Chapter 1.

GENERAL INTRODUCTION

Consumers are the final arbiters of beef eating quality and it is therefore paramount to produce beef which has consistent eating quality attributes and which meets, or better still, exceeds their expectations. In 1994, Sensory Market Analysis and Research Technology (SMART) conducted a large consumer study which identified that eating quality was the single most important factor contributing to the repurchase intent of consumers. When consumers eat cooked beef, they tend to form an overall perception of the eating quality which in essence determines their overall acceptance of the product (SMART, 1994).

In order to provide consumers with beef of desirable eating quality, it is essential to identify the attributes which are important. Substantial consumer research has identified three; tenderness, juiciness and flavour. Of these, tenderness has emerged as by far the most important (Gerrard, 1971; Bouton and Harris, 1972b, Brady and Hunecke, 1985; Dikeman, 1990; Monin and Ouali, 1991; SMART, 1994). Whilst this result does not mean that the juiciness and flavour can be ignored, the overriding importance of tenderness has been highlighted by the fact that 77% of Australian consumers surveyed would be prepared to buy more beef if it were consistently more tender (SMART, 1994).

Producing consistently tender beef is difficult because the mechanisms and factors which influence tenderness are numerous. These factors include breed, sex, finishing regime, age, carcase fatness and weight, marbling, post-slaughter chilling regime, electrical stimulation, ageing and cooking. Meat scientists are able to conduct experiments in which such factors are manipulated and hence, investigate their effects on eating quality attributes. This provides a number of options for the evaluation of cooked meat. Ultimately consumer evaluation is desirable, but humans are notoriously unreliable as measuring devices, being subject to an endless array of distractions, emotions, illness and sensitivities (Gillmore and Prescott, 1993). Consumer evaluations require large numbers of consumers and are expensive and time consuming.

In the laboratory, a number of devices have been developed to provide estimates of tenderness. However, despite the benefits of these measurements in cost, speed, ease and objectivity, the sensory evaluation of meat as interpreted by humans remains too complex for any device to interpret (Gillmore and Prescott, 1993). Therefore, the use of trained taste panels has become common. Trained taste panels have been reported to have greater acuity and sensitivity than consumers (eg. Bennett *et al.*, 1956). Given this, the effect of selection and training on panel sensory evaluations should be investigated. In particular, evaluations made by the panel should be compared to sensory evaluations made by consumers. It is also important to note, that the choice of method of evaluation to be employed is dependent on the specific question posed by the researcher.

In Australia, which is currently attempting to develop grading systems for the beef industry, two highly contentious issues are the effects of breed and intramuscular fat (marbling) on the eating quality of beef. The influence of *Bos indicus* content on eating quality remains equivocal. However, an industry bias against *Bos indicus* and *Bos indicus* cross cattle because of the perception that they produce tough meat is causing restricted use of such carcases in certain grading schemes (eg. McKinna *et al.*, 1995) and supermarket chains. Research has indicated that there is potential for *Bos indicus* content to interact with post-mortem treatments such as electrical stimulation and ageing (Johnson *et al.*, 1990a; Whipple *et al.*, 1990a; Wheeler *et al.*, 1990a,b). However, this potential interaction and consequent effects on consumer evaluations of beef tenderness remains unclear.

The influence of intramuscular fat, or marbling, on eating quality is the other current issue. There is debate on this issue, because on one hand, consumers actively discriminate against marbled meat (Hearnshaw *et al.*, 1992), primarily a result of perceived effects on health. However, on the other side, there is research which has shown increased marbling results in increased eating quality, and in particular, increased marbling protecting against the detrimental effects that overcooking has on tenderness and juiciness (Smith and Carpenter, 1974). Support for the theory that increased intramuscular fat acts as insurance in maintaining the eating quality of well cooked meat remains minimal. It is also not understood whether the suggested interaction between intramuscular fat and degree of doneness is dependent on the cooking method employed.

