THE EFFECT OF PRE-RIGOR INFUSION OF LAMB WITH KIWIFRUIT JUICE ON MEAT QUALITY

A thesis submitted in partial fulfilment

of the requirements for the degree

of

Master of Science (Food Science)

at

Lincoln University

By

Jin Han

LINCOLN UNIVERSITY

2008

An abstract of a thesis submitted in partial fulfilment of the requirement for the degree of Master of Science (Food Science)

THE EFFECT OF PRE-RIGOR INFUSION OF LAMB WITH KIWIFRUIT JUICE ON MEAT QUALITY

by

Jin Han

Tenderness, juiciness, colour and flavour are the most important meat quality attributes affecting the consumer acceptance. Maintaining the consistency of meat products by avoiding variable quality has become a major concern and great challenge to the meat industry. This in turn will also benefit meat end-users in the marketplace by having more tender meat. The present study was designed to evaluate the overall effects of pre-rigor infusion with kiwifruit juice, which contains the plant protease, actinidin, on lamb quality. A total of 18 lambs (12 months old) were divided into three treatment groups (6 lambs per each treatment). After exsanguination, lamb carcasses were infused (10% body weight) with fresh kiwifruit juice (Ac), water (W) and compared with a noninfusion treatment which acted as a control (C). Samples from different muscle/cuts (longissimus dorsi (LD) vs leg chops) at different post-mortem times (1 day post-mortem vs. 3 wks vacuum packaged storage at 2^{o}C) and display time (0 to 6 days after the post-mortem storage) were analysed to monitor the changes on meat physical properties (e.g., tenderness, temperature, drip and cooking loss, colour), biochemical changes (pH, proteins and lipids) and volatile flavour compounds after the infusion treatments.

The most tender meat (lowest shear force values) ($P < 0.001$) detected in the Ac carcasses post-mortem compared with C and W carcasses demonstrated that kiwifruit juice was a very powerful meat tenderizer, and could contribute to the meat tenderization process efficiently and effectively. Compared with C and W carcasses, the enhanced proteolytic activity ($P = 0.002$) resulting from the actinidin in kiwifruit juice in Ac carcasses caused degradation of the myofibrillar proteins and the appearance of new peptides during post-mortem ageing. A slight positive effect in a*-value (redness) and decreased lipid oxidation, found in leg chops, was thought to be caused by the natural antioxidants in kiwifruit juice.

Kiwifruit juice infused into the meat did not alter ($P > 0.05$) the volatile flavour compound profile indicating that the meat from Ac treated carcasses maintained its natural lamb flavour.

No treatment differences were found for the temperature decline ($P > 0.05$) between the infused treatments and C. The higher rate of pH decline ($P < 0.05$) found in W carcasses might have contributed to the higher drip and cooking loss. The unbound water in meat might contribute to the higher L*-values (lightness) found in W carcasses.

In summary, the proteolytic tenderizing infusion treatment using kiwifruit juice is a feasible approach for the commercial meat industry to increase profits, and also could satisfy the eating quality standards required by the consumers. In addition, tenderizing meat by using kiwifruit juice could also provide the kiwifruit processors an additional option for use of their product to gain a more profitable return.

Key words: actinidin, colour, eating quality, flavour, infusion, juiciness, kiwifruit juice, lamb, lipid oxidation, meat, protease, tenderness, volatile compounds.

Acknowledgements

This thesis would not have been completed without the generous encouragement and support from many people, to whom I am deeply indebted,

I would like to thank my supervisors, Dr. Alaa. El-Din Ahmed (Aladin) Bekhit and Dr. Jim Morton for their ongoing assistance and kindly guidance throughout the whole process of this work. Thank you very much for helping in the arrangements for me to go to the 53rd International Conference of Meat Science and Technology Conference (ICOMST 2007) in Beijing with two papers and two posters from my current research published in the conference proceedings and presented during the conference, respectively.

My special gratitude goes to Dr. Peter Jarvis who spend his valuable time correcting my English. Thank you, Peter, I am so grateful to have you back after your operation. Your encouragement and help always lifted me up whenever I had gloomy days, or needed academic advices during the studies. I would also like to express my sincere thanks to Dr. J. R. (Richard) Sedcole for helping me to go through all the statistical difficulties of this complicated experimental work.

Many thanks to the Lincoln University Cataract team, Dr. Lucinda Roberston, Dr. H. Y. Y. Lee, Karl Gately, and PhD student Matthew Muir. Thank you for being always readily available and keen to help me with your technical expertise. A special thanks to staff technician Jason Breitmeyer, not only for his technical support, but also for his wonderful and pleasant accompany during the GC-MS experiments. Together we are proud of being the first to analyse meat volatile flavour compounds using the GC-MS at Lincoln University. I can still remember some of the "slang" that you taught me to make me sound like a real kiwi. And your wonderful jokes cheered me up during those days. Also special thanks to the administrative staff in ALSD division for their helpfulness.

I would also like to take this opportunity to thank my dearest mum and dad who love me without any reason in this world, for their encouragement and spiritual support in my life.

Finally, I would like to express my appreciation to my life long friend in Singapore SL Chung, and our good family friends in New Zealand C. Yu and his wife J. Chu for all the help, encouragement and companionship from the time we got to know each other.

Table of Contents

List of Figures

List of Tables

Abbreviations

a*	redness
Ac	kiwifruit juice infusion
ADP	adenosine diphosphate
ATP	adenosine triphosphate
b*	yellowness
BCFAs	branched chain fatty acids
BHA	butylhydroxyanisole
BHT	butylated hydroxytoluene
BSA	bovine serum albumin
C	control
Ca^{2+}	calcium
CK	creatine kinase
CP	creatine phosphate
CAR/PDMS	stableflex carboxen/polydimethylsiloxane
DDH_20	deionized distilled water
DFD	dark, firm, dry
FID	flame ionization detector
GC	gas chromatography
hr	hour
HSSE	headspace sorptive extraction
L	litre
L*	lightness
LD	longissimus dorsi muscle
LM	longissimus muscle
LTL	longissimus thoracis et lumborum muscle
Mb	myoglobin
MDA	malondialdehyde
MetMb	metmyoglobin
MFI	myofibril fragmentation index
min	minute
MS	mass spectrophotometer
O_2	oxygen
OD	optical density

OxyMb	oxymyoglobin
pH_u	ultimate pH
PSE	pale, soft and exudative
REML	restricted maximum likelihood estimation
RT	retention time
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEMs	standard error of means
SM	semimembranosus muscle
SPME	solid phase microextraction
ST	semitendinosus muscle
TBA/TCA	thiobarbituric acid/trichloroacetic acid
TBARS	thiobarbituric acid reactive substance
TEMED	N,N,N'N'-tetramethylethylenediamine
W	water infusion
WBSF	Warner-Bratzler shear force
WHC	water holding capacity
wk	week
WT	weight

Chapter 1
Introduction

Meat tenderness, juiciness, colour and flavour are the most important quality attributes affecting the consumer acceptance of meat (Lawrie, 1998). In the USA, a survey showed that when consumers purchased beef from the supermarket to cook at home, they gave an unsatisfactory rating of its eating quality more than 20% of the time (Miller, 1992). Tenderness has been identified as the most important meat palatability characteristic, particularly in beef (Miller et al., 2001), and consumers are willing to pay a premium for more tender meat (Boleman et al., 1997; Shackelford et al., 2001). In addition, the red meat industry could also achieve higher financial gain from producing more tender meat because of the higher selling price associated with tender meat (e.g., fillet or porterhouse steak) compared with tough cuts (e.g., chuck steak) in the market. Therefore, improving customer satisfaction and maintaining the consistency of meat products is a major concern and challenge for the meat industry (Behrends et al., 2005).

A considerable amount of research and resources have been focused on investigations attempting to improve meat tenderness, and have achieved success to various degrees. The technologies used to improve meat tenderness include post-mortem ageing (Jayasooriya et al., 2007; Koohmaraie et al., 1986), mechanical tenderization (Anna et al., 2007; Bowker et al., 2007), electrical stimulation (Hopkins et al., 2006; Hwang et al., 2003), ionic chemical solution (Hunt et al., 2003; Koohmaraie et al., 1989) and addition of plant enzymes (Ashie et al., 2002; Lewis & Luh, 1988; Wada et al., 2002). Of these techniques, ageing of the meat (e.g., animal carcasses stored at chill temperatures for 10-14 days) has long been recognized and employed by the meat industry (Lawrie, 1998). However, the large storage space needed, and the energy consumption and labour costs make the meat products less competitive in the market place (Farouk et al., 1992b). Therefore, it is crucial to develop safe tenderization methods which can improve meat tenderness and consistency more efficiently and economically for the meat industry. This in turn will also benefit the meat end-users in the marketplace by having more tender meat.

It has been well documented that the calpain proteolytic system plays a key role in post-mortem proteolysis and meat tenderization processes by weakening and/or degrading the structure of myofibrillar proteins in the muscle cells (Geesink et al., 2006; Koohmaraie, 1992, 1994; Koohmaraie & Geesink, 2006; Koohmaraie et al., 1998; Koohmaraie et al., 1990). Attempting to make meat tender by using plant proteases such as ficin, papain, bromelin and actinidin (Arcus, 1959) which accelerate the meat tenderization process has long been of interest to meat scientists (Glazer & Smith, 1971; Sinku et al., 2003; Whittaker, 1994). The significant tenderization effects of actinidin, a cysteine protease in kiwifruit juice were evaluated and reported in previous research (Lewis & Luh, 1988; Wada et al., 2004; Wada et al., 2002). The advantages of actinidin as a potential meat tenderizer are mainly due to its mild tenderizing action compared with other plant proteases (Lewis & Luh, 1988).

Previous studies have considered tenderisation and protein solubilisation (Lewis & Luh, 1988; Wada et al., 2002) but not other quality characteristics, e.g. colour, juiciness and flavour, after meat has been treated with actindin. These characteristics contribute to the overall preference of consumers (Behrends et al., 2005). Thus the colour of meat displayed on the shelf contributes to the customer's initial purchasing decision, e.g., customers prefer bright, cherry-red colour for beef and lamb because this colour is believed to be related to the freshness of red meat. Meat juiciness and eating flavour are also considered as important eating characteristics when meat products are served (Behrends et al., 2005).

The technique of distributing substances by cardiovascular infusion, into muscle cell and adipose tissues efficiently and rapidly (Katsanidis et al., 2003) has been used in the previous meat tenderization studies (Hunt et al., 2003; Koohmaraie & Shackelford, 1991; Yancey et al., 2001).

The present study was designed to evaluate the overall effect on meat quality of pre-rigor infusion of lamb carcasses with kiwifruit juice. The objectives of this study were:

- To evaluate the effect of kiwifruit juice and water infusion treatments on post-mortem changes of lamb physical properties (e.g., tenderness, colour, etc.) and biochemical changes;
- To investigate the effect of kiwifruit juice and water infusion treatments on the post-mortem profiles of lamb volatile flavour compounds.

Chapter 2
Literature Review

2.1 Introduction

Skeletal muscle, smooth muscle and cardiac (heart) muscle are the three types of muscle found in vertebrates. Skeletal muscle is the principal muscle tissue, and is responsible for voluntary movement of animals (Lawrie, 1998). It is the source of the food known as meat.

Meat is primarily derived from muscle, plus variable quantities of all types of connective tissues, and some epithelial and nervous tissues (Forrest et al., 1975). The chemical composition of lean meat can be approximated broadly to 75% water, 19% protein, 3.5% soluble, non-protein substances and 2.5% fat (Lawrie, 1998). Muscle and connective tissue contribute to the qualitative and quantitative characteristics of meat (Lawrie, 1998).

The conversion of muscle to meat involves complex biochemical, biophysical and structural changes during post-mortem (Lawrie, 1998). Although it is well documented that post-mortem meat tenderization is mainly due to the proteolytic activity activated by the Ca^{2+}-dependent proteases (calpains) which denature the key myofibrillar proteins, there is some debate on the specific role of these proteases in contributing to the tenderization process during post-mortem (Boehm et al., 1998; Geesink et al., 2006; Geesink et al., 2005; Ilian et al., 2004; Koohmaraie & Geesink, 2006).

Meat eating qualities including tenderness, juiciness, meat colour and flavour have been considered as the most important palatability traits by consumers (Lawrie, 1998). Among these traits, tenderness has been rated as the most important meat quality characteristic (Miller et al., 2001). Maintaining the consistency of meat eating quality and eliminating the variability in meat tenderness has been a major concern to the consumers and the meat industry (Bindon & Jones, 2001). The enzymatic tenderizing treatment by using kiwifruit juice could be a feasible approach for the meat industry because of its mild tenderizing effects (Lewis & Luh, 1988). Using kiwifruit juice will benefit the consumers by having

more tender meat, and also help the kiwifruit processors who would have an additional outlet for their product.

In this chapter, muscle structure and composition will be described briefly. Subsequently, the post-mortem biochemical and biophysical changes, and their possible effects on meat eating quality, including tenderness, juiciness and display colour will be discussed. This will be followed by a summary of previous research focussed on the tenderizing infusion treatments by using different tenderizing substances. Finally, the use of kiwifruit juice and its protease actinidin, on meat will be reviewed.

2.2 Muscle Structure and Composition

Muscle fibres are the essential units of skeletal muscle tissue. About 60% of each muscle fibre consists of myofibrils. Myofibrils are long, cylinders, 1-2 μm in diameter, and extend the entire length of the muscle fibre. Myofibrils comprise approximately 75-80 percent thick and thin filaments, with the regulatory proteins as the remaining fractions (Figure 2-1). The thick and thin filaments of the myofibril which are referred to as the A band and I band, consist of myosin and actin as the main components respectively. In addition to myosin and actin, tropomyosin, troponin and α-actinin are also components of the myofibrillar proteins. The overlap of the myosin and actin accounts for the banding or striated appearance of the myofibril (Forrest et al., 1975). The thin line bisecting the I band is the Z line (Z disk). The unit of the myofibril between two adjacent Z lines is the sarcomere, which is composed of the thick filaments in the A band and the thin filaments that attach to the Z-lines (Huxley, 1957). The interaction of myosin and actin allows muscles to contract causing movement due to the globular heads of myosin in the thick filament forming cross-bridges that interact with the actin in the thin filaments. Two other large filamentous proteins titin and nebulin also play important roles in myofibril assembly (Labeit & Kolmerer, 1995) and regulation of the actin-myosin interactions (Taylor et al., 1995) (Figure 2-1).

Figure 2-1 Schematic diagram showing the structure levels and protein composition of a muscle (Greaser, 2001)

Myofibrils are surrounded by the intracellular colloidal substances known as sarcoplasm (Warriss, 2000). The sarcoplasm consists sarcoplasmic proteins which appear to be a complex mixture of about 50 components of which most are enzymes that are involved in the glycolytic and proteolytic cycles (Lawrie, 1998). Clarke et al. (1980) also reported that these enzymes were bound to the myofibrillar proteins and controlled the muscle enzymatic reactions. The sarcoplasmic reticulum which acts as a store for calcium ions, is the membranous system of tubules and cisternae attached around the outside of myofibrils (Warriss, 2000). The various levels of muscle organization are shown in Figure 2-1.

Figure 2-2 The structural hierarchy of a muscle modified from Tornberg et al. (1990)

Connective tissues present in the muscle are known as epimysium, perimysium and endomysium (Figure 2-2). Collagen and elastin are the main components that make up the connective tissue, which connects the various parts of the muscle fibres together. Collagen is the most abundant protein in the animal body and has a significant influence on meat tenderness. Elastin is a more rubbery protein than collagen and contributes less to the connective tissue protein. The relative proportion of connective tissue and muscle fibres differ among various muscles and as such, it contributes to the relative difference of meat tenderness (Lawrie, 1998).

2.3 Post-mortem Changes in Muscle Tissue

Meat differs from muscle due to significant biophysical and biochemical changes which occur between the slaughter of an animal and the consumption of the meat (Lawrie, 1998). During the conversion process of muscle to meat and the subsequent tenderization, a series of post-mortem changes occur. These changes in the muscles include the decline of pH and muscle temperature, the development of rigor mortis, and then the gradual reduction of rigor and increase in meat tenderization induced by the endogenous enzymes in the muscle.

2.3.1 Muscle pH, Temperature Fall and Rigor Mortis during Post-mortem

Adenosine triphosphate (ATP), which provides the energy for contraction and maintains the functional integrity of the muscle, is generated by mitochondrial oxidative metabolism or by anaerobic glycolysis. ATP is also needed to fuel the calcium pumps of the sarcoplasmic reticulum. In a living muscle, the hydrolysis of ATP produces energy by releasing the phosphate from ATP to give adenosine diphosphate (ADP) reversibly. Immediately after ATP is hydrolysed, the creatine phosphate (CP), which is abundant and active in muscle reacts with ADP to form ATP catalysed by the enzyme creatine kinase (CK). After exsanguination, oxygen deficit occurs in the muscle, which induces the breakdown of many substrates including ATP and the ATP is not regenerated under this condition. Instead, anaerobic glycolysis is stimulated and glycogen breaks down to lactic acid. The conversion of glycogen to lactic acid will continue until an ultimate pH (pH_u) is reached when the glycogen has been used up and/or enzymes affecting the glycogen breakdown become inactivated (Lawrie, 1998).

Figure 2-3 The pH decline during post-mortem in the normal meat, PSE meat and DFD meat. (Warriss, 2000).

The rate of meat pH fall depends on various factors including initial concentration of glycogen, glycolysis rate, animal species, pre-slaughter treatment (stress) and storage

temperature (Warriss, 2000). The rate of meat pH decline and the ultimate pH are important in determining meat quality (Teixeira et al., 2005), especially the meat tenderness (McGeehin et al., 2001). Normally the muscle pH gradually declines from approximately pH 7 to an ultimate pH (pH_u) range of 5.3-5.6 (depending on the animal species) after 24-36 hours post-mortem due to the accumulation of the lactic acid (Figure 2-3). For sheep, the acidification process usually takes about 12-24 hours before it reach its pH_u (Lawrie, 1998).

However, abnormal pH decline will influence the meat eating quality and induce the two major quality defects of PSE (pale, soft, exudative) and DFD (dark, firm, dry) meats. Rapid decline of the pH ($pH < 6$) at 45 mins post mortem leads to the characteristic meat condition known as PSE meat (Warriss, 2000) which is found mainly in pork (Figure 2-3). Conversely, if meat ultimate pH is still too high ($pH \geq 6$) measured after 12-48 post-mortem, this induces the forming of DFD meat (Warriss, 2000) which is mostly found in beef (Figure 2-3). In addition, Hunt et al. (2003) reported that rapid pH decline and elevated muscle temperature might favour protein denaturation, and thus result in the lightening of meat colour. Thus, a better understanding of the factors influencing pH decline and pH_u, and how to control them will reduce the variation of meat tenderness and colour, and improve the consistency of meat quality (Van Laack et al., 2001).

The carcass temperature is about 37-39^{o}C at slaughter, and the body temperature drops after dressing. The rate of meat temperature loss depends on various factors including the size of the carcass, the covering of subcutaneous fat and the post-mortem storage temperature. The rate of cooling of meat has implications for meat quality such as weight loss, colour and water holding capacity (WHC). There are two main methods of handling the carcasses after slaughter by the meat industry to improve WHC and uniformity of meat colour. Carcasses may be stored in chilled rooms after dressing or on the other hand, hot processing involves cutting the carcasses before chilling into smaller portions in order to save the storage capacity in the chiller and energy consumption in cooling the meat. The rate of carcasses' temperature loss can also affect the meat pH decline through lactic acid production, the disappearance of CP and ATP, and the speed of onset of rigor mortis due to the activity of enzymes in the muscle (Warriss, 2000).

Muscle is very tender at the time of slaughter, but it becomes progressively tougher with the development of rigor mortis which is induced by the formation of cross-bridges called actomyosin between the actin and myosin filament in the muscle after slaughter. Accompanying the development of rigor mortis, some physical changes such as loss of elasticity and extensibility, shortening, and an increase in tension of the muscle also occur. The time of onset of rigor will relate to the factors affecting the levels of glycogen and CP at death and the rate of post mortem muscle metabolism (Warriss, 2000). The water holding capacity is lower when the onset of rigor mortis happens in meat due to the drop of pH, and the possible effect of denaturation of the myofibrillar proteins during post-mortem (Lawrie, 1998).

2.3.2 Meat Tenderization Mechanisms

It is well established that post-mortem meat tenderization is a multifaceted process involving the fracturing of key myofibrillar proteins, which are responsible for maintaining the structural integrity of the myofibrils (Koohmaraie, 1994; McDonagh et al., 1999). Even though research has shown that improvement in meat tenderness is mainly due to the degradation of specific myofibrillar proteins in muscle during the post-mortem period (Boehm et al., 1998; Hopkins & Thompson, 2002a; Koohmaraie, 1992, 1996; Koohmaraie & Geesink, 2006), the mechanisms of post-rigor tenderization in meat have not yet been conclusively determined. Attention has been mainly drawn to two natural enzymatic systems, cathepsins and calpains. However, there is still some debate on whether the cathepsins and other enzymes systems are largely responsible for this degradation, or whether the calpains play an important role in myofibrillar muscle protein degradation (Geesink et al., 2005; Hopkins & Thompson, 2002b; Ilian et al., 2004)

2.3.2.1 Cathepsins

The cathepsins were the first enzymatic systems implicated in meat tenderization and were discovered in the 1950's (De Duve et al., 1955). Cathepsins B, D, H and L, located in the lysosomes (Goll et al., 1983), are proteolytic enzymes. It was thought that cathepsins were released from lysosomes and caused the breakdown of myofibrillar proteins, and thus contributed to the meat tenderization process. However, Pommier et al. (1987) found that the amount of free cathepsin D increased during ageing of meat with a fall in pH and

lysosomal rupture, but they concluded that this had no impact on meat tenderization. Conversely, Whipple et al. (1990) suggested that there was no lysosomal rupture during ageing and that therefore cathepsin did not contribute to the tenderization process. Different tenderization rates in lamb and beef were also reported by Koohmaraie et al. (1991) although similar cathepsin B and cathepsin B + L activities were detected at death. The relationship between cathepsin and tenderization was further questioned when it was observed that cathepsin L altered the density of a large number of different myofibrillar proteins including myosin and actin at the Z-lines under high temperature and low pH conditions (Mikami et al., 1987), although this was not correlated with meat tenderness (Taylor et al., 1995). There is not enough significant evidence to conclude that the cathepsins are the main contributor to the tenderization process. In particular enzymes escaping from lysosomes into the cytosol, the acidic conditions, and the need for a higher temperature environment require further investigation.

2.3.2.2 Calpains

Olson et al. (1977) first reported calpain activity related to meat tenderization. Since then, meat scientists have mainly focussed on calpain proteolytic activity in relation to the meat post-mortem tenderization. In skeletal muscle, calpains consists of at least three proteases, calpain I (μ-calpain), calpain II (m-calpain) and skeletal muscle-specific calpain 3 (calpain p94). In addition, calpastatin, which is an inhibitor of μ- and m-calpain, is also present (Murachi et al., 1981; Sorimachi et al., 1989). The calpains have different Ca^{2+} requirements and Warriss (2000) suggested that μ- and m-calpain required a Ca^{2+} concentration of at 50-100μM and 1-2mM for activation, respectively.

After rigor mortis, calcium ions, released from the sarcoplamic reticulum into the sarcoplasm, bathe the myofibrils (Jeacocke, 1993). This increased calcium concentration activates the calpains located at Z-disk which accelerated proteolysis (Warriss, 2000). It was proposed that calpains could meet all the characteristics of the possible proteases required during meat post-mortem tenderization (Koohmaraie, 1992). These characteristics are to: (a) be located within the skeletal muscle cell; (b) have access to the myofibrils and (c) have the ability to degrade the same proteins that are degraded during post-mortem storage (Koohmaraie, 1992). Thus calpains, activated by calcium (Ca^{2+}), could degrade myofibrillar proteins, resulting in a significant increase in muscle relaxation which in turn

increases meat tenderization (Goll et al., 2003; Huang & Wang, 2001; Koohmaraie, 1992; Koohmaraie & Geesink, 2006). This suggests that calpains are the major proteolytic enzymes involved in post-mortem tenderization of meat. Koohmaraie (1994) summarized the concept that the calpain proteolytic system is the best possible candidate for causing post-mortem proteolysis and tenderization by using the following logic: (1) μ- and m-calpain have an absolute requirement for calcium ions; elevated calcium ion concentration is responsible for the weakening of the myofibrillar proteins during post-mortem that result in tenderization; (2) calpains are localized primarily at the Z-disk where most proteolytic activity is thought to take place; (3) calcium has no effect on the activity of other proteases; (4) calcium not only does not stimulate cathepsin activity, but at 10mM inhibits cathepsin activity by 39% (Barrett, 1973) and (5) only the calpain proteolytic system, but not other potential naturally occurring proteolytic candidates in meat; can reproduce post-mortem changes under *in-vitro* conditions. This indicates that calpains maybe active in meat tenderisation but that cathepsin is unlikely to play a major role in this process.

Despite the evidence that it is proteolysis of key myofibrillar proteins by calpains that leads to increased tenderness of meat, there is still some debate on the specific role of μ- and m-calpain and calpastatin in post-mortem tenderization. Some studies showed that μ-calpain and not m-calpain was largely responsible for the post-mortem proteolysis, and thus contributed to the meat tenderization (Geesink et al., 2006; Koohmaraie, 1994; Morton et al., 1999; Veiseth et al., 2001). In lamb longissimus muscle, μ-calpain lost 42% and 95% of its activity after 1 day and 3 days post-mortem while there was no activity change on m-calpain during post-mortem storage (Veiseth et al., 2001). They also concluded that calcium concentration in post-mortem muscle was only high enough to activate μ-calpain. This was supported by Geesink et al. (2001) who used two different analytical methods and found that free calcium level in ovine longissimus muscle at 1 day was at only 60 and 106μM, which seemed only sufficient to activate μ-calpain. Additionally, Koohmaraie et al. (1989) claimed that m-calpain underwent intermolecular autolysis and became inactivated completely once exposed to sufficient calcium when ovine carcasses were infused with calcium chloride. Nevertheless, a recent study reported that some limited post-mortem proteolysis occurred due to m-calpain autolysis in murine skeletal muscle (Geesink et al., 2006).

In contrast to the concept that μ-calpain is solely, or largely responsible for the post-mortem tenderization hypothesis, Boehm et al. (1998) reported μ-calpain in bovine semimembranosus muscle was not extensively degraded during 7 days post-mortem storage but became increasingly bound to the myofibrillar fraction, and μ-calpain was proteolytically inactive after it was isolated from 7 days post-mortem muscle. Thus, the previous thought that μ-calpain was the major protease causing post-mortem tenderization was questioned. Based on increasing the concentration of free calcium from 100μM at 12 to 24 hrs to 600-900 μM at day 10 post-mortem, Boehm et al. (1998) further proposed that the m-calpain proteolysis increased with the increasing free calcium concentration and was sufficient for full activity, thus m-calpain could be activated and contribute to the meat tenderization.

The possible role of calpain 3 in post-mortem tenderization in meat is still not conclusive. It binds to two distinct sites on titin (Labeit & Kolmerer, 1995) contributing to proteolysis of the titin in the early post-mortem and seems to be very important in meat tenderization (Taylor et al., 1995). The observation, using Western blotting, that calpain 3 autolysed during post-mortem was reported by Ilian et al. (2004) through observing the up and down regulation of the lamb LTL tenderization rate by Ca^{2+} and Zn^{2+} infusion treatments. However, conflicting results were reported by Geesink et al. (2005) that post-mortem proteolysis was not affected by calpain 3 by comparing the effect of post-mortem storage on proteolysis and structural changes in muscle from normal (containing calpain 3) and "knockout" mice which had no calpain 3 activity.

During post-mortem, calpain activity can be regulated by the endogenous inhibitor calpastatin (Murachi et al., 1981), although at that time the inhibition mechanism remained to be illuminated. It is now known that calpastatin binds with calcium ions and thus inhibit calpains activity (Warriss, 2000). Variability in calpastatin activity decline has been observed in bovine semimembranosus muscle (Boehm et al., 1998), and it was proposed that the loss of calpastatin activity was due to proteolytic activity of the calpains in the presence of higher muscle cellular calcium concentration (Doumit & Koohmaraie, 1999). Some of its inhibitory activity still remained in meat even after extensive post-mortem proteolysis (Nakamura et al., 1989).

2.3.3 Degradation of Key Myofibrillar Proteins

There are three protein fractions including myofibrillar (salt-soluble), connective tissue (acid soluble), and sarcoplasmic (water soluble) proteins in muscle (Koohmaraie et al., 2002). The weakening and/or degradation of myofibrillar proteins, which are the major protein fraction of skeletal muscle is accelerated by the calpain's proteolytic system during the post-mortem period and this gives rise to improvement in meat tenderness (Boyer-Berri & Greaser, 1998; Koohmaraie, 1994; Taylor et al., 1995). Although very limited changes occurring in the connective tissue may not contribute dramatically to the meat tenderization during post-mortem storage, different proportions of connective tissues present in the meat can cause variation in tenderness (Koohmaraie, 1994, 1996). Sarcoplasmic proteins do not directly relate to the meat tenderness as they are not structural proteins in muscle.

The degradation of major myofibrillar proteins activated by proteases results in the loss of structural integrity in meat during post-mortem. These alterations included Z-line weakening and/or degradation of selected muscle proteins including myosin, actin, titin, nebulin, desmin, troponin T, vinculin, and filamin. This will be discussed later in this section.

Proteolytic cleavage of myosin and actin is accelerated by the cathepsins under high temperature and low pH conditions but does not contribute to the meat tenderization (Mikami et al., 1987) because meat is normally stored at chilled temperature during post-mortem. Additionally, it has been found that actin is resistant to degradation by cathepsins under chill storage conditions (Goll et al., 1991). These offered the possible reasons why cathepsins were not considered as the major proteases contributing to the meat post-mortem tenderization process (Koohmaraie, 1996). Calpains do not degrade undenatured myosin and actin (Bandman & Zdanis, 1988).

The degradation of titin, nebulin, desmin, troponin T, vinculin and filamin which are important in maintaining the functional capability of muscle (Taylor et al., 1995), contributes to meat tenderization significantly, even though the rate of their degradation varies between muscles and even between the same muscle in different carcasses (Hopkins & Thompson, 2002a). Titin, the large protein (3700 kDa) which connects myosin filaments

in the direction of the muscle fibres from the M-line to the Z-disk was degraded into two fragments (2100-2400 kDa and 1200 kDa) during post-mortem (Huff-Lonergan et al., 1996). Nebulin is another large protein which was found to be involved in the regulation of actin-myosin interactions and degraded in the region close to the Z-disk during post-mortem (Taylor et al., 1995). Both titin and nebulin degradation increased the fragility of myofibrils in the I-band region and contributed to the improvement of meat tenderization between 24 and 72 hrs post-mortem (Taylor et al., 1995).

The degradation of desmin was observed during meat post-mortem and its disappearance rate was highly correlated with the degradation of troponin T (Wheeler & Koohmaraie, 1999). A 30 kDa protein fragment detected after the degradation of troponin T (Claeys et al., 1995; Ho et al., 1994) was proposed to be a directly related to post-mortem tenderization (Taylor et al., 1995). However, whether the degradation of troponin T is associated with an increase of meat tenderness, or is just a result of proteolysis during post-mortem still needs to be further clarified (Huff-Lonergan et al., 1995; Wheeler & Koohmaraie, 1999). When meat is in a chill condition, vinculin is very susceptible to degradation in muscle and this is associated with the meat tenderness post-mortem (Taylor et al., 1995). It begins to degrade in the bovine semimembranosus muscle during the first day post-mortem, and almost 50% of it was observed to be degraded within that period of time (Taylor et al., 1995). A 90 kDa fragment appears when the vinculin is degraded (Winkler et al., 1996). Similar to vinculin, the degradation of filamin which is located at both Z-disk and M line might also be implicated in the tenderization of meat during post-mortem (Taylor et al., 1995).

2.4 Meat Quality Attributes

Consumers' acceptance of meat eating quality normally includes tenderness, juiciness, colour and flavour aspects, although the individual preference might be different in terms of diverse eating habits and life styles (Lawrie, 1998). These important features affecting palatability will be discussed in this section.

2.4.1 Meat Tenderness

Tenderness has been ranked as the most important palatability factor in meat (Miller et al., 2001), and tender meat is highly valued by the consumers, particularly for red meats (Jeremiah, 1982). Meat tenderness is an organoleptic quality, and involves the ability of meat to be sheared, penetrated, bitten, stretched and ground during consumption (Lawrie, 1998). Three categories of proteins in muscle including connective tissue, myofibril and sarcoplasm can be related to the degree of meat tenderness (Lawrie, 1998).

