss kinetics

The kinetics of cook-loss has been studied by several authors, including Oillic et al. (2011) who reported that it (approximately) follows a first order. Meat is of such prodigious complexity, that it is impractical to formulate a comprehensive, predictive model that considers all the components. As such, in formulating a model, a considerable number of assumptions are necessary of which only the key ones are stipulated below.

- The cook-loss is described by a single first-order reaction, Eq. (4.4)
- Each temperature was assumed to be at an isothermal temperature
- The difference between the rate constant at different temperatures is described by the (reparametrized) Arrhenius expression, Eq. (2.4).

- Universal equilibrium moisture content (MC<sub>eq</sub>), based on the highest recorded value was assumed.

$$\frac{dMC}{dt} = -k(MC - MC_{eq}) \quad \text{for } t > 0 \quad (4.4)$$

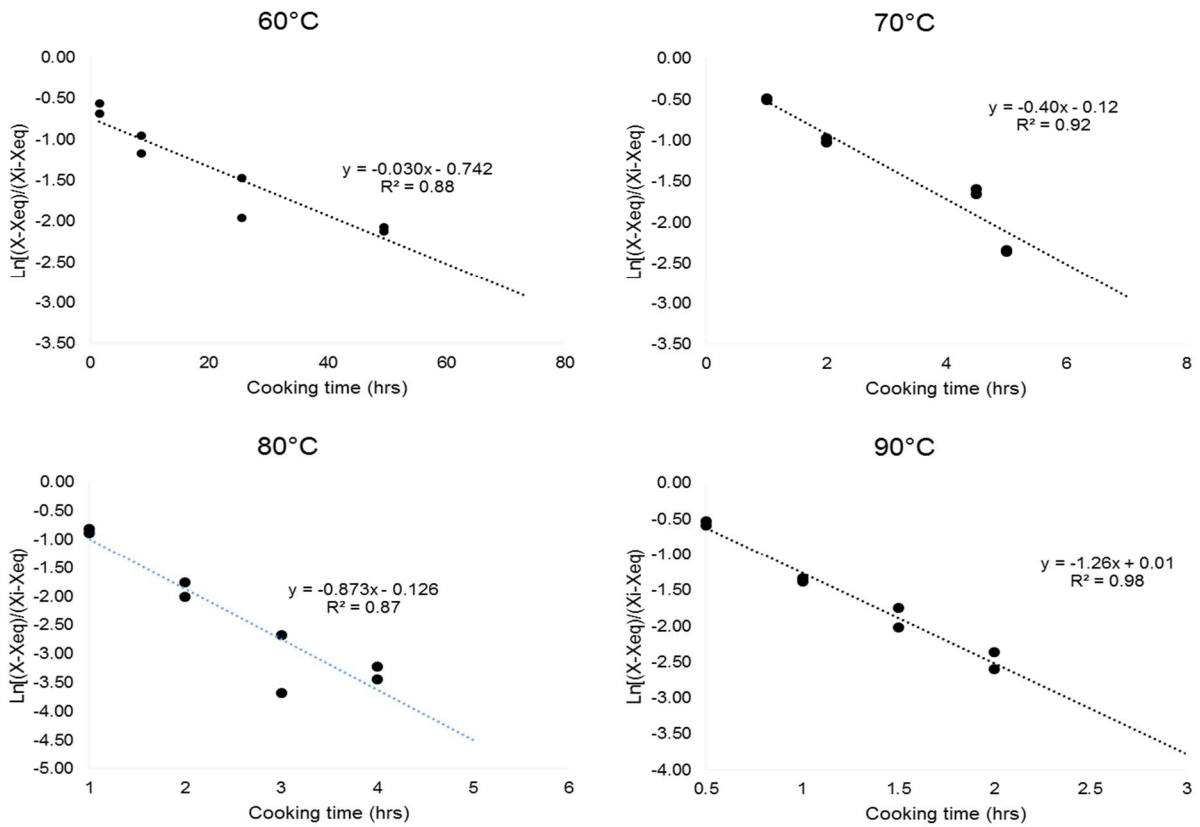
**Where:**

MC	=	the moisture content (g-water/g-solids) as a function of time.
t	=	time (hours)
k	=	rate constant (h <sup>-1</sup> )
MC <sub>eq</sub>	=	the equilibrium moisture content (g-water/g-solids)

$$k = k_r \cdot \exp \left[ -\frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T_r} \right) \right] \quad (2.4)$$

Integrating Eq. (4.4) yields Eq. (4.5) and a plot of  $\ln \left( \frac{MC - MC_{eq}}{MC_i - MC_{eq}} \right)$  vs  $t$  should give a straight line if the reaction is a first order. Indeed, Figure 4-7 does indicate that the system does (approximately) follow a first order. Therefore, a predictive model, Eq. (4.6), was developed by extracting the kinetics parameters ( $E_a$  and  $k_{ref}$ ) from Figure 4-7 and combining equations (2.4) and (4.5). The predictive model was plotted against the experimental data, but the fitting was very poor ( $R^2 \sim 75.3$ ).

$$\ln \left( \frac{MC - MC_{eq}}{MC_i - MC_{eq}} \right) = -kt \quad (4.5)$$



**Figure 4-7.**  $\ln((X - X_{eq})/(X_i - X_{eq}))$  as a function of time of the four temperatures.

$$MC = MC_{eq} + (MC_i - MC_{eq}) \exp\left(-k_r \cdot \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)\right] t\right) \quad (4.6)$$

One of the fundamental issues of this model is the applicability of the Arrhenius equation. As discussed in section 2.5.1, the application of the Arrhenius equation to solid systems is highly controversial as it was originally developed for gas systems. Nevertheless, it is still widely used in the scientific literature, principally for two reasons: (a) it often works well, even though the physical meaning of the kinetics parameters (particularly the pre-exponential factor) might not be understood, and (b) no alternative models which can be applied to the same extent have been developed.

To improve the model, the kinetic parameters ( $E_a$  and  $k_{ref}$ ) were treated as fitting parameters by maximising the Pearson  $R^2$  coefficient, Eq. (4.7), using the inbuilt Excel Solver.

$$R^2 = 1 - \frac{SSE}{SST} \quad (4.7)$$

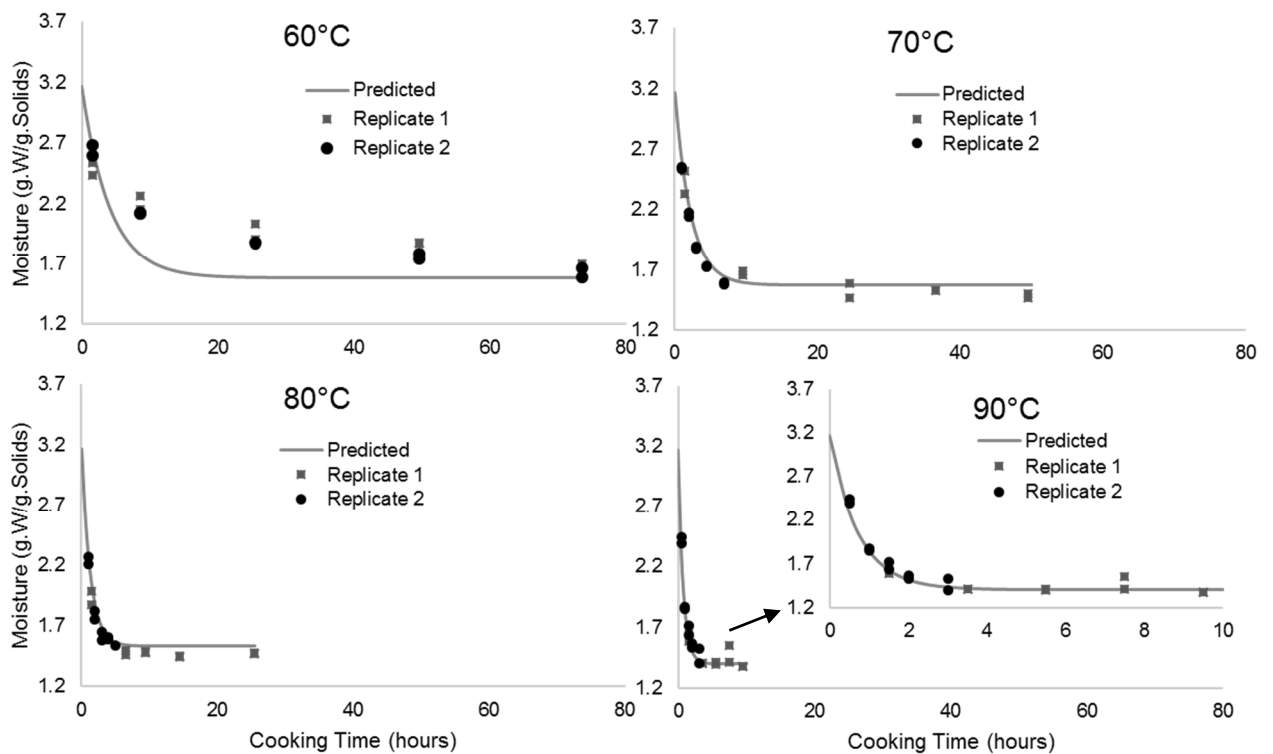
**Where:**

SSE = sum of squared errors (residual sum of squares).  
 SST = total sum of squares

Table 4-4 shows the values of the fitted parameters and Figure 4-8 shows the experimental data against the improved model prediction. As shown, overall, the model fits well and has an overall  $R^2$  of 87%. However, the model grossly under predicts the 60 °C data and the overall  $R^2$ , although considerably improved from its former value of 75.3%, is still rather low. This warranted further model improvements.

**Table 4-4. Fitted parameters.**

Fitted Parameter	Calculated Value	Units
$E_a$	58,054	$J mol^{-1} K^{-1}$
$k_r$	0.24	$h^{-1}$
$R^2$	87%	[--]
Reference temp	60	$^{\circ}C$



**Figure 4-8.** The experimental and the predicted (Eq. (4.6)) moisture content of the samples. The replicates represent two different experiments which themselves contain a duplicate.

As aforementioned, one of the key model assumptions an isothermal temperature, which is particularly questionable at higher temperatures where total reaction time approximates to heating time. To improve the model, this assumption was removed, and the temperature was incorporated into the model. As a result, the rate constant, Eq. (2.4), is now a function of temperature, which itself is a function of time, hence Eq. (4.4) could not be solved analytically and a numerical approach was chosen using MATLAB's Ode45 solver. The temperature was determined by calculating the mass average temperature of the meat using Eq. (4.11).

$$Y_x = \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp \left[ -(2m+1)^2 \frac{\pi^2}{4} \left( \frac{\lambda t}{c_p \rho R_x^2} \right) \right] \quad (4.8)$$

$$Y_y = \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp \left[ -(2m+1)^2 \frac{\pi^2}{4} \left( \frac{\lambda t}{c_p \rho R_y^2} \right) \right] \quad (4.9)$$

$$Y_{av} = Y_x \cdot Y_x \cdot Y_Y \quad (4.10)$$

$$T = T_a + Y_{av}(T_i - T_a) \quad (4.11)$$

**Where:**

$Y_x, Y_y, Y_{av}$	=	<i>fractional unaccomplished temperatures</i>
$t$	=	<i>time (seconds)</i>
$\lambda$	=	<i>meat thermal conductivity (W/m°C)</i>
$c_p$	=	<i>meat specific heat capacity (J/kg°C)</i>
$\rho$	=	<i>meat density (kg/m<sup>3</sup>)</i>
$R$	=	<i>meat half thickness (m)</i>

The results obtained were disappointing: even though the new R<sup>2</sup> (96%) was significantly higher than what was previously obtained, the poor fitting of the 60 °C data practically remained unchanged (data not shown), which strongly suggests that the initial isothermal assumption was valid.

Since the temperature incorporation failed to produce the desired results, other model assumptions were scrutinised. Thus far, the system has been modelled with a single reaction that obeys simple, a first-order reaction kinetics; however, as Vyazovkin (2006) suggested, complex systems, such as meat, cannot be approximated by a simple, first order kinetics. Rather, multiple reactions are present, and therefore a multi-reaction approach is appropriate (see section 2.5.1).

From the experimental data in Figure 4-8, it is evident that at least two reactions govern the cook-loss. In the first phase, the cook-loss is very rapid. In the second phase, the cook-loss is still occurring but at a much slower rate. Accordingly, two, parallel, non-isothermal reactions, were assumed, respectively represented by Eq. (4.12) and (4.13) and interlinked by Eq. (4.14). The two reactions are attributed to (i) myosin and (ii) collagen and actin denaturation. Between 40 and 60°C, myosin denatures, the muscle fibres shrink transversely, and significant water loss occurs, which is attributed to the observed initial steep gradient in, for example, Figure 4-5. At 60°C actin denatures very little, if at all, but collagen starts to denature and, as the temperature continues to rise, actin also denatures. The denaturation of collagen and actin cause the fibres to shrink longitudinally; and, as Tornberg (2005) stated, water is then squeezed out by the shrinkage action of the connective tissue.