When research is conducted to study eating quality, the cooking method to be used requires great consideration. Cooking methods such as use of a waterbath are often preferable in

terms of experimental control, but are not typically used by consumers. Consumers would be more likely to grill or fry beef steaks, but the use of these methods results in a decrease in the experimental control and increases the possibility of variation in the cooking procedure confounding eating quality results. This decision would be clearer if the effects of such cooking methods on tenderness and juiciness were identified.

Traditionally, high quality (low connective tissue) cuts of beef have been cooked at high temperatures for relatively short periods of time. However, there is some evidence which suggests that a longer cooking time at lower temperatures can be beneficial to tenderness and juiciness (Bramblett and Vail, 1964; Laakkonen *et al.,* 1970a). The potential for this theory to be applied for the cooking of steaks is not known.

Another decision that must be made by researchers investigating eating quality attributes is how well to cook the meat (the degree of doneness). Usually meat samples are cooked to one degree of doneness determined either by internal temperature or by cooking for a set time period. Consequently, not all consumers may be satisfied, as the meat is not cooked the way they prefer it cooked. For consumer evaluations, a less common option is to cook samples to the degree of doneness requested by individual consumers. This introduces the possibility of degree of doneness confounding results, but the benefits may outweigh this. The importance of preferred degree of doneness on consumer satisfaction and evaluations of eating quality has recently been documented. Work by Cox *et al.* (1997) showed that consumer tenderness evaluations decreased significantly if the steak they received was not cooked to the degree of doneness which they ordered. The effect was worse for steaks which had been overcooked. This finding highlights the importance of delivering meat to the degree of doneness requested by consumers. However, this is difficult for two reasons. First, individual perceptions of the degree of doneness vary enormously. Secondly, there is no method for accurately evaluating the degree of doneness (by internal colour) during the cooking process. Given the importance of degree of doneness on consumer satisfaction, it may become imperative to be able to determine the colour of cooked beef prior to the consumer cutting the steak, rather then the current system of relying solely on the performance of the chef.

The work reported in this thesis was designed to investigate some of the currently contentious issues which influence the sensory evaluation and consumer acceptability of beef. The specific areas of investigation are outlined below.

A taste panel trained for sensory evaluation of beef tenderness and juiciness was developed at UNE. Chapter 3 investigates the relationship between sensory evaluations made by untrained consumers and the taste panel both before and after training of the panel. Consequently, the effect of training on panellists' sensory evaluations could be determined. It was considered important to identify how the taste panel evaluated samples compared with consumers, because the trained taste panel was used in experiments conducted for this thesis and for other studies conducted by the department.

Chapter 4 examines the effect of different cooking methods and the rate of cooking on tenderness and juiciness. The laboratory waterbath method, preferred in some laboratories, was compared with cooking on a hot plate and with a vertical grill. In addition, the samples cooked on the hot plate and using the vertical grill were cooked at two temperatures. A high temperature (more traditionally used) and a low temperature were compared because the latter has shown to beneficial in terms of tenderness and juiciness.

The effects of cooking in the waterbath or vertical grill on tenderness and juiciness were further investigated in Chapter 5. In this study, meat which varied in the amount of intramuscular fat was cooked by these two methods to both rare and well done. This allowed the effects on tenderness and juiciness of intramuscular fat, cooking method and degree of doneness and their interactions to be determined.

Chapter 6 reports the results of a large consumer study designed to examine the effect of Brahman content, sex and finishing regime and their interaction with post-mortem treatments of electrical stimulation and ageing on tenderness. The electrical stimulation and ageing treatments were applied to each carcase which allowed these effects to be evaluated within animal, rather than including a between animal effect. This study also enabled the effect of Brahman content to be compared to the effects of electrical stimulation and ageing.

Chapter 2.

LITERATURE REVIEW

2.1 Muscle structure

Muscle comprises essentially two structural components which influence its palatability attributes. The physical components of the muscle are the meat fibres (myofibrils) and the connective tissue matrix, both of which contribute in a different manner to the ultimate tenderness, and to a lesser extent, juiciness. The myofibre structure is responsible for the contraction of muscle while the connective tissue performs the dual functions of holding the muscle fibres together and attaching them to the skeletal framework (Harris and Shorthose, 1988).