The tenderness of meat varies among different species, among animals within a specie, among different muscles and in muscles held for different post-mortem times (Lawrie, 1998). Cattle show higher muscle coarseness than sheep or pig because of the high content of connective tissue in beef. Veal is more tender than beef due to its higher water holding capacity (Lawrie, 1998) as well as the lower cross-linking in connective tissue in calves (Light et al., 1985). Different muscles from the same carcass may also vary significantly. A comparison of intramuscular connective tissue of beef longissimus dorsi (LD) and semitendinosus (ST) muscle at both 4 and 21 days post-mortem found that LD muscle was more tender than ST muscle due to its low collagen and high water holding capacity (Li et al., 2007).

The connective tissue of meat is stable during post-mortem (Lawrie, 1998). Hence, it does not contribute much to the tenderization process although the intramuscular connective tissue shows signs of structural changes after an extended period of storage (Nishimura et al., 1995). The improvement of meat tenderness is considered mainly to result from the endogenous calpain proteolysis system by softening the myofibrillar proteins during post-mortem (Koohmaraie, 1992; Koohmaraie & Geesink, 2006). During aging (e.g. meat stored at chill temperatures for 10-14 days), proteolysis of the myofibrillar proteins, loss of calcium ions, the uptake of potassium ions, and an increase in the water holding capacity of muscle could contribute to the improvement of meat tenderness (Lawrie, 1998).

Research associated with meat tenderness/toughness has been explored extensively for decades. Subjective assessments, using taste panels, are employed to convert the consumers' feelings of meat tenderness into measurable parameters. In parallel with this method, physical techniques of measuring the resistance of meat to shear forces have

proved useful in assessing the meat tenderness (Lawrie, 1998). The shear force values, measured in kilogram force units (kgF), related to consumers' perceptions of tenderness/toughness of beef and lambs measured using a MIRINZ tenderometer are illustrated in Table 2-1 (Bickerstaffe et al., 2001).

Table 2-1 Consumers' perceptions of tenderness of beef and lamb loins and their shear force category values (Bickerstaffe et al., 2001)

2.4.2 Meat Juiciness

Meat juiciness is one of the eating qualities of interest to consumers, especially for red meat (Lawrie, 1998). Juiciness is often associated with muscle water holding capacity (WHC), which is commonly defined as the ability of meat to retain its water during application of external forces such as cutting, heating, grinding, or processing (Forrest et al., 1975). This retained water contributes to the juiciness and palatability of meat as a food.

As mentioned earlier (Section 2.1), approximately 75% of fresh lean meat is water (Lawrie, 1998). The majority of the water in muscle is probably held in the spaces between the thick and thin filaments of the myofibrils (Lawrie, 1998). During the onset of rigor mortis, the contraction of myosin and actin will eventually lead to the movement of intracellular water towards the extracellular space in the myofibrils (Lawrie, 1998). This exudation of water from the extracellular space will tend to be partially lost through evaporation from the surface of the carcass, as well as by drip loss during aging and during cooking. Such water loss will decrease weight and are important because they may lead to lower meat yield and cause economic problems for the meat industry as meat is sold by weight. In addition, water loss during cooking may influence the size of meat that can be

served, and the juiciness and tenderness of the meat (Offer & Trinick, 1983; Warriss, 2000).

Although the relationship of juiciness with various biological parameters is still unclear and research data reported are often highly controversial, factors including animal species and age, and post-mortem meat pH fall rate, may result in different meat quality, and thus affect the water holding capacity of the meat (Lawrie, 1998). Water holding capacity in pork is higher than that of beef, and veal from calves has a higher water holding capacity than mature beef. However age does not seem to affect the water holding capacity of pork (Lawrie, 1998). During the conversion of muscle to meat, a rapid post-mortem pH drop will result in a lower water holding capacity which might lead to PSE meat (Forrest et al., 1975). A higher ultimate pH results in a higher water holding capacity, which leads to a juicy and more palatable meat product (Bouton et al., 1971), but the flavour is affected and not deemed acceptable by consumers.

2.4.3 Post-mortem Meat Colour

Meat colour acts as a key indicator of freshness and high quality when consumers purchase meat products (Hood, 1980). It is critically evaluated by consumers and often is the basis for their product selection or rejection at the point of sale (Faustman & Cassens, 1990). The colours correlated with good meat quality depend on the nature of the meat. For example, consumers like beef and lamb to appear a bright, cherry-red colour but like pork to be pink because they associate these colours with meat freshness. Hence, meat colour is of crucial importance to the consumers when the meat is displayed on the shelf for sale, as well as to the meat industry because prolonging fresh meat display shelf life by delaying colour deterioration will commercially benefit the industry (Zerby et al., 1999).

The pigment primarily responsible for the colour of meat is the protein myoglobin (Mb). Mb is a conjugated protein consisting of an iron-porphyrin compound haem, which is combined with the globin polypeptide. The haem pigments in Mb bind with oxygen (O_2) reversibly to form oxymyoglobin (OxyMb), which is bright red in colour. In living tissue, the reduced form of Mb, which is a purple-red colour, exists in equilibrium with its oxygenated form of OxyMb. In the case of meat, OxyMb can be autoxidised further to

metmyoglobin (MetMb), which appears as a discoloured brown colour. MetMb cannot bind oxygen but does bind a molecule of water instead. Although OxyMb autoxidation is a relatively slow reaction, the formation of MetMb in meat must be avoided because the consumer will not accept the brown colour when they purchase the meat products.

During the conversion of muscle to meat, there are three main factors affecting raw meat colour and colour stability after slaughter. They are, firstly the competition between metabolic O_2 consumption and O_2 binding by Mb with time (Lanari & Cassens, 1991). After slaughter, muscle as meat still needs oxygen to continue its metabolic respiration. This respiratory system in meat competes with Mb for the oxygen diffusing into the meat from the meat cut surface, thus influencing the colour of the meat (Young & West, 2001). Secondly the enzymatic activity of MetMb reductase in the living animals lowers the MetMb concentration by chemically reducing MetMb back to the oxygen-binding Mb ferrous form in the muscle tissue, and this imparts the red colour to the meat (Livingston et al., 1985). MetMb reductase can still maintain its activity in both surface or deeper in anaerobic tissue in meat (Echevarne et al., 1990). Ledward (1985) also reported that high MetMb reductase played a very important role in preventing meat discoloration. Finally, the generation of free radicals in meat resulting from the degradation of muscle cells in the meat, could accelerate MetMb formation which could eventually influence the colour of the meat post-mortem (Young & West, 2001). On the other hand, several natural antioxidants such as vitamin E (Yancey et al., 2001), vitamin C (ascorbic acid) (Okayama et al., 1987) have been under evaluation due to their possible ability to inhibit lipid oxidation, and thus contribute to the extension of the displayed shelf life of meat. However, their ability to maintain the colour and colour stability are dependent on the various methods used to incorporate them into animals or meat. Several synthetic antioxidants, such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), are currently permitted to be used during food processing under the food laws.

Apart from the presence of the oxidized state of Mb influencing the meat colour, the meat colour intensity also depends on the way the incident light is absorbed or scattered and this is influenced by pH (Swan, 1993). A slow pH fall at low temperature may result in high ultimate pH (pH > 5.8) in the meat during the onset of rigor mortis. This is typically found in DFD meat mainly in beef (Warriss, 2000). In this situation, meat becomes more translucent and allows less light scattering. Incident light can penetrate the meat deeply and

is strongly absorbed by Mb. As a consequence, the meat appears dark red (Swan, 1993). Additionally, the darker colour of the meat with a high pH also due to the water holding capacity, as meat at a high pH binds more water than meat at a low pH. This meat may not be accepted by the consumers due to its very dark colour appearance.

Meat colour is perceived when light is reflected in a diffuse way from the surface of the meat. Meat colour can be assessed by a sensory panel (subjective assessment) or measured by an instrument (objective assessment). Panel assessment techniques include triangle tests, intensity tests and preferences tests (Young & West, 2001).

Figure 2-4 The diagram of L* a* b* colour space. The tip of the thick arrow is defined by lightness (70 on a scale of 0 to 100), redness (+26 on a scale of -60 to +60), and yellowness (+15). (Young & West, 2001)

Objective instrumental measurements are based on the measurement of colour scale parameters including lightness (L*), redness (a*) and yellowness (b*) by using a reflectance colorimeter (CIE, 1986). The value of L* indicates lightness where 0 equals black and 100 equals white. The value of a* is the measure of redness and greenness, while b* is the measure of yellowness and blueness (Figure 2-4). The CIE-L*a*b* method for colour measurement of meat has been employed in previous research (Bekhit et al., 2005; Murphy & Zerby, 2004; Zerby et al., 1999).

2.4.4 Post-mortem Meat Flavour Changes

Meat flavour has two components including taste and aroma or smell (Warriss, 2000). Meat flavour is mostly generated on heating during the cooking process. There are two aspects to meat flavour: species-specific component and non-species-specific component (Warriss, 2000). The species-specific component is a function of the different meat flavour of various animal species such as beef, lamb, chicken and pork. (Warriss, 2000). The variations between different cuts/muscles, sample collection and preparation procedures and cooking conditions may also affect the generation of aroma compounds, and give significant variation in results (Fu & Ho, 1997; Priolo et al., 2001).

Hundreds of volatile constituents have been identified, but only a few of these compounds play a significant role in the overall aroma quality (Rochat & Chaintreau, 2005). A great number of research studies on meat flavour have been conducted using gas chromatography (GC) in conjunction with a flame ionization detector (FID) or mass spectrophotometer (MS) to identify volatile compounds in meat commodities (Hierro et al., 2004; Martin et al., 2000; Priolo et al., 2004; Wettasinghe et al., 2001). However, the different fibre types employed in the investigations by solid phase microextraction (SPME) combined with GC-MS might also give rise to different outcomes (Brunton et al., 2000; Marco et al., 2004).

Several studies reported that branched chain fatty acids (BCFAs) such as 4-methyloctanoic and 4-methylnonanoic acids are the characteristic volatile compounds in sheep meat and these are responsible for the typical “goaty” and “muttony” flavour, and the level of BCFAs detected will be influenced by differences in animal age, diet, and levels of hormone at slaughter (Priolo et al., 2001; Shahidi, 1998; Sutherland & Ames, 1996; Young et al., 1997). Sutherland and Ames (1996) analysed the level of 4-methyloctanoic and 4-methylnonanoic acids in adipose tissue, and found that these two compounds were not significantly different in rams and wethers at 12 wks of age. However, after 3 wks of age, 4-methyloctanoic and 4-methylnonanoic acids levels were 13- and 14- fold higher in rams than in the castrated wethers. Diet can alter the accumulation of BCFAs in the meat. More BCFAs were accumulated in meat fat after ruminants were fed pasture-based diets compared with a corn-based diets (Young et al., 1997). This was consistent with research that maize-concentrate diets significantly increased 4-methyloctanoic and 4-

methylnonanoic acids levels in subcutaneous fat of ram lambs compared with the animals fed green grass (Young et al., 2003). However, these two flavour compounds were not detected in lambs by Paleari et al. (2006) and Sebastiàn et al. (2003), and it was proposed that the analytical procedures or the headspace sorptive extraction (HSSE) procedure employed in the analysis might influence the detection results.

Aldehydes such as hexanal, heptanal, octanal, nonanal are the most significant flavour compounds derived from lipid oxidation (Paleari et al., 2006). Aldehydes not only contribute to the odour of meat, but they can also react with other compounds to produce flavour through amino-carbonyl reactions (Moody, 1983). Among the aldehyde compounds, hexanal which originates mainly from linoleic and arachidonic acids acts as the most prominent volatile, and has been reported to lead to a rancid odour (Martin et al., 2000). However, other flavours have been also reported for hexanal, for example it contributes fatty or fruity aromas to the meat flavour (Stanke, 1994).

Hexanal, together with other volatile aldehydes such as heptanal, octanal and nonanal, which are derived mainly from oleic acid and linoleic acid (Elmore et al., 1999), are considered very important to the cooked beef meat flavour (Machiels et al., 2004). The flavour of meat can depend on concentration of these compounds. They may impart a pleasant fruity flavour when their concentration was low (Machiels et al., 2004).

Although terpenes account for only a small percentage of the volatile compounds, the presence of terpenes in ruminant meat or dairy products can be considered as a marker of animals fed by green forage diets (Young et al., 2003). Several terpenoid molecules including phyt-2-ene, β-cariophyllene and limonene were detected in sheep/lamb meat, and were the main discriminating compounds of grass feeding detected (Priolo et al., 2004; Sebastiàn et al., 2003).

A recent study identified 16 terpenes out of the 42 compounds in dry-cured lamb ham (violino) (Paleari et al., 2006). Finely ground meat was analysed using the HSSE method. Among the 16 terpenes in this research, linalool was the most representative terpene and accounted for 16.01% of total volatile compounds followed by eucalyptol (3.71%), limonene (3.10%), sabinene (2.00%), α-pinene (1.70%) and β-caryophyllene (1.50%). However there is some doubt about the origin of some of the terpenes found in dry-cured

lamb ham, as they are recognized constituents of spices and condiments such as pepper, thyme, bay leaf and coriander (Chevance & Farmer, 1999), and may have originated from the spices in the curing mixture in this research. It is worth noting that many of the volatile compounds available in forage do not accumulate in animal cells, but their metabolized by-products do (Vasta & Priolo, 2005). Larick et al. (1987) found that some mono- and sesquiterpenes found in meat might have been derived as secondary metabolites from fresh forage plants in the diet. This has been confirmed in a recent study that examined the content of terpenes in subcutaneous fat from lambs fed on pasture (Priolo et al., 2004).

Alcohols are the most abundant compounds in cooked meat (Estévez et al., 2003). Alcohols mainly derived from lipid oxidation (Estévez et al., 2003; Wettasinghe et al., 2001). The flavour of alcohols has been reported as greenish, woody and fatty-floral (Peterson et al., 1975). Of alcohols, 1-octen-3-ol, derived from linoleic acid oxidation, has been identified as having a marked mushroom flavour, and contributes to the overall flavour due to its low threshold (Muriel et al., 2004).

Ketones mainly derived from fatty acids oxidation, might contribute the buttery aroma to cooked meats (Peterson et al., 1975). Rochat and Chaintreau (2005) reported that ketones were generated in reasonably large amounts during cooking in beef. However, Wettasinghe et al. (2001) indicated that the contribution of ketones to the flavour of meat was lower than that of aldehydes in chicken, and they did not contribute much to the flavour of beef shoulder muscles (Wettasinghe et al., 2001).

Sulphur volatile compounds are considered to be very potent contributors to meat flavour due to their low thresholds of sensory detection, even though their percentage concentration was very low (Drumm & Spanier, 1991). A number of studies stated that comparatively high levels of sulphur compounds were present in the animals fed by pasture diets (Raes et al., 2003; Young et al., 1997; Young et al., 2003). Two sulphur volatiles, dimethylsulphide and dimethylsulphone, from lamb fat were positively related to the grass feeding diet (Young et al., 1997; Young et al., 2003) . It is proposed that meat sulphur compounds occur from the reaction of hydrogen sulphide (breakdown by-product of the amino acid cysteine) with dicarbonyl compounds formed in the Maillard reaction during thermal processing (Mottram, 1998). The sulphur-containing volatiles may be affected by the meat's ultimate pH. The concentration of sulphur volatiles, including

furanthiols, mercaptoketones, aliphatic sulphides and thiophenes increased, whilst the concentrations of some other compounds such as thiazoles and thiophenones decreased with the decrease of pH values (Madruga & Mottram, 1995). A similar study also showed that with the decrease of meat pH from 6.81 to 6.26, the level of sulphur compounds dimethyl disulphide and dimethyl trisulphide increased in ruminant meat (Braggins, 1996).

Hydrocarbons have been reported in various meats, but they were not considered as major contributors to meat aroma (Hwang, 1999). Similarly to aldehydes and ketones, hydrocarbons are derived from the oxidation of fatty acids. Although hydrocarbons are volatile compounds formed via lipid degradation, they probably have no significant impact on flavour due to their relatively high odour threshold values (Drumm & Spanier, 1991).

Esters may make a small contribution to uncured meat aroma, and are generally associated with a fruity aroma (Cross & Ziegler, 1965).

Overall, the volatile flavour compounds of cooked meat from different kinds of animals were qualitatively and quantitatively different (Wettasinghe et al., 2001) even though the meat had essentially similar physical properties (Lawrie, 1998). Although the variations in aldehyde and alcohol concentrations might be responsible for the difference in the overall aroma characteristics of cooked meat, it is difficult to recognise a single volatile flavour component or several components as the main contributor(s) to the meat flavour (Wettasinghe et al., 2001).

2.5 Previous Infusion Tenderization Studies

Although the exact mechanism of post-mortem tenderization still remains controversial and uncertain, many studies have been carried out on the changes of myofibrillar proteins during post-mortem storage, the causes of these changes, and the relationship between these changes and meat tenderness. Many methods and different ingredients have been used in attempt to improve meat tenderness such as electrical stimulation (Hwang et al., 2003), mechanical tenderization (Jeremiah et al., 1999), aging (Lamare et al., 2002), proteolytic enzymatic tenderization (Lewis & Luh, 1988; Sinku et al., 2003; Wada et al., 2002) and ionic solution treatment (Hunt et al., 2003; Koohmaraie & Shackelford, 1991). The ionic solution and enzymes treatments have involved several techniques including

injection (Koohmaraie et al., 1998), marination (Gonzalez et al., 2001) and infusion (Koohmaraie & Shackelford, 1991; Koohmaraie et al., 1990).

The effects of cardiovascular infusion of carcasses immediately after exsanguination in relation to the meat quality changes have been reported extensively (Bekhit et al., 2005; Farouk & Price, 1994; Hunt et al., 2003; Ilian et al., 2004; Koohmaraie & Shackelford, 1991). Substances such as calcium ions (Koohmaraie & Shackelford, 1991), dextrose (Farouk & Price, 1994; Farouk et al., 1992b), glycerine (Farouk & Price, 1994; Farouk et al., 1992b), polyphosphate (Yancey et al., 2002b; Yancey et al., 1999), antioxidants (Dikeman et al., 2003; Yancey et al., 2001) and water (Ilian et al., 2004) have been used in various combinations as tenderization blends employed in the infused treatments. The observations from previous research which have investigated the effects of infusion treatment on meat palatability (including tenderness, sensory traits, exudation, colour, and post-mortem metabolic changes) by using different compounds/solutions during the post-mortem period have been summarised and are presented in Table 2-2.

Table 2-2 Summary of effects of various meat infusion tenderization treatments during post-mortem period

Compounds infused	Animal	Muscle /Cuts*	Experimental Conditions	Effects	Reference
0.3M $CaCl_2$	lamb	LTL	Wethers were fed with 4ppm β-Adrenergic Agonist (BAA) for 6 wks before slaughter. Carcasses were in cooler at -1.1°C for 24h, and then stored at 2°C.	$CaCl_2$ can overcome the negative effect of BAA. $CaCl_2$ increased meat tenderness, lean colour score, dressing percentage; but it had a negative effect on lean meat firmness.	(Koohmaraie & Shackelford, 1991)
0.3M $CaCl_2$	lamb	LTL	After artery infusion at 10% live weight, carcasses were placed in a cold room at 2°C. Samples were vacuum packed and stored at 1°C.	$CaCl_2$ infusion improved lamb tenderness at both 2 and 6 days post-mortem through activation of μ and m-calpain during post-mortem proteolysis and tenderization.	(Polidori et al., 2000)
0.3M $CaCl_2$	beef	LTL	After completion of artery infusion process (within 45 mins after slaughter), carcasses were placed in a cold room at 2°C.	$CaCl_2$ infusion improved beef tenderness through activation of μ- and m-calpain during post-mortem proteolysis and tenderization; $CaCl_2$ had no effect on sarcomere length.	(Polidori et al., 2001)
0.3M $CaCl_2$ (Ca); 50 mM$ZnCl_2$ (Zn); Water (W)	lamb	LTL	10% body weight solution was infused through artery. Carcasses were kept for 4h at 15°C, and then moved to 2°C chiller for 7 days.	$CaCl_2$ infusion had a negative effects on colour which showed lower L*, a* and b*, lower amounts of unbound water, shorter sarcomere length, higher lipid peroxidation, and lower NAD concentrations. Zinc and water-infusion had less lipid oxidation and improved colour and colour stability.	(Bekhit et al., 2005)
0.3M $CaCl_2$ (Ca); 50 mM$ZnCl_2$ (Zn); Water (W)	lamb	LTL	10% body weight solution was infused through artery. Carcasses were kept for 4h at 15°C, and then moved to 2°C chiller for 7 days.	$CaCl_2$ or Zn accelerated or inhibited meat tenderization and titin and nebulin degradation, respectively; W infusion improved meat tenderness. Calpain 3 may contribute to the meat tenderization through specific muscle structure proteins proteolysis.	(Ilian et al., 2004)
0.3M $CaCl_2$	pork	LTL	Rigor boning (RB) or accelerated boning (AB) with/without 10% body weight $CaCl_2$ solution was infused at either 0.5 or 6 hr post slaughter chilled at 0°C or 14°C water bath.	$CaCl_2$ increased meat tenderness; $CaCl_2$ had a negative effect on colour and water holding capacity; Infusion time had no effect on aging rate.	(Rees et al., 2002)

(continued on next page)

(continued)

Compounds infused	Animal	Muscle /Cuts*	Experimental Conditions	Effects	Reference
0.23% dextrose, 0.21% glycerine, 0.14% phosphate blend and 0.1% maltose (tenderizing blend)	dairy cows	SS; LTL; ST	After artery infusion (10% live weight), carcasses were kept in a holding cooler at 2-4^{o}C.	Retained infusion solution: SS >LTL > ST; Tenderness and protein extractability were improved after infusion; Infusion had no effect on water holding capacity.	(Farouk et al., 1992b)
0.23% dextrose, 0.21% glycerine, 0.14% phosphate blend and 0.1% maltose (tenderizing blend, NCa); Tenderizing blend+ 0.015M $CaCl_2$ (WCa)	lamb	LTL (loin); IS (shoulder); leg	After artery infusion (10% live weight), carcasses were kept in a holding cooler at 2-4^{o}C.	Retained infusion solution: shoulder > loin > leg; Drip & cooking loss: WCa > NCa > Control; Infusion had no effect on drip/cooking loss in refrigerated samples; Infusion frozen samples had higher thaw drip and cooking loss after being thawed; Infusion samples (both fresh and frozen) had higher lightness and yellowness than control; WCa had less red colour than NCa and Control; Infusion lowered carcasses temperature in first 3h post-mortem; Glycolysis completed within 6 hrs post-mortem in NCa, 12-24 hrs post-mortem in WCa, the glycolysis order was : NCa > WCa > Control.	(Farouk & Price, 1994)
0.3M $CaCl_2$; 98.52% water, 0.97% sacc-harides, 0.23% NaCl and 0.28% Phosphate blend (MPSC)	steer	LTL; ST; QF	Steer had been fed corn-based feedlot diet for 140-155 days before slaughter. After artery infusion (10% live weight) carcasses were chilled at 2^{o}C using a 1-min spray-chill cycle every 15 min for 8 hours after cooler entry followed by 16 hrs of air chilling.	MPSC and $CaCl_2$ had higher dressing percentage, and rapid pH decline rate before 24 hrs post than control; $CaCl_2$ had no effect on ST tenderness and flavour, but it decreased LL tenderness due to severe muscle contraction early post-mortem, and it reduced flavour intensity of LL steak and ground beef. $CaCl_2$ had no effect on ST tenderness or flavour; MPSC infusion had no effect on flavour quality.	(Dikeman et al., 2003)

(continued on next page)

(continued)

Compounds infused	Animal	Muscle /Cuts*	Experimental Conditions	Effects	Reference
0.3M $CaCl_2$; 98.52% water, 0.97% saccharides, 0.23% NaCl and 0.28% Phosphate blend (MPSC)	steer	LL; SM (ISM/OSM); PM	After artery infusion (10% live weight), carcasses were kept in a holding cooler at 2°C with a 1-min spray-chill cycle every 0.25 hr for 8 hrs before being chilled at 2°C for 16 hrs. Samples were taken at 24 hrs post-mortem.	LL and OSM steaks from MPSC infused carcasses had a lighter red initial colour; display colour stability similar to control samples; LL, ISM and OSM from both infusion treatments had higher L* than control; LL from $CaCl_2$ had lower a*, lower display colour stability and undesirable meat flavour; Infusion effects were not consistent among muscles.	(Hunt et al., 2003)
98.52% water, 0.97% saccharides, 0.23% NaCl and 0.28% phosphate blend (MPSC); MPSC+500ppm VitC (MPSC+C)	steer	LL; ST	After artery infusion in 10% live weight, carcases were placed in a spray-chill cooler at 0-2°C for 12h, and then placed in a cooler at 2°C.	MPSC infusion treatment had higher dressing percentage than control; MPSC and MPSC+C infusion increased carcass WT; Infusion had no effect on tenderness of LL/ST and purge loss from vacuum packaged muscles; Infusion had inconsistent effect on flavour profile of cooked beef.	(Yancey et al., 1999)
98.52% water, 0.97% saccharides, 0.23% NaCl and 0.28% phosphate blend (MPSC); MPSC+1000ppm VitC (MPSC+C)	steer	LL; ST; QF	After artery infusion in 10% live weight, carcases were placed in a spray-chill cooler at 0-2°C for 12h, and then placed in a cooler at 2°C.	MPSC infusion treatment had higher dressing percentage and organ weight than control; MPSC infusion had no effect on tenderness of LTL or ST; MPSC had inconsistent effects on flavour of cooked beef; Additional VitC to MPSC had no extra effect.	(Yancey et al., 2002a)
MPSC+500ppm VitC; MPSC+500ppm VitE; MPSC+500ppm VitC+500ppm VitE (MPSC+C+E)	steer	LT; PM; SM	After artery infusion in 10% live weight, carcases were spray chilled in a cooler at 1- 2°C for 12h, and then placed in a cooler at 1- 2°C.	No interaction between display time and treatment for L*, a* and b* value; At 0 time, LT from control had more uniform and cherry red colour than infusion treatment samples; Infusion had no effect on colour or display-colour stability of LT; Infusion solution with VitE improved display colour stability of ground beef.	(Yancey et al., 2001)

*BAA – β-adrenergic agonist
IS – infraspinatus
ISM – inside semimembranosus
LTL/LL – longissimus thoracis et lumborum
OSM – outside semimembranosus
PM – psoas major
QF – quadriceps femoris
SM – semimembranosus
SS – supraspinatus
ST – semitendinosus

Infusion of compounds pre-rigor into the carcasses to manipulate the post-mortem tenderization process in meat appeared feasible, and some recent infusion treatments illustrated in Table 2-2 will be discussed below according to the category of infusion substances employed.

Infusion of bovine carcasses with a volume equal to 10% of the body weight of live animals at 0.3M concentration calcium chloride ($CaCl_2$) could accelerate post-mortem tenderization (Ilian et al., 2004; Koohmaraie & Shackelford, 1991; Polidori et al., 2001; Rees et al., 2002). A combined effect of three different mechanisms induced by the pre-rigor calcium chloride infusion was proposed: calcium chloride (1) could accelerate the calpain's proteolytic activity to improve meat tenderness (Koohmaraie et al., 1989; Koohmaraie & Shackelford, 1991); (2) could cause extreme contraction of the muscle fibres resulting in disruption of the myofibrillar network (Morgan et al., 1991); (3) could cause protein to protein interactions due to the elevated ionic strength (Wu & Smith, 1987). The immediate tenderizing effect of infusion after slaughter also suggested that meat post-mortem storage ageing to ensure the meat tenderness might no longer be necessary for the meat industry (Koohmaraie et al., 1989; Koohmaraie et al., 1990). It was also suggested that increased pork tenderness after calcium chloride infusion might be due to an increase in the rate of pH decline post slaughter (Rees et al., 2002). This might be a result of the increased breakdown of ATP activated by the increasing activity of the calcium-dependent ATPase, which eventually stimulated the post-mortem glycolysis rate (Rees et al., 2002). However, it might have negative effect by giving a rise of possible PSE pork. In addition, $CaCl_2$ pre-rigor infusion also had negative effects on colour (Bekhit et al., 2005; Rees et al., 2002), water holding capacity (Rees et al., 2002), and meat flavour (Morgan et al., 1991). $CaCl_2$ pre-rigor infused pork was lighter in appearance and this, combined with a higher drip loss might result in a higher number of PSE carcasses (Rees et al., 2002). For beef, Wheeler et al. (1993) reported that a darker colour was observed after 3 days display time due to the calcium chloride infusion. A similar darker colour was also observed in lamb LM muscle, and it was proposed that this was caused by several factors including rapid pH decline and lower amounts of unbound water in meat, increasing the oxygen consumption rate of the mitochondria and the muscle contraction induced by the $CaCl_2$ pre-rigor infusion (Bekhit et al., 2005). Morgan et al. (1991) reported that bitter and metabolic off-flavours were detected with a 300mM $CaCl_2$ infusion treatment in mature beef cow meat indicating that the effects of $CaCl_2$ infusion needed further clarification.

Farouk et al. (1992b) reported that infusion of a tenderizing blend of 0.23% dextrose, 0.21% glycerine, 0.14% phosphate blend and 0.1% maltose significantly improved tenderness of longissimus dorsi (LD) steaks by 13% in bovine carcasses. It was proposed that the tenderization mechanisms induce by the infusion treatment were caused by (1) the infusion solution enhancing the proteolytic activity process; (2) disruption of the muscle structure caused by the volume and pressure when the infusion solution was introduced into the carcasses; and (3) the phosphate in the infusion solutions may contribute to the solubilization of actomyosin thus improving the meat tenderness (Farouk et al., 1992b). This improvement of meat tenderness was without any negative effect on water-holding capacity and protein extractability of the meat (Farouk et al., 1992b). Different compositions of the infusion solution may possibly induce different results due to differences in the rate of pH fall and the mechanical disruption of the musculature induced by the stimulating glycolytic enzymes during the infusion process (Farouk & Price, 1994; Stanton & Light, 1990). Farouk and Price (1994) conducted another experiment by adding 0.015M $CaCl_2$ to the tenderizing blend, and showed that samples frozen after infusion had higher drip loss and cooking loss (WCa > NCa > Control refer to Table 2-2) which might be caused by the increase in moisture retained and higher pH fall rate in the infusion treatment, or the increased pressure involved when the infusion solution was introduced into the carcasses. They also showed that infused, were lighter (L*) on display day 0 and yellower (b*) on display days 0 and 7 compared with control, lamb leg steak samples. They suggested that this was due to the increasing light scattering or dilution of pigments in the muscle caused by the infusion solution (Farouk & Price, 1994).

Another tenderizing solution, MPSC, developed by MPSC, Inc. (St. Paul, MN), consisting of 98.52% water, 0.97% saccharides, 0.23% sodium chloride and 0.27% phosphate blend was also employed to examine the effects on post-mortem carcasses (Dikeman et al., 2003; Hunt et al., 2003; Yancey et al., 2002a, 2002b; Yancey et al., 1999; Yancey et al., 2001). This solution was designed based on the hypothesis that saccharides in the solution could increase the rate of pH decline by providing sufficient glucose for anaerobic metabolism to continue after residual muscle glycogen was utilized, and thus prevent dark colouring in beef (Yancey et al., 2001). It was reported that this infusion treatment resulted in not only a more rapid pH decline, but also a higher dressing percentage (Dikeman et al., 2003; Hunt et al., 2003). The effect of infusion decreased the tenderness of longissiums lumborum (LL) muscle with a higher Warner Bratzler shear force (WBSF) value, whereas the

infusion treatment increased the tenderness of semitendinosus (ST) muscle with a lower WBSF value than those from control carcasses (Dikeman et al., 2003). In contrast, there was no effect of this MPSC infusion treatment on post-mortem meat tenderness of LL and ST muscle (Yancey et al., 2002a; Yancey et al., 1999), and the flavour profile was not consistent with the normal characteristics of cooked beef (Yancey et al., 2002a; Yancey et al., 1999).

Research into including antioxidants such as sodium ascorbate, vitamin C and vitamin E in the infusion blend has been investigated. It was reported that intravenous injection of sodium ascorbate, or sodium ascorbate combined with $CaCl_2$ reduced beef steak's discoloration due to the possible reduction in beef muscle oxidation (Hood, 1975; Wheeler et al., 1996). It was thought that vitamin E could be added to prevent meat rancidity and colour loss (Waylan et al., 2002). However, a recent study indicated that addition 500ppm vitamin E, 500ppm vitamin C, or 500ppm vitamin E plus 500ppm vitamin C to a MPSC solution had no effect on display colour stability of LD, psoas or semimembranosus (SM) muscles in beef (Yancey et al., 2002a, 2002b; Yancey et al., 1999; Yancey et al., 2001). There was an increased dressing percentage and minimal effects on other carcasses traits, or on WBSF value and flavour profile of beef (Yancey et al., 2002b). On the other hand, an addition of a MPSC solution including 500ppm vitamin E improved display colour stability of ground beef formulated from the quadriceps femoris (Yancey et al., 2001). Meanwhile, addition of 1000 ppm vitamin C to the MPSC solution did not contribute to the colour or display-colour stability of beef steaks (Yancey et al., 2002a).