In the proposed two reaction system, unlike the Vyazovkin approach, the pre-exponential factor,  $k_0$ , was not omitted and was instead addressed by using the reparametrized form of the Arrhenius expression, Eq. (2.4), section 2.5.1. The new fitting parameters are  $\alpha$ , the contribution of reaction (1) to the overall reaction,  $k_{r1}$ ,  $k_{r2}$ ,  $E_{a1}$  and  $E_{a2}$ , the respective reference rate constants and activation energies of the two reactions. Additionally, a universal equilibrium moisture content (MCE) of 1.4 (on dry basis) was assumed, which corresponded to the highest experimentally measured cook-loss of any sample (which was 90 °C sample in this instance). Ouilic et al. (2011) employed the same technique but assumed isothermal temperature and a single reaction that obeys a first order kinetics. The

assumption of a universal MCE was reasonable since the experimental data strongly suggested that the equilibrium cook-loss of samples (subjected to different treatments) appear to be very similar, Figure 4-6.

$$\frac{dX_1}{dt} = k_1(1 - X_1) \quad \text{for } t > 0 \quad (4.12)$$

$$\frac{dX_2}{dt} = k_2(1 - X_2) \quad \text{for } t > 0 \quad (4.13)$$

$$X = \alpha \cdot X_1 + (1 - \alpha)X_2 \quad (4.14)$$

**Where:**

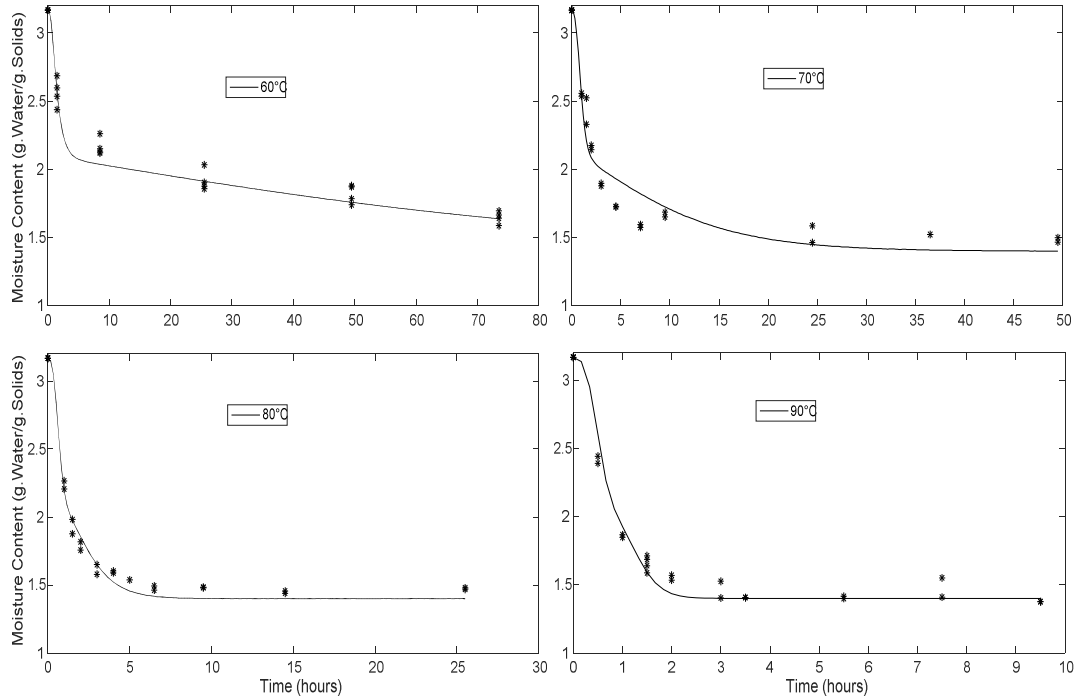
$X_1, X_2$  = extent of reactions 1 & 2  
 $\alpha$  = fractional contribution of reaction 1 to the overall reaction  
 $X$  = the overall extent of reaction.  $MC=f(X)$ , eq. (5.12).

As Table 4-5 shows the values of the fitted parameters and Figure 4-9 shows the results, which were produced using MATLAB's *Lsqnonlin* function (nonlinear least squares). The fitting of the 60 °C has substantially improved and the  $R^2$  is still relatively high 97.4%. Two additional observations can be drawn from the Figure 4-9: (i) the prediction of the 70 °C data has somewhat weakened, nonetheless, overall, the model has improved, and (ii) the apparent initial model lag (which is more visible in the 90 °C plot) is a consequence of the temperature incorporation and represents the heating of the meat samples.

**Table 4-5.** Fitted parameters for the two-reaction system.

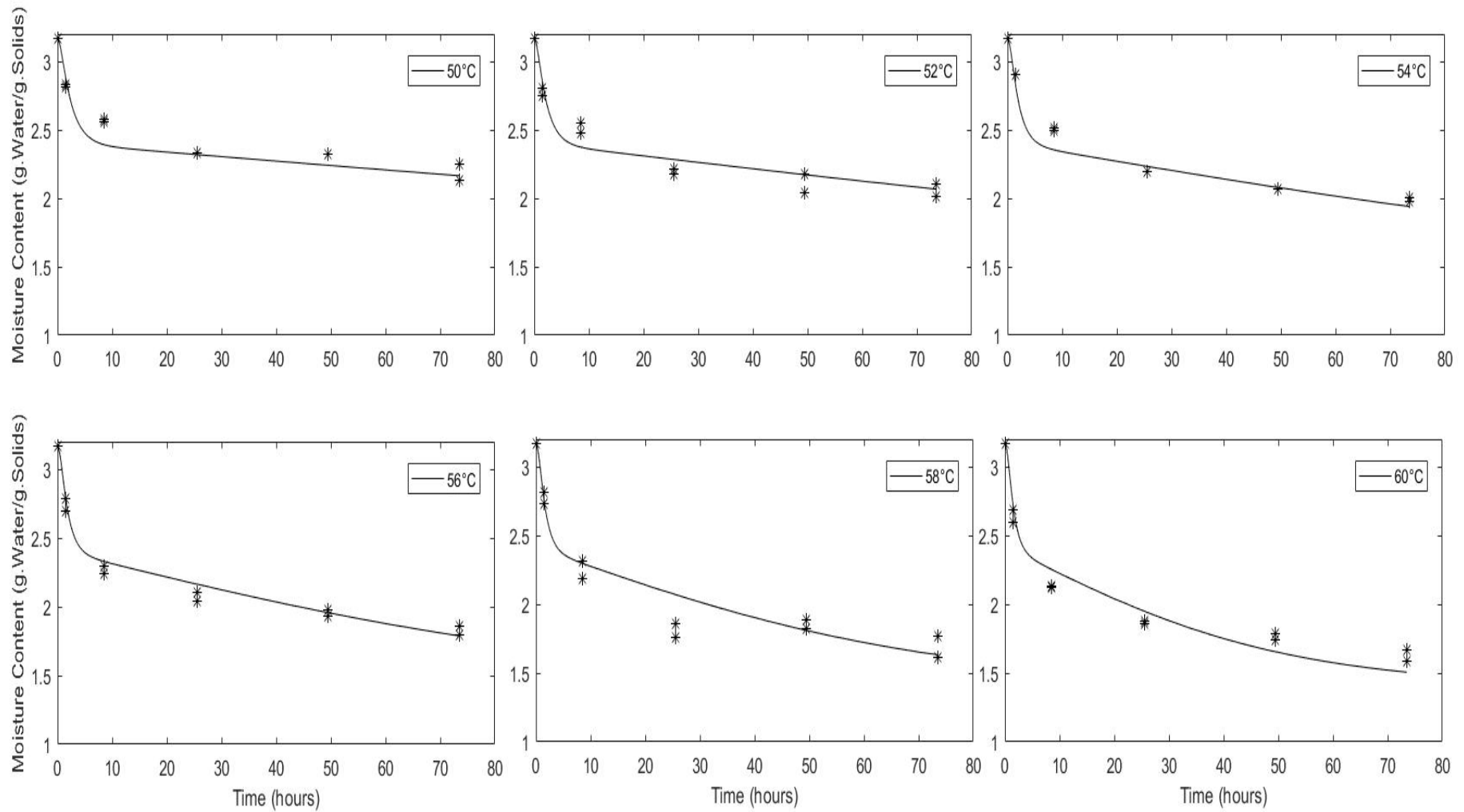
Parameter	Symbol	Value	Units
Contribution of reaction 1 to the overall reaction	$\alpha$	0.392	[--]
Reference rate constant for reaction 1	$k_{r1}$	0.0108	$h^{-1}$
Reference rate constant for reaction 2	$k_{r2}$	1.08	$h^{-1}$
Activation energy for reaction 1	$E_{a1}$	178,000	$J \text{ mol}^{-1}K^{-1}$
Activation energy for reaction 2	$E_{a2}$	93,920	$J \text{ mol}^{-1}K^{-1}$

Overall $R^2$ coefficient	$R^2$	97.6%	[--]
Reference temperature	$T_{ref}$	60	$^{\circ}\text{C}$



**Figure 4-9.** The experimental data (\*) against the two-reaction, non-isothermal model (–) for 60, 70, 80, and 90  $^{\circ}\text{C}$ . (note different axis scales are used)

The model was also applied to the data of samples treated at 50, 52, 54, 56, and 58 (which was collected in the final stages of the project). As Figure 4-10 shows, satisfactory results were obtained, which demonstrates the robustness of the model. Thus, it can be concluded that the model works just as well for temperatures outside of the range at which it was developed for.

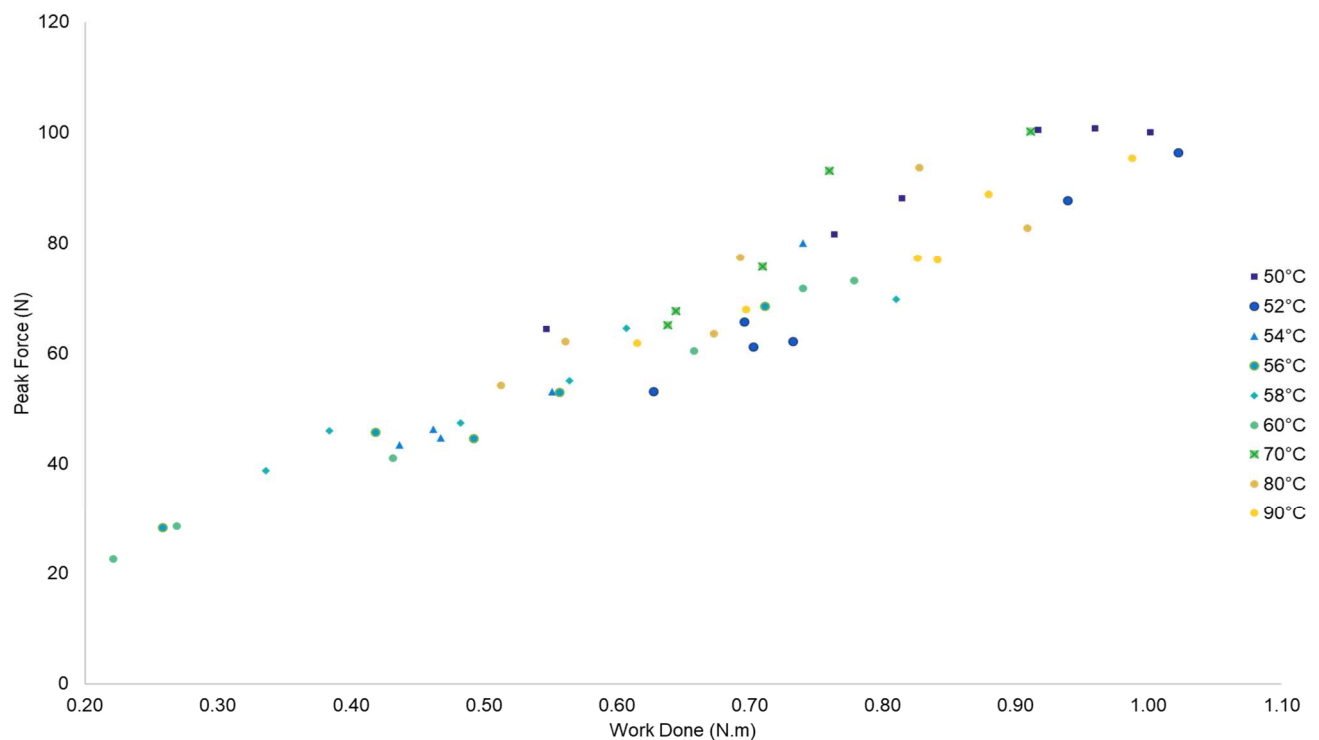


**Figure 4-10.** The experimental data (\*) against the two-reaction, non-isothermal model (-) for 60, 70, 80, and 90 °C.

### 4.6.3 Warner Bratzler Shear-force

Many studies have reported peak force (firmness) along with the toughness (work of deformation); however, as Figure 4-11 shows, there is a strong, positive correlation between the parameters ( $R^2=0.92$ ), meaning that it is sufficient to report only one parameter.

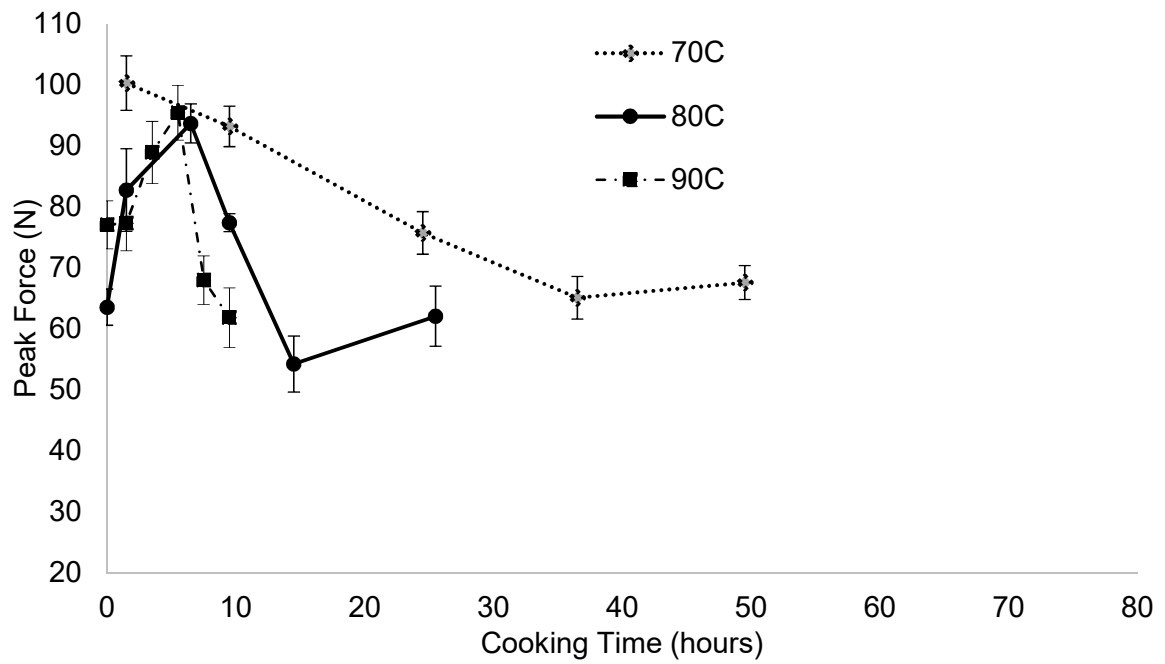
Therefore, it was decided that peak force would be used to report the results of the Warner Bratzler Shear –Force for the rest of this work.