Lawrie (1991) stated that the degree of tenderness can actually be related to three categories of protein in the muscle: 1) myofibrillar; 2) connective tissue and; 3) sarcoplasmic proteins. The importance of their relative contributions depends on circumstances such as degree of myofibrillar contraction, type of muscle and the nature of cooking. Although sarcoplasmic proteins are soluble and may seem that they could not contribute to tenderness, Lawrie (1991) provided evidence which indicated that their contribution cannot be entirely dismissed. However, the majority of tenderness research has focused on the myofibrillar and connective tissue structures and this review will basically consider these two components also.

2.1.1 Muscle fibres

The basic structural unit of the muscle is the muscle fibre (Lawrie, 1991). Each fibre is an independent structure which varies in length (Pearson and Young, 1989) and comprises a multinucleated cell composed of smaller subunits known as myofibrils. Purslow (1991) stated that each muscle fibre contains about 1,000 myofibrils. The regular striated appearance of the myofibril structures creates a regularly repeating structural unit along their length referred to as

sarcomeres. The sarcomere comprises the length of the fibre between two adjoining Z bands in the same muscle fibre (see figure 2.1.1) (Pearson and Young, 1989).

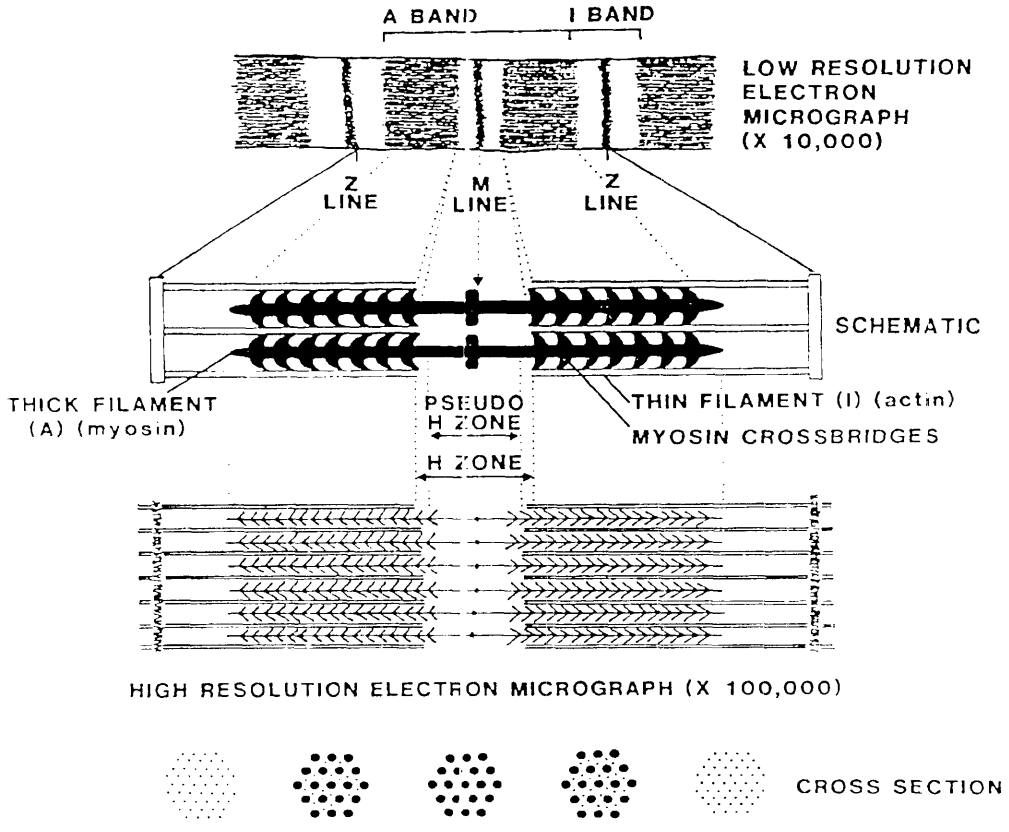

Figure 2.1.1 Fine structure of a single sarcomere along with portions of two adjacent sarcomeres. From Pearson and Young (1989).