Studies have also found that infusion of water can increase the tenderness of meat (Ilian et al., 2004). However, meat under the water infusion treatment might appear lighter than normal because of the light scattering, or pumping aqueous solutions through the cardiovascular system may dilute or remove muscle pigments, which might create an impression similar to the undesirable PSE meat (Farouk & Price, 1994). However, Hunt et al. (2003) found that there was no difference in the concentration of total pigment (myoglobin plus haemoglobin) in control and infusion treatment beef carcasses, and they suggested that the rapid pH decline played the main role in the higher lightness in beef. Bekhit el al. (2005) found that the most rapid pH decline in $CaCl_2$-infusion treatment carcasses resulted in darker LM colour compared with W carcasses after infusion treatment. Additionally, Wang et al. (1995) reported that water infusion of young bull with

10% or 20% water increased dressing percentage by 2 and 4%. However, most of the increased dressing percentage was lost by evaporation during the chilling process, which resulted in negligible weight gain.

2.6 Enzymatic Meat Tenderization Using Kiwifruit Juice

From a historical perspective, the Mexican Indians knew at least 500 years ago about the theory of enzymatic tenderization of meat when they wrapped meat in pawpaw leaves during cooking (Lawrie, 1998). Since then, various proteases from plant sources such as papain, ficin and bromelin, have been identified and employed as commercial meat tenderizers in an attempt to make meat tender artificially. This process has become more systematic in recent years (Glazer & Smith, 1971; Sinku et al., 2003; Whittaker, 1994). In order to avoid some unsatisfactory effects such as overtenderized surface, a mushy texture or sometimes unusual flavour, pumping the enzyme-containing solutions into the major blood vessels of the carcass pre-rigor could eventually assure a complete homogenous distribution via penetration of the enzymatic solution throughout the whole carcass (Lawrie, 1998). It was suggested that proteolytic enzymes of plant origin might degrade the collagen only when it is denatured by heat during cooking (Lawrie, 1998).

Among the various plant enzymes, papain is a protease derived from the papaya plant. After infusion, it became activated during the acidification of the muscles post-mortem and still remained active when the meat was cooked (Warriss, 2000). Papain showed a broad ability to hydrolyse a diverse range of myofibrillar proteins and amino groups. However, it was unsatisfactory at a commercial level because it over-tenderized the surface of the meat, or produced a mushy texture and unusual “fruity” flavour (Lawrie, 1998).

Kiwifruit (*Actinidia chinensis*) juice contains actinidin, a plant thiol protease which contains a free sulfhydryl group that is essential for enzyme activity (Arcus, 1959). Actinidin has a molecular weight of 23,500 D (Carne & Moore, 1978), and has the advantages over other proteases originating from plants, such as papain and bromelin, of a mild tenderizing action (Lewis & Luh, 1988). It shows significant activities against globular proteins such as myosin (Lewis & Luh, 1988) and fibrous proteins such as collagen (Wada et al., 2004) of muscle tissue. In addition to its protease activity, the high acid content of acid in kiwifruit juice might break the myofibrillar structure down by

possibly enhancing the action of collagenases and cathepsins which are active at low pH (Warriss, 2000).

Lewis and Luh (1988) compared the effect of actinidin and papain on the tenderization of bovine semitendinosus muscles, and found that treated steaks were significantly more tender than the non-treated samples. It required an activity of 175 U/mL and 18 U/mL of actinidin and papain-based meat tenderizer respectively to obtain Kramer shear values (Lee, 1983) of 91kg/20g. This indicated that actindin had a milder protyolytic activity compared with papain. Also actinidin treated samples did not produce off flavours or odours in the meat or excessive surface tenderization as papain did (Lewis & Luh, 1988). Similar results were also achieved by Samejima et al. (1991) who demonstrated that the shear forces for raw and cooked meats decreased gradually (ie became more tender) with the time of soaking when beef pieces were immersed in crude actinidin solution at 4^{o}C.

A decreasing shear force value was also reported in other research which investigated the tenderizing effect of kiwifruit proteolytic activity on semitendinosus muscle in beef (Wada et al., 2002). This tenderizing effect was proposed to be caused not only by the disorganization of the myosin and actin filaments, but also by the denaturation of connective tissue because little soluble α-chain collagen was present with the kiwifruit protease immersed samples under both cooked and unheated conditions (Wada et al., 2002). The solubilisation of cattle achilles tendon with actinidin was investigated further at 20^{o}C at pH's of 6.0 and 3.3 (Wada et al., 2004). They found that actinidin could degrade the insoluble collagen under unheated conditions, and actinidin could also digest elastin into peptide fragments (Wada et al., 2004), which suggested that kiwifruit tenderizing effects were also partially due to the degradation of the connective tissue in muscle. This was consistent with the research reported by Sugiyama et al. (2005) that kiwifruit juice can degrade denatured beef collagen during the cooking process. Therefore it would appear that meat tenderizing ability of kiwifruit juice could be a feasible option for the commercial meat industry and also would benefit the consumers (Lewis & Luh, 1988).

Chapter 3
The Effect of Pre-rigor Infusion of Lamb with Kiwifruit Juice on Meat Physical Properties and Biochemical Changes

3.1 Introduction

Consumer's acceptability of meat is strongly influenced by meat quality parameters including tenderness, juiciness, display colour and flavour (Lawrie, 1998). Consumers do not purchase meat products that fail to meet their quality expectations. The production of high quality meat of consistent tenderness and colour is becoming more crucial to the red meat industry in order to strengthen the consumers' confidence and to encourage them to return to purchase meat products again (Boleman et al., 1997).

Several tenderizing solutions (Farouk et al., 1992a; Hunt et al., 2003), plant proteases (Ashie et al., 2002; Lewis & Luh, 1988; Wada et al., 2002) and ions (Koohmaraie & Shackelford, 1991; Koohmaraie et al., 1998; Murphy & Zerby, 2004) have been used to accelerate meat tenderization through injection, marination or infusion. Papain and calcium chloride have been the most studied and are probably the most effective tenderizing agents. However, the negative effect of papain on over tenderization of the meat surface, which can lead to the undesirable "mushy" meat (Lawrie, 1998), has raised the concerns when using it as a commercial meat tenderizer for meat industry. Calcium ions on the other hand could reduce the colour stability of fresh meat and decrease the product shelf life (Bekhit et al., 2005; Hunt et al., 2003) although the infusion of $CaCl_2$ solution could improve meat tenderness (Koohmaraie et al., 1989; Koohmaraie et al., 1998). The advantages of the mild tenderizing effect of the kiwifruit protease, actinidin, over other plant proteases such as papain and bromlain (Lewis & Luh, 1988) has made it a possible candidate as a commercial meat tenderizer.

Kiwifruit juice contains actinidin (a cysteine protease) which contributes to tender meat post-mortem (Lewis & Luh, 1988; Wada et al., 2004; Wada et al., 2002). In addition to its tenderization effect, the high content of natural antioxidants (ascorbic acid, vitamin E and other polyphenols) in kiwifruit juice (Dawes & Keene, 1999; Nishiyama et al., 2004) might inhibit lipid oxidation in meat during retail display and thus possibly influence the meat colour. The cardiovascular infusion of kiwifruit juice into carcasses employed in this study could homogeneously introduce the infusion elements into muscle and adipose tissues efficiently and quickly (Katsanidis et al., 2003).

In this chapter, the possible effect of pre-rigor infusion of lamb carcasses with kiwifruit juice and water on meat physical properties and biochemical changes will be investigated. This will be discussed using the following measurements in this work: (1) pH and temperature decline profile; (2) live weight, hot carcass weight, cold carcass weight and chilling evaporative loss; (3) drip loss and cooking loss percentage; (4) shear force value reflecting the tenderization level; (5) meat proteolytic activity; (6) sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE); (7) casein zymography; (8) display colour and (9) lipid oxidation.

3.2 Materials & Methods

3.2.1 Animals and Infusion Treatment

A total of 18 lambs (12 months old, average live weight 40.98 ± 3.60 kg) were selected from the Lincoln University farm, and were divided into three similar groups (6 lambs in each group/treatment). Three treatments were employed: 50% kiwifruit fresh juice supernatant infusion (Ac), water infusion (W), and no infusion which acted as a control (C). The infusion procedure was carried out according to that described by Ilian et al. (2004).

The experimental animals were processed on two consecutive days in which three animals from each treatment were slaughtered. The live weight of each animal was measured, and the volume of infusion solution was determined at 10% of body weight. Animals were slaughtered humanely using standard captive bolt stunning procedures at the Lincoln University facilities. After death, animals were exsanguinated and then transferred to a

surgery table, and the neck area was dressed for easy access to blood vessels. The right carotid artery was exposed and prepared for cannulation by making a sharp cut in the artery wall. Then the artery wall was enlarged with the help of forceps, and the cannula was inserted gently into the artery. The other carotid artery was clamped to prevent blood back flow. Then the infusion process was started by using a high rate continuous pump (MHRE, Watson-Marlow Ltd, Cornwall, England) at a flow rate of 240mL/min. The dead animal was transferred to the processing room for dressing by removing the pelt and viscera immediately after the infusion process was completed. Control animals receiving no infusion (n = 6) were also slaughtered by using standard captive bolt stunning procedures followed by exsanguination and dressing.

After dressing, the weight of individual carcasses was measured for all the three treatments. Carcasses were kept at room temperature (10^{o}C) for 5 hours before they were moved to a chiller at 2^{o}C and stored for another 6 days.

3.2.2 Kiwifruit Juice Preparation

Fresh kiwifruit (K1W1 Green, New Zealand) purchased from a local store were stored at 4^{o}C before making juice. After pressing the kiwifruit, using a bladder press, the juice was removed and filtered through very fine filter cloth, and then centrifuged at 8500g for 20 mins at 4^{o}C. The supernatant from this kiwifruit juice was diluted (50%) by adding an equal volume of distilled water for the infusion treatment solution. The kiwifruit juice infusion solution was made fresh each day before the experiment. A sample (10mL) of diluted kiwifruit juice infusion solution was stored in the freezer at -30^{o}C for the enzyme activity assay.

3.2.3 Sample Preparations

The left LD muscle and left leg were excised from each carcass at 1 day post-mortem and the muscles were divided into two parts (Figure 3-1). One part was used to examine the treatment effects on colour, lipid oxidation and volatile flavour compound at 1 day post-mortem, and the other part was vacuum packed (VP) and was stored at 2^{o}C to examine the effects of storage treatment on colour, lipid oxidation and volatile flavour compound after 3 wks vacuum storage. Samples from each part were cut into 2.5 cm thick pieces and

placed in polystyrene trays covered with O_2 permeable polyvinyl chloride film (O_2 permeability > 2000 mL m^{-2} atm^{-1} $24hr^{-1}$ at 25°C, AEP FilmPac (Ltd), Auckland, New Zealand) and displayed under fluorescent light in a cold room for 6 days at 4°C.

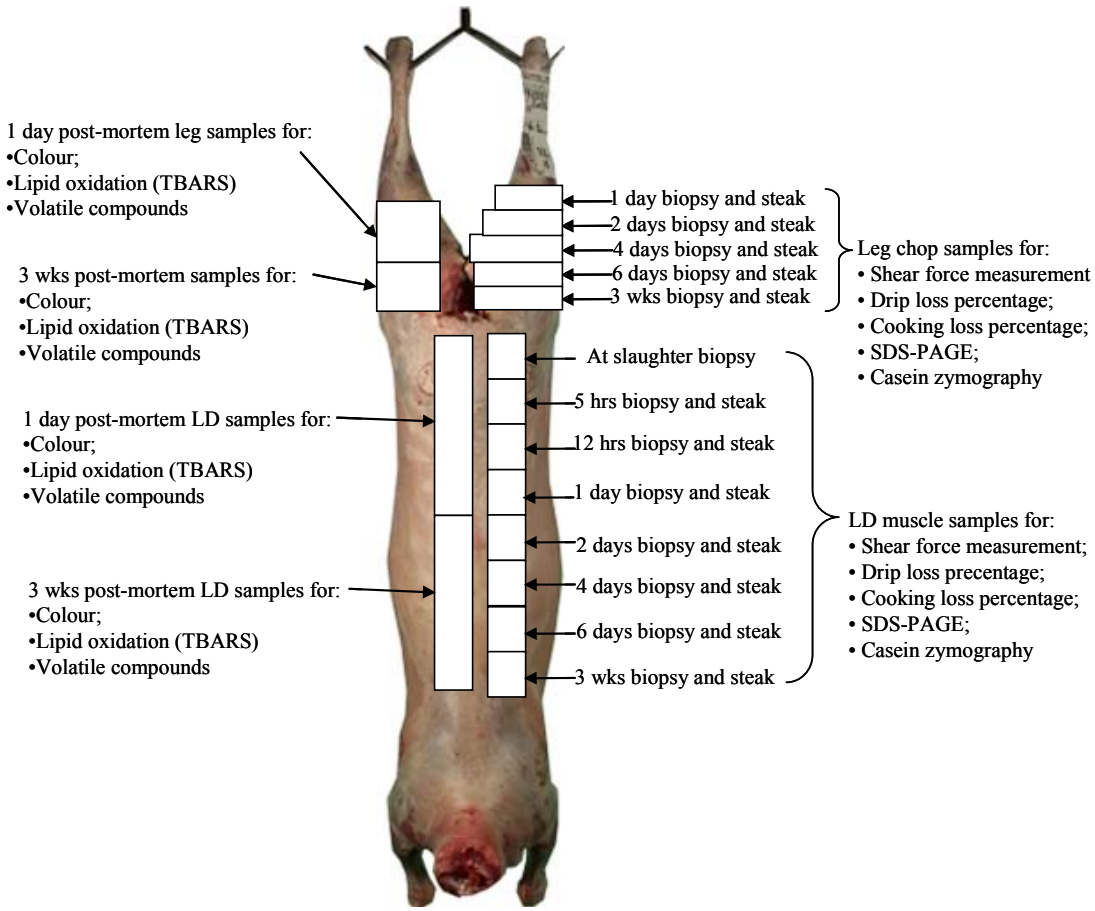

Figure 3-1 A diagram representing the sampling locations for the present study

The right LD and right leg muscles (Figure 3-1) were used to examine the various factors including tenderness measurement, percentage of drip loss, percentage of cooking loss and myofibrillar protein alteration during post-mortem. The LD muscle samples, which were cut into 5cm thick portions were taken at slaughter (0 time), and after 5 hours, 12 hours, 1 day, 2 days, 4 days, 6 days and 3 wks vacuum packaged storage at 2°C. The leg was cut into 2.5 cm thick chop samples, after 1 day, 2 days, 4 days, 6 days post-mortem and 3 wks vacuum packaged storage at 2°C. After the samples were taken they were then individually vacuum packed, frozen rapidly in liquid nitrogen to stop further biochemical changes and stored (approximately 1 month) at -30°C until analyses.

3.2.4 pH and Temperature Measurements

The pH and temperature profile of lamb carcasses during onset of rigor was determined as described by Bekhit et al. (2005). Carcass pH and temperature were measured every 30 minutes during the first 8 hours post-mortem, and then at 12 hour, 1 days and 2 days post-mortem in the longissimus dorsi muscle (LD) starting immediately after dressing. The ultimate pH values of individual muscles in the leg chops (including rectus femoris and other muscles, semimembranosus and adductor, biceps femoris and semitendinosus muscles shown in Figure 3-2) of the three treatments were measured at 1 day post-mortem stored at 4°C. An InLab 427 pH combination puncture electrode (Mettler-Toledo Process Analytical Inc., Wilmington, MA, U.S.A.) attached to a Hanna HI 9025 pH meter (Hanna Instruments Inc., Woonsocket, U.S.A.) was inserted into the centre of the muscles for pH measurements.

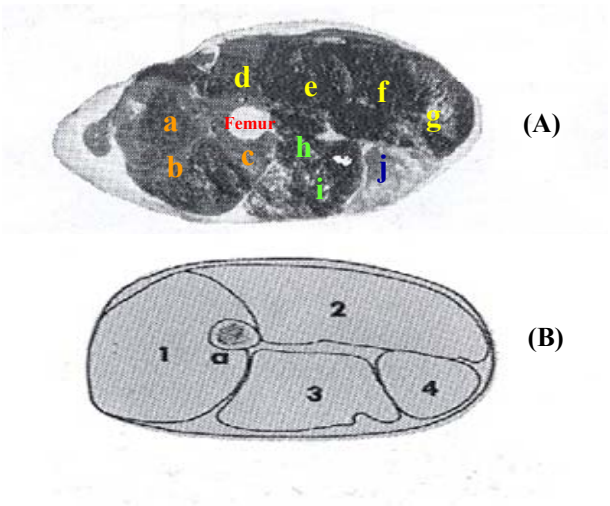

Figure 3-2 The image of lamb leg chop slice (a. femur in the centre; 1. rectus femoris and other muscles (sirloin tip muscle) including muscles numbered a, b and c in Figure 3-2 (A); 2. seminembranosus and adductor (top round muscle) including muscles numbered d, e, f and g in Figure 3-2 (A); 3. biceps femoris (bottom round muscle) including muscles numbered h and i in Figure 3-2 (A) and 4. semitendinosus (eye of round muscle) numbered j in Figure 3-2 (A)) (Forrest et al., 1975)

3.2.5 Hot Carcass Weight, Cold Carcass Weight and Chilling Evaporative Loss

After dressing, the carcasses from the various experimental treatments were weighed (hot carcass weight) and kept at room temperature at about 10°C for 5 hrs before being moved

to a chiller at 2°C. The weight of each carcass was measured again 1 day later after they had been placed in the chiller (cold carcass weight). The differences between hot carcass weights and cold carcass weights were calculated as the chilling evaporative loss:

Chilling evaporative loss = Hot carcass weight – Cold carcass weight

3.2.6 Drip Loss and Cooking Loss Percentage

Samples from the right LD muscle and right leg chops were used for the determination of drip loss and cooking loss (Figure 3-1). Meat samples (5cm width steaks) from the right LD muscles were excised at 5 hours, 12 hours, 1 day, 2 days, 4 days, 6 days and 3 wks post-mortem, and the leg chop samples (2.5 cm thickness) from the right leg were obtained at 1 day, 2 days, 4 days, 6 days and 3 wks post-mortem. Meat samples were weighed (WT before freezing) before the samples were stored directly at -30°C for further experiments. After the meat samples were thawed overnight at 2°C, the surface of the meat samples was blotted gently using paper towels and the samples were reweighed (WT after thawing). The drip loss was calculated as follows:

Drip loss (%) = (WT before freezing – WT after thawing) / WT before freezing*100

The samples were cooked individually in plastic bags immersed in a water bath at 90°C until they reached an internal temperature of 75°C. A Fluke type K thermocouple attached to Fluke 52 meter (John Fluke MFG CO., Washington, U.S.A.) was introduced into the centre of the meat samples during cooking. The cooked steaks were cooled on ice, blotted with paper towels and weighed (WT after cooking). The cooking loss was calculated as follows:

Cooking loss (%) = (WT after thawing – WT after cooking) / WT after thawing*100

3.2.7 MIRINZ Shear Force Measurements

Following the drip and cooking loss measurements, each cooked sample was cut into 8 – 10 strips (10mm x 10mm square) parallel to the muscle fibre direction. The meat strips were placed into the metal wedge of a MIRINZ tenderometer (AgResearch MIRINZ,

Hamilton, New Zealand). The tenderness values were obtained by measuring the pressure required to sever the meat strip and values were read in kPa. Results were then converted into kiloforce units (kgF) and then to Newton (N) using the equations.

kgF = kPa*0.2 - 1.7

N = (kPa*0.2 - 1.7)* 9.8.

3.2.8 Proteolytic Activity of Kiwifruit Juice and Meat Samples Assay

In order to examine the effects of the three treatments, the proteolytic activity of meat samples was measured. The proteolytic activity of fresh kiwifruit juice was also measured. The enhanced proteolytic activity level of meat samples which had been infused with kiwifruit juice, which contains the protease (actinidin), indicated that the infusion treatment was successful.

Two methods of measuring proteolytic activity in kiwifruit juice and in meat samples were employed: a spectrophotometric assay (using p-nitrophenol) and a fluorometric assay (using BODIPY-FL casein).

3.2.8.1 Spectrophotometric Assay (Using p-nitrophenol)

Protease activity was measured by comparing the release of p-nitrophenol from CBZ-ONP-Lysine in the presence of kiwifruit juice, with the spontaneous release of p-nitrophenol in a control of buffer according to the method of Lewis and Luh (1988).

The enzyme assay buffer was prepared in 0.07M potassium phosphate (0.2mM EDTA, 1.6% acetonitrile, pH 6.95). The buffer was made weekly and stored at 4^{o}C. A 0.02M p-nitrophenol stock solution was prepared in distilled water for the standard curve using concentrations from 0 – 0.5µmol. The 0.01M substrate solution was prepared by dissolving 10.5mg of N-α- benzyloxycarbony-L-lysine p-nitrophenol ester (CBZ-Lys-ONP) in 0.1mL of distilled water and 1.9mL of acetonitrile. This solution was freshly prepared for each assay and was kept in ice during the assay. An ATI UNICAM UV spectrophotometer

linked to ATI UNICAM Vision computer software was used to measure the absorbance values and the rates of degradation in this assay.

To assay the proteolytic activity of the kiwifruit juice, 5mL frozen diluted kiwifruit juice infusion solution as described in Section 3.2.2 was thawed, and kept in an ice box ready for the assay.

To assay the proteolytic activity of the meat samples from the treatment carcasses, a minced meat sample (1g) from LD muscle was placed in a 50ml test tube, and homogenized with 5ml of enzyme assay buffer using a Polytron homogeniser (Type PT-MR 3100, Kinematica AG, Littau, Switzerland) at 15000rpm for 20 seconds. The homogenate was then centrifuged at 37000g for 30 mins at 4^{o}C. After centrifugation, the supernatant was weighed and stored in the ice box ready for the assay.

The spectrophotometer was zeroed by using enzyme assay buffer. A 2.98mL aliquot of buffer was added to 50μL of the CBZ-Lys-ONP substrate solution in a 3mL plastic cuvette. The initial degradation rate of the substrate was measured at a wavelength of 348nm for 30 seconds due to the background non-enzymatic hydrolysis of substrate. Thereafter 50μL of kiwifruit juice solution or meat sample supernatant was added, and the rate of substrate degradation was measured for a further 30 seconds.

A standard curve of absorbance vs. concentration of p-nitrophenol was described by a linear equation ($y = mx + c$) generated using SigmaPlot. The proteolytic activity of kiwifruit fresh juice infusion solution or meat samples from the control and infused carcasses could be calculated from the concentration of p-nitrophenol. The initial non-enzymatic hydrolysis rate of substrate was subtracted from the enzyme activity rate to calculate the enhanced proteolytic activity induced by the kiwifruit fresh juice or infusion treatments. One unit of proteolytic activity was the amount of enzyme (actinidin contained in kiwifruit juice infusion solution in mL, or in meat in gram) which produced 1μmol of product (p-nitrophenol) per minute.

3.2.8.2 Fluorometric Assay (Using BODIPY-FL Casein)

A second assay was designed to confirm the proteolytic activity of kiwifruit juice used in this work and the proteolytic activity changes occurring in the lamb muscle after the infusion treatment. Activity was calculated by using the fluorescence changes caused by the enzymatic activity. BODIPY-FL was purchased from Molecular Probes (Eugene, OR, USA) and the BODIPY-FL casein was prepared as described by Thompson et al. (2000). Black microtiter plates were used in this assay.

The preparation of the kiwifruit juice infusion solution and the meat sample supernatant used in this assay were the same as described in section 3.2.8.1.

In a typical assay, 50µL of kiwifruit juice, or meat sample supernatant was combined with 50µL buffer (20 mM Tris, pH7.5; 1 mM EDTA; 1 mM EGTA; 2 mM DTT) and 100µL BODIPY-FL casein substrate (5000 µL dilution buffer (40 mMTris, pH 7.5; 2 mM EDTA; 200 mM EDTA; 200 mM KCl); 3965 µL DDH_2O; 10 mM $CaCl_2$; 50 µL BODIPY-FL casein stock; 0.1% β-mercaptoethanol). The blank reference pool contained 100 µL buffer and 100 µL BODIPY-FL casein substrate. Sample fluorescence was read immediately and after every minute for 10 minutes in a BMG ELISA plate reader (Fluostar, BMG Labtechnologies GmbH. Offenburg, Germany) with an excitation wavelength of 485 nm, emission 538 nm and a gain of 60. The software used was version 3.02-0.

The fluorescence changes caused by kiwifruit juice or meat sample supernatant activity were calculated, and expressed as Units per mL of kiwifruit juice or Units per gram of meat sample, respectively.

3.2.9 SDS Polyacrylamide Gel Electrophoresis

3.2.9.1 Sarcoplasmic and Myofibrillar Isolation

Sarcoplasmic and myofibrillar fractions of LD proteins from samples at-death, 5 hours, 12 hours, 1 day, 2 days, 4 days and 6 days post-mortem time were separated according to the procedure described by Ilian et al. (2001). Muscle tissue (2g) was cut into small pieces and homogenized in three volumes of a homogenizing buffer (100 mM Tris-HCl, 5 mM

EDTA, 0.05% β-mercaptoethanol, pH 8.3) at 2^{o}C for 30 seconds at 13500rpm using a Polytron homogeniser (Type PT-MR 3100, Kinematica AG, Littau, Switzerland). The homogenate was centrifuged at 5500g for 30 mins at 4^{o}C. After centrifuging, 1mL of the supernatant containing the sarcoplasmic protein fraction was decanted into an eppendorf tube and stored at 4^{o}C for use in the SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) assay. Another 1mL of the supernatant was decanted into an eppendorf tube, and was frozen for further use in zymographic PAGE analysis. The rest of the supernatant was discarded. The pellet was washed twice with 15mL of homogenizing buffer and was centrifuged at 3500rpm for 10 minutes at 4^{o}C. The washed pellet was then re-suspended with 15mL of myofibril fragmentation index (MFI) buffer (50 mM Tris-HCl pH 6.8, 1% SDS, 2% MCE, 3% glycerol) and was heated at 80^{o}C in a water bath for 3 minutes. The hot sample was then vortexed and filtered through Whatman No. 1 filter paper, 1mL of the separated solution was stored at 4^{o}C for myofibrillar protein analysis using SDS-PAGE.

3.2.9.2 Protein Assay

The protein concentration of the various fractions was determined according to the method of Karlsson et al. (1994). Bovine serum albumin (BSA) standards (0, 0.0625, 0.125, 0.25, 0.5μg/μL) were prepared from a 2mg/mL stock standard solution. Both sarcoplasmic and myofibrillar protein samples prepared (see section 3.2.9.1) were diluted 50x with distilled water. Triplicate 150μL aliquots of each standard and sample were applied to a microtiter well of a Falcon 96 well microplate. Then 100μL of 60% TCA (w/v, filtered) was added to each of the microtiter wells with a multichannel pipette, and the microplate was left at room temperature for 30 minutes. The plate was measured at 570nm using a BMG Lab Technologies Ltd Fluostar fluorometer (type 0403). A standard curve was generated from which sample protein concentrations were determined.

3.2.9.3 SDS Polyacrylamide Gel Electrophoresis

Sarcoplasmic and myofibrillar protein samples were analysed using SDS-PAGE (procedure according to Mini-PROTEAN 3 Cell Instruction Manual, BioRad). Samples were prepared with a 6 x SDS dissociation buffer (0.35M Tris-HCl pH 6.8, 10.28% SDS (w/v), 30% (v/v) glycerol, 5% (v/v) β-mercaptoethnanol and 0.012% bromophenol blue), and protein fractions were denatured by heating at 95^{o}C for 4 minutes. A 10μL BioRad

Precision Protein Pre-stained Broad Range molecular marker was loaded onto a 12% (w/v) polyacrylamide gel (The running gel contained: 1.7 mL DDH_2O, 1.3 mL 1.5M Tris-HCl pH 8.8, 0.05 mL 10% w/v SDS, 2 mL 30% degassed acrylamide /Bis, 25 µL 10% APS, 2.5 µL TEMED (N,N,N'N'-Tetramethylethylenediamine); (The stacking gel contained: 1.14 mL DDH_2O, 0.47 mL 0.5M Tris-HCl pH 6.5, 17.5 µL 10% w/v SDS, 0.25 mL 30% degassed acrylamide/Bis, 12.5 µL 10% APS and 1.88 µL TEMED). Each sample (20µg) with the protein concentration (as determined in the prior protein assay) was loaded onto the gel. Electrophoresis was performed at 150V for 70 minutes in a Mini-PROTEAN 3 Cell (Bio-Rad) with the 1 x SDS running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS).

Protein bands on the gels were stained for one hour with Gelcode Blue (Pierce, USA). The gels were then destained in distilled water overnight to reveal the bands.

3.2.10 Zymographic PAGE Analysis

A 10% polyacrylamide gel co-polymerised with 0.05% casein (The running gel contained: 2.06 mL DDH_2O, 0.75 mL 1.5M Tris-HCl pH 7.5, 1.67 mL 30% degassed acrylamide /Bis, 0.5 mL 0.5% Casein/1.5M Tris-HCl pH 7.5, 30 µL 10% APS, 3 µL TEMED; The stacking gel contained: 1.24 mL DDH_2O, 0.5 mL 0.5M Tris-HCl pH 6.8, 0.26mL 30% degassed acrylamide/Bis, 20 µL 10% APS, 2 µL TEMED) were pre-run with buffer containing 25 mM Tris-HCl (pH 8.3), 192 mM glycine, 1 mM EGTA and 1 mM DTT for 15 mins at 125 V at 4^{o}C. Then, 10 µL of each Kiwifruit juice (50% concentration) , µ-calpain and m-calpain, and 12 µL sarcoplasmic protein samples (samples preparation refer to section 3.2.9.1) were loaded and run at 125 V for 2.5 hr at 4^{o}C. Subsequently, the gels were incubated overnight at room temperature with slow shaking in buffer (20 mM Tris-HCl, 10 mM DTT, pH 7.4) with or without 20 mM calcium chloride. Gels were rinsed thoroughly with distilled water 3 times for 10 mins each, and then gels were stained with Gelcode Blue (Pierce, USA) for at least 1 hr.

3.2.11 Colour Measurement

The left LD muscle and left hind leg were excised from the carcasses at 1 day post-mortem. The left LD muscle was divided into two portions. The caudal section was used to

measure the colour of the treatment effects at 1 day post-mortem, whereas the cranial section was vacuum-packaged to examine the treatment effects after 3 wk of vacuum packaged storage at 2^{o}C (Figure 3-1). Both the left LD muscle and left hind leg were cut into 2.5-cm-thick chops before vacuum-packaged storage at 2^{o}C. Samples from left LD and left leg muscles, used for colour evaluation (Figure 3-1), were placed in polystyrene trays, and wrapped with an O_2-permeable polyvinyl chloride film (O_2-permeability > 2000 mL m^{-2} atm^{-1} $24hr^{-1}$ at 25^{o}C, AEP FilmPac (Ltd), Auckland, New Zealand) and displayed under fluorescent lighting in the cold room at 4^{o}C for 6 days. Colour was evaluated on these samples objectively, using a colorimeter (MiniScan XE Plus, Hunter Associates Laboratory, Inc., Virginia, U.S.A.). HunterLab values of lightness (L*), redness (a*) and yellowness (b*) were recorded.

3.2.12 Lipid Oxidation

Changes in lipid oxidation were determined by analysis of 2-thiobarbituric acid reactive substances (TBARS) method as described by Nam & Ahn (2003). Minced meat samples (3g) were placed in a 50ml test tube and homogenized with 9ml of deionized distilled water (DDH_2O) using a Polytron homogeniser (Type PT-MR 3100, Kinematica AG, Littau, Switzerland) at 14,000rpm for 30 s. Meat homogenate (1 ml) was transferred to a small tube, and 50µL butylated hydroxytoluene (BHT, 7.2%) and 2mL thiobarbituric acid/trichloroacetic acid (20mM TBA and 15% (w/v) TCA) solution were added. The mixture was vortexed and then incubated in a water bath at 90°C for 15 min to develop colour. After cooling for 10 min in cold water, the samples were vortexed and centrifuged at 3000 x g for 15 min at 5°C. The absorbance of the resulting upper layer was read at 531 nm against a blank prepared with 1 ml DDH_2O and 2 ml TBA/TCA solution. The TBARS test determines the amount of malondialdehyde (MDA) which is the major secondary by-products generated from lipid oxidation in meat. The amount of TBARS was expressed as mg MDA per kg of meat.

3.2.13 Statistical Analysis

Generally, the restricted maximum likelihood estimation (REML) routines in GenStat Statistical Software Package Version 9 (VSN International Ltd, UK) were used to analyse the data owing to the unbalanced nature of the design with only some display days for

some muscles/cuts being used. Where the data formed a balanced set, the general linear model routine in Minitab software (Minitab version 11, State College, PA) was used. Some time-dependent results were sub-analysed by calculating derived variables from an exponential decay model and the estimated parameters further analysed to elucidate trends.