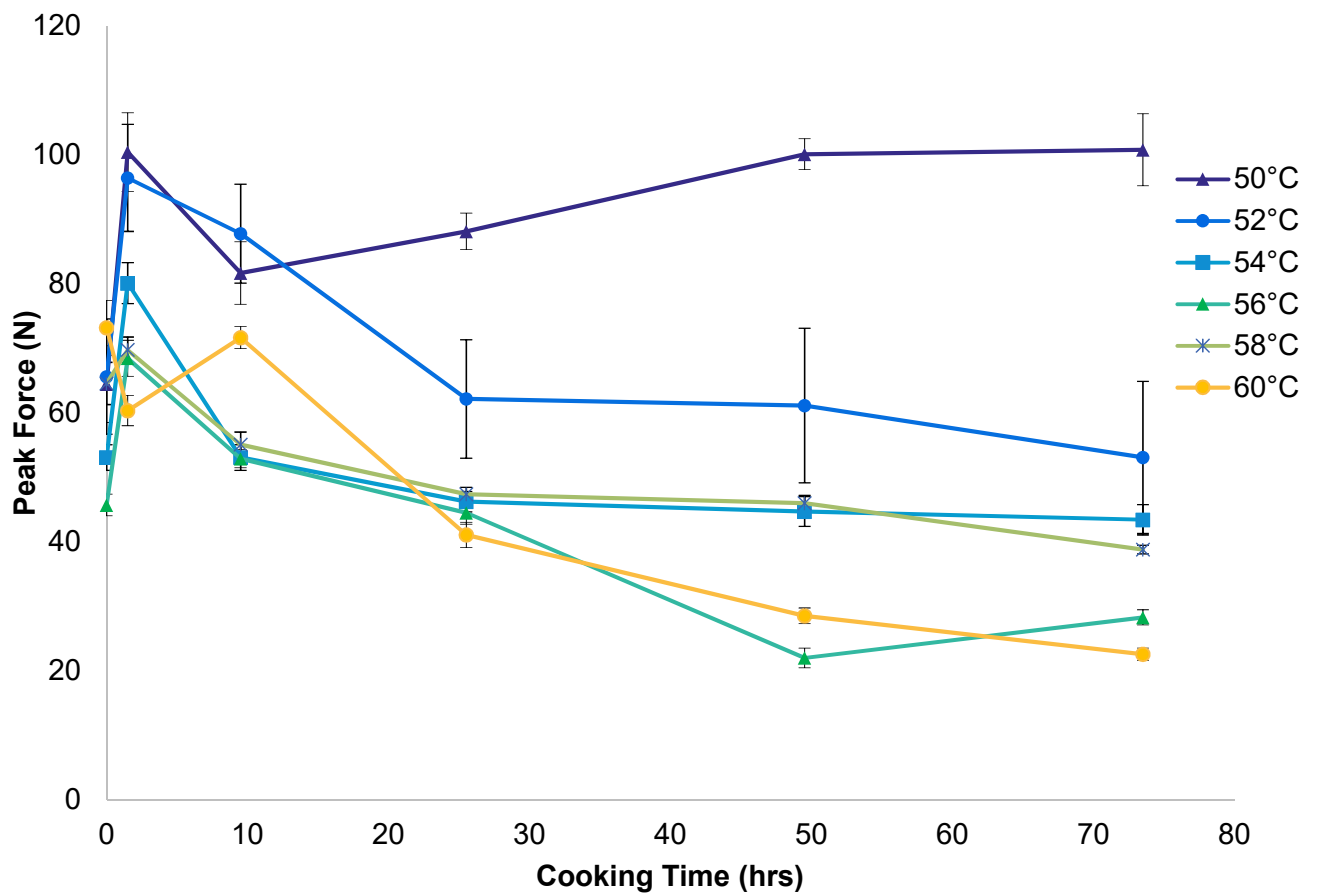
**Figure 4-11.** Assessing the correlation between the peak force and the work done.

Figure 4-12 and Figure 4-13 show the peak force measurements, where the zeroth measurements correspond to the raw samples. The peak force of all the samples initially increased with cooking which is attributed to greater fibre density caused by the water expulsion. However, as the cooking progresses, the mechanical properties of the meat weaken and consequently the force needed to cut through the meat fibres decreases. Moreover, the observed changes appear to be temperature dependent: the higher the temperature, the faster the change in peak force – see Figure 4-12, for example.

The peak force profile of samples cooked at 60 °C behaved slightly differently compared to the other samples: it decreased from raw, then increased, and finally continued to decrease until the end of the experiment (Figure 4-12). Most of the samples increased from raw then decreased over time. No conclusion can be stated about the initial profile of the samples cooked at 70 °C, since, due to insufficient samples, no measurements were taken for the raw samples. Nonetheless, it is apparent that, as time progressed, the peak force of the 70 °C samples continued to decrease similar to the behaviour of the other samples. The peak force of samples treated at 50 °C was the most different: it rose from raw, then experienced a decline, but then (unlike any other samples) it continuously increased with time. This suggests that the structure integrity of the collagen is not being degraded; compounded by the presence of cook-loss, which results in an increased in the fibre density. Bouton and Harris (1981) and Christensen et al. (2013) reported very similar results for beef samples heated at 50 and 53 °C (respectively). But as clearly shown, the results of the 53 °C do not agree what is reported by Christensen et al. (2013). The possible explanation is that the samples treated by Christensen et al. (2013) were from older cows (4-6 years), which had a greater intramuscular connective tissue cross-linking; hence a higher resistance to heat. In fact, samples of young bulls (10—12 months) heated at 53 °C resulted a similar profile to samples cooked at 52 °C (Christensen et al., 2013).

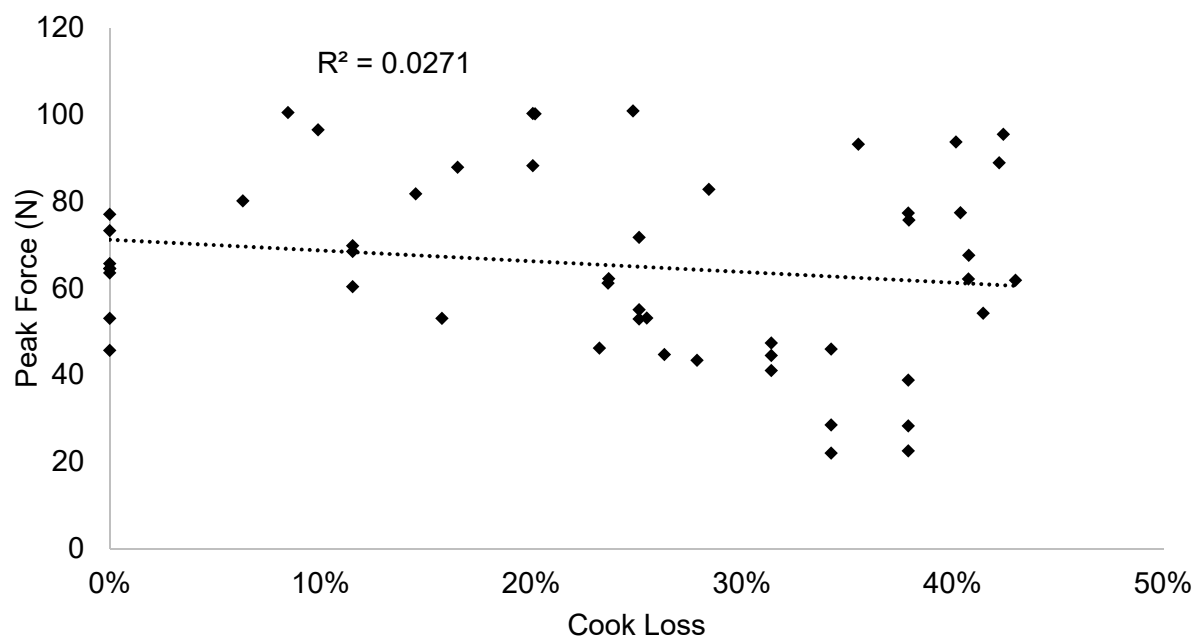


**Figure 4-12.** Peak force measurement for samples cooked at 70, 80, 90 °C



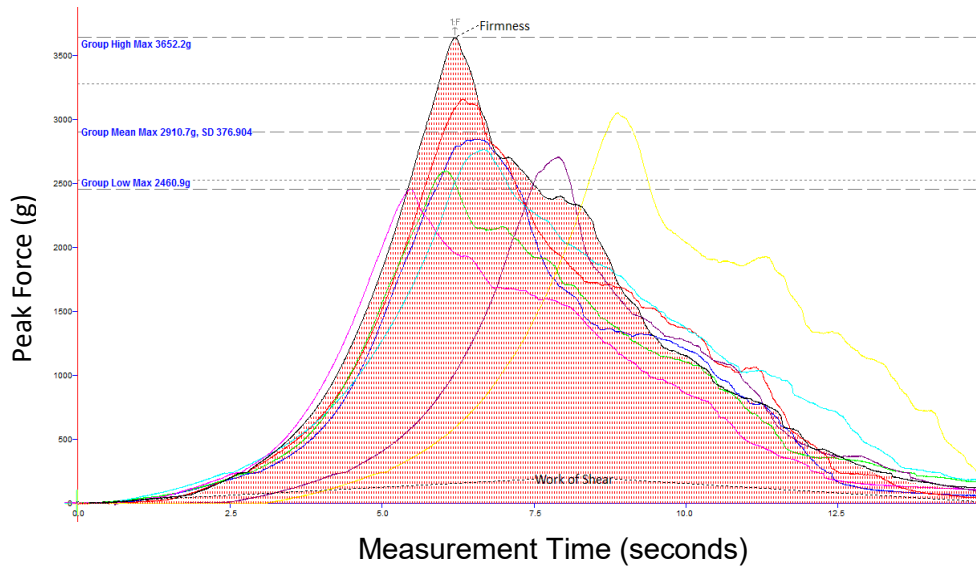
**Figure 4-13.** Peak force measurement for samples cooked at 60 °C.

There is no correlation between the cook-loss and the peak force (Figure 4-14), which suggests that cook-loss has very limited role in meat tenderness. But as reported, the cook-loss is highly correlated with the sarcomere length (see Figure 2-18). As discussed, in section 2.3.3, the calpain enzymes denature at low temperature (> 50 °C) and are very unlikely to have played any role in meat tenderness under the tested conditions. The cathepsin, however, are still active at elevated temperatures and would be expected to contribute to the meat tenderisation.



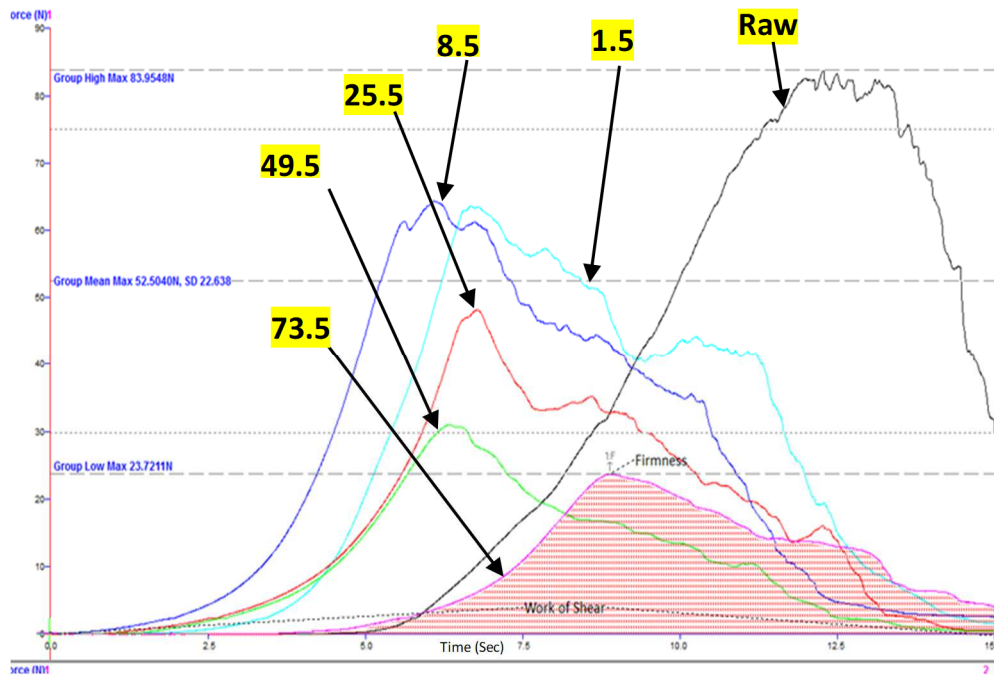
**Figure 4-14.** Correlation between the peak force and the cook-loss.

Due to sample heterogeneity and dimensional variability (among others), it is virtually impossible to reproduce consistent force measurements – even within individual small meat strips. This is exemplified by Figure 4-15, where none of the measurements are the same. Consequently, this introduces inevitable errors. The use of replicates and the subsequent calculations of standard errors has somewhat mitigated this problem.