Upon magnification of myofibrils it is evident that there are two types of filaments present, thick and thin. The thick filaments are also referred to as myosin filaments as they contain mainly myosin proteins. The thin filaments are known as actin filaments as they are composed primarily of actin proteins (Forrest *et al.*, 1975). The major function of the actin and myosin proteins is their role in muscle contraction.

In relaxed muscle, myosin and actin exist in an extremely weak binding state. However, during contraction the cross bridges on the myosin filament heads become engaged with a specific site for interaction on the thin filaments and result in the formation of actomyosin (Pearson and Young, 1989). Actomyosin formation results in a rigid and relatively inextensible

condition in the contractile muscle which is then broken during muscle relaxation. Other proteins in the actin filaments are troponmyosin and troponin which bind to each other and their major function is to regulate contraction by regulating the interaction between actin and myosin through the mediation of Ca^{2+} (Pearson and Young, 1989). Apart from inherent characteristics, the length of the sarcomere in any particular myofibre will depend on the state of contraction in the muscle. Pearson and Young (1989) and Lawrie (1991) provide detailed descriptions of the myofibrillar structure and the proteins within the structures.

2.1.2 Connective tissue

The connective tissue within muscle exists in a variety of forms which all differ in their native properties. Essentially three connective tissue sub-classes have been identified and detailed discussion is provided elsewhere (eg. Bailey, 1984, 1988).

Surrounding the muscle as a whole is the connective tissue sheath known as the epimysium, which is continuous with the tendon (Purslow, 1991). From the inner surface of the epimysium, septa of connective tissue penetrates into the muscle separating the muscle fibres into bundles and this is known as the perimysium (Lawrie, 1991). From the perimysium, a fine connective tissue framework passes further inwards to surround each muscle fibre and this is referred to as the endomysium (Lawrie, 1991). Underneath the endomysium is the sarcolemma, the membrane of the muscle cell.

The relative proportions of connective tissue varies between muscles and the amount of connective tissue usually parallels its physical activity (Forrest *et al.*, 1975). Muscles with a high connective tissue content will be tougher than muscles with a low connective tissue content and this decline in quality is reflected in a decrease in the price of beef cuts. The major component and principal structural protein of connective tissue is collagen. Other minor proteins include elastin, reticulum, large proteoglycans and specialised glycoproteins (Bailey, 1988). Although collagen fibres are relatively inextensible, the arrangement of connective tissue fibres within the muscle network must be such as to accommodate changes in muscle dimensions (Shorthose and Harris, 1991).

The intermolecular crosslinks within collagen fibres are important in terms of tenderness because upon heating the properties and extent of crosslinks determine the tensile strength of the

collagen fibre (Bailey, 1984). The collagen crosslinks can be formed by two different pathways, known as the allysine and hydroxyallysine pathways (Kurth, 1993). In simplistic terms, crosslinks formed from the allysine pathway are referred to as immature crosslinks or bonds. Those formed by the hydroxyallysine pathway are referred to as mature crosslinks. It is the relative proportions of the immature and mature crosslinks which confer the largest effects on collagen related toughness.

The immature crosslinks have been demonstrated to be reducible and heat and acid labile (Lawrie, 1991). This solubility allows the connective tissue to break down upon heating (for long periods above *ca.* 60-65°C). The collagen is converted to gelatin which results in increased tenderness. However, mature crosslinks have been shown to be resistant to heat due to the formation of non reducible links (Lawrie, 1991). This heat stability, or maturation is achieved by further reaction of the intermediate crosslinks to form multivalent crosslinks which crosslink several molecules (Bailey, 1988). This results in greater tension and a marked increase in meat toughness due to the increased thermal and mechanical stabilities of the collagen. Unlike the immature crosslinks, these mature crosslinks can not be denatured with heating, so there is no way of avoiding toughness caused by this type of chemical bonding.