3.2.13.1 Statistical Analysis for pH-Temperature Measurements

The pH-temperature value for the three treatments over time was analysed by using the REML routine in GenStat to calculate the expected means and standard error of means (SEMs). Then the means of pH-Temperature were analysed by fitting an exponential decay curve ($Y = Y_0 + a*\exp(-b*X)$). An exponential model was fitted to the REML-estimated means to analyse the decay over post-mortem time of the pH or temperature responses.

3.2.13.2 Statistical Analysis for Proteolytic Activity of Kiwifruit juice and Meat Samples Measurements

For the spectrophotometric assay by using p-nitrophenol, measurements in triplicate were made at each concentration of p-nitrophenol (0, 0.1, 0.2, 0.3, 0.4 and 0.5 μmol) and means were calculated by using Minitab. A linear regression was fitted where:

The means of absorbance (at 348nm) = 0.8889 * concentration + 0.0063 (SEMs = 0.0135)

For the fluorometric assay by using BODIPY-FL casein, measurements in triplicate were made, and means and SEMs of the estimated enhancement of the proteolytic activities were calculated by using Minitab.

3.2.13.3 Statistical Analysis for Shear Force Measurement

The shear force data for the three treatments over time were analysed by using the REML routine in GenStat. Animal and animal x time terms were considered to be the random effect, and muscle/cut, treatment and time period terms to be the fixed effects. An exponential model was fitted to the REML-estimated means to analyse the decay over post-mortem time of the shear force responses.

3.2.13.4 Statistical Analysis for Hot Carcass Weight, Cold Carcass Weight, Chilling Evaporative Loss, Drip Loss and Cooking Loss

Data was tabulated in Microsoft Excel spreadsheets, means and SEMs of the measurements were calculated by using Minitab. The significance of differences between means was determined at the 5% significance level.

3.2.13.5 Statistical Analysis for Colour Measurement

Data were analysed as a split-plot design with the REML routine in Genstat. In the REML analysis, treatment, muscle/cut, post-mortem time and display time were taken to be fixed effects, while animals and post-mortem time within animals were set as random factors. The significance of treatment terms and their interactions were determined by Wald tests.

3.2.13.6 Statistical Analysis for Lipid Oxidation

REML routine in GenStat was used to analyse the lipid oxidation. Animals were considered to be the random term, and muscle/cut, post-mortem time, display time and treatment were the fixed effects. An exponential model was fitted to the REML-estimated means to analyse the decay over days of the lipid oxidation responses.

3.3 Results and Discussion

3.3.1 Meat Physical Property Studies

3.3.1.1 Live Weight, Hot Carcass Weight, Cold Carcass Weight and Chilling Evaporative Loss

There were no live weight differences ($P > 0.05$) among lambs assigned to the different treatments (Table 3-1), nor were there differences ($P > 0.05$) between the infused treatments and control for the hot carcass weight, cold carcass weight and chilling evaporative loss in the lamb carcasses (Table 3-1).

Table 3-1 Effects of vascular infused treatments on live weight, hot carcass weight, cold carcass weight and chilling evaporative loss in lamb carcasses (means ± SEMs)

Treatment	C	W	Ac
Live WT (kg)	41.00 ± 1.83	40.85 ± 1.44	41.08 ± 1.39
Hot carcass WT (kg)	20.55 ± 1.09	20.66 ± 0.96	19.41 ± 0.52
Cold carcass WT (kg)	19.54 ± 1.02	19.86 ± 0.95	18.60 ± 0.50
Chilling evaporative loss (kg)	1.01 ± 0.18	0.84 ± 0.07	0.82 ± 0.06

C: control W: water infusion Ac: kiwifruit juice infusion

The absence of hot carcass weight differences found in this experiment were not expected because higher dressed carcass yields were reported in previous vascular infusion treatments when beef carcasses were infused with MPSC solution which consist of 98.52% water, 0.97% saccharides, 0.23% NaCl and 0.28% phosphate blend (refer to Table 2.2) or $CaCl_2$. Higher dressed carcass weight could contribute positively to the meat industry as meat is sold by weight (Dikeman et al., 2003; Yancey et al., 2002a, 2002b). Undoubtedly some of the infused solutions would be located in the organs and viscera as was shown to be the case by Yancey et al. (2002a; , 2002b). When these were removed from the carcass during dressing much of the infused solutions might also have been lost, accounting for the similar dressed weights from the carcasses given different treatments. Wang et al. (1995) also claimed that the increased dressing percentage resulted from the infusion treatments might not be important because extra water was lost via evaporation during the chilling process, which resulted in negligible net cold weight gain in beef carcasses.

3.3.1.2 Post-mortem pH and Temperature Fall

The treatment effects on rate of pH fall and the rate of temperature fall on post-mortem lamb carcasses are shown in Figure 3-3 (A) and (B), respectively. The initial pH measured at slaughter differed ($P < 0.05$) between the three treatments (the means of initial pH were at 6.71, 6.20 and 6.49 for C, W and Ac carcasses, respectively). All infused treatment carcasses had lower ($P < 0.05$) pH values than C carcasses during the initial 12 hrs post-mortem (Figure 3-3 (A)). This was in similar to the results reported by Bekhit et al. (2005) that carcasses had lower pH values after $CaCl_2$, water and $ZnCl_2$ solutions (10% of live weight) were infused into lamb carcasses compared with control during the first 12 hrs post-mortem. A more rapid pH decline occurred in W carcasses compared with the C and

Ac carcasses, and W had a significantly lower pH values ($P < 0.05$) than C and Ac carcasses during the first day post-mortem.

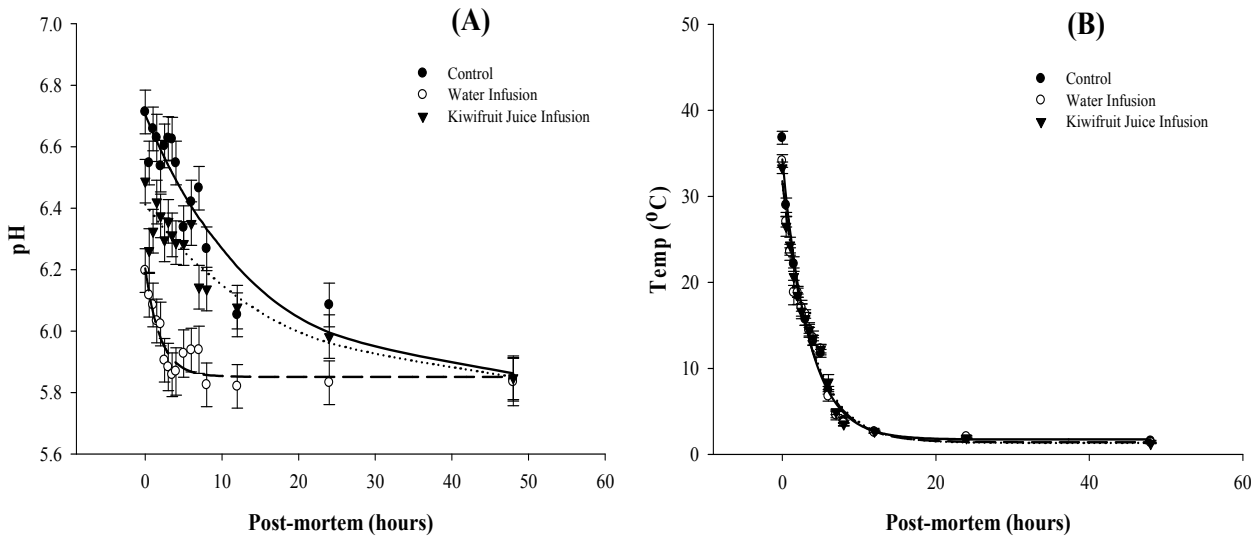

Figure 3-3 Effect of control, water infusion and kiwifruit juice pre-rigor vascular infusion treatment on both (A) the rate of pH fall; (B) the rate of temperature fall in lamb carcasses during 48 hrs post-mortem (means ± SEMs)

The different pH decline rates ($P < 0.05$) shown in W and Ac carcasses were thought to be caused by the different effects of infusion substances (water vs kiwifruit juice) used in this research. In addition, the stimulation of glycolytic enzymes due to the mechanical disruption of the muscle structure caused by the infusion process (Farouk & Price, 1994) might also contribute to the pH differences between infusion carcasses and C during the first 12 hrs post-mortem.

Water infusion treatment carcasses achieved their ultimate pH (pH_u) by 8 hrs post-mortem, whereas both C and Ac carcasses reached their pH_u of about 5.84 ± 0.07 by 48 hrs post-mortem. Similar pH_u results with W carcasses were also reported by Bekhit et al. (2005). Although in the current study, it took longer for C and Ac carcasses to reach pH_u, the value of pH_u was the same as that in the W treatment. This was in agreement with Murphy and Zerby (2004) that water infusion had no effect on ultimate pH. The post-mortem pH values are reported to be good indicators of the muscle glycolysis rate (Newbold & Small, 1985),

therefore the rate of glycolysis of the three treatments in the current study would be: W > Ac > C.

The infusion treatment showed no significant effect ($P > 0.05$) on temperature changes in post-mortem carcasses except in the initial 2 hrs after the completion of the infusion process compared with the C carcasses (Figure 3-3 (B)). This phenomenon might be induced by the lower temperature of the infused solutions which was stored at chilled temperature at 4^{o}C before infusion. A similar result was reported by Farouk and Price (1994). They also proposed that the initial lowering of the carcasses temperatures might retard the rate of glycolysis in the infused carcasses which might lead to a slower pH decline (Farouk & Price, 1994). However, the initial decrease in body temperature in infusion treatment carcasses in the present study did not appear to interact with their pH decline rate, which might indicate that the post-mortem carcasses temperature changes did not play the main role in the pH decline differences which occurred with the three treatments in the current research.

When carcasses achieved their pH_u, the temperatures of W carcasses were moderately high (17.1^{o}C) compared with Ac and C carcasses (1.5 and 1.3^{o}C for Ac and C, respectively) (Figure 3-3 (B)) due to the rapid pH decline occurred in the LD muscles of the W carcasses. High rate of pH decline at a relatively high carcass temperature in W carcasses might accelerate protein denaturation (Hunt et al., 2003), and have resulted in abnormal meat texture and low water holding capacity, similar to PSE pork (Wang et al., 1995), and alter the meat colour by modifying the metmyoglobin formation (Ledward, 1985).

Table 3-2 Effect of control, water infused and kiwifruit juice pre-rigor vascular infused treatment on the ultimate pH of the muscles for leg chops in lamb carcasses at 1 day post-mortem stored at 4°C (means ± SEMs)

Muscle numbers*	Ultimate pH		
	C	W	Ac
a	5.93 ± 0.02	6.07 ± 0.12	6.12 ± 0.07
b	6.33 ± 0.16	6.25 ± 0.11	6.16 ± 0.04
c	6.12 ± 0.15	6.26 ± 0.21	5.93 ± 0.00
d	6.19 ± 0.15	ND	6.00 ± 0.01
e	6.13 ± 0.15	6.03 ± 0.14	6.02 ± 0.13
f	6.33 ± 0.13	6.15 ± 0.02	6.19 ± 0.16
g	6.47 ± 0.15	6.33 ± 0.08	6.39 ± 0.19
h	6.80 ± 0.25	6.57 ± 0.07	6.46 ± 0.06
i	6.16 ± 0.13	6.05 ± 0.16	6.26 ± 0.10
j	6.43 ± 0.14	6.11± 0.08	6.25 ± 0.16
Mean	6.29 ± 0.24	6.20 ± 0.17	6.18 ± 0.17

* Muscle numbers refer to the leg chop image in Figure 3-2;
C: control W: water infusion Ac: kiwifruit juice infusion
ND = not determined

There were no significant differences ($P > 0.05$) in the ultimate pH values measured at 1 day post-mortem between the leg chops in the 3 treatments (Table 3-2). However the pH_u of LD muscles ($pH_u = 5.84$) was much less ($P < 0.05$) than that of leg chops ($pH_u = 6.22$) in lamb carcasses in the present work. This was consistent with earlier reports that variations in glycolysis of different muscles within a beef carcass were due to the differences in muscle fibre composition and their different abilities to undergo glycolysis in different muscles (Tarrant & Mothersill, 1977; Warriss, 2000). Post-mortem glycolysis is a complex process which might be influenced by various factors such as animal species, animal age, type of muscle, pre-slaughter handling, slaughter conditions and post-slaughter handling and temperature, and thus might have resulted in different pH_u of a given muscle (Lawrie, 1998).

3.3.1.3 Drip Loss and Cooking Loss Percentage

Statistically, there was no significant effect ($P > 0.05$) of treatment, post-mortem time and their interactions on percentage of drip loss for LD muscles (Figure 3-4 (A)). However, variation between samples might preclude a significant result from being determined. It was observed that LD muscles from W carcasses had higher percentage of drip loss than C carcasses during day 1 to day 6 post-mortem storage. Post-mortem time had no significant

effect (P > 0.05) on the percentage of leg chop drip loss of the three treatments (Figure 3-4 (B)). The leg chops from Ac carcasses had the lowest (P < 0.001) percentage of drip loss of the three treatments, while the leg chops from W carcasses was the highest (P < 0.001).

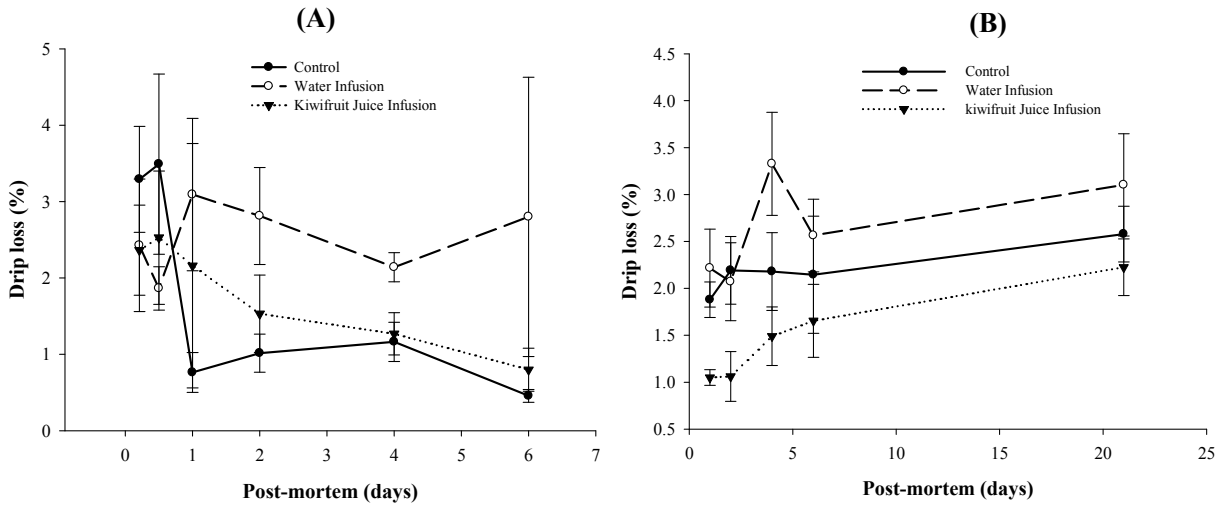

Figure 3-4 Effects of vascular infusion treatments on drip loss (%) in (A) LD muscles; (B) leg chops in lamb carcasses (means ± SEMs)

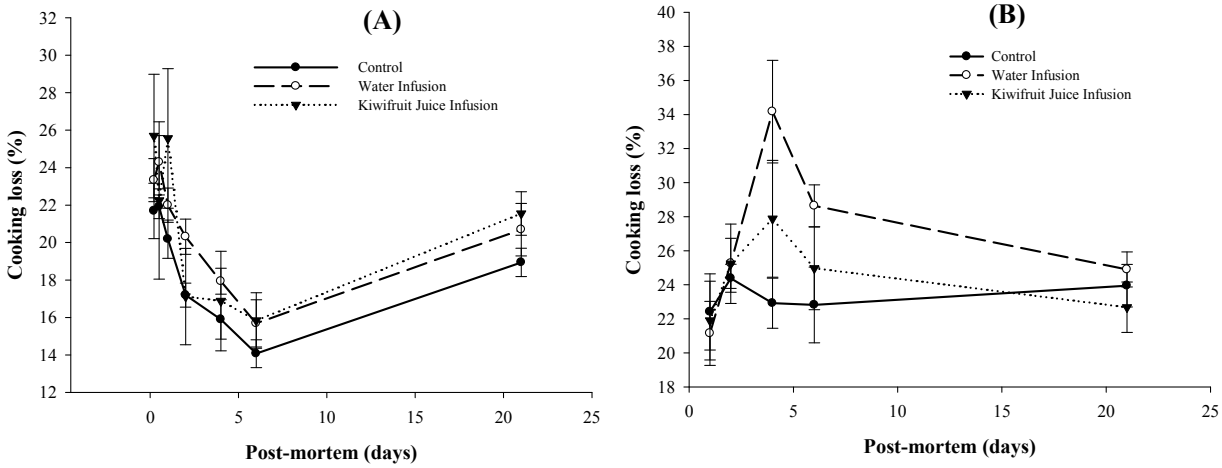

Figure 3-5 Effects of vascular infusion treatments on cooking loss (%) in (A) LD muscles; (B) leg chops in lamb carcasses (means ± SEMs)

Both treatment and post-mortem time had significant effect (P < 0.001) on percentage of cooking loss of LD muscles and leg chops (Figure 3-5). The LD muscles from C carcasses had the lowest percentage of cooking loss of the three treatments, while the W carcasses were the highest. The percentage of cooking loss of LD muscles from the three treatments

decreased consistently during the first 6 days display time, and achieved their lowest value at day 6 post-mortem. Higher percentage of cooking loss of LD muscles of the three treatments was shown after 3 wks vacuum packaged storage at 2^{o}C. It was interesting to note that leg chops of W carcasses had a higher ($P < 0.01$) percentage of drip loss (Figure 3-4 (B)) and cooking loss (Figure 3-5 (B)) than C at day 4 post-mortem.

Meat juiciness is generally a reflection of the water holding capacity (WHC) of the meat. Drip loss and cooking loss have been considered as important markers of WHC and the amount of the unbound water in meat (Joo et al., 1999). In general, W carcasses showed the highest percentage of drip and cooking loss in the current research. This might be mainly due to the lower WHC in the W carcasses resulted from the rapid pH decline at relatively high temperature (Forrest et al., 1975) (refer to Section 3.3.1.1). The excess drip loss might result in an unattractive appearance of fresh meat, and large amount water loss on cooking might have a negative effect on consumer satisfaction by reducing the size of meat that can be served (Joo et al., 1999).

3.3.2 Meat Tenderness Studies

3.3.2.1 MIRINZ Shear Force Measurements

The effects of pre-rigor infusion treatment on MIRINZ shear force values of the LD muscles and leg chops from the lamb carcasses are presented in Figure 3.6 (A) and (B), respectively. Muscle cut (LD muscle vs leg chops), post-mortem time, infusion treatments and their pair interactions significantly ($P < 0.001$ for all) affected MIRINZ shear force values. The shear force values of both LD muscles and leg chops from the various treatments decreased with time during ageing. The LD muscles from Ac carcasses were significantly more tender ($P < 0.001$) than C and W carcasses (Figure 3-6 (A)) with a significantly lower shear force values after initial 5 hrs post-mortem and throughout the following 6 days post-mortem period after the kiwifruit juice infusion process. By 3 wks, the vacuum packaged meat stored at 2^{o}C from the three treatment carcasses was of similar tenderness due to the ageing process. During this period, leg chops from the Ac carcasses showed a non-significantly lower tenderness than from the C and W carcasses. Ageing is a common process used by the meat industry to ensure the meat tenderness.

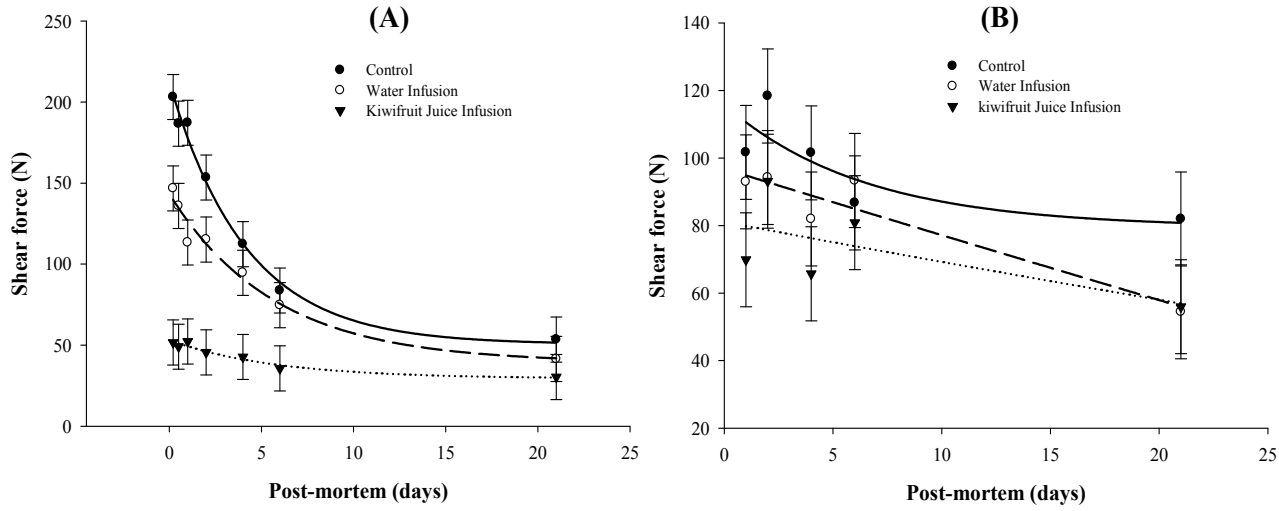

Figure 3-6 Effects of vascular infusion treatments on the MIRINZ shear force values of (A) LD muscles and (B) leg chops during post-mortem storage at 2°C (means ± SEMs)

Based on a consumer survey (n = 2313) from over three hundreds retail outlets in New Zealand, consumers' perceptions of the tenderness of lamb were allocated into five categories (very tender; tender; acceptable; tough, very tough) according to the MIRINZ shear forces values (< 5.0; 5.0 - 7.9; 8.0 - 10.9; 11.0 - 14.9; ≥ 15.0 kgF) of the meat, respectively (Bickerstaffe et al., 2001) (refer to Table 2-1 in Section 2.4.1). In the current study, the MIRINZ mean shear force values for LD muscle in C, W and Ac carcasses at 5 hrs post-mortem were 203.10, 146.74 and 51.64 N (20.75, 14.97 and 5.27 kgF) (Figure 3-6 (A)), which would be rated as very tough, tough and tender meat, respectively. The tenderness of LD muscles in C carcasses would be rated as acceptable (83.71 N equals to 8.54 kgF) after 6 days post-mortem. After 3 wks vacuum packaged ageing at 2°C, their shear force value was 53.44 N (5.45 kgF) which was similar to the shear force value of LD muscle from Ac carcasses measured after the initial 5 hrs post-mortem. During post-mortem ageing, the shear force value of LD muscle from Ac carcasses decreased approximately 75%, 57% and 43% compared with the C carcasses after 5 hrs, 6 days and 3 wks vacuum packaged storage at 2°C, respectively (Figure 3-6 (A)).

The shear force value of leg chops in C, W and Ac carcasses were 101.69, 92.94 and 69.86 N (10.38, 9.48 and 7.13 kgF) at day 1 post-mortem which would be rated as acceptable, acceptable and tender, respectively. After 3 wks vacuum packaged ageing stored at 2°C,

the tenderness of leg chops in C carcasses were rated acceptable with the shear force value decreasing from 101.69N to 81.93 N (8.36 kgF), whereas the shear force of leg chops in W and Ac carcasses decreased to 54.47 and 55.95 N (5.55 and 5.71 kgF) which both of them would be rated tender. During post-mortem ageing, the shear force value of leg chops from Ac carcasses decreased approximately 30% after initial 1 day post-mortem and after 3 wks vacuum packaged storage at 2^{o}C (Figure 3-6 (B)).

The tender meat obtained in both W and Ac carcasses at the initial post-mortem period was consistent with the report by Farouk et al. (1992a) that the disruption of the muscle structure was induced by the infusion pressure when the solution was pumped into the carcasses. This could contribute to the increasing tenderness of the meat. In addition, the most tender meat shown for both LD muscle and leg chops was from the Ac carcasses (5 hrs and 1 day post-mortem, respectively) compared with C and W carcasses. This strongly suggests that infused kiwifruit juice contributed to the meat tenderization process effectively and efficiently after completion of infusion process. These results were in accord with previous research by Lewis and Luh (1988), who reported that actinidin treated beef steaks were significantly more tender than the non-treated samples due to the weakening of the myofibrillar proteins by the proteolytic activity of actinidin in kiwifruit juice. A decreasing shear force value was also observed by Wada et al. (2002) when beef semitendinosus muscles were immersed with kiwifruit protease. In addition to its proteolytic activity by degrading the myofibrillar proteins which contributed to the tender meat, the degradation effects of the actinidin on the meat connective tissue was also reported by Wada et al. (2004).

3.3.2.2 Proteolytic Activity Measurement of Kiwifruit Juice and Meat Samples from Control and Infusion Treatment Carcasses

3.3.2.2.1 Spectrophotometric Assay (Using p-nitrophenol)

In order to show that the kiwifruit juice might act as a protease and contribute to the meat tenderization in kiwifruit juice infused carcasses, it was vital to confirm that the kiwifruit juice actually enhanced the proteolytic activity. One method employed in this research was to determine the release of p-nitrophenol from CBZ-ONP-Lysine in the presence of actinidin (Lewis & Luh, 1988).

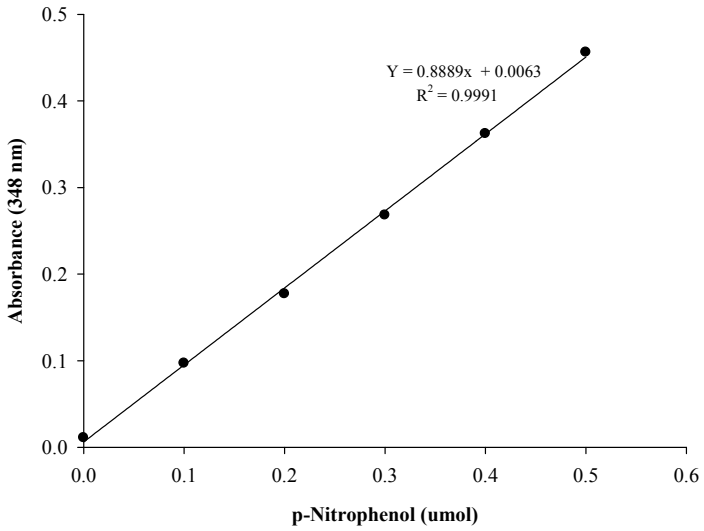

Figure 3-7 Standard curve for p-nitrophenol absorbance at 348 nm

The linear regression equation of absorbance vs. concentration of p-nitrophenol (Absorbance = 0.8889 * concentration + 0.0063 (R^2 = 0.9991)) was generated from data in Figure 3-7. The proteolytic activity of meat samples from the control and infused carcasses were calculated and presented in Figure 3-8.

The mean proteolytic activity in the kiwifruit juice employed in this research was 47.17 ± 4.51 units/mL. After the infusion treatment, the proteolytic activity of the LD muscle from Ac carcasses was 7.47 ± 1.72 units/g meat, which was significantly higher (P = 0.002) than both C (1.63 ± 0.39 units/g meat) and W (1.39 ± 0.63 units/g meat) samples. There was no significant difference (P > 0.05) between the proteolytic activity of water infused and control LD samples.

The higher proteolytic activity in lamb samples from Ac suggested that the enhanced proteolytic hydrolysis of muscle protein was induced by the actinidin which could degrade the myofibrillar structural proteins and eventually result in tender meat (Lewis & Luh, 1988). It was also reported that actinidin improved the tenderness of bovine semitendinosus steaks after they were incubated with actinidin solution for 30 mins (Wada et al., 2002). In addition, several studies confirmed the solubilisation effects of kiwifruit protease on beef collagen which would also contribute to the improvement of meat tenderness (Sugiyama et al., 2005; Wada et al., 2004).

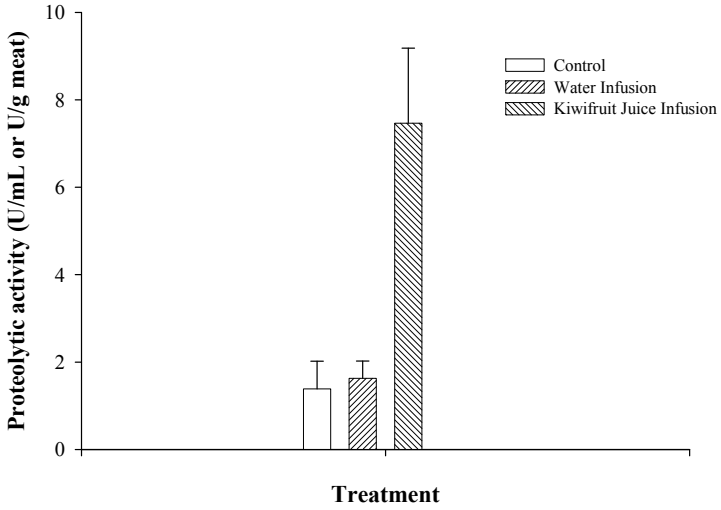

Figure 3-8 The comparison of proteolytic activity of meat samples from the control and infused treatment carcasses determined by using p-nitrophenol (means ± SEMs)

Actinidin activities were studied by Lewis & Luh (1988) to determine the optimum level of actinidin activity for effective tenderization. Using p-nitrophenol, they found that actinidin had no effect upon tenderization when its activity was 18 units/mL, while the optimum actinidin activity level was reported to be more than 300 units/mL. The mean proteolytic activity level of the kiwifruit juice used in this study was 47.17 ± 4.51 units/mL, which was about 1/6 of the tenderizing effective level indicated by Lewis & Luh (1988). This might be due to the different preparation and concentration of kiwifruit juice used in these two experiments, thus influence their activity levels. Nevertheless, the tender meat achieved in Ac carcasses indicated that the tenderization effect of the kiwifruit juice used in the current work was very effective.

3.3.2.2.2 Fluorometric Assay (Using BODIPY-FL casein)

The second method used to measure the proteolytic activity of kiwifruit juice or the proteolytic activity due to the kiwifruit juice infusion in meat samples from the three treatments was a fluorometric assay using BODIPY-FL casein. The changes in the fluorescence from BODIPY-FL casein within the first 10 mins of incubation were monitored, and the results are presented in Figure 3-9.

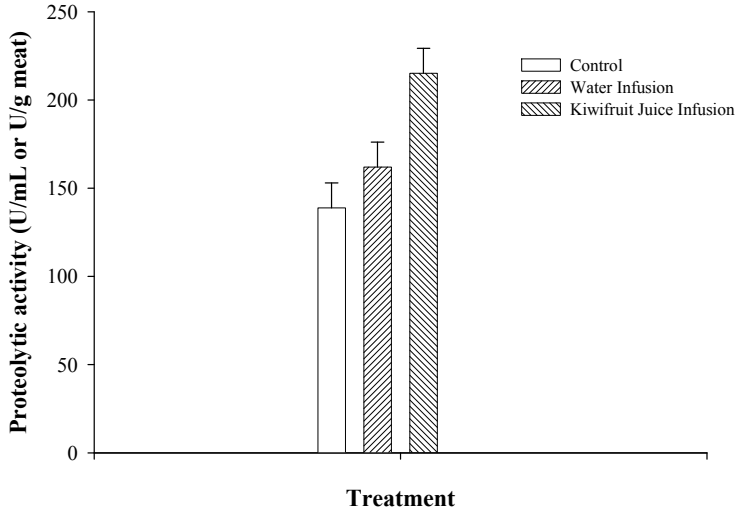

Figure 3-9 The comparison of proteolytic activity of meat samples from the control and infused treatment carcasses determined using BODIPY-FL casein assay (mean ± SEMs)

The means of proteolytic activity of the kiwifruit juice was 896.70 ± 21.98 units/mL. After the pre-rigor infusion treatment, the means of proteolytic activity of LD muscle from Ac carcasses was 215.22 ± 14.18 units/g meat, which showed significantly higher (P = 0.01) activity than the samples from C and W carcasses. These results reiterate that a higher proteolytic activity occurred in the Ac treated carcasses due to the actinidin contained in kiwifruit juice, (Lewis & Luh, 1988; Wada et al., 2002). There was no significant difference (P > 0.05) between the proteolytic activity in water infusion treatment samples (162.00 ± 14.18 units/g meat) and the control samples (138.78 ± 14.18 units/g meat) (Figure 3-9).

3.3.2.3 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

It is well accepted that calpains, found naturally in muscle, play an important role in meat tenderisation by weakening/degrading the structural integrity of the myofibrillar proteins during the post-mortem ageing period (Geesink et al., 2006; Koohmaraie & Geesink, 2006; Veiseth et al., 2001). In this research, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to analyse the myofibrillar fraction (refer to Section 3.2.9.1) from LD samples taken at slaughter, or stored at 2^{o}C for 1 day and 6 days post-mortem (Figure 3-10). The changes of the myofibrillar protein fractions were

compared for the carcasses from the three treatments with subunit molecular marker weights ranging from 10 kDa to 250 kDa. Electrophoretic analysis was performed in triplicate, and a representative SDS-PAGE profile is presented in the results (Figure 3-10).