**Figure 4-15.** Peak force measurement for samples cooked at 60 °C.

As Figure 4-16 shows, the amplitude and the profile of the shear force measurements changed with time. The raw samples displayed the most distant profile: the samples were first compressed until a critical point was reached and the samples were cut, causing the force to rapidly decline. As collagen is dissolved and more-and-more moisture is lost with increasing cooking time, the meat becomes denser and more homogenous, hence the shape of the force becomes more symmetrical (Figure 4-16). These observations were also present in samples cooked at other temperatures.



**Figure 4-16.** Peak force measurement for samples cooked at 60 °C for 0 (Raw) to 73.5 hours.

#### 4.6.4 Chapter conclusion

A rapid cook-loss is observed in the initial phase of the cooking process followed by a slower rate of cook-loss, which are likely due to the denaturation of myofibrillar proteins and actin and collagen – these are further explored in the next chapter. The kinetics of cook-loss is satisfactorily described by a two-reaction system. The peak force of sous vide initially increases then eventually declines as the cook process continues for all temperatures. No obvious explanations could be manufactured for the slightly higher peak force of the 70°C treated samples, except sample variability.

## Chapter 5 Collagen solubilisation

### 5.1 Introduction

In Chapter 2, the importance of collagen on tenderness was highlighted: it supports and holds the meat structure in place. Therefore, any changes in the organisation of its structure (due to, for instance, thermal, enzymatic, or mechanical stresses) is very likely to have profound influences on the properties of the resulting meat. The cook-loss and the tenderness are of particular importance. As explained in section 4.6.2, when collagen denatures, it contracts and expels the water held between the meat fibres, thereby reducing the meat's water holding capacity (WHC). Furthermore, because collagen is a key contributor to meat toughness, by virtue of its denaturation and ultimate dissolution, the toughness is reduced; although this reduction is subject to the processing conditions of temperature and time. Thus, it is important to investigate how varying conditions influences the denaturation and dissolution of collagen.

The objectives of this chapter were to quantify the total collagen in raw samples and the dissolved collagen in cook-loss samples, for meat subjected to different time and temperature combinations.

### 5.2 Sample preparation

#### ***Total Collagen***

One of the raw samples (described in section 4.2) was thoroughly homogenised with a food processor, then 4 g of homogenised meat was withdrawn and the total collagen was determined according to the method of Kolar (1990), which is described in section 5.3. Due to the laboriousness of collagen quantification methods, it was not practical to undertake large number of replications which would have enable the calculations of standard error and standard deviations. This underscores the need to develop better collagen quantification methods.

### ***Solubilised Collagen in the cook-loss***

In the sous vide experiment outlined above in Chapter 4, samples of the cook-loss after different time/temperature combinations were stored frozen at – 18 °C. In this work, the dissolved collagen in these samples was measured. The cook-loss samples were thawed to 4 °C, filtered through a 60 µm sieve, cooled down to about 2 °C, and centrifuged at 5069xg for 15 minutes (Sigma 6—16S). Thereafter, 5 ml samples were withdrawn and the soluble collagen was determined as per the method of Kolar (1990)

### ***Soluble collagen within the meat***

Cooking solubilises collagen within the meat structure of which some of this exits with the cook-loss. The remaining solubilised collagen within the meat is not, however, easily quantifiable and no existing method was found in the surveyed literature. Therefore, an attempt was made at developing a new method to do this. The approach taken was to flush out all the dissolved collagen from the samples and to measure the remaining residual insoluble collagen. Based on this the solubilised collagen in the meat was calculated using Eq. (5.1), where all concentrations are expressed on grams of original meat.

$$C_{DM} = C_{Total} - C_R - C_{CL} \quad (5.1)$$

***Where:***

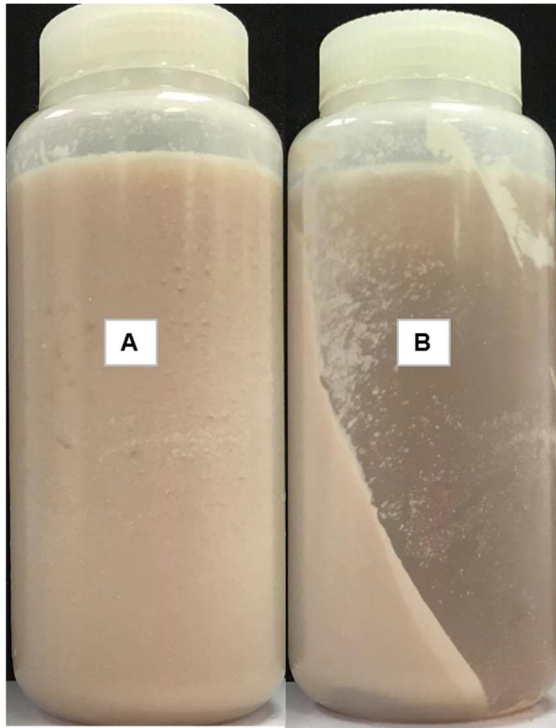
$C_{DM}$	=	<i>dissolved collagen within the meat</i>
$C_{Total}$	=	<i>total collagen (soluble + insoluble)</i>
$C_R$	=	<i>residual collagen after cooking and washing</i>
$C_{CL}$	=	<i>solubilised collagen in cook-loss</i>

Previously frozen blocks of meat cooked at 58, 70, 80 and 90 °C were thawed and cut into small pieces with a knife and were subsequently re-frozen. Next, the samples were pulverised in a 5-speed blender (*Brabantia*) as shown in Figure 5-1 (A). To wash out all the solubilised collagen, the samples were transferred into stacked 300 and 40 µm sieves and washed with copious amounts of water ( Figure 5-1, B). Next, the washed samples were (with the aid of ultrapure water) further homogenised in a blender (to flush out any solubilised collagen entrapped within the bigger particles, which were ~ 2—3 mm in size).

The homogenate was divided into portions of 100 g and was transferred into 500 ml centrifuge tubes and filled to volume with ultrapure water. The tubes were capped, vigorously shaken, and centrifuged at 7,000xg for 10 mins (Figure 5-2). The water was discarded, and the various pellets were recombined and mixed thoroughly. Four samples (~4 g each) were withdrawn and the collagen was determined in the manner described in the next section (5.3). An additional sample was taken for moisture analysis. This method is an improvement of an earlier one which did not involve the pulverisation step nor the washing in a sieve. The earlier method was applied to samples cooked at 60 °C for 1.5 and 73.5 hours.



**Figure 5-1.** Pulverised cooked meat (A) washed in a 300 µm sieve (B).



**Figure 5-2.** Blender homogenised cooked meat (**A**) centrifuged (**B**) for 10 min at 7,000xg.

### 5.3 Collagen determination via the measurement of hydroxyproline

#### **Reagents**

Kolar (1990) described the preparation of the reagents as follows – where changes have been made is noted in italics.

- (a) “Sulphuric acid – 3.5M. Add 750 mL H<sub>2</sub>O to a 2 L volumetric flask. Slowly add, with agitation, 375 mL H<sub>2</sub>SO<sub>4</sub> (1.84 g/mL). Cool to room temperature and dilute to volume with water.” *Note: wherever H<sub>2</sub>O is mentioned, ultrapure (type 2) water was used.*
  
- (b) “Buffer solution – pH 6.0. Dissolve 30 g citric acid monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>•H<sub>2</sub>O), 15 g NaOH, and 90 g sodium acetate trihydrate (CH<sub>3</sub>COONa•3H<sub>2</sub>O) in approximately 500 mL H<sub>2</sub>O. Transfer solution to 1 L volumetric flasks. Add 290 mL of 1-propanol. *Check pH with meter and adjust if necessary with acid or base.* Dilute to volume with water.” *Note: A small quantity (<10 ml) of concentrated acetic acid was used to adjust the pH.*
  
- (c) “Oxidant solution – Dissolve 1.41 g chloramine-T reagent in 100 mL buffer solution, (b)”. *Note: The solution was kept in a dark bottle at 2°C.*
  
- (d) “Colour reagent – Dissolve 10g 4-demethylaminobenzaldehyde in 35 mL perchloric acid (60% w/w). Slowly add, with agitation, 65 mL 2-propanol.” *Note: The solution was prepared on the day of use.*

(e) Hydroxyproline standard solutions:

- (i) "Stock solution – 600 µg/mL. Dissolve 60 mg hydroxyproline in water in 100 mL volumetric flask. Dilute to volume with water." *Note: the solution was stored in a fridge at 2°C for up to two months.*
- (ii) "Intermediate solution – 6 µg/mL. Pipet 5 mL stock solution into 500 mL volumetric flask. Dilute to volume with water." *Note: the solution was prepared on the day of use.*
- (iii) "Working standard 0.6 – 2.4 µg/ml: Pipet 10, 20, 30 and 40 mL portions of intermediate solution (ii) into 100 mL volumetric flasks. Dilute each to volume with water. Working standard solutions contain 0.6, 1.2, 1.8, and 2.4 µg hydroxyproline / mL, respectively." *Note: this was also prepared on the day of use.*

### ***Hydrolysis***

For the total collagen, four samples (each 4 g) of homogenised meat were transferred to an Erlenmeyer flask and 30 ml of H<sub>2</sub>SO<sub>4</sub> (3.5M) was added to each flask. The samples were covered with aluminium foil and hydrolysed in an oven at 105 ± 1 °C for at least 20 hours. For the soluble collagen, 5 ml of cook-loss was combined with 10 ml of H<sub>2</sub>SO<sub>4</sub> (3.5M) in a test tube. The test tubes were screwed loosely, and the samples were hydrolysed in the same manner as for the total collagen.

### ***Hydroxyproline determination***

The total collagen hydrolysate was diluted to 500 ml with ultrapure water and filtered through a Whatman filter (125 mm, LBS0001.125), then 5 ml of the filtrate was further diluted to 100 ml with water. The soluble collagen hydrolysate was treated similarly except it was first diluted to 100 ml and then 5 ml of the filtrate to 50 ml with water.

2 ml of the final hydrolysate diluents and the standard solutions were pipetted into test tubes and 1 ml of oxidant solution was added to each tube. The tubes were capped, shaken, and let stood for 20 minutes at room temperature. Then, 1 ml of colour reagent was added to each test tube, capped, mixed vigorously, and immediately placed in a water bath (Grant T100) at 60 °C for 15 minutes. The tubes were then removed from the water bath and

cooled in an ice bath for 3—4 minutes. Finally, the absorbance was measured with a spectrophotometer (Genesys 10S UV-VIS) with a wavelength of 558 nm.

The collagen was calculated from the hydroxyproline (H) measurements, Eq. (5.3)

### **TOTAL COLLAGEN (RAW SAMPLES)**

$$H = \left( \frac{h \times \text{Dilution1} \times \text{Dilution2}}{\text{sample weight} \times \text{filtrate}} \right) \times \left( \frac{1 \text{ mg}}{1,000 \mu\text{g}} \right)$$

$$H = \left( \frac{h \left( \frac{\mu\text{g}}{\text{ml}} \right) \times 500 \left( \frac{\text{ml}}{1} \right) \times 100 \left( \frac{\text{ml}}{1} \right)}{m(\text{g}) \times V(\text{ml})} \right) \left( \frac{1(\text{mg})}{1,000\cancel{\mu\text{g}}} \right)$$

$$H = \frac{h \times 50}{m \times V} \left( \frac{\text{mg. hydroxyproline}}{\text{g. meat}} \right) \quad (5.2)$$

**Where:**

- $h$  = the hydroxyproline ( $\mu\text{g/ml}$ ) that is read off the calibration curve
- $m$  = weight of sample (g) taken for hydrolysis
- $V$  = volume (ml) of hydrolysate diluted to 100 ml

$$\text{Total Collagen} = 8 \times H \left( \frac{\text{mg. collagen}}{\text{g. meat}} \right)^1 \quad (5.3)$$

Note: '8' is obtained by assuming that connective tissue contains 12.5% hydroxyproline (i.e.  $100/12.5=8$ ) (Kolar (1990)).

### **SOLUBLE COLLAGEN IN THE COOK-LOSS**

$$H = \left( \frac{h \times \text{Dilution1} \times \text{Dilution2}}{\text{sample volume} \times \text{filtrate}} \right) \times \left( \frac{1 \text{ mg}}{1,000 \mu\text{g}} \right)$$

$$H = \frac{h \left( \frac{\mu\text{g}}{\text{ml}} \right) \times 100(\text{ml}) \times 50(\text{ml})}{V_{\text{Cl}}(\text{ml}) \times V(\text{ml})} \times \frac{1}{1000} \left( \frac{\text{mg}}{\mu\text{g}} \right)$$

$$H = \frac{h \times 5}{V_{\text{Cl}} \times V} \left( \frac{\text{mg} \cdot \text{hydroxyproline}}{\text{ml cook} - \text{loss}} \right) \quad (5.4)$$

**Where:**

$h$  = the hydroxyproline ( $\mu\text{g}/\text{ml}$ ) per sample, read off the calibration curve

$V_{\text{Cl}}$  = volume of cook-loss (ml) taken for hydrolysis

$V$  = volume of filtrate diluted to 50 ml.

$$\text{Sol. Collagen} = 8 \times H \left( \frac{\text{mg collagen}}{\text{ml cook} - \text{loss}} \right)^1 \quad (5.5)$$

## **INSOLUBLE COLLAGEN WITHIN THE MEAT**

$$H = \left( \frac{h \times \text{Dilution1} \times \text{Dilution2}}{\text{sample weight} \times \text{filtrate}} \right) \times \left( \frac{1 \text{mg}}{1,000, \mu\text{g}} \right)$$

$$H = \frac{h \left( \frac{\mu\text{g}}{\text{ml}} \right) \times 100(\text{ml}) \times 50(\text{ml})}{m(\text{g}) \times V(\text{ml})} \times \frac{1}{1000} \left( \frac{\text{mg}}{\mu\text{g}} \right)$$

$$H = \frac{h \times 12.5}{m \times V} \left( \frac{\text{mg} \cdot \text{hydroxyproline}}{\text{g} \cdot \text{cooked meat}} \right) \quad (5.6)$$

**Where:**

$h$  = the hydroxyproline ( $\mu\text{g}/\text{ml}$ ) per sample, read off the calibration curve

$m$  = mass of homogenised and centrifuged cooked meat

$V$  = volume of filtrate diluted to 50 ml.