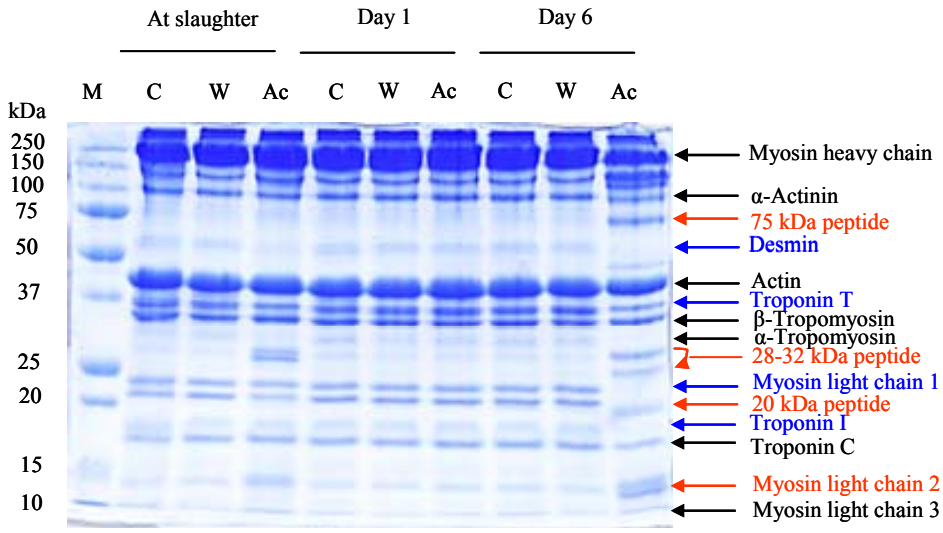

Figure 3-10 A representative SDS-PAGE profiles of myofibrillar proteins extracted on 12% SDS-PAGE from Lamb LD muscle at slaughter, day 1 and 6 post-mortem. M = molecular weight markers (BioRad, Precision Protein Standard, Prestained Broad Range), C = control, W = water infusion, Ac = kiwifruit juice infusion.

In general, more changes were observed on bands from the Ac treated myofibrillar fraction at slaughter and day 6 post-mortem compared with C and W treatments (Figure 3-10). These changes in the Ac myofibrillar fraction on the gel at slaughter showed degradation of desmin, a more intense band thought to be the myosin light chain 2 and the appearance of peptides at approximately 28-32 kDa. More bands indicating proteins degradation were observed for the Ac myofibrillar proteins compared with C and W samples at 6 day post-mortem. These band changes included: the disappearance of troponin I, myosin light chain 1 and desmin (shown with blue arrows in Figure 3-10); degradation of troponin T (shown with blue arrows in Figure 3-10); a band thought to be the myosin light chain 2 was more intense; and subsequently more new peptides were observed at approximately 20, 28-32 and 75 kDa (shown with red arrows in Figure 3-10).

The bands believed to be the troponin I and myosin light chain 1 disappeared by day 6 post-mortem only in Ac treatment, which suggested that enhanced proteolytic activity of

actinidin in kiwifruit juice infused carcasses occurred during aging. A new peptide at approximately 20 kDa appeared in Ac samples at day 6 post-mortem compared with C and W. This was believed to be consistent with the observation reported by Lewis and Luh (1988) that a 21 kDa band appeared in kiwifruit treated beef steak at 2 days post-mortem.

The 28-32kDa polypeptide fragments which are used as indicators of proteolysis (Ho et al., 1994) are closely associated with tender steak (McBride & Parrish, 1977). They appeared in the Ac myofibrillar fraction, at slaughter and day 6 post-mortem but not in the C and W samples (Figure 3-10). These results possibly suggest that a higher degree of proteolysis in kiwifruit juice infusion carcasses occurred immediately after infusion process and that enhanced proteolysis was induced by actinidin at day 6 post-mortem. The 28-32kDa polypeptides were reported to be the proteolytic products derived from troponin T in post-mortem muscle (Claeys et al., 1995; Ho et al., 1994). Loss of intensity of the troponin T band was also observed at day 6 for the LD samples from Ac carcasses, but was not obvious in C and W.

The band which was believed to be desmin, the 55kDa cytoskeletal protein was clearly detectable in C and W, whereas it was much reduced immediately after the kiwifruit juice was infused into carcasses at slaughter, and absent at 6 days post-mortem, indicating extensive degradation occurred (Figure 3-10). Desmin degradation can be considered as a good indicator of post-mortem proteolysis during meat tenderisation, and the degradation of desmin during ageing of meat has been reported previously (Koohmaraie & Shackelford, 1991; O'Halloran et al., 1997; Wheeler & Koohmaraie, 1999). This further supported the suggestion that a higher degree of proteolytic activity occurred due to the infusion of kiwifruit juice and that this contributed to the meat tenderization process (Lewis & Luh, 1988)

A strong band of unknown origin at 75 kDa in LD samples from Ac carcasses at day 6 post-mortem was observed (Figure 3-10). The presence of this band together with several bands migrating between 100-150 kDa in Ac at day 6 post-mortem were thought to be derived from myosin heavy chain degradation mainly due to the proteolytic activity resulting from kiwifruit juice infusion (McDowall, 1970).

The intensity of the bands of the major myofibrillar proteins, such as myosin and actin, were reduced only in the Ac LD samples at day 6 post-mortem, but not in C and W samples (Figure 3-10). This was consistent with the report that natural calpains in meat do not degrade undenatured myosin and actin (Bandman & Zdanis, 1988). Additionally, actin was also resistant to degradation under chill storage conditions (Goll et al., 1991). Thus, the degradation of myosin and actin due to the actinidin in kiwifruit juice infused in this study plays an important role in contributing to the tender meat in Ac carcasses due to the higher proteolysis of structural myofibrillar proteins induced by actinidin which could accelerate the tenderization process in meat (Lewis & Luh, 1988; Wada et al., 2002).

Degradation of higher molecular weight bands such as titin and nebulin during post-mortem aging contributes to meat tenderisation significantly (Hopkins & Thompson, 2002a; Ilian et al., 2004). However, the intensities of these protein bands were not able to be separated clearly due to the range of molecular weight markers (from 10 to 250 kDa) employed in this work.

3.3.2.4 Zymography

Calpains, μ-calpain rather than m-calpain, found naturally in muscle, play an important role in meat tenderisation by weakening the structural integrity of the myofibrillar proteins when they are exposed to sufficient calcium concentration in post-mortem muscle (Geesink et al., 2006; Koohmaraie & Geesink, 2006; Veiseth et al., 2001). However, the activity of m-calpain has not been conclusively correlated with the degrading of myofibrillar proteins or the consequent contribution to the meat tenderization process (Boehm et al., 1998). In the current study, measurement of actinidin and calpain activity using casein zymography in the sarcoplasmic fraction of LD muscle was used to evaluate the effect of enhanced proteolytic activity of actinidin in the kiwifruit juice, and to study the effect of infusion treatments on μ- and m-calpain during 6 days post-mortem storage.

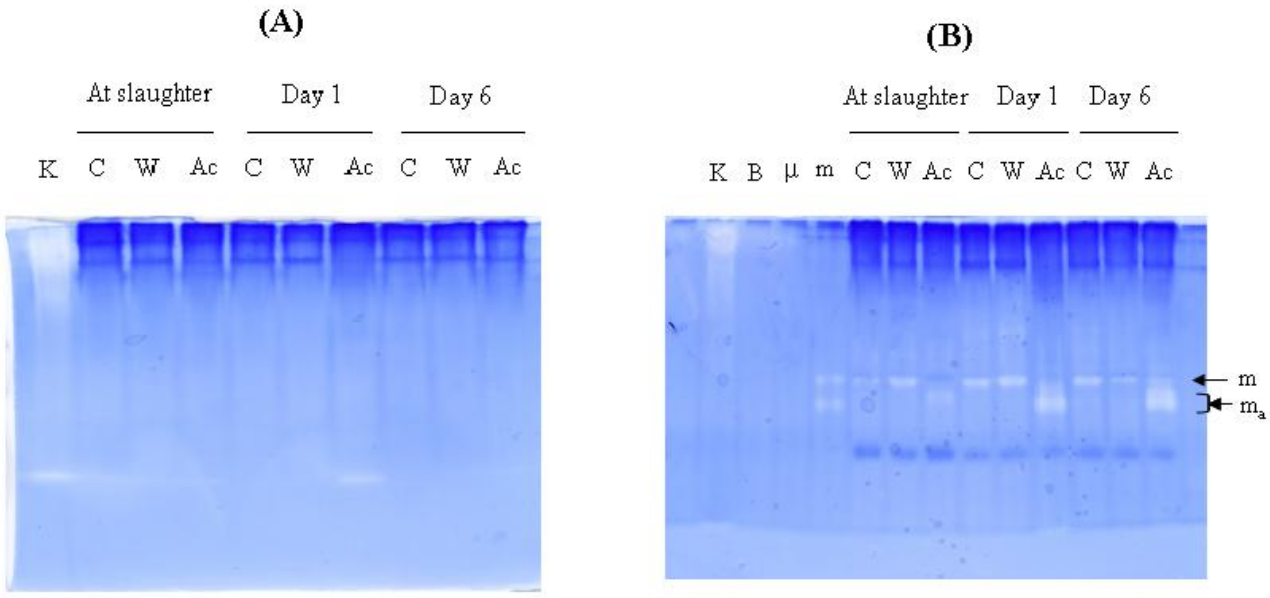

Figure 3-11 Representative casein zymography gels of sarcoplasmic proteins extracted from lamb LD muscle using various infusion treatments sampled at slaughter, day 1 and day 6 post-mortem, respectively. (A) Incubation without Ca^{2+} and reducing agent; (B) Incubation with Ca^{2+} and reducing agent to activate calpains. K = kiwifruit juice, B = Blank, μ = μ-calpain, m = m-calpain, m_a = m-calpain autolysed form, C = control, W = water infusion, Ac = kiwifruit juice infusion

The clear areas on the gels are indicative of proteolytic activity induced by proteases including calpains in the meat and the actinidin in the kiwifruit juice (Figure 3-11). The casein gel, presented in Figure 3-11 (A) was incubated without calcium after completion of electrophoresis. A clear band was shown at the bottom of the gel loaded with pure kiwifruit juice indicates actinidin activity. Clear bands were also found in the kiwifruit infused meat samples, but not in the control and water infused samples (Figure 3-11 (A)). These results confirm that actinidin acts as a protease and could accelerate proteolytic activity in meat during post-mortem storage.

To determine the effect of infusion treatments on μ- and m-calpain in the meat, the casein gel was incubated with calcium and a reducing agent. Both μ- and m-calpain and their autolysed form were used as markers to determine their activity after infusion treatments (Figure 3-11 (B)). Light bands running in the same position as m-calpain and its autolysed form were found in all the Ac samples at slaughter, day 1 and 6 post-mortem, but the m-calpain autolysed form was not found in the C and W samples. This indicated that the actindin had an important role in the autolysis of m-calpain in the carcasses during post-

mortem storage. The light bands at day 1 and 6 post-mortem were brighter than those at slaughter indicating that m-calpain autolysis extended significantly with time during storage after the kiwifruit juice had been infused into the carcasses.

While the direct contribution of m-calpain to the meat tenderization was not measured in the current experiment, the actual role of m-calpain in post-mortem proteolysis that results in meat tenderization still remain an open question (Veiseth et al., 2001). The activation of μ-calpain and m-calpain has been shown to be responsible for post-mortem proteolysis and tenderization in meat after the carcasses had been treated with $CaCl_2$ (Polidori et al., 2001; Polidori et al., 2000). Ilian et al. (2004) reported that m-calpain was activated in LTL muscle after lamb carcasses had been infused with 0.3M $CaCl_2$ solution.

It was expected that the μ-calpain marker which should be obvious at 80 kDa would appear on the gel after Ca^{2+} incubation in this work, unfortunately no band of μ-calpain and/or its autolysed form (76 kDa) (Boehm et al., 1998) could be detected in the marker lane or in the LD samples in various treatments after post-mortem (Figure 3-11 (B)). The contribution of extensive autolysis of μ-calpain has been reported to be one of the proteolytic activation mechanisms to contribute to meat tenderization (Boehm et al., 1998).

There are several possible reasons why μ-calpain was not detected. If Ca^{2+} concentration was not present in sufficient amounts, the μ-calpain would not be activated. But because m-calpain activity was detected in the casein gel in the presence of $CaCl_2$, it was thought to be unlikely that Ca^{2+} was the reason that limiting the expression of the μ- calpain as Ca^{2+} concentration in the gel should be more than sufficient to activate the proteolytic activity of μ-calpain as it requires a lower Ca^{2+} concentration than m-calpain for its activation (Veiseth et al., 2001). They also reported that μ-calpain contributed to the meat tenderization by losing 42 and 95% of its activity at 1 day and 3 days post-mortem during storage (Veiseth et al., 2001). This was a distinct possibility as Geesink and Koohmaraie (1999; , 2000) reported that μ-calpain was activated immediately once it was incubated with high calcium concentration, and started to autolyse and became very unstable, and then resulted in the inactivation of the μ-calpain in post-mortem muscle.

3.3.3 Post-mortem Colour Studies

3.3.3.1 Fresh Meat Colour

The Hunter L*, a* and b*-values are indicators of lightness, redness and yellowness, respectively (AMSA, 1991). The infusion treatment effects on L*, a* and b*-values of the LD muscle and leg chops from control and infused lamb carcasses during simulated 6 days refrigerated retail display at 4^{o}C either at day 1 post-mortem or at 3 wks of vacuum packaged storage are shown in Figures 3-12, 3-13 and 3-14, respectively.

3.3.3.1.1 Lightness (L*)

For L*-values (Figure 3-12), both LD muscle and leg chops from W carcasses had significant higher L*-values ($P < 0.001$) than C and Ac carcasses during the 6 days display time in either 1 day post-mortem or after 3 wks vacuum packaged ageing stored at 2^{o}C. After 3 wks vacuum packaged ageing stored at 2^{o}C, leg chops of the three treatments showed significantly higher L*-values ($P < 0.001$) throughout the 6 days display time compared with the LD muscle . Generally, a decreasing trend within 6 days display time for both LD muscle and leg chops was observed in 1 day post-mortem and after 3 wks of vacuum packaged storage (Figure 3-12).

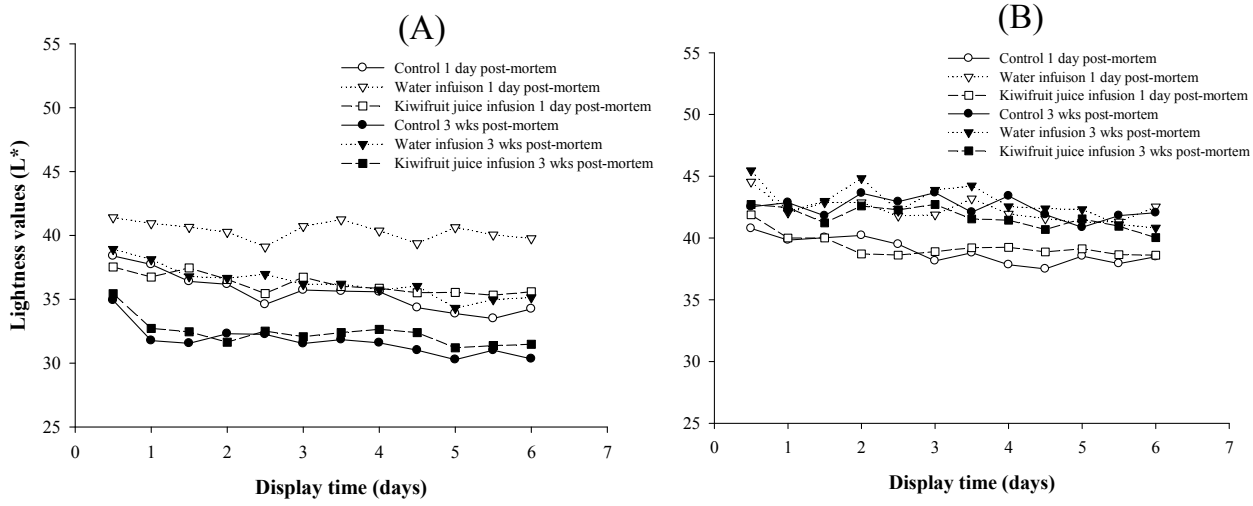

Figure 3-12 The effect of control and pre-rigor infusion treatment on lightness (L*) of (A) LD muscles; (B) leg chops during 6 days refrigerated display at 4^{o}C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2^{o}C. Higher L*-values indicate a lighter colour. SEMs (L*) = 1.22.

These results were partially consistent with the observations reported by Bekhit et al. (2005) that LM (same as LD) muscle aged 1 day or 3 wks from water infused carcasses had higher L*-values than the C and other infusion treatment carcasses. The lighter colour shown in the W lamb carcasses may be a result of light scattering or the dilution of muscle pigments by the infusion solution (Farouk & Price, 1994). Hunt et al. (2003) proposed that the rapid pH decline in beef might contribute to the higher lightness.

3.3.3.1.2 Redness (a*)

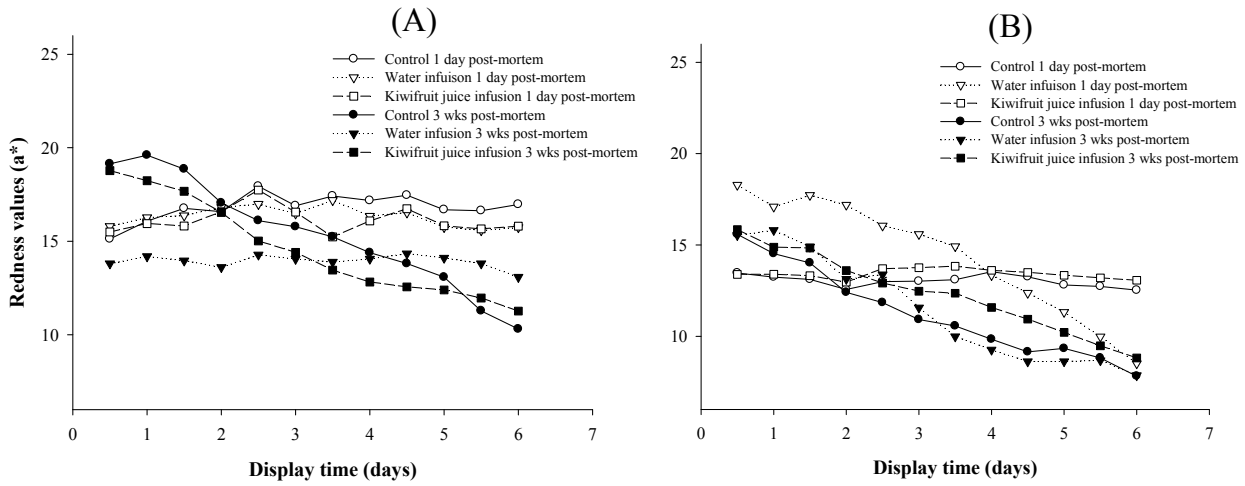

Figure 3-13 The effect of control and pre-rigor infusion treatment on redness (a*) of (A) LD muscles; (B) leg chops during 6 days refrigerated display at 4^{o}C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2^{o}C. Higher a*-values indicate a redder colour. SEMs (a*) = 1.21.

Consumers prefer a bright red colour for red meat. Generally, there were significant effects of infusion treatment ($P = 0.006$), display time ($P < 0.001$), muscle cuts ($P < 0.001$) and post-mortem time ($P < 0.001$) on a*-values (redness). There were significant interactions between treatment and post-mortem time ($P < 0.001$), post-mortem time and display time ($P < 0.001$) and treatment and cut ($P < 0.001$) (Figure 3-13). The a*-value remained constant for both LD muscles and leg chops during the display time at 1 day post-mortem for all the three treatment carcasses except a decreasing trend was shown in leg chops in water infusion treatment (Figure 3-13 (B)). After 3 wks vacuum packaged storage at 2^{o}C, the a*-values decreased with the display time for both LD muscles and leg chops for all the three treatment carcasses.

The LD samples from C and Ac carcasses showed a redder initial colour after 3 wks vacuum packaged storage than the 1 day post-mortem samples (Figure 3-13 (A)). Thus vacuum packaging in combination with optimum storage temperature maintains the attractive red colour of meat during extended chilled meat storage (Jeyamkondan et al., 2000). In addition, during the 3 wks vacuum packaging storage, respiration in the meat decreased, so less oxygen was required for respiration so more oxygen could diffuse into the meat from the meat cut surface when it was exposed to air and resulted in redder colour (Young & West, 2001). In the current research, a faster discolouration was shown in both LD muscles and leg chops during the 6 days display time when the meat was exposed to air after 3 wks vacuum packaged storage than those at 1 day post-mortem. This was in agreement with the reports that a faster redness discolouration was found in 3 wks vacuum packaged samples than the 1 day post-mortem samples during the 6 days display time (Bekhit et al., 2001; Bekhit et al., 2005).

In the current work, a higher but non-significant a*-values (redness) of the leg chops were shown in the Ac carcasses than in the other treatments during the 6 days display time after 3 wks vacuum packaged storage at 2^{o}C. This might be a result of the considerable amounts of antioxidants including ascorbic acid and other polyphenols present in the kiwifruit juice (Sale, 1983). Higher a*-value were reported in ascorbic acid treated ground beef in the earlier research (Shivas et al., 1984). Okayama et al. (1987) also reported that beef steak treated with a dip solution containing ascorbic acid and α-tocopherol had higher a*-values.

3.3.3.1.3 Yellowness (b*)

The yellowness (b*) values of meat samples shown in Figure 3-14 was significantly affected by treatment ($P < 0.001$), muscle cuts ($P < 0.001$), post-mortem time ($P < 0.001$) and display time ($P < 0.001$). There were significant interactions between post-mortem time, display time and muscle cuts ($P < 0.001$), and post-mortem time and treatment ($P < 0.001$) (Figure 3-14). Both LD samples and leg chops in 1 day post-mortem and 3 wks vacuum packaged storage constantly exhibited similar trends during the display time. Both LD muscle and leg chops from W carcasses had higher ($P < 0.001$) b*-values throughout the 6 days display time compared with the C and Ac. Similar results were also reported by Bekhit et al. (2005). Similar to a*-values shown in Figure 3-13, a lower b*-value was shown in both LD muscle and leg chops in 3 wks vacuum packaged storage than in 1 day

post-mortem samples from samples given 2 to 6 days display time. This shows the higher discolouration rate in vacuum packaged meat samples when meat was exposed to air. Similar b*-values were found after both 1 day and 3 wks post-mortem, but b*-values decrease steady during the 6 days display time only in the samples that had been stored for 3 wks.

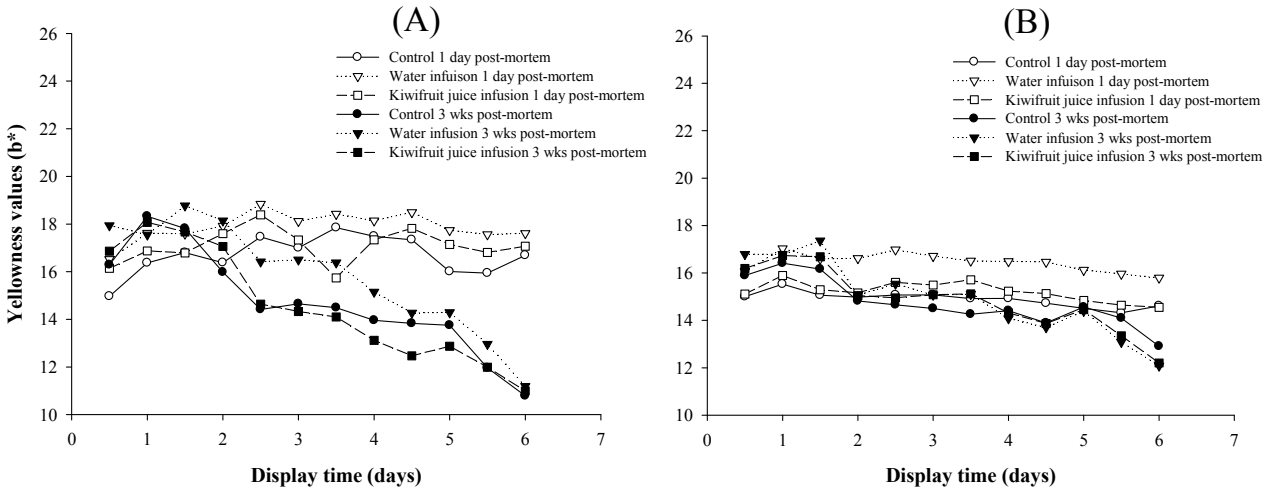

Figure 3-14 The effect of control and pre-rigor infusion treatment on yellowness (b*) of (A) LD muscles; (B) leg chops during 6 days refrigerated display at 4°C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2°C. Higher b*-values indicate a more yellow colour. SEMs (b*) = 0.81.

3.3.3.2 Lipid oxidation

Lipid oxidation is a major cause of meat deterioration, and is a critical factor in determining meat storage life (Ross & Smith, 2006). Measurement of TBARS (thiobarbituric acid-reactive substances) value of meat has been commonly used as an assay to determine the lipid oxidative status during meat post-mortem storage period (Brunton et al., 2000).

Comparing the initial TBARS value in 1 day post-mortem and after 3 wks vacuum packaged stored at 2°C in both LD muscle and leg chops, showed that the lipid oxidation did not occur during the vacuum storage, but when the meat was displayed it increased upon exposure to the air (Figure 3-15 and Figure 3-16). A similar result was also described by Bekhit et al. (2005), and they proposed that the possible explanation for this was that

the vacuum packaged ageing inhibited the oxidation of the lipids. On exposure to the air (oxygen) during display, higher TBARS values occurred (Cheng & Ockerman, 2004; St. Angelo et al., 1991) possibly due to natural depletion of endogenous antioxidants during the storage (Bekhit et al., 2005).

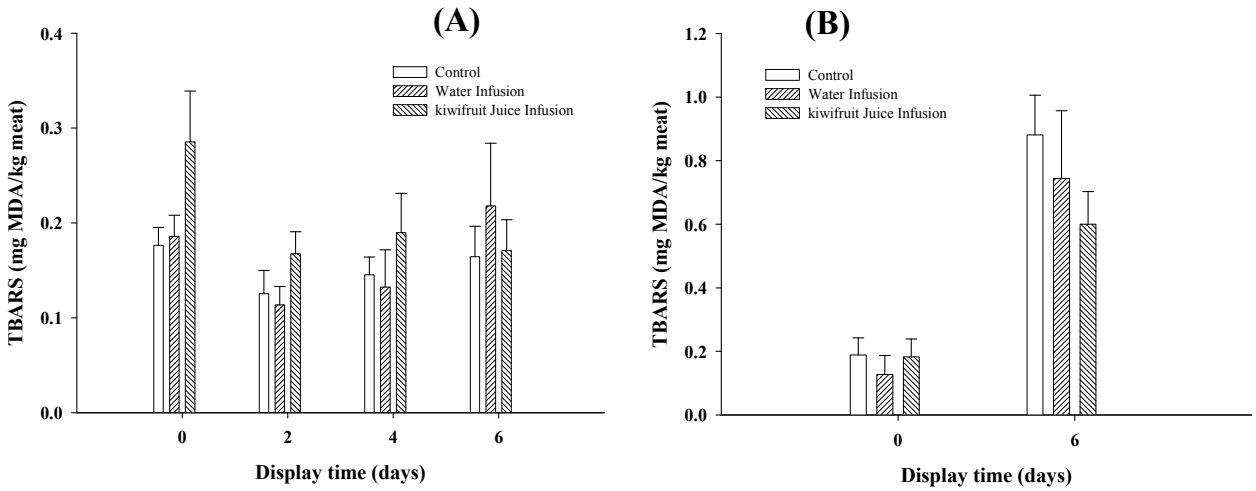

Figure 3-15 Interaction effects of pre-rigor infusion treatments and display time on the values of thiobarbituric acid reactive substances (TBARS, mg MDA/kg meat) of the LD muscles in (A) 1 day post-mortem; (B) after 3 wks vacuum packaged storage at 2^{o}C (means ± SEMs)

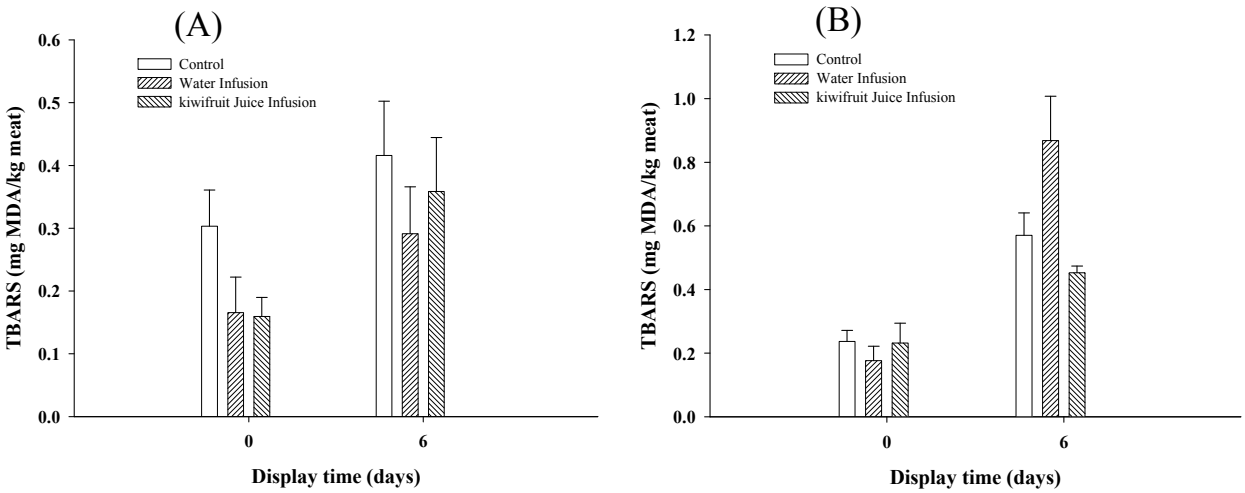

Figure 3-16 Interaction effects of pre-rigor infusion treatments and display time on the values of thiobarbituric acid reactive substances (TBARS, mg MDA/kg meat) of the leg chops in (A) 1 day post-mortem; (B) after 3 wks vacuum packaged storage at 2^{o}C (means ± SEMs)

Both LD muscles and leg chops in 3 wks vacuum packaged storage at 2°C from Ac lamb carcasses had non-significant lower TBARS value than C or W samples after 6 days display time (Figure 3-15 (B) and Figure 3-16 (B)). This may be due to the large amount of antioxidants (ascorbic acid and other polyphenols) (Dawes & Keene, 1999; Nishiyama et al., 2004; Sale, 1983) in the kiwifruit juice used in the current experiment. After infusion treatment, these antioxidants might be introduced into muscle and adipose tissues quickly, and preserved in the muscle during the vacuum packaging storage at 2°C, and play the role of inhibiting lipid oxidation during the display time. St. Angelo et al. (1991) reported that antioxidant-prooxidant balance was improved by using calcium chloride and vitamin C after infusion in lamb carcasses. This was similar to the report by Katsanidis et al (2003) that TBARS values of ground beef were lower when vitamin C and E or vitamin E was added into MPSC solution after infusion. However, they suggested that the addition vitamin E in the infusion solution played the key role in lower TBARS values rather than vitamin C. In the current research, the effect of these antioxidants in the kiwifruit juice did not show significant and consistent results on the TBARS values of all the samples from Ac carcasses, and thus the contribution to the inhibition of lipid oxidation status need further investigation.

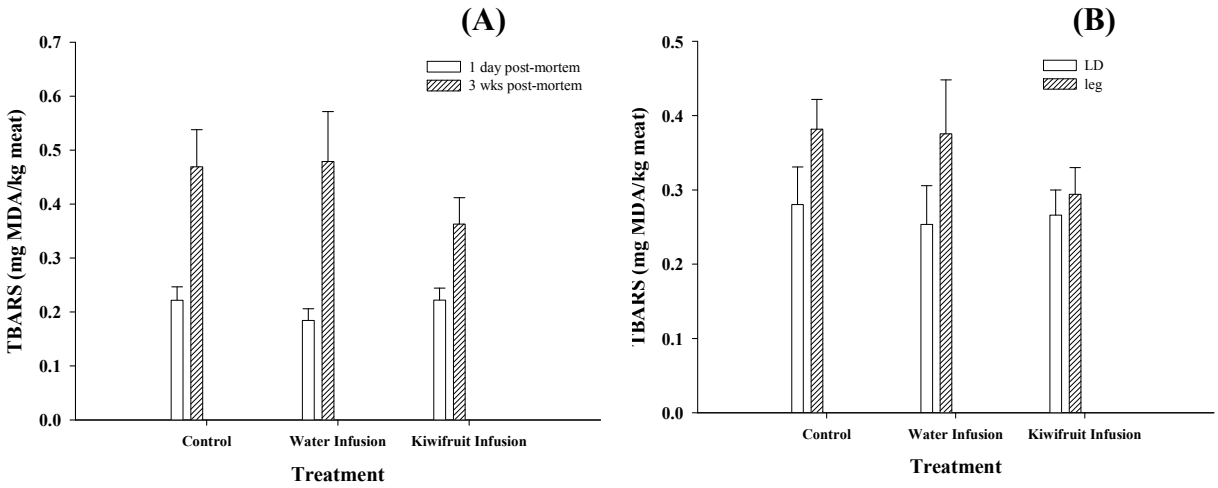

Figure 3-17 Main effect of pre-rigor infusion treatments and (A) different post-mortem time (1 day post-mortem vs 3 wks vacuum packaged storage at 2°C; (B) different muscle/cut (LD muscles vs leg chops) on the values of thiobarbituric acid reactive substances (TBARS, mg MDA/kg meat) (means ± SEMs)

In the current research, the infusion treatment had no effect ($P > 0.05$) on the TBARS value. There were significant interactions between post-mortem time and display time ($P < 0.01$), and muscle/cut, post-mortem time and display time ($P < 0.01$). TBARS values increased ($P < 0.001$) with display time and post-mortem time (1 day post-mortem vs 3 wks vacuum packaged storage (Figure 3-17(A)). LD muscle had marginal lower TBARS values ($P = 0.05$) than leg chops (Figure 3-17(B)). Morcuende et al. (2003) reported that TBARS values depended on muscle types. They found that the TBARS values in masseter muscles were significantly higher than LD muscle and serratus ventralis muscles in pork.

In this chapter, the effects of Ac and W treatments compared with C treatment on post-mortem changes of lamb quality characters including physical properties, tenderness and colour have been investigated. The significantly more tender meat found in the Ac carcasses indicated that kiwifruit juice can be employed as a meat tenderizer and contributes to the meat tenderization process effectively after infusion. The Ac treatment might have slight positive effects on the maintaining display colour and inhibition of lipid oxidation due to its natural antioxidants content.

In addition to these physical and biochemical factors, customer satisfaction surveys of beef have shown that meat flavour also plays an important role in the consumer satisfaction after meat tenderness (Behrends et al., 2005). Hence, the effects of the infusion treatment on the meat volatile flavour profile will be studied in the next chapter.

Chapter 4
The Effect of Pre-rigor Infusion of Lamb with Kiwifruit Juice on Meat Volatile Flavour Compound

4.1 Introduction

Meat flavour is an essential component of the eating quality, and usually has two components including taste and aroma compounds (Warriss, 2000). Meat flavour is thermally derived, because uncooked meat has little or no aroma. During cooking, the volatile compounds generated between non-volatile components of lean and fatty tissues through a complex series of thermally induced reactions, contribute to the aroma attributes and characteristic flavours of meat (Mottram, 1998). More than a thousand flavour compounds have been reported to be generated during cooking (Warriss, 2000) and these consist of volatile and non-volatile compounds (Huang & Ho, 2001). The volatile compounds in meat flavour include aldehydes, alcohols, ketones, hydrocarbons, phenols, carboxylic acids, esters, lactones, furans, pyrans, pyrroles, pyridines, pyrazines, oxazoles, oxazolines, thiophenes, thiazoles, thiazolines and other nitrogen or sulphur containing compounds (Mottram, 1994).

Studies that deal with the meat flavour and aroma components have been undertaken since the 1930s (Howe & Barbella, 1937). Sophisticated separation and analysis techniques have been introduced so that trace quantities of materials can be identified (Wasserman, 1979). The meat flavour components are formed from thermal breakdown of the compounds normally found in meat such as fats, proteins and carbohydrates (Huang & Ho, 2001). A variety of factors including different animal species, breed, sex, age, nutrition, and processing conditions are known to influence the meat flavour. Although no single factor, or group of factors, can be identified as the principal influence, there is no doubt that heating processes significantly affect the overall meat flavour (Sink, 1979).

In addition to the effects of cooking conditions on meat aroma formation, it has been suggested that biochemical mechanisms involved during refrigerated storage and display

time also had effects on the generation of volatile compounds via enzymatic oxidation of unsaturated fatty acids, and further interactions with proteins, peptides and amino acids (Huang & Ho, 2001). These biochemical mechanisms include proteolysis caused by proteases, lipolysis induced by various enzymes and factors that control of the enzymatic activities in meat (Toldra, 1998). For example the generation of lipid-derived aldehydes was affected by biochemical mechanisms, thus influencing the overall meat flavour (Sucan et al., 2002).

In Chapter 3, the effects of pre-rigor lamb with kiwifruit juice which acts as a source of actinidin (a protease) on the meat quality aspects such as pH and temperature changes, water loss, tenderness and colour changes after infusion treatment were discussed. The objective of this study was to investigate the effects, during ageing, of pre-rigor infusion treatment with kiwifruit juice and water on the volatile flavour compounds of lamb using solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) and identifying compounds which might contribute to the overall meat flavour.

4.2 Materials and Methods

4.2.1 Determination of Flavour Volatiles using GC-MS

Both LD muscle and leg chop samples were prepared as detailed in section 3.2.3. Only 3 samples from the 6 animals were used because of the cost of the GC-MS analyses. In preparation for volatile compound analysis, 5g samples of the raw meat were cut into small thin pieces and placed into a 10mL headspace glass vial (SUPELCO, USA), capped tightly with a polytetrafluoroethylene/silicone liner tin plate seal (SUPELCO, USA), cooked in a water bath at 90°C for 30 minutes, and then stored in a refrigerator (4°C, for no longer than 6 hrs) until headspace analysis.

Samples, removed from the refrigerator, were prepared for analysis using solid-phase microextraction gas chromatography-mass spectrometry (SPME) by the injection of 10µL of an internal standard mixture (2-methyl-2-pentenal at a concentration of 8.8 mg/L and ethyl trans-3-hexenoate at a concentration of 8.3 mg/L) into the vial, followed by equilibration of the sample vial in a water bath at 40°C for 5 mins. Volatile components in the samples were adsorbed onto a 85µm stableflex carboxen/polydimethylsiloxane

(CAR/PDMS) fibre (SUPELCO, USA) inserted into the headspace of the vial while at 40°C for 40 minutes. The fibre was initially conditioned at 280°C for 90 minutes at the start of the experiment, and then conditioned at 280°C for 10 minutes each day prior to use.

Headspace volatile compounds were analysed using a Shimadzu GC-2010 GC (Shimadzu, Japan) with flame ionization detector (FID) (Shimadzu, Japan) fitted with a Restek-5MS fused silica capillary column (30.0m x 0.25mm i.d. x 0.25µm, Shimadzu, Japan). After extraction, the SPME (CAR/PDMS) fibre was removed from the sample vial and immediately inserted into the injection port (splitless mode); and the adsorbed volatile components were desorbed in the injector port of the GC-FID at 280°C for 5 minutes. After desorption, the column oven was held at 40°C for 8 minutes, then heated to 150°C at 4°C/min, and finally heated up to 300°C at 30°C/min and held at this temperature for 7 minutes. Helium was used as the carrier gas with the constant linear velocity set at 30.5 cm/sec in the splitless mode.

The separation of volatile compounds was achieved using a gas chromatograph mass spectrometer (GC-MS Shimadzu QP2010) fitted with a Restek-5MS fused silica capillary column (30.0m x 0.25mm i.d. x 0.25µm, Shimadzu, Japan). The conditions used for the GC-MS were the same as those used on the GC-FID. The mass spectrometer (MS) was operated in electron impact ionization mode with 70eV and mass range of 33 to 450 m/z. The temperature of the capillary interface was 310°C, with the source temperature set at 260°C.

A selection of samples were analysed using the GC-MS conditions to establish identification of the volatile compounds. Volatile compounds were identified by matching mass spectra with the spectra of reference compounds in the NIST EPA/NIH Mass Spectral Library database (National Institute of Standards and Technology, NIST2005, Version 2.0d). Further confirmation of identification was obtained by using Retention Index information.

The relative quantities of the volatile compounds detected in the samples were determined by comparison of the peak area ratio of the volatile compound with the internal calibration standard of 2-methyl-2-pentenal. Another internal standard of ethyl trans-3-hexenoate was used as an identification and retention time marker for further confirmation of the

identification of the volatile compounds detected in this work. The retention time (RT) of the two internal standards remained consistent throughout all the samples.

4.2.2 Statistical analysis

Data were subjected to analysis of variance (ANOVA), using Minitab Release 14.0 Statistical Software. The results were presented as means of the triplicate samples and were analysed for variance and multiple mean comparisons (Pairwise comparisons, Tukey method) to compare the 3 treatment in various post-mortem time and display time. The significance of differences between means was determined at the 5% significance level.

4.3 Results and discussion

In this study, the SPME-GC-MS technique was applied to characterise the volatile flavour profiles of cooked lamb samples to compare the treatments and muscle cuts at different post-mortem and display times. A total of 48 volatile flavour compounds (Table 4-1 and Table 4-2) consisting of 8 aldehydes, 8 ketones, 11 alcohol, 4 terpenes, 2 sulphur compounds, 2 hydrocarbons, 5 alkanes, 6 esters, 1 carboxylic acid and 1 ether (Table 4-3 and Table 4-4) were identified in lamb LD muscles and leg chops, although some of the identified substances were not present in both of samples analysed. Additionally, another 70 unknown compounds were detected. These were present only in small amounts, and were not able to be identified by comparing MS and RT data with the NIST EPA/NIH Mass Spectral library database.

Table 4-1 Ratio of volatile compounds (compared with internal standard) in LD muscles of 6 days refrigerated display at 4°C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2°C

				LD muscle (ratio to internal standard) 1 day post-mortem						LD muscle (ratio to internal standard) 3 wks post-mortem					
				0 day display time			6 days display time			0 day display time			6 days display time		
Peak No	Compound	RT (min)	RI	C	W	Ac	C	W	Ac	C	W	Ac	C	W	Ac
1	n-butan-1-ol	3.228	662	0.057a	0.065a	0.055a	0.079a	0.107a	0.082a	0.114a	0.195a	0.376a	9.540b	0.266a	2.050a
2	2-methyl-1-butanal	3.298	643	-	-	-	-	-	-	-	-	-	-	0.024	0.063
3	1-penten-3-ol	3.516	671	0.035	0.046	0.019	0.036	0.025	0.018	0.048	0.049	0.020	0.115	0.081	0.090
4	pentan-2-one	3.624	654	0.0132a	0.056a	0.011a	0.026a	0.033a	0.036ab	0.045ab	0.027a	0.012a	0.255bc	0.187b	0.073ab
5	n-pentanal	3.762	707	0.087a	0.331a	0.142a	0.203a	0.387ab	0.128a	0.135a	0.096a	0.0491a	0.116a	0.116a	0.059a
6	3-hydroxy-2-butanone	4.295	717	-	0.067	-	-	0.154	0.031	-	0.365	-	-	-	-
7	3-methyl-1-butanol	4.696	697	-	-	-	-	-	-	-	-	-	-	2.357b	3.91b
8	2-methyl-1-butanol	4.824	697	-	-	-	-	-	-	-		-	0.799ab	0.602ab	1.544b
9	dimethyl disulfide	4.941	772	-	-	-	-	-	-	0.011	-	-	0.707	0.408	1.860
10	propanoic acid	5.295	676	0.037	0.094	-	-	0.007	-	-	-	-	0.098	0.110	0.009
11	toluene	5.641	794	0.031	0.038	0.054	0.039	0.079	0.054	0.029	0.037	0.025	0.022	0.014	0.010
12	1-pentanol	5.878	761	0.023	0.030	0.017	0.056	0.047	0.029	0.072	0.057	0.022	0.027	0.032	0.019
13	3-hepten-2-one	6.597	861	-	-	-	-	-	-	-	0.014	-	0.012	0.008	0.008
14	octane	7.012	816	-	-	-	-	0.061	0.023	0.035	0.071	0.021	0.021	0.067	0.064
15	hexanal	7.133	806	0.636ab	1.240b	0.398ab	0.364ab	0.383ab	0.280ab	0.733ab	0.488ab	0.190a	0.071a	0.135a	0.099a
16	2,3 butanediol	8.627	743	-	-	-	-	-	-	-	-	-	-	-	-
17	4-hydroxy-4methyl-2-pentanone	10.863	845	-	-	-	-	-	-	-	-	-	0.014	-	-
18	styrene	12.167	883	-	-	0.014	-	-	-	-	-	-	-	-	-
19	2-heptanone	12.396	853	-	-	-	0.013a	0.040a	0.019a	-	-	-	0.139b	0.081ab	0.023a
20	cis-4-hepten-1-al	12.87	913	0.046a	0.049a	0.024a	0.063a	0.094a	0.062a	0.040a	0.058a	0.017a	0.066a	0.036a	0.014a
21	heptanal	12.953	905	-	-	-	-	-	-	-	-	-	0.034	-	-
22	acetic acid, pentyl ester	13.762	884	0.060	0.033	0.063	-	0.086	0.036	0.092	0.039	0.077	0.018	0.017	0.036
23	2-butoxy-ethanol	13.812	936	0.034	-	-	0.016	-	-	-	-	-	0.013	-	0.028
24	butyrolactone	14.224	825	0.016a	0.034a	0.019a	0.009a	-	0.018a	-	-	0.023a	-	-	-
25	α-pinene	14.418	948	-	-	0.012a	0.024ab	0.026ab	0.012a	0.029ab	0.050abc	0.024ab	0.012a	0.013a	0.007a
26	benzaldehyde	15.933	982	0.060ab	0.093ab	0.059ab	0.064ab	0.078ab	0.053ab	0.115abc	0.153bc	0.088ab	0.069ab	0.063ab	0.054ab
27	dimethyl trisulphide	16.265	972	0.018a	0.069ab	0.052ab	0.035a	0.023a	0.021a	0.032a	0.047ab	0.026a	0.085ab	0.067ab	0.043ab
28	cis hept-4-enol	16.454	968	-	-	-	-	-	-	0.029a	0.056b	0.024a	0.014a	-	0.006a
29	β-pinene	16.579	943	-	-	-	-	-	-	0.038	0.073	0.036	-	0.028	-
30	1-heptanol	16.645	960	-	0.025	0.036	-	-	0.011	0.026	0.037	0.017	0.031	0.028	0.014
31	1-octen-3-ol	17.045	969	-	-	-	0.027	-	-	0.024	0.030	0.012	0.017	0.046	0.007
32	2-methyl-3-octanone	17.249	988	0.017	0.032	0.028	0.036	0.047	0.024	0.042	0.027	0.016	0.044	0.045	-

Continue on next page

Continued

Peak No	Compound	RT (min)	RI	LD muscle (ratio to internal standard) 1 day post-mortem 0 day display time C	W	Ac	6 days display time C	W	Ac	LD muscle (ratio to internal standard) 3 wks post-mortem 0 day display time C	W	Ac	6 days display time C	W	Ac
33	(3E)-2,7-dimethyl-1,3,7-octatriene	17.47	958	0.027	0.017	0.012	0.028	0.039	0.013	0.036	0.047	0.017	0.022	0.033	0.009
34	2,5-dimethylnonane	17.836	986	-	-	-	-	-	-	-	-	-	0.022	0.025	-
35	octanal	18.087	1005	-	-	-	-	-	-	-	-	-	0.024	0.035	0.024
36	1-hexyl acetate	18.63	984	-	-	-	-	-	-	-	-	-	-	-	-
37	2-ethyl-1-hexanol	19.312	995	-	-	-	1.563ab	1.782ab	0.647a	0.066a	0.091a	0.044a	2.863ab	2.980ab	2.508ab
38	pentanoic acid, 2,4-dimethyl-, t-butyl ester	19.983	1069	-	-	0.031ab	0.017ab	-	-	0.026ab	0.054b	0.028ab	0.011a	0.014a	0.010a
39	5-ethyl-2,2,3-trimethylheptane	20.218	1001	-	-	0.012	-			0.016	0.030	0.024	0.011	0.012	0.009
40	1-octanol	21.258	1059	-	-	-	-	-	-	0.010	0.022	0.011	-	0.011	-
41	1-heptyl-2-methylcyclopropane	21.903	1078	-	-	-	-	-	-	-	-	-	0.017	0.009	0.020
42	2-nonanone	22.021	1052	-	-	-	-	-	-	-	-	-	0.018	-	0.020
43	nonanal	22.524	1104	0.049abc	0.062abc	0.027a	0.056abc	0.055abc	0.039abc	0.045abc	0.057abc	0.028ab	0.025a	0.022a	0.019a
44	(2Z)-6-methyl-2undecene	22.971	1158	-	-	-	0.018	0.017	0.011	-	-	-	-	0.016	0.007
45	2-ethyl-1-hexanol acetate	24.413	1118	-	-	-	-	-	-	-	-	-	-	0.017	0.017
46	2-methyl-2-pentyicyclohexane	27.516	1287	0.044	-	-	-	-	-	-	-	-	-	-	-
47	propanoic acid, 2-methyl-,2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	31.737	1347	-	-	-	0.030	-	-	-	-	-	0.017	0.020	0.013
48	3-hydroxy-2,2,4-trimethylpentyl ester of isobutanoic acid	32.42	1331	-	-	-	0.022	-	-	-	-	-	-	-	-

C: control; W: water infusion; Ac: kiwi fruit juice infusion;
- = not detected; RT: retention time; RI: retention index;
a–d: letters indicate that both LD muscle and leg chop samples in the same group differ significantly ($P < 0.05$);

Table 4-2 Ratio of volatile compounds (compared with internal standard) in leg chops of 6 days refrigerated display at 4°C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2°C

				Leg chop (ratio to internal standard) 1 day post-mortem						Leg chop (ratio to internal standard) 3 wks post-mortem					
				0 day display time			6 days display time			0 day display time			6 days display time		
Peak No	Compound	RT (min)	RI	C	W	Ac	C	W	Ac	C	W	Ac	C	W	Ac
1	n-butan-1-ol	3.228	662	0.047a	0.030a	0.041a	0.090a	0.058a	0.076a	0.156a	0.065a	1.570a	3.840ab	0.201a	2.494a
2	2-methyl-1-butanal	3.298	643	-	-	-	-	-	-	0.013	-	0.027	-	-	-
3	1-penten-3-ol	3.516	671	0.025	0.021	0.042	0.067	0.081	0.055	0.030	0.025	0.027	0.090	0.036	0.081
4	pentan-2-one	3.624	654	0.019a	0.019a	0.037a	0.029a	0.030a	0.020a	0.017a	0.010a	0.010a	0.177b	0.07ab	0.250bc
5	n-pentanal	3.762	707	0.104a	0.120a	0.126a	0.382ab	0.675b	0.736b	0.188a	0.113a	0.086a	0.129a	0.152a	0.160a
6	3-hydroxy-2-butanone	4.295	717	0.029	0.024	0.021	0.052	0.041	-	0.039	0.008	0.268	0.240	0.245	0.244
7	3-methyl-1-butanol	4.696	697	-	-	0.048a	0.028a	0.024a	0.054a	-	-	-	-	-	-
8	2-methyl-1-butanol	4.824	697	0.056a	0.035a	-	-	-	-	-	0.008a	0.029a	0.183a	0.072a	0.290a
9	dimethyl disulfide	4.941	772	0.063	0.037	-	-	-	-	4.738	-	-	-	0.015	-
10	propanoic acid	5.295	676	-	0.044	-	-	-	-	-	-	-	0.054	0.045	0.060
11	toluene	5.641	794	0.023	0.019	0.021	0.042	0.057	0.036	0.019	0.011	0.014	0.033	0.026	0.009
12	1-pentanol	5.878	761	0.014	0.017	0.029	0.066	0.055	0.072	0.024	0.038	0.021	0.045	0.052	0.694
13	3-hepten-2-one	6.597	861	-	-	-	-	0.022	-	-	-	-	0.008	0.012	-
14	octane	7.012	816	0.019	0.020	0.019	0.025	0.050	0.026	0.037	0.050	0.020	0.055	0.050	0.026
15	hexanal	7.133	806	0.220a	0.334ab	0.375ab	0.959ab	1.226b	1.059ab	0.374ab	0.203a	0.244a	0.209a	0.333ab	0.138a
16	2,3 butanediol	8.627	743	-	0.163	0.191	-	-	-	0.134	0.180	0.069	-	-	0.032
17	4-hydroxy-4methyl-2-pentanone	10.863	845	-	-	-	-	-	-	-	-	-	-	0.010	0.014
18	styrene	12.167	883	-	-	-	-	0.019	-	-	-	-	-	-	-
19	2-heptanone	12.396	853	-	-	-	0.020a	0.022a	0.020a	-	-	-	0.046a	0.014a	0.050a
20	cis-4-hepten-1-al	12.87	913	0.050a	0.033a	0.048a	0.381b	0.418b	0.472b	0.094a	0.037a	0.044a	0.133a	0.108a	0.035a
21	heptanal	12.953	905	0.062	-	-	-	-	-	-	-	-	-	-	0.321
22	acetic acid, pentyl ester	13.762	884	0.057	0.070	0.072	0.090	0.099	0.123	0.053	0.044	0.055	0.075	0.028	0.031
23	2-butoxy-ethanol	13.812	936	-	-	-	-	-	-	-	-	-	-	-	0.033
24	butyrolactone	14.224	825	0.016a	0.023a	0.035a	0.011a	0.022a	-	0.017a	0.044a	0.011a	0.042a	-	0.259b
25	α-pinene	14.418	948	0.061bc	0.093c	0.013a	-	-	0.011a	0.022a	0.042abc	0.015a	0.032ab	0.013a	0.011a
26	benzaldehyde	15.933	982	0.038a	0.043a	0.053ab	0.199c	0.079ab	0.115abc	0.047a	0.024a	0.038a	0.052a	0.064ab	0.050a
27	dimethyl trisulfide	16.265	972	0.105b	-	-	-	0.092b	0.085b	-	-	-	0.026a	0.017a	0.013a
28	cis hept-4-enol	16.454	968	-	0.020a	-	-	-	-	0.015a	0.017a	0.016a	0.018a	0.016a	0.010a
29	β-pinene	16.579	943	-	-	-		0.017	0.018	-	-	-	-	-	-
30	1-heptanol	16.645	960	-	-	-	0.020	0.024	0.024	0.021	0.021	0.013	0.025	0.020	0.013
31	1-octen-3-ol	17.045	969	0.014	0.019	0.022	0.021	0.022	0.017	0.011	0.010	0.013	0.014	0.017	0.007
32	2-methyl-3-octanone	17.249	988	0.016	0.015	0.032	0.017	0.041	0.031	0.058	0.016	0.024	0.051	0.037	0.023

Continue on next page

Continued

Peak No	Compound	RT (min)	RI	Leg chop (ratio to internal standard) 1 day post-mortem 0 day display time C	W	Ac	6 days display time C	W	Ac	Leg chop (ratio to internal standard) 3 wks post-mortem 0 day display time C	W	Ac	6 days display time C	W	Ac
33	(3E)-2,7-dimethyl-1,3,7-octatriene	17.47	958	0.011	0.027	0.026	0.036	0.029	0.040	0.020	0.015	0.022	0.028	0.034	0.020
34	2,5-dimethylnonane	17.836	986	-	-	-	0.040	-	-	-	-	-	-	-	-
35	octanal	18.087	1005	-	-	0.036	-	-	-	0.075	-	-	0.062	-	-
36	1-hexyl acetate	18.63	984	-	0.01579	-	-	-	-	-	-	-	-	-	-
37	2-ethyl-1-hexanol	19.312	995	0.012a	0.048a	-	8.810c	8.160c	5.015bc	0.023a	0.030a	0.016a	0.224a	0.694a	0.116a
38	pentanoic acid, 2,4-dimethyl-, t-butyl ester	19.983	1069	-	0.015ab	-	-	-	-	-	-	-	-	-	0.007a
39	5-ethyl-2,2,3-trimethylheptane	20.218	1001	-	0.013	-	-	-	-	-	-	-	-	-	-
40	1-octanol	21.258	1059	-	-	-	-	-	-	-	0.018	-	-	-	-
41	1-heptyl-2-methylcyclopropane	21.903	1078	-	-	-	-	-	-	-	-	-	-	-	-
42	2-nonanone	22.021	1052	-	-	-	-	-	-	-	-	-	-	-	-
43	nonanal	22.524	1104	0.029a	0.048abc	0.059abc	0.094bc	0.096c	0.082abc	0.023a	0.021ab	0.027a	0.025a	0.035abc	0.025a
44	(2Z)-6-methyl-2undecene	22.971	1158	0.011	-	-	-	-	-	0.008	0.008	0.009	-	-	0.012
45	2-ethyl-1-hexanol acetate	24.413	1118	-	-	-	-	-	-	-	-	-	-	-	-
46	2-methyl-2-pentyicyclohexane	27.516	1287	-	0.011	0.018	0.017	0.032	0.013	-	-	-	-	-	-
47	propanoic acid, 2-methyl-,2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	31.737	1347	-	-	-	-	-	-	-	-	-	-	-	-
48	3-hydroxy-2,2,4-trimethylpentyl ester of isobutanoic acid	32.42	1331	-	-	0.016	0.023	0.024	0.013	-	-	-	-	-	-

C: control; W: water infusion; Ac: kiwi fruit juice infusion;
- = not detected; RT: retention time; RI: retention Index;
a–d: letters indicate that both LD muscle and leg chop samples in the same group differ significantly ($P < 0.05$);

Table 4-3 Ratio of chemical classes of volatile flavour (compared with internal standard) in LD muscles of 6 days refrigerated display at 4°C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2°C

	LD muscle (ratio to internal standard) 1 day post-mortem						LD muscle (ratio to internal standard) 3 wks post-mortem					
	0 day display time			6 days display time			0 day display time			6 days display time		
Compound Group	C	W	Ac	C	W	Ac	C	W	Ac	C	W	Ac
Aldehydes	0.878ab	1.776ab	0.65a	0.75ab	0.995ab	0.562a	1.07ab	0.851ab	0.373a	0.365a	0.392a	0.291a
Ketones	0.0461	0.100	0.042	0.054	0.119	0.055	0.064	0.041	0.045	0.297	0.176	0.063
Alcohol	0.099a	0.139a	0.104a	1.724ab	1.960ab	0.780ab	0.297a	0.487a	0.483a	13.400d	4.570abc	10.140cd
Terpenes	0.027a	0.017a	0.021a	0.039a	0.044a	0.021a	0.065ab	0.146b	0.048a	0.030a	0.036a	0.011a
Sulphur Compounds	0.018a	0.069a	0.052a	0.035a	0.023a	0.021a	0.035a	0.047a	0.026a	0.321ab	0.203ab	1.290b
Hydrocarbons	0.031ab	0.038ab	0.054ab	0.051ab	0.090b	0.062ab	0.029ab	0.037ab	0.025ab	0.022a	0.019a	0.015a
Alkanes	0.044b	-	0.012a	-	0.061bc	0.023a	0.043b	0.091c	0.037ab	0.056b	0.089bc	0.093bc
Esters	0.0598	0.033	0.094	0.035	0.086	0.036	0.072	0.093	0.070	0.026	0.052	0.047
Carboxylic Acid	0.037	0.094	-	-	0.007	-	-	-	-	0.098	0.110	0.009
Ether	0.034	-	-	0.016	-	-	-	-	-	0.013	-	0.028

C: control; W: water infusion; Ac: kiwi fruit juice infusion;
- = not detected; RT: retention time; RI: retention Index;
a–d: letters indicate that both LD muscle and leg chop samples in the same group differ significantly ($P < 0.05$);

Table 4-4 Ratio of chemical classes of volatile flavour (compared with internal standard) in leg chops of 6 days refrigerated display at 4°C in either 1 day post-mortem or after 3 wks of vacuum packaged storage at 2°C

	Leg chop (ratio to internal standard) 1 day post-mortem						Leg chop (ratio to internal standard) 3 wks post-mortem					
	0 day display time			6 days display time			0 day display time			6 days display time		
Compound Group	C	W	Ac	C	W	Ac	C	W	Ac	C	W	Ac
Aldehydes	1.120a	0.577a	0.670a	2.015ab	2.493b	2.464b	0.767a	0.391a	0.453a	0.579a	0.693a	0.490a
Ketones	0.035	0.028	0.087	0.070	0.084	0.058	0.070	0.047	0.026	0.173	0.109	0.204
Alcohol	0.100a	0.180a	0.157a	6.120abcd	8.400bcd	5.289abc	0.283a	0.252a	1.660ab	4.340abc	1.105ab	3.630bc
Terpenes	0.067ab	0.060ab	0.020a	0.053ab	0.047ab	0.053ab	0.032a	0.050ab	0.022a	0.040a	0.031a	0.027a
Sulphur Compounds	0.094a	0.037a	-	-	0.092a	0.085a	-	-	-	0.026a	0.022a	0.013a
Hydrocarbons	0.030ab	0.019a	0.021a	0.042ab	0.057ab	0.036ab	0.021a	0.010a	0.017a	0.033ab	0.026ab	0.015a
Alkanes	0.036ab	0.022a	0.019a	-	0.065bc	0.039bc	0.037abc	0.050abc	0.020a	0.055bc	0.050abc	0.026a
Esters	0.057	0.034	0.058	0.083	0.115	0.135	0.053	0.044	0.055	0.075	0.028	0.033
Carboxylic Acid	-	0.044	-	-	-	-	-	-	-	0.054	0.045	0.060
Ether	-	-	-	-	-	-	-	-	-	-	-	0.033

C: control; W: water infusion; Ac: kiwi fruit juice infusion;
- = not detected; RT: retention time; RI: retention Index;
a–d: letters indicate that both LD muscle and leg chop samples in the same group differ significantly ($P < 0.05$);

Table 4-5 Ratio of volatile compounds (compared with internal standard) in fresh kiwi fruit juice

Peak No	Volatile compound	RT (min)	RI	Ratio to internal standard
1	1-penten-3-ol	3.502	671	0.183
2	n-pentanal	3.787	707	0.006
3	ethyl propanoate	4.108	686	0.019
4	4-methyl-1-penten-3-ol	4.219	706	0.275
5	methyl butanoate	4.383	686	0.160
6	1-butanol, 3-methyl-	4.791	697	0.180
7	ethyl 2-methylpropanoate	5.499	721	0.122
8	(2E)-2-penten-1-ol	6.002	769	0.114
9	n-hexanal	7.123	806	1.284
10	ethyl butanoate	7.28	785	1.204
11	ethyl .alpha.-methylbutyrate	10.125	820	0.083
12	(E)-2-hexen-1-al	10.279	814	0.842
13	(4Z)-4-hexen-1-ol	10.501	868	0.818
14	(E)-2-hexen-1-ol	11.183	868	5.276
15	n-hexan-1-ol	11.323	860	5.518
16	n-heptanal	12.943	905	0.034
17	alpha.-pinene	14.429	948	0.080
18	camphene	15.166	943	0.010
19	1-isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	15.498	879	0.013
20	benzaldehyde	15.983	982	0.010
21	beta.-pinene	16.595	943	0.195
22	2-propyl-1-pentanol	16.783	995	0.010
23	hexanoic acid	17.55	974	0.045
24	ethyl 3-hexenoate	18.317	992	0.118
25	1,2-diisopropenylcyclobutane	19.133	934	0.011
26	2-ethyl-1-hexanol	19.36	995	2.102
27	.beta.-linalool	22.342	1082	0.021
28	n-nonanal	22.523	1104	0.106
29	1,1,3-trimethyl-3-cyclohexene-5-one	23.357	1097	0.050
30	n-decanal	26.501	1204	0.011
31	4-isopropenyl-1-methylcyclohexanol	27.414	1158	0.028
32	1-methyl-2-pentylcyclohexane	27.517	1239	0.124
33	m-di-tert-butylbenzene	28.256	1334	0.075
34	propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	31.714	1347	0.754
35	3-hydroxy-2,2,4-trimethylpentyl ester of isobutanoic acid	32.412	1331	0.675

RT: retention time;
RI: retention index;

The individual volatile compounds for LD muscle and leg chop samples are listed in Table 4-1 and Table 4-2 according to their RT sequence, respectively. The volatile compounds are also classified according to their chemical classes: e.g. aldehydes, ketones, alcohols, terpenes, sulphur compounds, hydrocarbons, alkanes, esters, carboxylic acid and ether, and the combined class data are presented in Table 4-3 and Table 4-4 for LD muscle and leg chop, respectively.