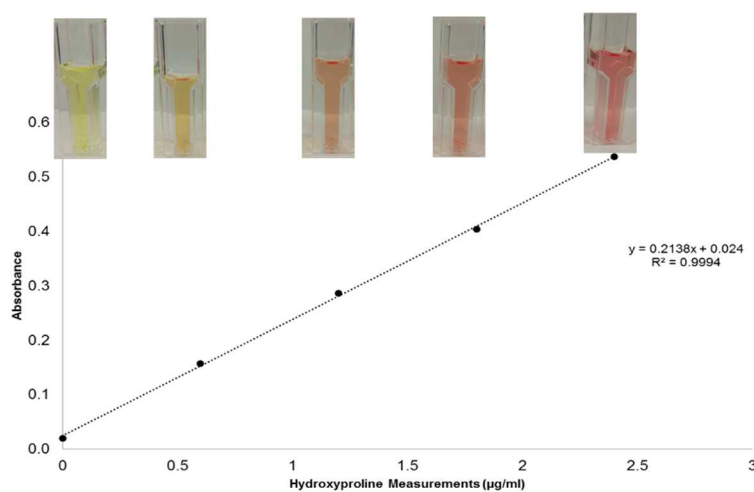
**Insol. Collagen**

$$= 8xH \left( \frac{\text{mg collagen}}{\text{g. cooked meat}} \right)^1 \quad (5.7)$$

## 5.4 Results and discussion

### 5.4.1 Total collagen

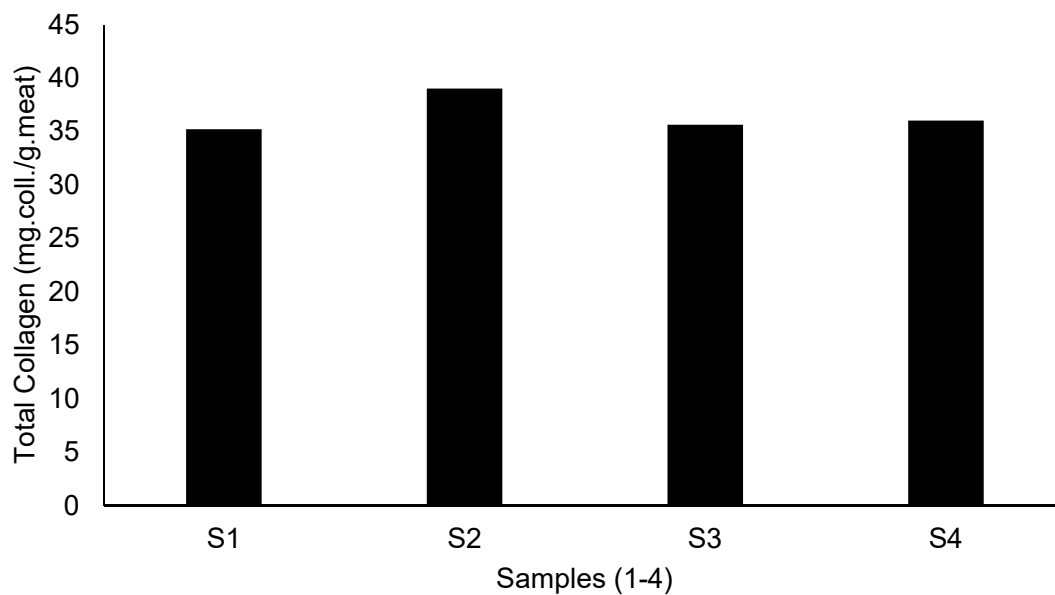
Excellent hydroxyproline calibration curves were consistently obtained. Figure 5-3 is an example of such a curve, which was obtained when measuring the soluble collagen in the cook-loss of a sample cooked at 60 °C. Since the hydroxyproline in the unknown sample is calculated using the calibration curve, the accuracy and consistency of the calibration curves are essential for obtaining reliable results.



**Figure 5-3.** Hydroxyproline calibration curve.

**Figure 5-4** shows the measured total collagen for the beef *semitendinosus* muscle. The samples closely agree, apart from sample two which is slightly higher than the other values. The observed differences could be due to several sources of error. One of the most apparent is the irregular distribution of connective tissue within the meat sample. The homogenisation step has reduced this irregularity, but since the samples were not liquified, differences will always remain even within very small samples. The second source of error is the hydrolysis step: it was observed that small particles always remained, an indication of incomplete hydrolysis. Both the time and the quantity of the hydrolysing acid were increased but to little or no advantage. A third major source of experimental error is the collagen extraction process. Because collagen is an extracellular protein, it cannot be easily

accessed, isolated, and quantified. As such, complex experimental methods (including the one used here) have been developed which involve many steps and reagents. Each of these steps introduces an error; which, if considered individually, might not amount to much but collectively become significant. Other sources of error include glassware, chemicals, machinery, etc. In light of these many possible errors, it is entirely reasonable to assume the overall experimental error to be an upward of  $\pm 15\%$ .



**Figure 5-4.** Total collagen for the raw *semiteudinosus* samples.

As Table 5-1 shows, the reported total collagen for beef *semiteudinosus* wildly varies amongst different studies. Some of these differences will be due to the experimental factors previously discussed, others would be due to the age of the animals and their level of physical fitness. In any case, it seems unreasonable to obtain such a variation. The values obtained in the current work (Figure 5-4) are higher than any value reported in the surveyed literature (Table 5-1), which suggested at the time that these results were wrong. This was investigated by sending a sample to an approved lab, which employed an advanced HPLC method to quantify the total collagen. The lab returned with a value of 39.7 mg-collagen/g-meat and agreed well with what is portrayed in Figure 5-4. It was therefore concluded the

measured values were correct, but no explanation was found as to why it is so much higher than what is previously reported in the literature.

**Table 5-1.** Total collagen in beef *semitendinosus* muscle.

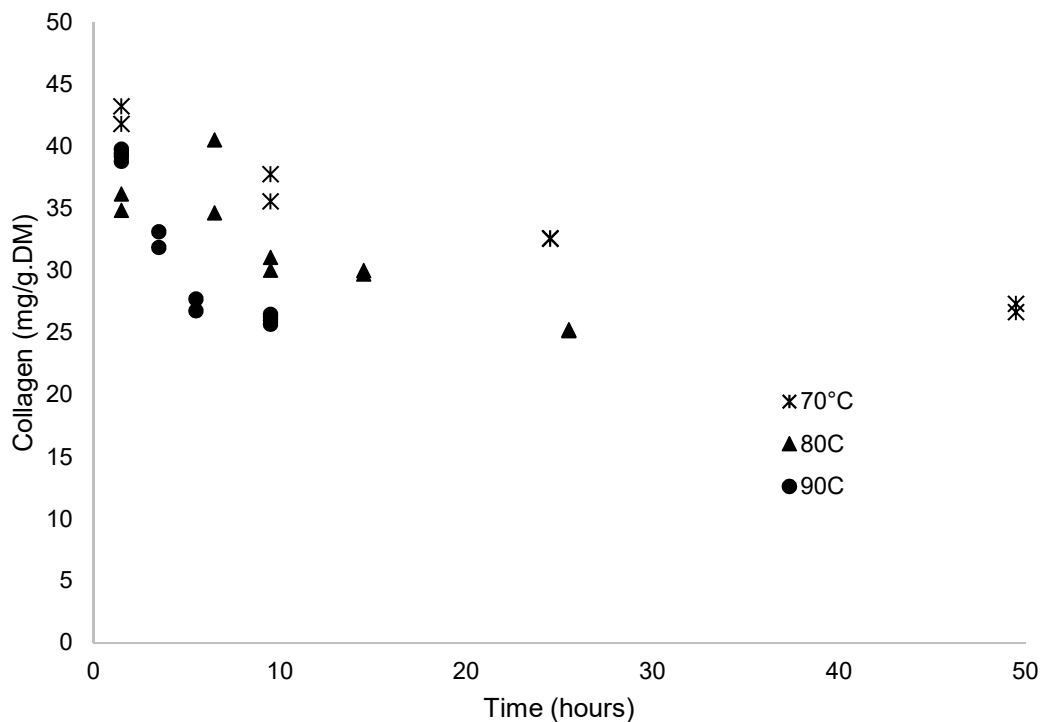
Collagen Content (mg-collagen/g-meat)	Animal	Source
17.4	Bulls (25-30 months)	Cho et al. (2016)
5.5	Beef	Booren et al. (2005)
6.9	Bulls (15-24 months)	Ba et al. (2014)
9.1	Beef	Oillic et al. (2011)**
0.79	Bulls (18 months)	Palka et. Al (2003)

\*\* Average value in the surveyed literature.

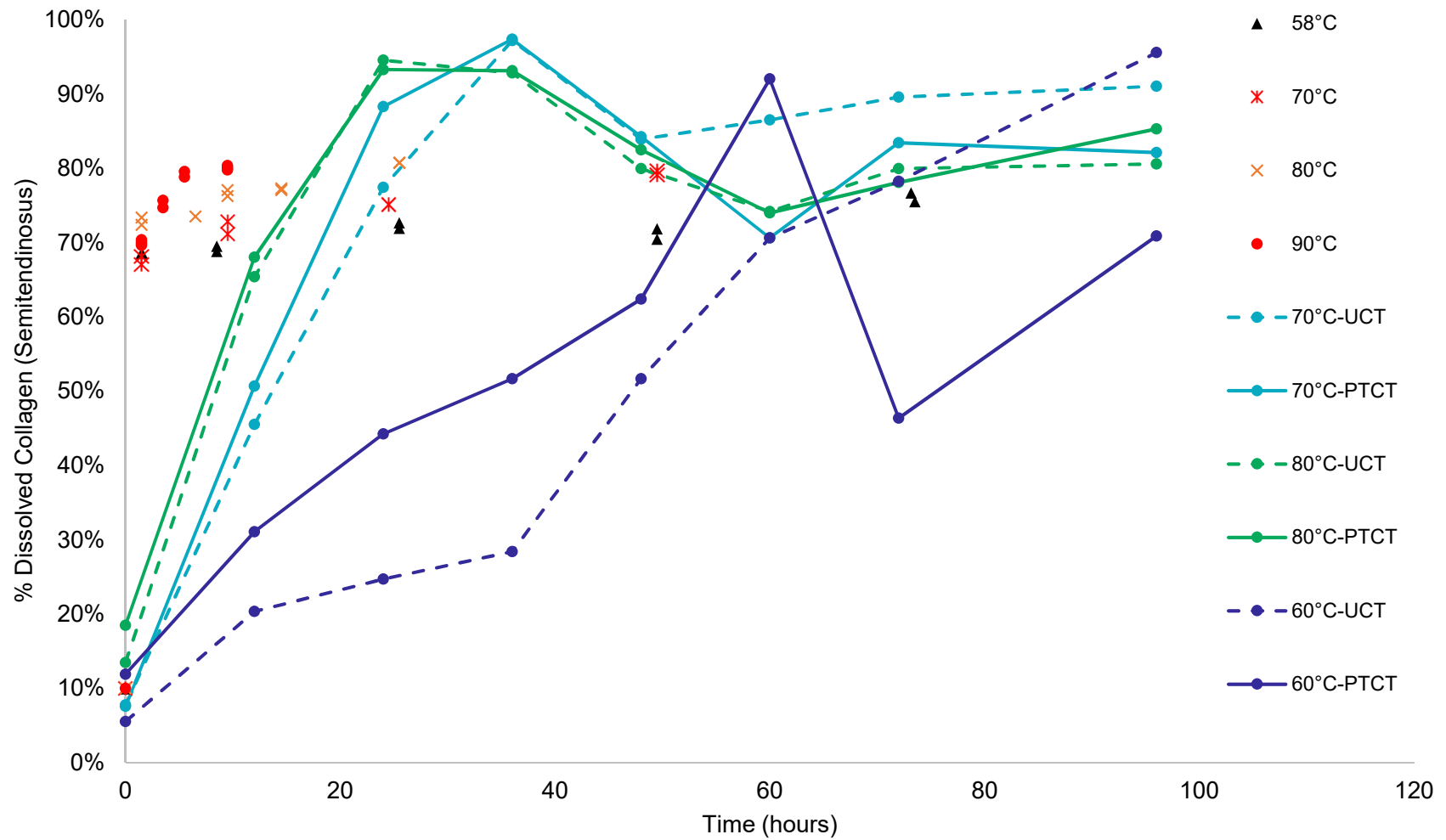
#### 5.4.2 Solubilised collagen within the meat

Alahakoon et al. (2017) circumvented the difficulty of measuring the solubilised collagen within cooked meat samples by first extracting pieces of collagen from beef brisket (deep *pectoralis*) and dividing them into two groups: a control group (untreated connective tissue, UCT) and pulse electrical field treated connective tissue (PTCT). The purpose of the PEF is to pre-tenderise the meat and to reduce the required sous vide cooking time. Next, the samples were suspended in agar gels, intended as meat analogues. Finally, the samples were sous vide cooked in a water bath at 60, 70, and 80 °C for 12—96 hours (in intervals of 12 hours). Figure 5-6 illustrates the measured dissolved collagen as well as the results of Alahakoon et al. (2017). As shown, although the equilibrium dissolved collagen was similar, the initial collagen dissolution rates between the two studies were significantly different. For example, after 1.5 hours of cooking, the measured dissolved collagen was approximately 70% whereas the Alahakoon et al. (2017) values had a maximum of 25%. Also, unlike the Alahakoon et al. (2017) results, which showed very clear differences, there were no significant differences in the measured dissolved collagen between samples cooked for 1.5 hours at different temperatures. The discrepancy (between the two studies) might be due to several factors. (1) Different samples: *semitendinosus* vs. brisket; (2) nature of samples:

isolated vs intact – it is unlikely that the agar gels accurately simulate the native structure of meat; collagen behaves differently when in isolation compared to when in situ; and (3) method of measurement: the developed method has not been thoroughly tested and was not independently evaluated nor compared with other methods (since there were no known methods). One of the obvious weaknesses of this method is the use of sieves. Even though the smallest sieve was 40  $\mu\text{m}$ , it is possible that small insoluble collagen particles were washed out with the water; but if this was the case, the differences should have been also observed in the equilibrium values (i.e. the measured equilibrium values would have been higher than those reported by the other study). In any case, until an accurate, verifiable method is developed, any results obtained through this method should be received with caution. Nonetheless, as Figure 5-5 shows, the measured insoluble collagen within the meat decreases with time as expected (meaning the method shows promise).



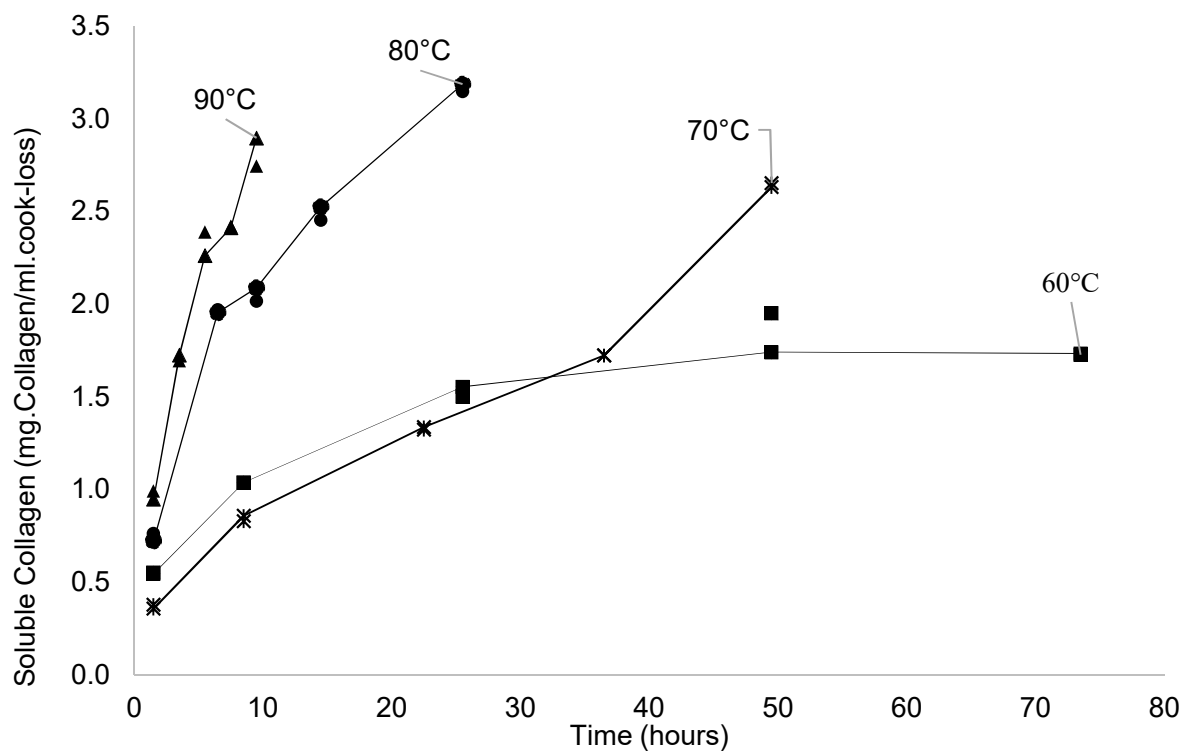
**Figure 5-5.** insoluble residual collagen within the meat of in sous vide cooked samples.



**Figure 5-6.** Measured dissolved collagen (pullets) and those reported by Alahakoon et al. (2017). UCT=*un-treated connective tissue*, PTCT=*PEF treated connective tissue*. The dissolved collagen of raw samples is about 10% of the total collagen (Li et al., 2008).

### 5.4.3 Soluble collagen in the cook-loss

Figure 5-7 depicts the measured soluble collagen in the cook-loss. The soluble collagen bears similarities with the cook-loss. For example, a steep initial slope is observed as well as an increasing solubilised collagen with increasing temperature (for a given time). On the other hand, unlike the cook-loss measurements, the soluble collagen did not equilibrate, with the lone exception of samples treated at 60 °C – this is the exact opposite of what is observed in the cook-loss data.



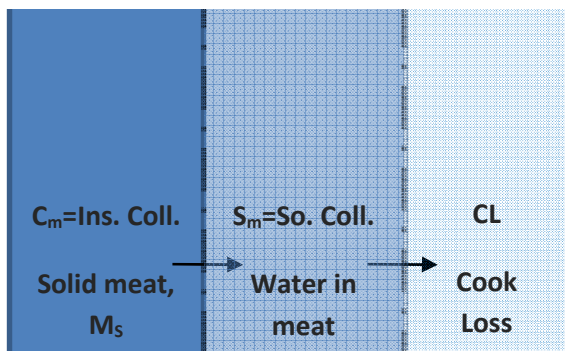
**Figure 5-7.** Soluble collagen in the cook-loss.

The measured dissolved collagen in the cook-loss is not an accurate reflection of the total dissolved collagen within the meat. This is so because of two main reasons. First, as shown in Figure 5-4, the total collagen in the meat is about 35 mg-collagen/g-meat, but as Figure 5-7 shows, the highest measured dissolved collagen is only about 3.15 mg-collagen/ml-cook-loss. It is, therefore, difficult to accept that only 10% of the total collagen was dissolved. Second, the peak force measurements of samples cooked at 60 °C (Figure 4-13, section 4.6.3) continues to decline even after the soluble collagen has equilibrated, Figure 5-7.

Although collagen is not the only factor governing the peak-force, it is a major factor. As such, once collagen stopped dissolving, the effect should have been reflected upon the peak force measurements.

At the initial stage of the cooking process, significant water loss occurs (for example as Figure 4-5 shows) but it is theorised that little collagen is dissolving, although if sufficient time is given, significant amount of collagen can diffuse out. As the cooking process proceeds, the rate of cook-loss slows but collagen continues to dissolve – most likely at an accelerated rate. The collagen expulsion from the meat is caused by the cook-loss; hence, as the cook-loss stops, the dissolved collagen in the cook should stabilise; however, Figure 5-7 shows the contrary – that is, collagen continues to dissolve. Two possible explanations were formulated for this observation: (1) There is a small background cook-loss, which enables the collagen to seep out of the meat into the cook-loss – furthermore, it is very probable that this background cook-loss contains much higher levels of dissolved collagen, and (2) the meat is physically in contact with the cook-loss thereby enables the collagen to diffuse out – it is, of course, possible that both mechanisms are occurring simultaneously.

The dissolution and eventual expulsion of collagen is obviously a complex process that is difficult to model. Therefore, a simplified conceptual model is presented (Figure 5-8). In this model, the meat is divided into three regions: the solid meat, the water within the meat, and the cook-loss. At time zero, there is no (measurable) cook-loss.



**Figure 5-8.** Conceptual model of collagen dissolution and migration.

Using the above conceptual model, the system was modelled as follows:

The insoluble collagen in the meat ( $C_m$ ) is assumed to dissolve as a first order release dependent on the insoluble collagen concentration, Eq. (5.8):

$$\frac{dC_m}{dt} = -kC_m \text{ for } t > 0 \quad (5.8)$$

The solubilised collagen in the water within the meat ( $S_m$ ) (i.e. middle region) is obtained as follows:

$$\begin{aligned} \text{Rate of Collagen} &= \text{rate of collagen} - \text{rate of collagen exiting} \\ \text{accumulation in the water} &= \text{dissolution} - \text{with the cook loss} \\ \frac{dS_m M_w}{dt} &= kC_m M_s - S_m \frac{dM_{CL}}{dt} \end{aligned}$$

Where  $M_s$ ,  $M_w$  represent the weight (g) of the solids and water in the original meat sample and  $M_{CL}$  is the weight (g) of the cook-loss.

Applying the product rule to the above equations and simplifying, yields Eq. (5.9):

$$\begin{aligned} M_w \frac{dS_m}{dt} + S_m \frac{dM_w}{dt} &= kC_m M_s - S_m \frac{dM_{CL}}{dt} \\ M_w \frac{dS_m}{dt} &= kC_m M_s - S_m \frac{dM_{CL}}{dt} - S_m \frac{dM_w}{dt} \\ \frac{dS_m}{dt} &= \frac{1}{M_w} \left[ kC_m M_s - S_m \left( \frac{dM_{CL}}{dt} + \frac{dM_w}{dt} \right) \right] \end{aligned}$$

But the rate of water loss = rate of cook loss, i.e.  $\frac{dM_w}{dt} = -\frac{dM_{CL}}{dt}$

$$\therefore \frac{dS_m}{dt} = \frac{M_s}{M_w} kC_m \text{ for } t > 0 \quad (5.9)$$

Finally, the soluble collagen in the cook-loss ( $C_{CL}$ ) is obtained as follows:

$$\frac{dM_{CL} C_{CL}}{dt} = S_m \frac{dM_{CL}}{dt}$$

Applying the product rule and simplifying, yields an expression for  $C_{CL}$ , Eq. (5.10):

$$M_{CL} \frac{dC_{CL}}{dt} + C_{CL} \frac{dM_{CL}}{dt} = S_m \frac{dM_{CL}}{dt}$$

$$\frac{dC_{CL}}{dt} = \frac{1}{M_{CL}} \frac{dM_{CL}}{dt} (S_m - C_{CL}) \quad \text{for } t > 0 \quad (5.10)$$

Eq. (5.10) contains the rate of cook-loss,  $dM_{CL}/dt$ , which can be calculated from the moisture content. In section 4.6.2, the cook-loss was model as with a system of two reactions, where the extent of reaction,  $X$ , was the dependent variable. By knowing the initial and the equilibrium moisture contents ( $MC_i$  &  $MC_e$ ), the moisture content ( $MC$ ) as a function of time was calculated; this method can be extended to yield an expression for the rate of cook-loss as a function of the extent of reaction. This is demonstrated as follows:

The weight of cook-loss is given by Eq. (5.11):

$$M_{CL} = M_s(MC_i - MC)$$

$$M_{CL} = M_s MC_i - M_s MC \quad (5.11)$$

**Where:**  $M_s$  = mass of the solids in the meat (g), and  $MC_i$  &  $MC$  are the initial and the time depend moisture contents, respectively. Note:  $MC=f(t)$ .

The rate of cook-loss is the derivative of Eq. (5.11).

$$\frac{M_{CL}}{dt} = -M_s \frac{dMC}{dt} \quad (\text{Since } M_s \cdot MC_i \text{ is a constant, it goes to zero when differentiated})$$

The relationship between the extent of reaction,  $X$ , and the moisture content,  $MC$ , is given by the following expression, which, when rearranged gives, Eq. (5.12).

$$X = 1 - \frac{MC - MC_{eq}}{MC_i - MC_{eq}}$$

$$MC = MC_i + (MC_e - MC_i)X \quad (5.12)$$

Substituting Eq. (5.12) into Eq. (5.11) and simplifying gives an expression for the rate of cook-loss as a function of the extent of reaction.

$$\frac{M_{CL}}{dt} = -M_s \frac{d[MC_i + (MC_e - MC_i)X]}{dt}$$

$$\frac{M_{CL}}{dt} = -M_s \left[ \frac{dMC_i}{dt} + (MC_e - MC_i) \frac{dX}{dt} \right]$$

$$\frac{M_{CL}}{dt} = -M_s \left[ 0 + (MC_e - MC_i) \frac{dX}{dt} \right]$$

$$\frac{M_{CL}}{dt} = M_s(MC_i - MC_e) \frac{dX}{dt}$$

But as discussed in 4.6.2, X is the overall extent of reaction and is composed of two distinct extent of reactions ( $X_1, X_2$ ) that are paired by a fractional fitting factor,  $\alpha$ . Substituting these back to the equation above and simplifying, yields:

$$X = \alpha X_1 + (1 - \alpha) X_2$$

$$\frac{M_{CL}}{dt} = M_s(MC_i - MC_e) \frac{d[\alpha X_1 + (1 - \alpha) X_2]}{dt}$$

$$\frac{M_{CL}}{dt} = M_s(MC_i - MC_e) \left[ \alpha \frac{dX_1}{dt} + (1 - \alpha) \frac{dX_2}{dt} \right]$$

Recall that  $X_1$  and  $X_2$  are respectively given by equations (4.12) and (4.13). A final expression can therefore be obtained for the rate of cook-loss, (5.13).

$$\frac{dX_1}{dt} = k_1(1 - X_1) \quad \text{for } t > 0$$

$$\frac{dX_2}{dt} = k_2(1 - X_2) \quad \text{for } t > 0$$

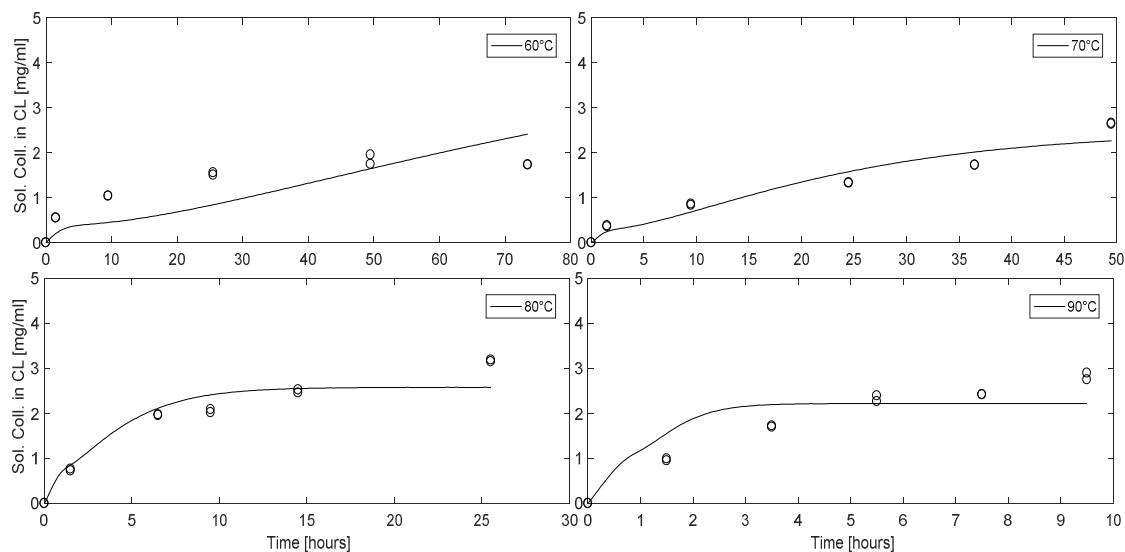
$$\frac{M_{CL}}{dt} = M_s(MC_i - MC_e) [\alpha k_1(1 - X_1) + (1 - \alpha) k_2(1 - X_2)] \quad \text{(5.13)}$$

The model was validated by using the experimentally measured solubilised collagen in the cook-loss. The only fitting parameter, which was fitted independently to each temperature (60, 70, 80, 90°C), was the rate constant for collagen solubilised within the meat, i.e. the rate constant in Eq. (5.8). Table 5-2 and Figure 5-9 show the fitted rate constants and the model predictions. In general, the model predictions were moderately satisfactory. As shown, at low temperatures the rate constant is rather smaller (particularly at 60 °C) and the predicted profile is more of a linear than exponential and below the experimental data. At 90 °C, the

opposite profile is observed, where the model over predicts in the first part of the cooking process.