4.3.1 Aldehydes

The aldehydes were the chemical family displaying consistently high concentrations in both LD muscles and leg chops (Table 4-3 and Table 4-4). This was consistent with the view of Cramer (1983) that aldehydes were responsible, in a major way, for cooked lamb aroma. Aldehydes were not significantly affected by the infusion treatments, but post-

mortem time ($P < 0.001$), display time ($P = 0.045$) and muscle type ($P = 0.05$) did have a significant effect. For LD muscles, the concentration of the aldehydes decreased during the 6 days display time for both 1day and 3 wks post-mortem. However there was a significant increase ($P < 0.05$) after the leg chops had been displayed for 6 days after 1 day post-mortem, and a slight non significant increase was shown after 6 days display time in the leg chops that had been stored vacuum packaged for 3 wks at 2^{o}C. Aldehydes, in general, are not stable (Shahidi & Pegg, 1994), and can easily react with other compounds to produce different compounds which have different flavours (Moody, 1983) and thus eventually contribute to the development of overall meat flavour.

Of the eight aldehydes (2-methyl-1-butanal, n-pentanal, hexanal, cis-4-hepten-1-al, heptanal, benzaldehyde, octanal and nonanal) present in both of the LD muscle and leg chop samples, hexanal was detected in the greatest amounts. There was no difference in hexanal concentration ($P > 0.05$) for both LD muscle and leg chop samples after the 3 wks vacuum packaged storage at 2^{o}C, indicating that no or little lipid oxidation occurred during the vacuum packaging storage at chilled temperature. In leg chop samples, but not in the LD muscles (Table 4-1 and Table 4-2), the hexanal level increased about 4 fold after 6 days display time after 1day post-mortem whereas there was no significant change after the samples were displayed for 6 days after the lamb leg chops had been vacuum packed and stored for 3 wks at 2^{o}C. This was similar to the results reported by Shahidi and Pegg (1994) that hexanal increased in concentration in cooked pork loins during the first 6 days of storage before it started to decline. A similar increase in hexanal levels during the first 5 days of storage were also found in broiler cooked chicken (Ang & Lyon, 1990), cooked beef (Drumm & Spanier, 1991) and cooked dark and light mackerel fish muscles (Shahidi & Spurvey, 1996). There was a non-significant decrease in the hexanal concentration after 6 days display time for the LD muscles after both 1 day post-mortem and after 3 wks vacuum packed storage at 2^{o}C. The different hexanal concentration development trend with the display and post-mortem time between LD muscles and leg chops could be explained due to the different muscle cuts. Morcuende et al. (2003) reported that different muscle cuts behaved differently. They showed that hexanal concentration in masseter muscle had a significant increase after 10 days display time at 4^{o}C, but this was not found in the LD or serratus ventralis muscles in pork. In addition, Shahidi and Pegg (1994) reported that hexanal concentration levels increased dramatically in the initial 6 days display time, and then started to decrease in LD muscle of pork. In the present study, the

hexanal concentration for both LD muscle and leg chop samples were only analysed at initial and after display for 6 days for both 1 day post-mortem and 3 wks vacuum packaged storage at 2^{o}C. Thus, more measurements on hexanal concentration changes within and after the 6 days display time needs further studies.

Hexanal generated initially in meat can be continuously being oxidized (Shahidi & Pegg, 1994). Shahidi and Pegg (1994) suggested that it combined with other components in the meat matrix. Palamand and Dieckmann (1974) studied the autoxidation of hexanal which was held at 70^{o}C for 48 hrs with a stream of air passing through. They found that the volume of hexanoic acid increased when hexanal was undergoing autoxidation. Subsequently, hexanal and hexanoic acid generated were then broken down via acid-catalysed reactions, and resulted in the formation of various compounds including esters, lactones, carbonyl compounds, acids, alcohols and hydrocarbons. In the current research, no hexanoic acid was identified and only a small amount of carboxylic acid (propanoic acid) was found (Table 4-3 and Table 4-4), which might suggest that the hexanal and acids were converted into other compounds such as alcohols, accounting for the reduced concentrations of the former compounds. In support of this, significant increasing levels of alcohol were found after 6 days display time at 1 day and 3 wks post-mortem time for both LD muscle and leg chop samples (alcohol discussion refer to Section 4.3.3). In a recent research by Hierro et al. (2004), hexanal was also reported to have an effect on the meat flavour and can be modulated simultaneously by other accumulated aromatic compounds. Furthermore, the variations in factors including different animal species, cuts/muscles, experimental design, sample collection and preparation procedures and cooking conditions might alter the level of hexanal detected (Estévez et al., 2003; Fu & Ho, 1997; Morcuende et al., 2003; Priolo et al., 2001) in LD muscle and leg chops in the current work, and thus give rise to the variation in overall volatile results.

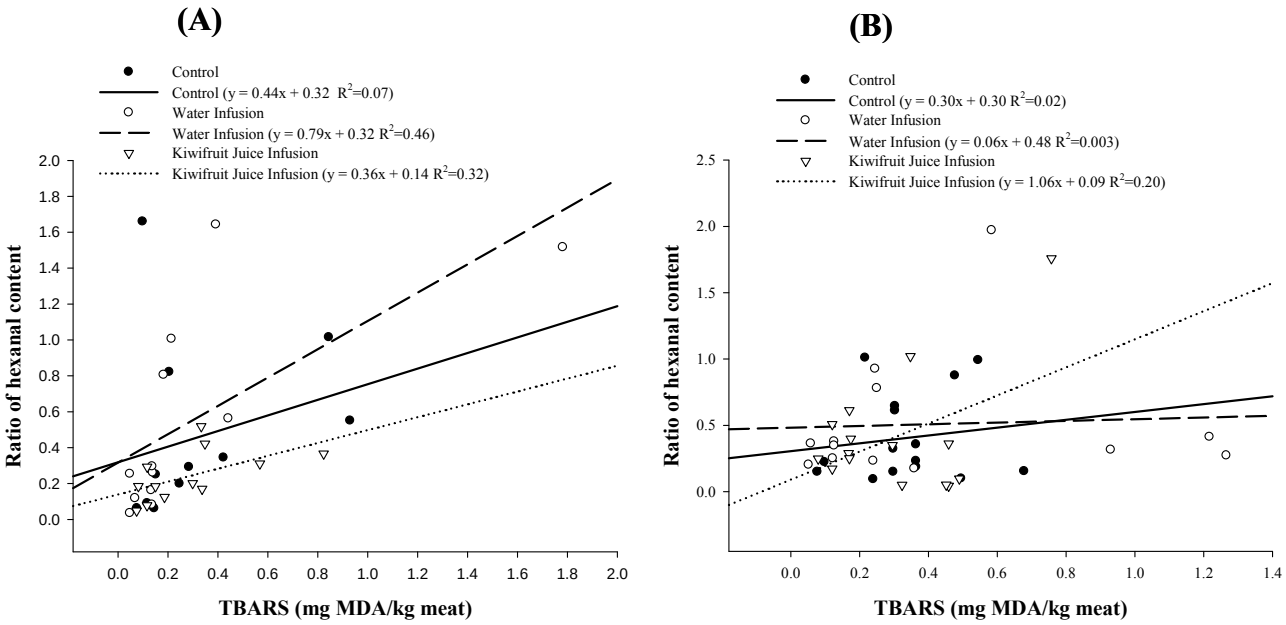

Figure 4-1 Relationships between the value of TBARS and the ratio of hexanal content of (A) LD muscles, (B) leg chops in lamb carcasses post-mortem after control and infused treatments

Results linking hexanal concentration and lipid oxidation in a linear relationship between hexanal and TBARS values was reported in cooked beef (Shahidi et al., 1987). It was also suggested that hexanal might serve as an indicator in monitoring oxidative processes of lipids (Brunton et al., 2000; Frankel et al., 1994). However, the usefulness of hexanal as a lipid oxidation indicator in meat has not been shown conclusively. In an attempt to further investigate whether hexanal could be considered as an indicator of lipid oxidation in cooked lamb, the relationship between the hexanal and the TBARS value which indicate the oxidation status of the meat (see section 3.3.3.2) for both LD muscle and leg chop samples were plotted and is presented in Figure 4-1 (A) and (B), respectively. A poor linear relationship was obtained (with a low R^2 value range of 0.003 – 0.46) for both LD muscle and leg chop from all three treatment carcasses. This suggested that hexanal cannot be considered as an indicator of the oxidative status in meat. This finding was consistent with previous reports (Brunton et al., 2000; Shahidi & Pegg, 1994)that caution was required when suggesting that hexanal could be employed as a indicator to represent the status of lipid oxidation with time.

In addition to hexanal, the other aldehydes including n-pentanal, heptanal, octanal, and nonanal were also detected in the lamb samples (Table 4-1 and Table 4-2). These aldehydes were reported to be present in beef and lamb ham (Machiels et al., 2004; Paleari

et al., 2006). However, in the present work they were only found in small quantities and no conclusions can be drawn about their contribution to the overall picture.

Branched aldehydes (cis-4-hepten-1-al) and aromatic aldehydes (benzaldehyde) result from proteolysis and amino acid degradation rather than lipid degradation. They appeared at a considerably higher level after the 6 days display time for both LD muscles and leg chops at 1 day and 3 wks post-mortem time, particularly for the leg chop samples of three treatments after 6 days display time after 1 day post-mortem. This suggested that these compounds increased with display time as a result of increased protein degradation during ageing (Table 4-2).

4.3.2 Ketones

The contribution of ketones to total volatile flavour of meat was lower than that of aldehydes (Table 4-3 and Table 4-4). However, Rocthhat and Chaintreau (2005) reported that ketones also represented a reasonably large proportion of aroma compounds formed during cooking in beef. Ketones, like aldehydes, also result from lipid oxidation. There were 8 ketones including pentan-2-one, 3-hepten-2-one, 3-hydroxy-2-butanone, 4-hydroxy-4-methyl-2-pentanone, 2-heptanone, butyrolactone, 2-methyl-3-octanone, 2-nonanone found in the lamb samples (Table 4-1 and Table 4-2). Ketones derived from fatty acids degradation might contribute the buttery aroma to cooked meats (Peterson et al., 1975). In the current work, content of the ketones was not affected by infusion treatment, but increased with the display time ($P = 0.016$) in the three treatment samples at different post-mortem and display times. In particular, a dramatic increase was found after the samples were displayed for 6 days after 3 wks post-mortem time in both LD and leg muscles, which indicated that more lipid oxidation occurred with longer display time. 3-hydroxy-2-butanone was believed to be a quantitative indicator compound for raw ground beef due to its steady increase in LD samples during 10 days storage at $10^{o}C$ (Chen et al., 2004). However, noticeable amounts of 3-hydroxy-2-butanone were only found in leg chop samples after the 6 days display time of the 3 wks post-mortem samples in the current work.

Other ketones also detected were 2-heptanone and pentan-2-one. Generally, a slightly increasing trend was found after the samples were displayed for 6 days. This is consistent

with their being produced by lipid oxidation with time. Another volatile compound present in samples in the current research was 2-methyl-3-octanone. The concentration of 2-methyl-3-octanone showed a slightly increasing trend for C and W samples in both LD and leg muscles after the samples had been displayed for 6 days in both 1 day and 3 wks post-mortem time, which might suggest that these ketones were lipid degradation products which increased with the display time in meat.

4.3.3 Alcohols

Alcohols, mainly derived from lipid oxidation (Estévez et al., 2003; Wettasinghe et al., 2001), were found in high concentrations in the present study (Table 4-3 and Table 4-4). A similar result was also found in pigs (Estévez et al., 2003). There was no significant effect of kiwifruit juice on alcohol production during this experiment. Eleven different alcohols (including n-butan-1-ol, 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 2, 3 butanediol, cis hept-4-enol, 1-heptanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-octanol) were detected in this research. It was apparent that the alcohols found in both LD muscles and leg chops of the three treatment samples increased with the display time (Table 4-3 and Table 4-4), which is similar to the result reported by Watanabe and Sato (1971). The increase in alcohols during the storage days (0 to 4 days) at 4^{o}C was also reported in cooked chicken meat by Byrne et al. (2002). The increasing alcohol concentration with display time in the current research suggested that alcohols generated during the display time may be derived from the aldehydes, which are not stable, during the development of volatile flavour compounds (refer to Section 4.3.1).

Of the alcohols, n-butan-1-ol was present in the greatest amount, and increased with time (up to a 95 fold increase in some treatments). It was interesting to note that the samples from the C treatment showed the most dramatic increase. An explanation for this is not immediately obvious. 2-ethyl-1-hexanol and 3-methyl-1-butanol were the other alcohols found in relatively large concentrations, and similarly showed an overall increase with time (Tables 4-1 and Table 4-2). This is at variance with the results reported by Chen et al. (2004) that 2-ethyl-1-hexanol was present in beef LD muscle samples stored at 10^{o}C and 4^{o}C, but little change occurred during storage time.

3-methyl-1-butanol, 2-ethyl-1-hexanol and 1-penten-3-ol were detected as the dominating fractions in fresh Kiwi fruit juice (Table 4-5), but the samples from kiwifruit juice infusion were not significantly different ($P > 0.5$) from the other treatment samples. This indicated that there was no residual effect of kiwifruit juice on the volatile flavour compounds in the meat in this experiment, and presumably means that the flavour of the meat was not changed by the infusion of kiwifruit juice.

4.3.4 Terpenes

Four terpenes including styrene, α-pinene, β-pinene and (3E)-2,7-dimethyl-1,3,7-octatriene were found in small amounts in the various treatment samples at different post-mortem and display times. This is consistent with the work done by Priolo et al. (2004) that terpenes account for a small percentage in adipose tissue of sheep. In their research, they also reported that certain terpene compounds were indicative of animals fed with green forage diets (Priolo et al., 2004). Interestingly, α-pinene & β-pinene were also detected in Kiwi fruit juice (Table 4-5). However, there were no significant ($P > 0.05$) differences between the Ac treatment samples and the C and W treatment samples at various post-mortem and display times even though an enhanced presence of α-pinene and β-pinene in kiwifruit juice treated samples might have been expected. Thus, these results suggested that the deposition of α-pinene and β-pinene in the muscle from kiwifruit juice was minimal.

4.3.5 Sulphur volatiles

Two sulphur volatile compounds (dimethyl disulphide and dimethyl trisulphide) that have been reported to be derived from sulphur-containing amino acid degradation (Mottram, 1998) were also detected in this study (Table 4-3 and Table 4-4).

In general, the sulphur compound concentrations were very low. However sulphur volatile compounds can be considered as very potent contributors to the meat flavour due to their low thresholds of sensory detection, even though their concentration may be very low (Drumm & Spanier, 1991). Sulphur compounds increased significantly in the Ac treated LD muscle after 3 wks vacuum packed storage and after 6 days display time. This might suggest that the degradation of sulphur-containing amino acids increased with display time due to the post-mortem proteolysis of myofibrillar proteins during ageing. This, in turn,

might indicate that actinidin in the kiwifruit juice infusion treatment acts as a protease that breaks down proteins and releases these sulphur based degradation products with time, and that this might contribute to the flavour compound profile. Sulphur volatile compounds can be considered as very potent contributors to the meat flavour due to their low thresholds of sensory detection, even though their concentration was very low (Drumm & Spanier, 1991). Sulphur containing compounds dimethyl disulfide and dimethyl trisulfide were also found as important flavour compounds in cooked beef since some heterocyclic sulphur compounds were described as possessing meat like aromas (Mottram, 1994). Sulphur-containing compounds could contribute roasted meat-like, cabbage-like and onion-like flavours to the overall meat aroma (Pittet & Hruza, 1974). It was surprising that an increase in sulphur compounds did not occur in the leg chops and this may be a further indication that different muscles have different properties.

4.3.6 Hydrocarbons

Similar to aldehydes and ketones, hydrocarbons are also mainly derived from fatty acid degradation (Hwang, 1999). In the current study, the effect of hydrocarbons to the meat aroma was minor as they were only found in very low concentration in the samples. Similar results were also reported that hydrocarbons was not considered as an important compound contribute to the cooked meat due to their low concentrations (Hwang, 1999) and relatively high odour threshold values (Drumm & Spanier, 1991). There was little difference between the various display and post-mortem times for the two hydrocarbons ((2Z)-6-methyl-2undecene and toluene) in both LD and leg muscles between the various display and post-mortem times. The presence of hydrocarbons such as toluene has been reported to be found in cooked beef (MacLeod & Seyyedain-Ardebili, 1981).

4.3.7 Esters

Esters showed no significant differences among the samples from different treatments in the present work. Esters have been reported to be associated with branched aldehydes and cause the ripened flavour in cured meat products (Barbieri et al., 1992). Esters are generally associated with fruity aroma but are unlikely to have much impact on uncured meat aroma (Cross & Ziegler, 1965).

4.3.8 Alkanes, Carboxylic Acids and Ethers

Alkanes were detected in low concentrations in both LD muscle and leg chop samples, and showed only small concentration differences with display and post-mortem times. Carboxylic acids and ethers were only present at a low concentration in muscle samples. There was no significant effect of these compounds on the overall flavour compound profiles in the present study.

4.3.9 Branched Chain Fatty Acids (BCFAs)

BCFAs have been reported as the main compounds responsible for the sheep and goat flavour (Shahidi, 1998) and mutton flavour (Wong et al., 1975). None of the branched chain fatty acids (BCFAs) such as 4-methyloctanoic and 4-methylnonanoic acids were detected in the current research. Sutherland and Ames (1996) reported that the level of 4-methyloctanoic and 4-methylnonanoic acids in sheep fat was higher in older aged animals when they were slaughtered. Therefore, it was suggested that the lambs, being slaughtered at about 12 months in current study might account for not detecting the branched chain fatty acids in any of the three treatments. In addition, it was also suggested that analytical procedures (Paleari et al., 2006) and extraction methods (Sebastiàn et al., 2003) might also influence the detection of the BCFAs and that this might affect the results in the current study.

4.4 Summary

A total of 48 known volatile compounds, and 70 unknown volatile compounds, associated with aroma were found in both LD muscle and leg chop samples during 6 days display time after 1 day post-mortem and after 3 wks vacuum packed storage at 2^{o}C, by combined use of SPME headspace analysis with GC-FID and GC-MS. Of the overall volatile compound profile detected, 8 aldehydes, 8 ketones, 11 alcohols, 4 terpenes, 2 sulphur compounds, 2 hydrocarbons, 5 alkanes, 6 esters, 1 carboxylic acid and 1 ether were identified and discussed in this chapter.

Quantatively, aldehydes and alcohols were the two dominant fractions contributing to the overall volatile flavour compound profile of LD muscle and leg chop lamb samples in the

present study. In general, infusion treatment had no effect on their concentrations, which indicated that infusion treatment had no effect on the overall flavour. Of the aldehydes, hexanal was present in the greatest concentration compared with the other aldehydes. The decrease in aldehydes concentration with time, particularly the decrease in the hexanal concentration in the present work might indicate that hexanal generated initially was continuously being oxidized, or combined with other components in the meat matrix resulting in the production of alcohols. Significant increasing levels of alcohol were found with display time for both LD muscle and leg chop samples. This might account for the observation that hexanal concentration can not be considered as an indicator of oxidative processes of lipids. This was confirmed through the poor correlation between total hexanal and lipid oxidation TBARS value in current research.

It was also observed that various ketones and sulphur containing compounds were of importance for the flavour of the lamb meat. In agreement with previous research, lipid oxidation and amino acid degradation were the main pathways producing the precursors required for the generation of volatile compounds during the display time for the post-mortem meat samples.

In the current research, differences were shown for the overall volatile compounds with the muscle cut, display time and post-mortem time in the major volatile compound groups, but there was no effect of the infusion treatments. Treatment by using infusion of carcasses with water and kiwifruit juice for tenderization had only a minor impact on the aroma compounds changes of lamb, and this is an advantage in that they did not change the natural lamb flavour. This will meet the preference of consumers by having more tender meat yet still enjoy the ordinary lamb flavour.

Chapter 5
General Discussion and Future Work

5.1 General Discussion

Meat eating qualities including meat tenderness, juiciness, colour and flavour are the major characteristics affecting consumers' satisfaction of meat (Lawrie, 1998). Tenderness is usually considered as one of the most important palatability traits by consumers (Miller et al., 2001). In the current study, the use of an infusion technique ensures a homogeneous and efficient distribution of infusates (in the current experiment, water and kiwifruit juice) throughout the carcass (Katsanidis et al., 2003). The outstanding lower shear force values (more tender) detected in the Ac carcasses post-mortem compared with C and W carcasses demonstrated that kiwifruit juice was a very powerful meat tenderizer and could contribute to the meat tenderization process efficiently and effectively. More weakening and/or degradation of structural integrity of the myofibril proteins and the simultaneous appearance of new peptides were found in Ac carcasses compared with C and W carcasses during post-mortem ageing. These changes were believed mainly due to the enhanced proteolytic activity resulting from the actinidin in kiwifruit juice, which acts as a protease and thus contributes to the improvement of meat tenderness. Previous reports of the tenderization of meat by actinidin have required very high levels of the protease (Lewis & Luh, 1988) or pressure treatment (Wada et al., 2002).

Tough meat cuts are usually sold at a relatively low price compared with tender meat, such as fillet steak. Meat of both LD and leg chops from Ac carcasses was significantly more tender than from the same cuts from the C treatment, when measured at the initial stages of post mortem. This tenderizing effect observed during the initial period (5 hrs to 1 day) of post-mortem after the Ac treatment indicated that the extended length of time commonly used by the red meat industry for ageing during meat post-mortem storage may be reduced by the use of kiwifruit juice, and still ensure the meat tenderness. This will potentially lead to additional profit for the meat industry from selling more tender meat soon after slaughter

and thus saving the storage, energy and labour costs, and yet still having meat of good quality.

In the current study, no "mushy" meat texture and volatile flavour compound profile changes were found in Ac carcasses. This was explained by Lewis and Luh (1988) that actinidin acted in a more gentle way on muscle structural proteins rather than degrading all the proteins in meat which might then alter the meat texture and mouthfeel, as found with papain. The fact that the Ac treatment did not alter the volatile flavour compound profile in the current work was an additional bonus, in that consistently tender meat still had the preferred original lamb flavour.

Unlike the negative effects of calcium chloride on meat display colour (Bekhit et al., 2005; Rees et al., 2002), there were no major changes in the colour of post-mortem meat during display in the Ac carcasses compared with the C and W carcasses. In fact, in Ac carcasses, there was a slight, non significantly higher a*-value (redness) in the leg chops during the 6 days display time after 3 wks vacuum packaged storage at 2^{o}C. This may be associated with a decreased lipid oxidation that occurred in the leg chops during the same post-mortem period. This reduced lipid oxidation could be caused by the natural antioxidants including ascorbic acid and other polyphenols in the kiwifruit juice.

In the current study, the use of the W treatment was introduced to investigate whether the effects on tenderness were mainly caused by the kiwifruit juice or the effect of the infusion itself. The meats from Ac treated carcasses were the most tender of the three treatments, and this is attributed to the enhanced proteolytic activity of the actinidin in the kiwifruit juice. Water infused treatment carcasses were more tender than the C carcasses during the initial post-mortem period, but the tenderization effect was not as pronounced as in the Ac carcasses. This indicated that the infusion technique itself might have an effect on the tenderization of the meat. This might be because of the disruption of the muscle microstructure caused by the infusion pressure when the infusion substances were introduced into carcass as suggested by Farouk et al. (1992b).

In order to minimise the variation of technique/procedures in the present study, lambs assigned to the three treatments were of similar weight. No treatment differences ($P > 0.05$ for all) existed for the chilling evaporative loss and rate of temperature decline between the

infusion treatments or C treatment. Six lambs per treatment were thought to be a minimum to obtain statistical significance, but this may not have been enough, given the wide variation found in many of the results in this experiment. In the measurement of the volatile compounds, meat samples from only 3 animals were analysed and the variation between samples/animals almost certainly played a role in determining statistical significance of these results. However, it was not possible to use more animals because of the cost.

In summary, proteolytic tenderizing infusion treatment using kiwifruit juice is a feasible approach for the commercial meat industry, and also could satisfy the eating quality standards required by the consumers. This is brought about because of its enhanced proteolytic activity, outstanding tenderization effects, lacking of excessive surface tenderization in the meat, maintenance of natural lamb flavour, and the positive effect on colour and inhibition of lipid oxidation during post-mortem. In addition, tenderizing meat by using kiwifruit juice could also provide the kiwifruit processors an additional option for consumption of their products to gain a more profitable return, especially using those kiwifruit which were not up to the grade to be sold in the market.

5.2 Future works

- Further research will be essential to determine the optimal infusion concentration and volume of the kiwifruit juice used to maximize tenderization effects, and minimize the percentage of the drip and cooking loss while still maintaining the display meat colour stability and natural lamb flavour after the infusion treatment.

- Techniques involved in the infusion process might still need to be developed. It took about 10 minutes per lamb for the infusion treatment pre-rigor in the current study. This would be too long for commercial purposes. Therefore, methods and procedures which impart an effective and efficient commercial practice will be required to make this process viable for meat processors.

- In the current study, the volatile flavour compound profile was measured objectively. In order to convert this into consumer preference in a more subjective manner, it will be necessary to complement the results by running a taste panel to discover the

preferred taste when consumer eats the meat products derived from kiwifruit juice infusion techniques.

- Some people display an allergic reaction to kiwifruit (Bublin et al., 2004) although the prevalence of kiwifruit allergy is less common than with many other foods. Further studies on the possibility of allergic reactions for the consumers after they eat the meat treated with kiwifruit juice may be useful.

References

AMSA. (1991). *Guidelines for meat colour evaluation*: The American Livestock and Meat Board, Chicago, IL.

Ang, C. Y. W. & Lyon, B. G. (1990). Evaluations of warmed-oven flavour during chill storage of cooked broiler breast, thigh and skin by chemical, instrumental and sensory methods. *Journal of Food Science, 55*, 644-648, 673.

Anna, A. M., Lakshmanan, V., Radha, K., Singh, R. P., Mendiratta, S. K. & Anjaneyulu, A. S. R. (2007). Effect of blade tenderization and sodium bicarbonate on quality of buffalo rumen meat. *Journal of Food Science and Technology, 44*(4), 437-439.

Arcus, A. C. (1959). Proteolytic enzyme of actinidia chinensis. *Biochemical et Biophysical Acta, 33*, 242-244.

Ashie, I. N. A., Sorensen, T. L. & Nielsen, P. M. (2002). Effects of papain and a microbial enzyme on meat proteins and beef tenderness. *Journal of Food Science, 67*(6), 2138-2142.

Bandman, E. & Zdanis, D. (1988). An immunological method to access protein degradation in post-mortem muscle. *Meat Science, 22*(1), 1-19.

Barbieri, G., Bolzoni, L., Parolari, G. & Virgile, R. (1992). Flavor compounds of dry-cured ham. *Journal of Agricultural and Food Chemistry, 40*, 2389-2394.

Barrett, A. J. (1973). Human cathepsin B1: Purification and some properties of the enzyme. *Biochemical Journal, 131*, 809-822.

Behrends, J. M., Goodson, K. J., Koohmaraie, M., Shackelford, S. D., Wheeler, T. L., Morgan, W. W., Reagan, J. O., Gwartney, B. L., Wise, J. W. & Savell, J. W. (2005). Beef customer satisfaction: factors affecting consumer evaluation of calcium chloride-injected top sirloin steaks when given instructions for preparation. *Journal of Animal Science, 83*, 2869-2875.

Bekhit, A. E. D., Geesink, G. H., Morton, D. J. & Bickerstaffe, R. (2001). Metmyoglobin reducing activity and colour stability of ovine longissimus muscle. *Meat Science, 57*, 412-435.

Bekhit, A. E. D., Ilian, M. A., Morton, J. D., Vanhannan, L., Sedcole, J. R. & Bickerstaffe, R. (2005). Effect of calcium chloride, zinc chloride, and water infusion on metmyoglobin reducing activity and fresh lamb colour. *Journal of Animal Science, 83*, 2189-2204.

Bickerstaffe, R., Bekhit, A. E. D., Robertson, L. J., Roberts, N. & Geesink, G. H. (2001). Impact of introducing specifications on the tenderness of retail meat. *Meat Science, 59*, 303-315.

Bindon, B. M. & Jones, N. M. (2001). Cattle supply, production systems and markets for Australian beef. *Australian Journal of Experimental Agriculture, 41*, 861-877.

Boehm, M. L., Kendall, T. L., Thompson, V. F. & Goll, D. E. (1998). Changes in the calpains and calpastatin during post-mortem storage of bovine muscle. *Journal of Animal Science, 76*, 2415-2434.

Boleman, S. J., Boleman, S. L., Miller, R. K., Taylor, J. F., Cross, H. R., Wheeler, T. L., Koohmaraie, M., Shackelford, S. D., Miller, M. F., West, R. L., Johnson, D. D. &

Savell, J. W. (1997). Consumer evaluation of beef of known categories of tenderness. *Journal of Animal Science, 75*, 1521-1524.

Bouton, P. E., Harris, P. V. & Shorthose, W. R. (1971). Effect of ultimate pH upon water-holding capacity and tenderness of mutton. *Journal of Food Science, 36*, 435.

Bowker, B. C., Liu, M. N., Solomon, M. B., Eastridge, J. S., Fahrenholz, T. M. & Vinyard, B. (2007). Effects of hydrodynamic pressure processing and blade tenderization on intramuscular collagen and tenderness-related protein characteristics of top rounds from Brahman cattle. *Journal of Muscle Foods, 18*(1), 35-55.

Boyer-Berri, C. & Greaser, M. L. (1998). Effect of post-mortem storage on the Z-line region of titin in bovine muscle. *Journal of Animal Science, 76*(4), 1034-1044.

Braggins, T. J. (1996). Effect of stress-related changes in sheep meat ultimate pH on cooked odour and flavour. *Journal of Agricultural and Food Chemistry, 44*(8), 2352-2360.

Brunton, N. P., Cronin, D. A., Monahan, F. J. & Durcan, R. (2000). A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food Chemistry, 68*, 339-345.

Bublin, M., Mari, A., Ebner, C., Knulst, A., Scheiner, O., Hoffmann-Sommergurber, K., Breiteneder, H. & Radauer, C. (2004). IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *Journal of Allergy and Clinical Immunology, 114*(5), 1169-1175.

Byrne, D. V., Bredie, W. L. P., Mottram, D. S. & Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour in chicken meat. *Meat Science, 61*(2), 127-139.

Carne, A. & Moore, C. H. (1978). The amino acid sequence of the tryptic peptides from actinidin, a proteolytic enzyme from the fruit *Actinidia chinensis*. *Biochemistry Journal, 173*, 73-83.

Chen, Q., Panigrahi, S., Balasubramanian, S., Logue, C., Marchello, M., Gu, H., Jelen, H. & Nord, R. (2004). *GC-MS techniques for characterization of meat products*. Paper presented at the 2004 ASAE/CSAE Annual International Meeting.

Cheng, J. H. & Ockerman, H. W. (2004). Effect of ascorbic acid with tumbling on lipid oxidation of precooked roast beef. *Journal of Muscle Foods, 15*(2), 83-93.

Chevance, F. V. & Farmer, L. J. (1999). Identification of major volatile odour compounds in frankfurters. *Journal of Agricultural and Food Chemistry, 47*, 5151-5160.

CIE. (1986). *Colorimetry 2nd ed. Commission International de 1'Eclairage*. Vienna: Publication CIE 15.2.

Claeys, E., Uytterhaegen, L., Buts, B. & Demeyer, D. (1995). Quantification of beef myofibrillar proteins by SDS-PAGE. *Meat Science, 39*(2), 159-318.

Clarke, E. M., Shaw, F. D. & Morton, D. J. (1980). Effect of electrical stimulation post-mortem of bovin muscle on the binding of glycolytic enzymes: functional and structural implications. *Biochemical Journal, 186*, 105-109.

Cramer, D. (1983). Chemical compounds implicated in lamb flavor. Overview. *Food Technology, 37*(5), 249-257.

Cross, C. K. & Ziegler, P. (1965). A comparison of the volatile fractions of cured and uncured meats. *Journal of Food Science, 30*, 610-614.

Dawes, H. M. & Keene, J. B. (1999). Phenolic composition of kiwifruit juice. *Journal of Agricultural and Food Chemistry, 47*(6), 2398-2403.

De Duve, C., Gianetto, R., Wattiaux, E. & Appelmans, F. (1955). Tissue fractionation studies. 6. intracellular distribution patterns of enzymes in rat-liver tissue. *Biochemical Journal, 60*, 604-617.

Dikeman, M. E., Hunt, M. C., Addis, P. B., Schoenbeck, H. J., Pullen, M., Kstsanidis, E. & Yancey, E. J. (2003). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science, 81*, 156-166.

Doumit, M. E. & Koohmaraie, M. (1999). Immunoblot analysis of calpastatin degradation: evidence for cleavage by calpain in post-mortem muscle *Journal of Animal Science, 77*, 1467-1473.

Drumm, T. D. & Spanier, A. M. (1991). Changes in the content of lipid autoxidation and sulphur-containing compounds in cooked beef during storage. *Journal of Agricultural and Food Chemistry, 39*(2), 336-343.

Echevarne, C. M., Renerre, M. & Labas, R. (1990). Metmyoglobin reductase activity in bovine muscles. *Meat Science, 27*, 161-172.

Elmore, J. S., Mottram, D. S., Enser, M. & Wood, J. D. (1999). Effect of polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry, 47*, 1619-1625.