**Table 5-2.** Fitted rate constants *in Eq. (5.8).*

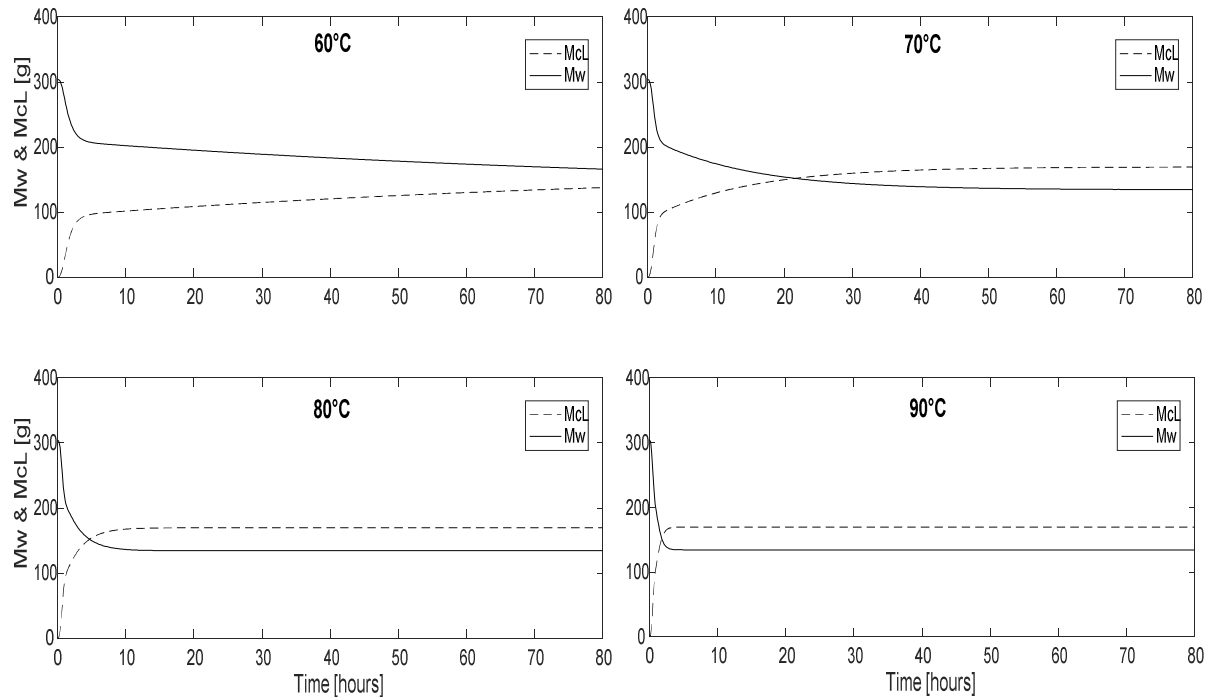
Temperature (°C)	Fitted Rate Constant, $k$ , ( $\text{h}^{-1}$ )
60	0.0195
70	0.0252
80	0.1030
90	0.1910



**Figure 5-9.** Predicted and measured dissolved collagen in the cook-loss (CL).

The weakness in the model may be due to model simplicity. It was assumed the only mechanism by which collagen is exuded is with the cook-loss. Other mechanisms may, however, be at play; if, for instance, the meat had a higher level of dissolved collagen concentration (than the cook-loss) and the meat was in contact with the cook-loss, the collagen would diffuse out from the meat to the cook-loss. This supposition is supported by the fact after the cook-loss has stopped (Figure 5-10), the collagen concentration in the cook-loss continued to increase (Figure 5-9). The other possibility, by which dissolved collagen is diffusing into the cook-loss, is the presence of a small continuing cook-loss but with high dissolved collagen concentration. A third possibility is that the collagen dissolution

does not follow a first order kinetics. The incorporation of either of these mechanisms into the model is difficult and outside of the scope of the current work.



**Figure 5-10.** Mass of water (Mw) and mass of cook-loss (Mcl) at different temperatures.

## 5.5 Chapter conclusion

Both the total and the dissolved collagen in the cook-loss were successfully quantified. The total collagen of the beef *semitendinosus* was found to be (approximately) 35 mg-collagen/g-meat, which is higher than what is reported in the literature. An independent, accredited lab has, however, confirmed the results to be correct. Additionally, the figures reported in the literature are highly variable, which suggests variation in experimental variability and or source material. The dissolved collagen in the cook-loss is not an accurate measure of the total collagen dissolved within the meat. The model developed for predicting the collagen dissolution produced moderate results.

Thus far, most of the measurements and observations made were largely considered on individual basis but is important to consider these collectively in an effort to elucidate the underlying mechanism in meat tenderisation. This is accomplished in the following chapter.

## Chapter 6 General discussions, conclusions and recommendations

### 6.1 Introduction

In the preceding chapters, a literature review, a financial feasibility study, kinetics, tenderness measurements, and collagen solubilisation were presented. In this chapter, the various concepts and results are brought together to construct an overall picture and to summarise the key factors and how they influence meat tenderisation. Also in this chapter are recommendations for future studies.

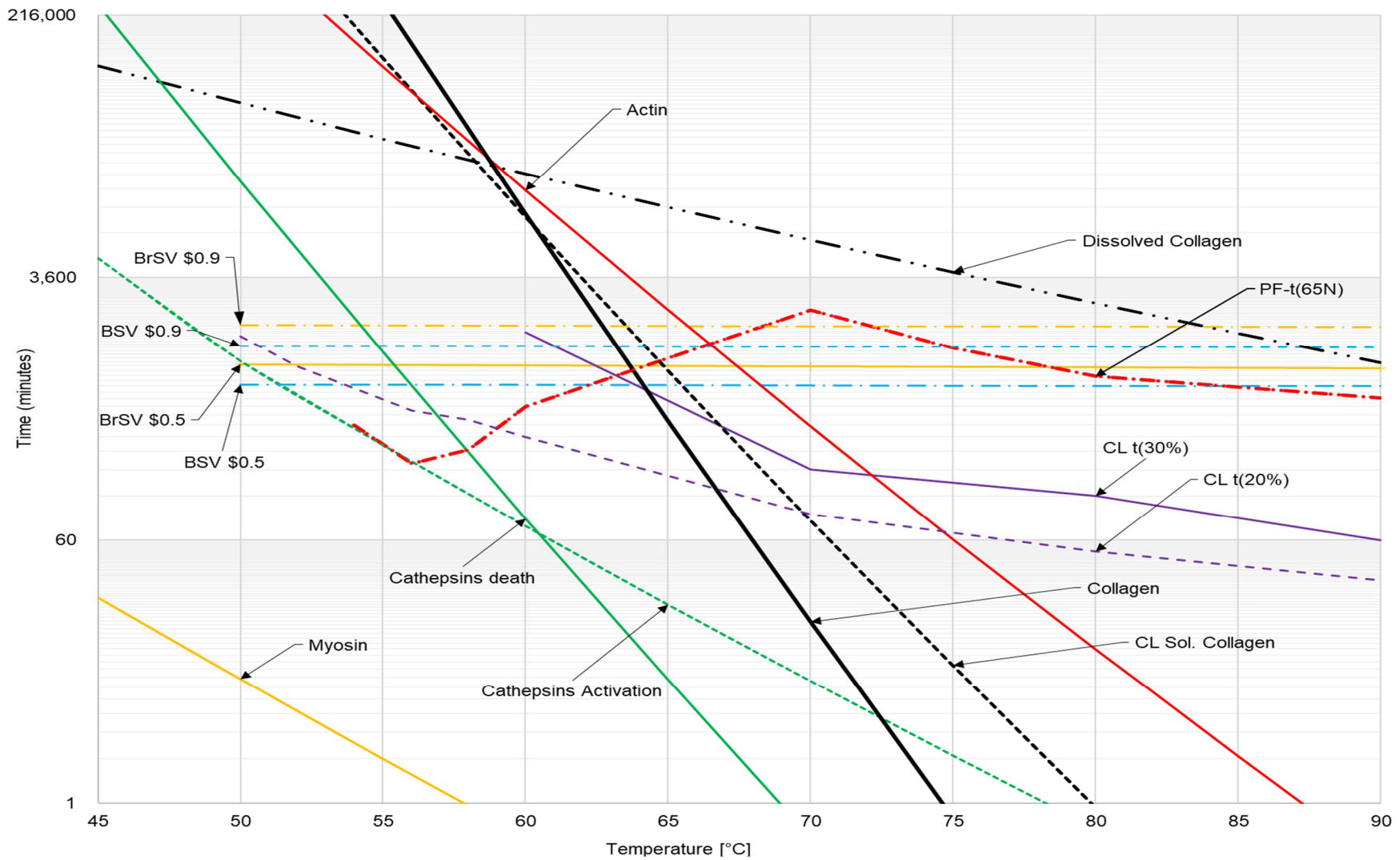
### 6.2 Process mapping

As afore explained, meat is exceedingly complex material that is composed of numerous components. Therefore, any of its measurable properties – such as the Warner-Bratzler peak force – is likely to be governed by multiple factors. But because it is not practical to consider every such contributor, the current work was constrained to the main factors, namely the denaturation and dissolution of collagen, the denaturation of myofibrillar proteins, the cook-loss, and the cathepsins enzymatic activity (the calpains, the other main form of enzymes in meat, were not considered as they denature at low temperatures and are considered not a significant contributor to meat tenderisation in the temperature ranges of interest). To bring all these together, Figure 6-1 was constructed, which contains iso line for the followings (iso-lines that show the temperature and time combination required to bring about the same extent of change):

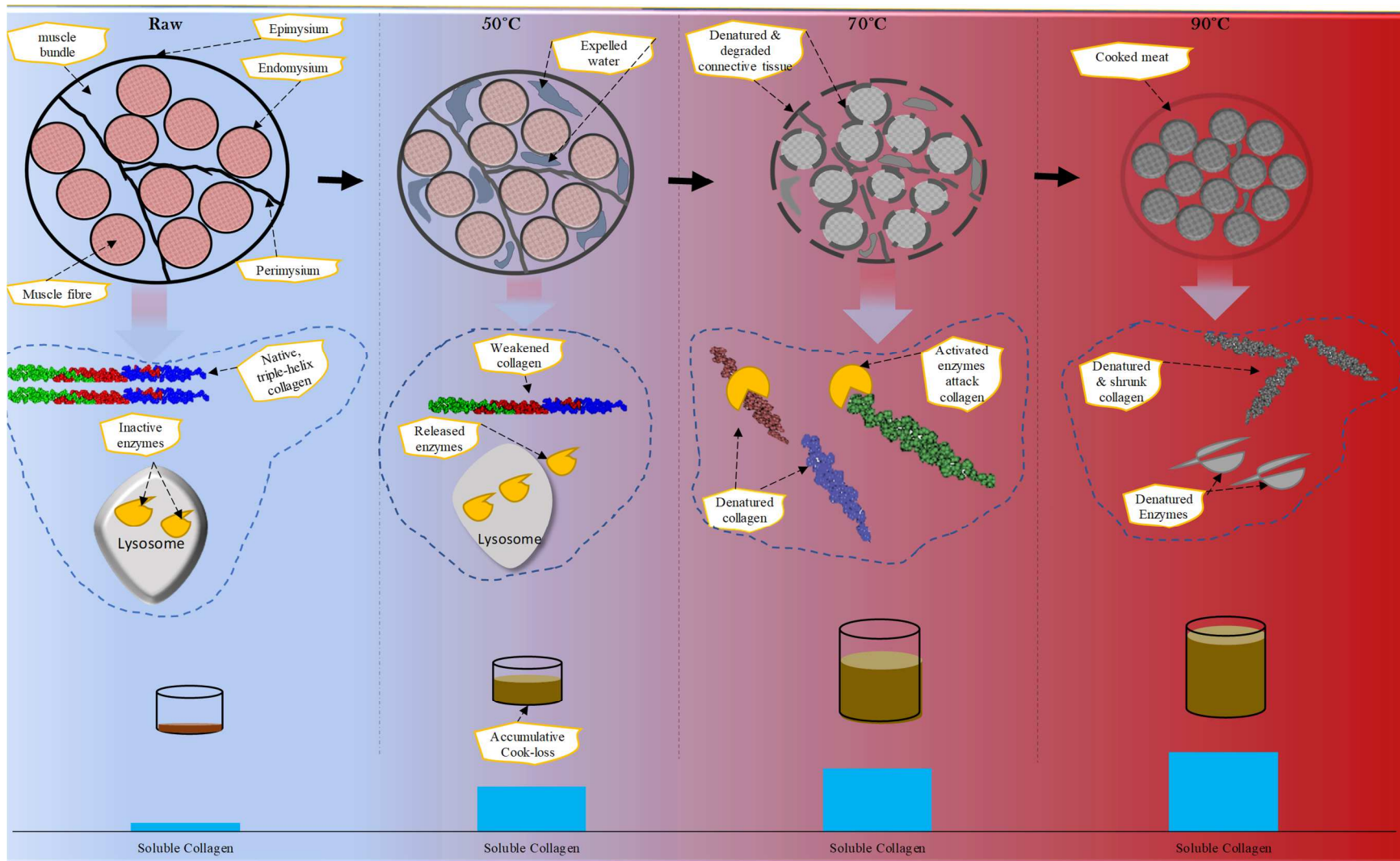
- Iso-extent of reaction lines representing the kinetic profiles of myofibrillar proteins (myosin and actin) denaturation, cathepsins enzymes activation and inactivation, native collagen denaturation, the dissolved collagen formation within cooked meat and in the cook-loss (i.e. the 'heat soluble collagen').

- Iso-cook-loss lines of 20 and 30% (*the maximum cook-loss was 42%, hence these correspond to 47.6 and 71.4% of the total cook loss, respectively*)
- Iso-peak force line of 65 Newtons in the decline phase of the Warner-Bratzler shear force measurements (Figure 4-12, Figure 4-13)
- The required cooking time for the overall cost of basic sous vide (BSV, case (1)) and the brisket-like sous vide (BrSV, case (2)) to be \$0.5 and \$0.9.

Also, based on the results obtained and what was previously reported in the literature, a conceptual model – which shows the key changes in meat during the cooking process – has been proposed (Figure 6-2). The model illustrates what happens as the meat is heated from raw to resulting meat, as a function of heating temperature. The model is composed of three basic elements: the changes which occur in the macro level (top layer), the micro level (middle-layer), and the changes in the measurable properties of the meat (lower region).



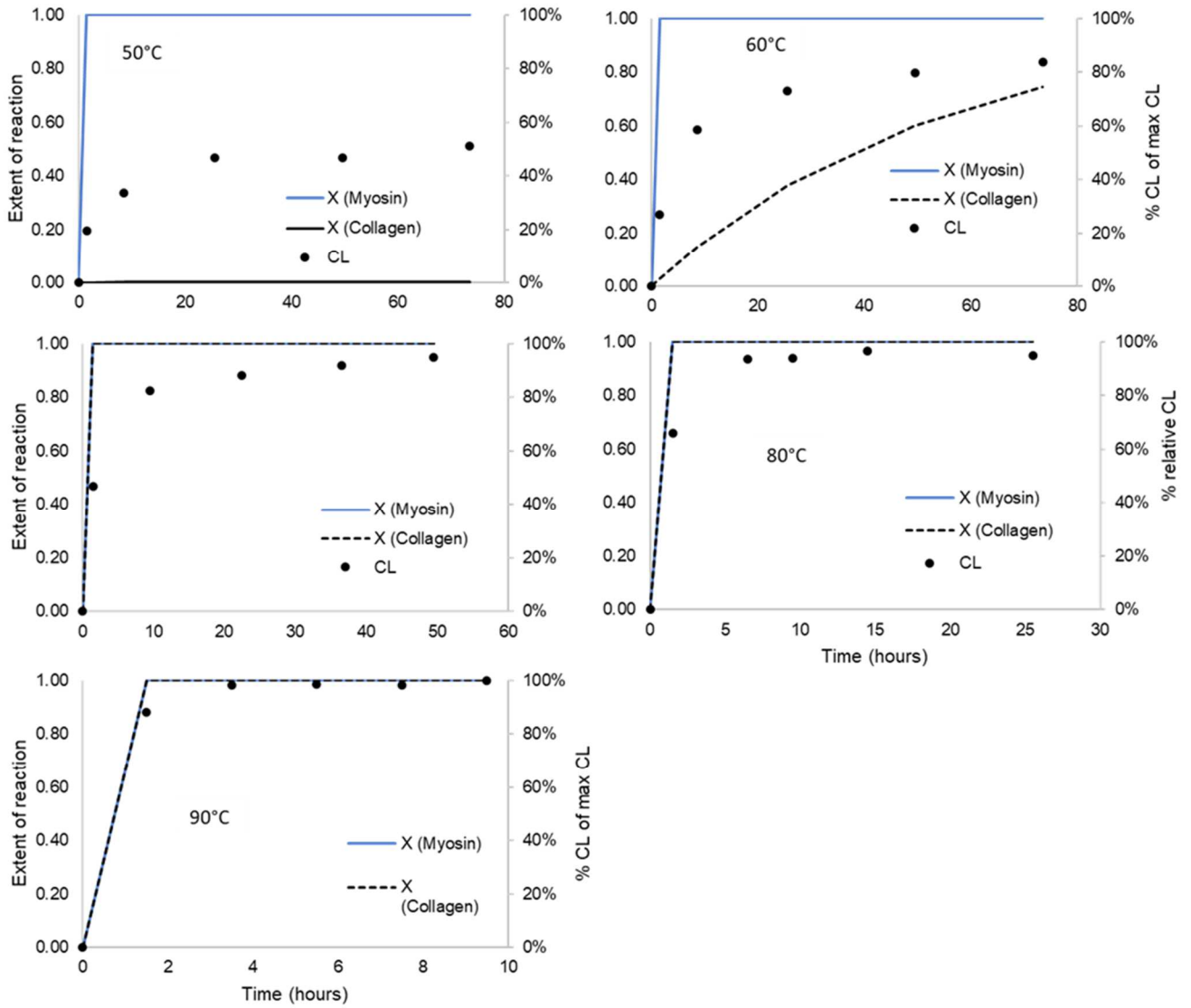
**Figure 6-1.** Time for 95% of kinetic changes in meat components as well as cost, cook-loss, and peak force isolines. BSV= basic sous vide, BrSV = brisket like sous vide.



**Figure 6-2.** Proposed conceptual model for the changes that occur during meat cooking. This is only a conceptual model and the indicated processes do not necessarily occur only at the indicated temperature points – it is more probable that these occur over range of temperature. It is hoped that future studies will elucidate these temperatures.

Of the components thought to influence meat tenderness, the myosin component of the myofibrillar proteins undergoes changes well before any of the other components (Figure 6-1). Because the meat fibres hold a substantial fraction of the overall water within the meat, the denaturation of myosin causes significant water losses, which is shown as the initial, rapid cook-loss in Figure 4-5 (also as Figure 6-3 shows, this amount accounts for approximately 50% of the measured cook-loss). Consequently, the overall density of the meat increases as does the meat toughness (this is not shown on Figure 6-1 since the plotted iso-peak force was post the first peak). In the conceptual model (Figure 6-2), this is depicted as a reduction in the diameter of the meat fibres, the appearance of 'expelled water', the liberation of (still inactive) enzymes from the lysosomal structure, and an increase in the cook-loss. At medium temperatures, the relative cook-loss increases, but the increase in the peak force is moderated by the activation of cathepsins, which attack the collagen structure. It is probable that the calpain enzymes are already activated at lower temperatures and might be contributing to the tenderisation. As the temperature increases further, the enzymes are denatured, and collagen denatures (and shrinks), which causes further water losses, resulting an increase in the time required to reach a peak force of 65 N. Again, as can be seen from Figure 6-3, the denaturation of collagen leads to increased cook loss, and by the time collagen is completely denatured, more than 80% of the total cook-loss has occurred (the remaining ~ 20% cook-loss is likely due to the denaturation of actin). Eventually, the temperature goes high enough for collagen to solubilise, consequently, the time required to reach a peak force of 65 N reduces. Although the pH and enzymes inhibitors were not the focus of this work, they are deemed to be important for enzyme activity and should be considered in future studies.

As Figure 6-2 shows, the cook-loss is influenced by the heating temperature and time (though to a lesser extent). The figure also shows (with respect to cost), it is the cooking time which dominates the cost of the sous vide process and, as the horizontal lines show, the cooking temperature (for a given cooking time) has little influence on the overall cost of the process – the power contribution to the overall cost is very small.



**Figure 6-3.** The extent of reaction (X) for myosin and collagen, and % relative cook-loss (CL) at different temperatures. The % relative CL is defined by eq. (6.1).

$$\% \text{ relative CL} = \frac{CL(t, T)}{CL_{\text{Max}}} \quad (6.1)$$

**Where:**

$CL(t, T) =$  the measured % Cook-loss (CL) as a function of time and temp  
 $CL_{\text{max}} =$  the maximum experimentally recorded cook-loss

### 6.3 Overall conclusions and recommendations

The literature review of the current work has clearly shown there are clear gaps in the scientific understanding of sous vide. Because the sous vide process is relatively new, the scientific description of sous vide is still at its infancy and the approach to sous vide cooking has been largely of art than concise science. Accordingly, the current work was intended to characterise the key process in sous vide so that, ultimately, the process can be optimised both in quality and economic metrics.

The financial analysis has shown that high pressure processing (HPP) is uneconomical and thus should not be entertained unless significant value can be added through other functional changes. Similarly, pulse electric field (PEF) was found to be uneconomical; however, given the novelty of the technology – particularly its application to meat – it is likely to experience appreciable capital price drop to a point where it may become economical and a suitable supplementary technology for sous vide. The enzyme process was found to be most economical and thus the best technology to supplement the sous vide process. The economic analysis was done using only formulated models (as discussed in Chapter 3) and no pilot or industrial trials were conducted. The next step in the process should therefore be the validation of the theoretical calculations through pilot and industrial trials. The economic analysis has also shown that improvements can be realised through improved sous vide. That is, reducing the sous vide cooking time, would improve the economics of the process. Significant cook losses were observed for all temperatures (50–90 °C), which was found to be both time and temperature dependent. Similarly, all temperatures showed a steep cook-loss gradient in the initial phase of the cooking process, attributed to myofibrillar protein denaturation, followed by a gradual cook-loss increase. Cooking at 70, 80, and 90 °C showed similar, ultimate equilibrium cook-loss of approximately 40–42% (previous studies have also reported similar values), which suggests that the maximal cook-loss (irrespective of time and temperature) is around these values; but this statement is only applicable to 70

°C and above, as lower cooking temperature (50—60 °C) either did not equilibrate or had much lower equilibrium values. Cook-loss is undesirable outcome of the cooking process and appears to be largely unavoidable. Future studies should, nevertheless, focus on ways of minimising it. Marinades and salts alter the pH and the ionic strength of the meat and are known to increase the water uptake of the meat, which may serve as a potential remedy for excessive cook-loss. Mechanistically, these loosen the collagen fibrils and enhance collagen solubilisation. The shrinkage of collagen squeezes out water entrapped between the muscle fibres; thus, if collagen can be solubilised without shrinking it first, water losses can be reduced.

A kinetic model was derived for the cook-loss and was found to be adequate for predicting the cook-loss at both low (50—60°C) and high temperatures (70—90°C) for beef *semitendinosus* samples. The Warner-Bratzler (WB) shear force of all samples (except samples sous vide at 50 °C) showed a similar profile of rapid peak force rise (from the raw samples) then a rapid drop, followed by a slow decline – unlike the cook-loss, the peak force measurements did not reach an equilibrium values and continued to decrease.

The Warner Bratzler method has been traditionally used for quantifying meat tenderness/toughness, but the use of this method might not be adequate or accurate in many situations. For example, when attempting to gauge the in-situ connective tissue contribution to the overall observed meat toughness. The issues lie with how the connective tissue is more or less randomly dispersed within the meat and how the WB blade comes in contact with the samples relative to the meat fibre direction. Almost universally, the blade cuts the samples perpendicular to the fibre direction; as such, in one measurement the blade might cut a piece of connective tissue (hence the peak force is very high) but it might not encounter any connective tissue in the next measurement resulting in a lower peak force value. This leads to highly variable results between successive measurements. Also, the perpendicular arrangement leads to complications when attempting to quantify the ability of the connective tissue to hold the meat fibres in place. It appears, that the Warner-Bratzler

method heavily favours the contribution of the myofibrillar protein, thus, future studies should either develop novel methods (for quantifying the connective tissue contribution) or modify the existing method by for example measuring the toughness parallel to the fibre direction.

The total collagen in the raw beef semitendinosus samples was found to be approximately 35 mg-collagen/g-meat, which was higher than what was previously reported in the literature. The solubilised ('heat soluble') collagen in the cook-loss was also quantified and was found to be much lower than expected, which suggests that the dissolved collagen in the cook-loss is not an accurate reflection of the actual solubilised collagen within the meat. Measuring the dissolved collagen within the meat is rather difficult and no existing methods were found. A method was therefore devised for such a requirement. The method was successfully applied, but it has clear weaknesses that future studies should address. For instance, the use of sieves (of which the smallest was 40  $\mu\text{m}$ ) may allow the washing out of small undissolved, collagen particles, resulting in over estimation of the actual dissolved collagen.

The current work has sufficiently characterised the key processes in sous vide; hence, the primary focus of future studies should be process optimisation. That is, (i) improving product profitability, by reducing the cooking time, and (ii) maximising product quality, by maximising connective tissue solubilisation, optimising myofibrillar protein denaturation, minimizing drip loss, and identifying the best marinades to alter the pH and the ionic strength of the meat. The end goal should be the ability to select the appropriate processing conditions for a given raw material with known characteristics.

Even though the work carried out in this study, including the feasibility analysis, the development of a novel method (for quantifying residual collagen within cooked meat), and the development of kinetic models and a conceptual meat tenderisation model, represent an obvious and important contribution to the existing knowledge, still much research is warranted in sous vide and meat science in general. Further work is required to enhance the

understanding of meat tenderisation that will allow improved and new products and processes to meet consumers' demand for better products.

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