Estévez, M., Morcuende, D., Ventanas, S. & Cava, R. (2003). Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS. *Journal of Agricultural and Food Chemistry, 51*, 3429-3435.

Farouk, M. M. & Price, J. F. (1994). The effect of post-exsanguination infusion on the composition, exudation, colour and post-mortem metabolic changes in lamb. *Meat Science, 38*, 477-496.

Farouk, M. M., Price, J. F. & Salih, A. M. (1992a). Post-exsanguination infusion of ovine carcasses: Effect on tenderness indicators and muscle microstructure. *Journal of Food Science, 57*, 1311-1315.

Farouk, M. M., Price, J. F., Salih, A. M. & Burnett, R. J. (1992b). The effect of postexsanguination infusion of beef on composition, tenderness, and functional properties. *Journal of Animal Science, 70*, 2773-2778.

Faustman, C. & Cassens, R. G. (1990). Influence of aerobic metmyoglobin reducing capacity on colour stability of beef. *Journal of Food Science, 55*, 1278-1283.

Forrest, J. C., Alberle, E. D., Hedrick, H. B., Judge, M. D. & Merkel, R. A. (1975). Properties of fresh meat. In *Principles of meat science*: W. H. Freeman and Company: USA.

Frankel, E. N., Parks, E. J., Xu, R., Schneeman, B. O., Davies, P. A. & German, J. B. (1994). Effect of n-3 fatty acid-rich fish oil supplementation on the oxidation of low density lipoproteins. *Lipids, 29*, 233-236.

Fu, H. Y. & Ho, C. T. (1997). Mechanistic studies of 2-(1-hydroxyethyl)-2, 4, 5-trimethyl-3-oxazoline formation under low temperature in 3-hydroxy-2-butanone/ammonium acetate model systems. *Journal of Agricultural and Food Chemistry, 45*, 1878-1882.

Geesink, G. H. & Koohmaraie, M. (1999). Post-mortem proteolysis and calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended post-mortem storage. *Journal of Animal Science, 77*, 1490-1501.

Geesink, G. H. & Koohmaraie, M. (2000). Ionic strength-induced inactivation of μ-calpain in post-mortem muscle. *Journal of Animal Science, 78*, 2336-2343.

Geesink, G. H., Kuchay, S., Chishti, A. H. & Koohmaraie, M. (2006). μ-Calpain is essential for post-mortem proteolysis of muscle proteins. *Journal of Animal Science, 84*, 2834-2840.

Geesink, G. H., Talylor, R. G. & Koohmaraie, M. (2005). Calpain 3/p94 is not involved in post-mortem proteolysis. *Journal of Animal Science, 83*, 1646-1652.

Geesink, G. H., Taylor, R. G., Bekhit, A. E. D. & Bickerstaffe, R. (2001). Evidence against the non-enzymatic calcium theory of tenderisation. *Meat Science, 59*, 417-422.

Glazer, A. M. & Smith, E. L. (1971). Papain and other sulfhydryl proteolytic enzymes. In *Enzymes.* (3rd ed., Vol. 3, pp. 501): P. D. Boyer. Ed.

Goll, D. E., Dayton, W. R., Singh, I. & Robson, R. M. (1991). Studies of the α-actinin/actin interaction in the Z-disk by using calpain. *Journal of Biological Chemistry, 266*, 8501-8510.

Goll, D. E., Otsuka, Y., Nagainis, P. A., Shannon, J. D., Sathe, S. K. & Mugurma, M. (1983). Role of muscle proteases in maintenance of muscle integrity and mass. *Journal of Food Biochemistry, 7*(137-177).

Goll, D. E., Thompson, V. F., Li, H., Wei, W. & Cong, J. (2003). The calpain system. *Physiological Reviews, 83*, 731-801.

Gonzalez, C. B., Salitto, V. A. & Carduza, F. J. (2001). Effect of calcium chloride marination on bovine cutaneus trunci muscle. *Meat Science, 57*, 251-256.

Greaser, M. L. (2001). Post-mortem muscle chemistry. In Y. H. Hui, W. K. Nip, R. W. Rogers & O. A. Young (Eds.), *Meat science and applications* (pp. 21-37). New York: Marcel Dekker, Inc.

Hierro, E., Hoz, L. D. L. & Ordóñez, J. A. (2004). Headspace volatile compounds from salted and occasionally smoked dried meats (cecinas) as affected by animal species. *Food Chemistry, 85*, 649-657.

Ho, C. Y., Stromer, M. H. & Robson, R. M. (1994). Identification of the 30 kDa polypeptide in post-mortem skeletal muscle as a degradation product of troponin-T. *Biochimie, 76*(5), 369-375.

Hood, D. E. (1975). Pre-slaughter injection of sodium ascorbate as a method of inhibiting metmyoglobin fomation in fresh beef. *Journal of the Science and Food Agriculture, 26*, 85-90.

Hood, D. E. (1980). Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. *Meat Science, 4*, 247-265.

Hopkins, D. L., Shaw, F. D., Baud, S. & Walker, P. J. (2006). Electrical currents applied to lamb carcasses - effects on blood release and meat quality. *Australian Journal of Experimental Agriculture, 46*(6-7), 885-889.

Hopkins, D. L. & Thompson, J. M. (2002a). The degradation of myofibrillar proteins in beef and lamb meat using denaturing electrophoresis - an overview. *Journal of Muscle Foods, 13*, 81-102.

Hopkins, D. L. & Thompson, J. M. (2002b). Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact on tenderisation in beef and sheep meat. *Australian Journal of Agricultural Research, 53*, 149-166.

Howe, P. E. & Barbella, N. G. (1937). The flavour of meat and meat products. *Journal of Food Science, 2*(3), 197-202.

Huang, T. C. & Ho, C. T. (2001). Flavours of meat products. In Y. H. Hui, W. K. Nip, R. W. Rogers & O. A. Young (Eds.), *Meat science and applications* (pp. 71-102). New York: Marcel Dekker, Inc.

Huang, Y. & Wang, K. (2001). The calpain family and human disease. *Trends in Molecular Medicine, 7*, 355-362.

Huff-Lonergan, E., Mitsuhashi, T., Parrish, F. C. J. & Robson, R. M. (1996). Sodium dodecylsulfate-polyacrylamide gel electrophoresis and western blotting comparisons of purified myofibrils and whole muscle preparations for evaluating titin and nebulin in post-mortem muscle. *Journal of Animal Science, 74*, 779-785.

Huff-Lonergan, E., Parrish, J. F. C. & Robson, R. M. (1995). Effects of post-mortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science, 73*(4), 1064-1073.

Hunt, M. C., Schoenbeck, J. J., Yancey, E. J., Dikeman, M. E., Loughin, T. M. & Addis, P. B. (2003). Effects of postexsanguination vascular infusion of carcasses with calcium chloride or a solution of saccharides, sodium chloride, and phosphates on beef display-colour stability. *Journal of Animal Science, 81*, 669-675.

Huxley, H. (1957). The double array of filaments in cross-striatd muscle. *Journal of Biophysical and Biochemical Cytology, 3*, 631-647.

Hwang, I. H., Lee, J. M. & Thompson, J. M. (2003). The role of electrical stimulation in meat tenderness *Food Science and Biotechnology, 12*(2), 191-197.

Hwang, K. T. (1999). Hydrocarbons detected in irradiated pork, bacon and ham. *Food Research International, 32*, 389-394.

Ilian, M. A., Bekhit, A. E. D., Stevenson, B., Morton, J. D., Isherwood, P. & Bickerstaffe, R. (2004). Up- and down-regulation of longissimus tenderness parallels changes in the myofibril-bound calpain 3 protein. *Meat Science, 67*(3), 433-445.

Ilian, M. A., Morton, J. D., Bekhit, A. E. D., Roberts, N., Palmer, B., Sorimachi, H. & Bickerstaffe, R. (2001). Effect of preslaughter feed withdrawal period on longissimus tenderness and the expression of calpains in the ovine. *Journal of Agricultural and Food Chemistry, 49*(4), 1990-1998.

Jayasooriya, S. D., Torley, P. J., D'Arcy, B. R. & Bhandari, B. R. (2007). Effect of high power ultrasound and ageing on the physical properties of bovine semitendinosus and longissimum muscles. *Meat Science, 75*(4), 628-639.

Jeacocke, R. E. (1993). The concentration of free magnesium and free calcium ions both increase in skeletal muscle fibres entering rigor mortis. *Meat Science, 35*, 27-45.

Jeremiah, L. E. (1982). Factors affecting consumption, selection and acceptability of meat purchases. *Journal of Consumer Studies and Home Economics, 6*, 137-154.

Jeremiah, L. E., Gibson, L. L. & Cunningham, B. (1999). The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International, 32*(8), 585-591.

Jeyamkondan, S., Jayas, D. S. & Holley, R. A. (2000). Review of centralized packaging system systems for distribution of retail-ready meat. *Journal of Food Protection, 63*, 796-804.

Joo, S. T., Kauffman, R. G., Kim, B. C. & Park, G. B. (1999). The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Science, 52*, 291-297.

Karlsson, J., Ostwald, K., Kabjorn, C. & Anderson, M. (1994). A method for protein assay in Laemmli buffer. *Animal Biochemistry, 219*, 144-146.

Katsanidis, E., Meyer, D. C., Addis, P. B., Jancey, E. J., Dikeman, M. E., Tsiamyrtzis, P. & Pullen, M. (2003). Vascular infusion as a means to improve the antioxidant-prooxidant balance of beef. *Journal of Food Science, 68*(4), 1149-1154.

Koohmaraie, M. (1992). The role of Ca2+-dependent proteases (calpains) in post-mortem proteolysis and meat tenderness. *Biochimie, 74*, 239-249.

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science, 36*(1-2), 93-104.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderisation processes of meat. *Meat Science, 43 (Suppl. S)*, S193-S201.

Koohmaraie, M., Crouse, J. D. & Mersmann, H. J. (1989). Acceleration of post-mortem tenderization in ovine carcasses through infusion of calcium chloride: effect of concentration and ionic strength. *Journal of Animal Science, 67*, 934-942.

Koohmaraie, M. & Geesink, G. H. (2006). Contribution of post-mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science, 74*, 34-43.

Koohmaraie, M., Kent, M. P., Shackelford, S. D., Veiseth, E. & Wheeler, T. L. (2002). Meat tenderness and muscle growth: is there any relationship. *Meat Science, 62*, 345-352.

Koohmaraie, M., Schollmeyer, J. E. & Dutson, T. R. (1986). Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *Journal of Food Science, 51*(1), 28-32.

Koohmaraie, M. & Shackelford, S. D. (1991). Effect of calcium chloride infusion on the tenderness of lambs fed a β-adrenergic agonist. *Journal of Animal Science, 69*, 2463-2471.

Koohmaraie, M., Shackelford, S. D. & Wheeler, T. L. (1998). Effect of prerigor freezing and postrigor calcium chloride injection on the tenderness of callipyge longissimus. *Journal of Animal Science, 76*, 1427-1432.

Koohmaraie, M., Whipple, G. & Crouse, J. D. (1990). Acceleration of post-mortem tenderization in lamb and brahman-cross beef carcases through infusion of calcium chloride. *Journal of Animal Science, 68*, 1278-1283.

Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D. & Mersmann, H. J. (1991). Post-mortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science, 69*, 617-624.

Labeit, S. & Kolmerer, B. (1995). Titins: giant porteins in charge of muscle ultrastructure and elasticity. *Science, 270*, 293-296.

Lamare, M., Taylor, R. G., Farout, L., Briand, Y. & Briand, M. (2002). Changes in proteasome activity during post-mortem aging of bovine muscle. *Meat Science, 61*(2), 199-204.

Lanari, M. C. & Cassens, R. G. (1991). Mitochondrial activity and beef muscle colour stability. *Journal of Food Science, 56*, 1476-1479.

Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., Garner, G. B. & Morrow, R. E. (1987). Flavour constituents of beef as influenced by forage and grain feeding. *Journal of Food Science, 52*, 245-251.

Lawrie, R. A. (1998). Lawrie's meat science (6th Ed). In: Woodhead Publishing Ltd.: England.

Ledward, D. A. (1985). Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science, 15*, 149-171.

Lee, Y. B. (1983). A modified sample preparation method for measuring meat tenderness by the Kramer shear press. *Journal of Food Science, 48*, 304.

Lewis, D. A. & Luh, B. S. (1988). Application of actinidin from kiwifruit to meat tenderization and characterization of beef muscle protein hydrolysis. *Journal of Food Biochemistry, 12*(3), 147-158.

Li, C. B., Zhou, G. H. & Xu, X. L. (2007). Comparisions of meat quality characteristics and intramuscular connective tissue between beef longissimus dorsi and semitendinosus muscles from Chinese yellow bulls. *Journal of Muscle Foods, 18*(2), 143-161.

Light, N., Champion, A. E., Voyle, C. & Bailey, A. J. (1985). The role of epimysical, perimysial and endomysial collagen in determining texture in six bovine muscles. *Meat Science, 13*(3), 137-149.

Livingston, D. J., McLachlan, S. J., La Mar, G. N. & Brown, W. D. (1985). Myoglobin: cytochrome b_5 interactions and the kinetic mechanism of metmyoglobin reductase. *Journal of Biological Chemistry, 260*, 15699-15707.

Machiels, D., Istasse, L. & Van Ruth, S. M. (2004). Gas chromatography-olfactometry analysis of beef meat originating from differently fed Belgiam Blue, Limousin and Aberdeen Angus bulls. *Food Chemistry, 86*, 377-383.

MacLeod, G. & Seyyedain-Ardebili, M. (1981). Natural and simulated meat flavours (with particular reference to beef). *CRC Critical Reviews in Food Science and Nutrition, 14*, 309-437.

Madruga, M. S. & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooking meat system. *Journal of the Science of Food and Agriculture, 68*(3), 305-310.

Marco, A., Navarro, J. L. & Flores, M. (2004). Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chemistry, 84*, 633-641.

Martin, L., Timon, M. L., Petron, M. J., Ventanas, J. & Antequera, T. (2000). Evolution of volatile aldehydes in Iberian Ham matured under different processing condition. *Meat Science, 54*, 333-337.

McBride, M. A. & Parrish, J. F. C. (1977). The 30,000-dalton component of tender bovine longissimus muscle. *Journal of Food Science, 42*, 1627-1629.

McDonagh, M. B., Fernandez, C. & Oddy, V. H. (1999). Hind-limb protein metabolism and calpain system activity influence post-mortem change in meat quality in lamb. *Meat Science, 52*(1), 9-18.

McDowall, M. A. (1970). Anionic proteinase from actinidin chinensis. *European Journal of Biochemistry, 14*, 214-221.

McGeehin, B., Sheridan, J. J. & Butler, F. (2001). Factors affecting the pH decline in lamb after slaughter. *Meat Science, 58*, 79-84.

Mikami, M., Whiting, A. M., Taylor, M. A. J., Maciewicz, R. A. & Etherington, D. J. (1987). Degradation of myofibrils from rabbit, chicken and beef by cathepsin I and lysosomal lysates. *Meat Science, 21*(2), 81-97.

Miller, B. (1992). Understanding customers. *Beef Today, 8*, 40.

Miller, M. F., Carr, M. F., Ramsey, C. B., Crockett, K. L. & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science, 79*, 3062-3068.

Moody, W. G. (1983). Beef flavour - a review. *Food Technology, 37*(5), 227-232 238.

Morcuende, D., Estévez, M., Ruiz, J. & Cava, R. (2003). Oxidative and lipolytic deterioration of different muscles from free-range reared Iberian pigs under refrigerated storage. *Meat Science, 65*, 1157-1164.

Morgan, J. B., Miller, R. K., Mendez, F. M., Hale, D. S. & Savell, J. W. (1991). Using calcium chloride injection to improve tenderness of beef from mature cows. *Journal of Animal Science, 69*, 4469-4476.

Morton, J. D., Bickerstaffe, R., Kent, M. P., Dransfield, E. & Keeley, G. M. (1999). Calpain-calpastatin and toughness in M. longissimus from electrically stimulated lamb and beef carcasses. *Meat Science, 52*(1), 71-79.

Mottram, D. S. (1994). Meat flavour. In J. R. Piggott & A. Paterson (Eds.), *Understanding natural flavours* (pp. 140-163): Glasgow: Chapman & Hall.

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

Murachi, T., Tanaka, K., Hatanaka, M. & Murakami, T. (1981). Intracellular Ca2+-dependent protease (calpain) and its high-molecular-weight endogenous inhibitor (calpastatin). In G. Weber (Ed.), *Advances in Enzyme Regulation, Vol. 19* (pp. 407-424): Pergamon Press: New York.

Muriel, E., Antequera, T., Petrón, M. J., Andrés, A. I. & Ruiz, J. (2004). Volatile compounds in Iberian dry-cured loin. *Meat Science, 68*, 391-400.

Murphy, M. A. & Zerby, H. N. (2004). Prerigor infusion of lamb with sodium chloride, phosphate, and dextrose solutions to improve tenderness. *Meat Science, 66*, 343-349.

Nakamura, M., Inomata, M., Imajoh, S., Suzuki, K. & Kawashima, S. (1989). Fragmentation of an endogenous inhibitor upon complex formation with high- and low-Ca^{2+}-requiring forms of calcium-activated neutral proteases. *Biochemistry, 28*, 449-455.

Nam, K. C. & Ahn, D. U. (2003). Combination of aerobic and vacuum packaging to control lipid oxidation and off-odour volatiles of irradiated raw turkey breast. *Meat Science, 63*, 389-395.

Newbold, R. P. & Small, L. M. (1985). Electrical stimulation of post-mortem glycolysis in the Semitendinosus muscle of sheep. *Meat Science, 12*(1), 1-16.

Nishimura, T., Hattori, A. & Takahashi, K. (1995). Structural weakening of intramuscular connective tissue during conditioning beef. *Meat Science, 39*, 127-133.

Nishiyama, I., Yamashita, Y., Yamanaka, M., Shimohashi, A., Fukuda, T. & Oota, T. (2004). Varietal difference in vitamin C content in the fruit of kiwifruit and other Actinidia species. *Journal of Agricultural and Food Chemistry, 52*(17), 5472-5475.

O'Halloran, G. R., Troy, D. J. & Buckley, D. J. (1997). The relationship between early post-mortem pH and the tenderisation of beef muscles. *Meat Science, 45*(2), 239-251.

Offer, G. & Trinick, J. (1983). On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Science, 8*, 245-281.

Okayama, T., Imai, T. & Yamanoue, M. (1987). Effect of ascorbic acid and alpha-tocopherol on storage stability of beef steaks. *Meat Science, 21*, 267-273.

Olson, D. G., Parrish, F. C., Dayton, W. R. & Goll, D. E. (1977). Effect of post-mortem storage and calcium activated factor on myofibrillar proteins of bovine skeletal muscle. *Journal of Food Science, 42*, 117-124.

Palamand, S. R. & Dieckmann, R. H. (1974). Autoxidation of n-hexanal. Identification and flavour properties of some products of autoxidation. *Journal of Agricultural and Food Chemistry, 22*, 503-506.

Paleari, M. A., Moretti, V. M., Beretta, G. & Caprino, F. (2006). Characterization of a lamb ham: fatty acids and volatile compounds composition. *Journal of Muscle Foods, 17*, 398-412.

Peterson, R. J., Izzo, H. J., Jungermann, E. & Chang, S. S. (1975). Changes in volatile flavour compounds during the retorting of canned beef stew. *Journal of Food Science, 40*, 948-954.

Pittet, A. O. & Hruza, D. E. (1974). Comparative study of flavour properties of thiazole derivatives. *Journal of Agricultural and Food Chemistry, 22*, 264-269.

Polidori, P., Trabalza-Marinucci, M., Fantuz, F. & Polidori, F. (2001). Post mortem proteolysis and tenderization of beef muscle through infusion of calcium chloride. *Animal Research, 50*, 223-226.

Polidori, P., Trabalza, M., Fantuz, F., Renieri, C. & Polidori, F. (2000). Tenderization of wether lambs meat through pre-rigor infusion of calcium ions. *Meat Science, 55*, 197-200.

Pommier, S. A., Postes, L. M. & Butler, G. (1987). Effect of low voltage electrical stimulation on the distribution of cathepsin D and the palatability of the longissimus dorsi from Holstein vel calves fed a corn or barley diet. *Meat Science, 21*, 203-218.

Priolo, A., Cornua, A., Prachea, S., Krogmanna, M., Kondjoyanb, N., Micol, D. & Berdague, J. L. (2004). Fat volatiles tracers of grass feeding in sheep. *Meat Science, 66*, 475-481.

Priolo, A., Micola, D. & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research, 50*, 185-200.

Raes, K., Balcaen, A., Dirinck, P., De Winne, A., Claeys, E. & Demeyer, D. (2003). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Science, 65*(4), 1237-1246.

Rees, M. P., Trout, G. R. & Warner, R. D. (2002). Effect of calcium infusion on tenderness and ageing rate of pork m. longissimus thoracis et lumborum after accelerated boning. *Meat Science, 61*, 169-179.

Rochat, S. & Chaintreau, A. (2005). Carbonyl odorants contributing to the in-oven roast beef top note. *Journal of Agricultural and Food Chemistry, 53*(24), 9578-9585.

Ross, C. F. & Smith, D. M. (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. *Comprehensive Reviews in Food Science and Food Safety, 5*(1), 18-25.

Sale, P. R. (1983). Kiwifruit culture. In D. A. Williams (Ed.). Wellington: P. D. Hasselberg.

Samejima, K., Choei, L. S., Isioroshi, M. & Hayakawa, T. (1991). Hydrolysis of muscle proteins by actinidin. *Journal of the Japanese Society of Food Science and Technology, 38*(9), 817-821.

Sebastiàn, I., Viallon, C., Berge, P., Dransfield, E. & Berdague, J. L. (2003). Analysis of the volatile fraction and the flavour characteristics of lamb: relationship with the type of feeding. *Sciences des Aliments, 23*, 497-511.

Shackelford, S. D., Wheeler, T. L., Meade, M. K., Reagan, J. O., Byrnes, B. L. & Koohmaraie, M. (2001). Consumer impressions of Tender Select beef. *Journal of Animal Science, 79*, 2605-2614.

Shahidi, F. (1998). Sheep meat odour and flavour. In O. A. Young & T. J. Braggins (Eds.), *In flavour of meat, meat products and seafoods* (pp. 101-130). London: Chapman & Hall: U. K.

Shahidi, F. & Pegg, R. B. (1994). Hexanal as an indicator of meat flavour deterioration. *Journal of Food Lipids, 1*, 177-186.

Shahidi, F. & Spurvey, S. A. (1996). Oxidative stability of fresh and heat-processed dark and light muscles of mackerel. *Journal of Food Lipids, 3*, 13-25.

Shahidi, F., Yun, J., Rubin, L. J. & Wood, D. F. (1987). The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. *Canadian Institute of Food Science and Technology Journal, 20*, 104-106.

Shivas, S. D., Kropf, D. H., Hunt, M. C., Kastner, C. L., Kendall, J. L. A. & Dayton, A. D. (1984). Effects of ascorbic acid on display life of ground beef. *Journal of Food Protection, 47*, 11-15.

Sink, J. D. (1979). Symposium on meat flavour factors influencing the flavour of muscle foods. *Journal of Food Science, 44*(1), 1-5.

Sinku, R. P., Prasad, R. L., Pal, A. K. & Jadhao, S. B. (2003). Effect of plant proteolytic enzyme on the physical-chemical properties and lipid profile of meat from culled, desi and broiler chicken. *Asian-Australasian Journal of Animal Sciences, 16*(6), 884-888.

Sorimachi, H., Imajoh-Ohmi, S., Emori, Y., Kwasaki, H., Ohno, S., Minami, Y. & Suzuki, K. (1989). Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and u-types. Specific expression of the mRNA in skeletal muscle. *Journal of Biological Chemistry, 264*, 20106-20111.

St. Angelo, A. J., Koohmaraie, M., Crippen, K. L. & Crouse, J. (1991). Acceleration of tenderization/inhibition of warmed-over flavour by calcium chloride-antioxidant infusion into lamb carcasses. *Journal of Food Science, 56*, 359-362.

Stanke, L. H. (1994). Aroma components from fermented dried sausages fermented with staphylococcus xylosus. *Meat Science, 38*, 39-53.

Stanton, C. & Light, N. (1990). The effects of conditioning on meat collagen: Part 4 - The use of pre-rigor lactic acid injection to accelerate conditioning in bovine meat. *Meat Science, 27*, 141-159.

Sucan, M. K., Byerly, E. A., Grun, I. U., Fernando, L. N. & Trivedi, N. B. (2002). Effects of enzyme hydrolysis and thermal treatment on bioactive flavour compounds in pork-based flavouring ingredients. *Bioactive Compounds in Foods Acs Symposium Series, 816*, 187-205.

Sugiyama, S., Hirota, A., Okada, C., Yorita, T., Sato, K. & Ohtsuki, K. (2005). Effect of kiwifruit juice on beef collagen. *Journal of Nutritional Science and Vitaminology, 51*(1), 27-33.

Sutherland, M. M. & Ames, J. M. (1996). Free fatty acid composition of the adipose tissue of intact and castrated lambs slaughtered at 12 and 30 weeks of age. *Journal of Agricultural and Food Chemistry, 44*(10), 3113-3116.

Swan, J. E. (1993). pH variability in muscle: a literature review. Meat Industry Research Institute of New Zealand, Hamilton, NZ. MIRINZ publication No. 925 1-40.

Tarrant, P. V. & Mothersill, C. (1977). Glycolysis and associated changes in beef carcasses. *Journal of the Science of Food and Agriculture, 28*(8), 739-749.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M. & Goll, D. E. (1995). Is Z-disk degradation responsible for post-mortem tenderization? *Journal of Animal Science, 73*, 1351-1367.

Teixeira, A., Batista, S., Delfa, R. & Cadavez, V. (2005). Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. *Meat Science, 71*, 530-536.

Thompson, V. F., Saldana, S., Cong, J. & Coll, D. E. (2000). A bodipy fluorescent microplate assay for measuring activity of calpains and other proteases. *Analytical Biochemistry, 279*, 170-178.

Toldra, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science, 49*, 101-110.

Tornberg, E., Olsson, A. & Persson, K. (1990). The structural and interfacial properties of food proteins in relation to their function in emulsions. In K. Larsson & S. E. Friberg (Eds.), *Food emulsions* (pp. 247). New York: Marcel Dekker, Inc.

Van Laack, R. L. J. M., Kauffman, R. G. & Greaser, M. L. (2001). Determinants of ultimate pH of meat. In Proceeding of 47th International Congress of Meat Science and Technology, Warsaw, Poland. 22-26.

Vasta, V. & Priolo, A. (2005). Ruminant fat volatiles as affected by diet. A review. *Meat Science, 55*, 149-159.

Veiseth, E., Shackelford, S. D., Wheeler, T. L. & Koohmaraie, M. (2001). Effect of post-mortem storage on μ-calpain and m-calpain in ovine skeletal muscle. *Journal of Animal Science, 79*, 1502-1508.

Wada, M., Hosaka, M., Nakazawa, R., Kobayashi, Y. & Hasegawa, T. (2004). The solubilization of unheated cattle achilles tendon with actinidin under neutral and acidic conditions. *Food Science and Technology Research, 10*(1), 35-37.

Wada, M., Suzuki, T., Yaguti, Y. & Hasegawa, T. (2002). The effects of pressure treatments with kiwi fruit protease on adult cattle semitendinosus muscle. *Food Chemistry, 78*, 167-171.

Wang, Y., McGinnis, D. S., Segado, R. R. & Jones, S. D. M. (1995). Vascular infusion of beef carcasses: Effects on chilling efficiency and weight changes. *Food Research International, 28*(4), 425-430.

Warriss, P. D. (2000). *Meat Science: an introductory text*: CABI Publishing: UK.

Wasserman, A. E. (1979). Symposium on meat flavour chemical basis for meat flavour: A review. *Journal of Food Science, 44*(1), 6-11.

Watanabe, K. & Sato, Y. (1971). Gas chromatography and mass spectral analysis of heated flavour compounds of beef fats. *Agricultural and Biological Chemistry, 35*, 736-739.

Waylan, A. T., O'Quinn, P. R., Unruh, J. A., Nelssen, J. L., Goodband, R. D., Woodworth, J. C., Tokach, M. D. & Koo, S. I. (2002). Effects of modified tall oil and vitamin E on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. *Journal of Animal Science, 80*(6), 1575-1585.

Wettasinghe, M., Vasanthan, T., Temelli, F. & Swallow, K. (2001). Volatile flavour composition of cooked by-product blends of chicken, beef and pork: a quantitative GC-MS investigation. *Food Research International, 34*, 149-158.

Wheeler, T. L. & Koohmaraie, M. (1999). The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. *Journal of Animal Science, 77*(9), 2444-2451.

Wheeler, T. L., Koohmaraie, M., Lansdell, J. L., Sinagusa, G. R. & Miller, M. F. (1993). Effects of post-mortem injection time, injection laeve and concentration of calcium chloride on beef quality traits. *Journal of Animal Science, 71*, 2965-2974.

Wheeler, T. L., Koohmaraie, M. & Shackelford, S. D. (1996). Effect of vitamin C concentration and co-injection with calcium chloride on beef retails display colour. *Journal of Animal Science, 74*(8), 1846-1853.

Whipple, G., Koohmaraie, M., Dikeman, M. E., Crouse, J. D., Hunt, M. C. & Klemm, R. D. (1990). Evaluation of attributes that affect longissimus muscle tenderness in Bos taurus and Bos indicus cattle. *Journal of Animal Science, 68*, 2716-2728.

Whittaker, J. R. (1994). *Principles of enzymology for the food science* (2nd ed.): Marcel Dekker Inc. New York.

Winkler, J., Lunsdorf, H. & Jockusch, B. M. (1996). The ultrastructure of chicken gizzard vinculin as visualized by high-resolution electron microscopy. *Journal of Structural Biology, 116*(2), 270-277.

Wong, E., Nixon, L. N. & Johnson, C. B. (1975). Volatile medium chain fatty acids and mutton flavour. *Journal of Agricultural and Food Chemistry, 23*(3), 495-498.

Wu, F. Y. & Smith, S. B. (1987). Ionic strength and myofibrillar protein solubilization. *Journal of Animal Science, 65*, 597-608.

Yancey, E. J., Dikeman, M. E., Addis, P. B., Katsanidis, E. & Pullen, M. (2002a). Effects of vascular infusion with a solution of saccharides, sodium chloride, and phosphates with or without vitamin C on carcass traits, warner-bratzler shear force, flavor-profile, and descriptive-attribute characteristics of steaks and ground beef from Charolais cattle. *Meat Science, 60*, 341-347.

Yancey, E. J., Dikeman, M. E., Addis, P. B., Katsanidis, E. & Pullen, M. (2002b). Effects of vascular infusion with a solution of saccharides; sodium chloride; phosphates; and vitamins C, E, or both on carcass traits, Warner-Bratzler shear force, and palatability traits of steaks and ground beef. *Journal of Animal Science, 80*, 1904-1910.

Yancey, E. J., Dobbels, T. E., Dikeman, M. E. & Katsanidis, E. (1999). Effects of vascular infusion of cattle after exsanguination with a solution of saccharides, sodium cholride, and phosphates, with or without vitamin C, on carcass and meat palatability traits. *Journal of Animal Science, 77 (Supplement 1)*, 47.

Yancey, E. J., Hunt, M. C., Dikeman, M. E., Addis, P. B. & Katsanidis, E. (2001). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, phosphates, and vitamins C, E, or C+E on meat display-colour stability. *Journal of Animal Science, 79*(2619-2626).

Young, O. A., Berdague, J. L., Viallon, C., RoussetAkrim, S. & Theriez, M. (1997). Fat-borne volatiles and sheep meat odour. *Meat Science, 45*(2), 183-200.

Young, O. A., Lane, G. A., Priolo, A. & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture, 83*(2), 93-104.

Young, O. A. & West, J. (2001). Meat colour. In Y. H. Hui, W. K. Nip, R. W. Rogers & O. A. Young (Eds.), *Meat Science and Application*. New York: Marcel Dekker, Inc.

Zerby, H. N., Belk, K. E., Ahola, J. K., Sofos, J. N., Schaefer, D. M., Morgan, J. B. & Smith, G. C. (1999). Effects of muscle α-tocopherol level and surface microbiological contamination on retail case life of fresh beef from the US, Japan, and Australia. *Meat Science, 52*, 111-118.

Publications/posters arising from this thesis

1*. Han, J., Bekhit, A. E. D., Morton, J. D., Sherlock, R. & Breitmeyer, J. (2007). Analysis of volatile compounds in lamb muscles infused with kiwifruit juice. The proceeding of the 53rd International Conference of Meat Science and Technology (ICOMST), Beijing, China.

2*. Bekhit, A. E. D., Han, J., Morton, J. D. & Sedcole, J. R. (2007). Effect of kiwifruit juice and water pre-rigor infusion on lamb quality. The proceeding of the 53rd International Conference of Meat Science and Technology (ICOMST), Beijing, China.

* Two posters from these two papers were also displayed during the 53rd International Conference of Meat Science and Technology (ICOMST), Beijing, China.