2.1.3 The conversion of muscle to meat

The initial step in the conversion of muscle to meat is the slaughter and ex-sanguination of the animal. The cessation of blood circulation around the body causes numerous changes in the muscular tissue. The first of these is the removal of the oxygen supply to muscles. Consequently, there is a fall in the oxidation - reduction potential in the muscle and the reactions within the muscle become anaerobic.

Geesink (1993) outlined two phases which occur during the conversion of muscle to meat. First, immediately post-slaughter, the energy rich compounds are almost completely depleted. The second phase involves the gradual improvement in tenderness during meat storage (post-mortem), known as ageing. The mechanisms involved in the ageing process and its effect on tenderness are discussed in section 2.4.2.2.

In the first phase, creatine-phosphate is used to convert ADP to ATP. This occurs without any reconversion of ATP to ADP as with aerobic metabolism. Consequently, the ATP level falls which initiates the anaerobic conversion of glycogen to lactate. As this reaction

proceeds, the concentration of lactic acid increases which in turn results in a pH fall from about 7.2 to 5.5-5.4 (in normal muscle). The conversion of glycogen to lactic acid will continue until a pH is reached when the enzymes effecting the breakdown become inactive (Lawrie, 1991). The fall in pH will also be dependent on intrinsic and extrinsic factors such as the amount of glycogen in the muscle prior to slaughter for conversion to lactic acid and the rate of carcase cooling. The final pH attained is referred to as the ultimate pH.

As post-mortem glycolysis proceeds, the muscle becomes inextensible and this stiffening has long been referred to as rigor mortis (Lawrie, 1991). The onset of rigor mortis occurs as ATP is depleted from the muscle. In the absence of ATP, actin and myosin combine to form rigid chains of actomyosin. Lawrie (1991) contended that the fall of ATP during the onset of rigor mortis is believed to result in some leakage of Ca^{2+} ions back into the sarcoplasm, whereby the contractile system is stimulated. In relaxed aerobic muscle, the availability of ATP is responsible for stopping the interaction between actin and myosin (Jolley *et al.*, 1982). The toughness of muscles increases as they enter rigor and reaches a maximum at about 24 hours post mortem (Dransfield and Rhodes, 1975).

The rate of post mortem glycolysis and pH decline have been shown to have large effects on meat quality and are influenced by the temperature at which carcases are chilled. Monin and Ouali (1991) stated that the rate of pH fall reflects the intensity of the immediate post mortem muscle metabolism. The temperature at which carcases are chilled can have a large effect on tenderness via its effect on the temperature at which carcases enter rigor mortis, which in turn affects the contractile state of the muscle fibres.

Hertzman *et al.* (1993) reported that muscles which entered rigor at high temperatures (above approximately 30°C) suffer heat shortening, causing significantly greater toughness compared with muscles entering rigor at 15°C.

Muscles entering rigor between about 20°C-28°C have been reported to be significantly more tender (and lower ultimate pH) compared with muscles entering rigor below about 5°C (Parrish *et al.*, 1973a; Koohmaraie *et al.*, 1988b; Whipple *et al.*, 1990b; Kerkeb and Lepitit, 1992). Koohmaraie (1992a) claimed that high temperatures (during the first 24 hours post-mortem) can produce equivalent to 86% of the tenderisation which occurs during ageing. According to Koohmaraie (1992a), about 50% of tenderisation within the first 24 hours after slaughter. Bruce and Ball (1990) believed the increased tenderness was due to increased proteolytic enzyme activity at the higher temperatures and slow pH decline.

If ATP depletion and actomyosin complex formation take place at normal chilling temperatures (15-20°C), the degree of muscle shortening is insignificant in terms of the resulting tenderness (Forrest *et al.*, 1975). In practice, rapid chilling of carcases is desirable so as to reduce carcase temperature quickly to effectively minimise carcase weight loss, restrict microbial growth and increase throughput. Consequently, many commercial abattoirs place carcases into chillers before rigor is completed and this can have a detrimental effect on tenderness via cold shortening.

Pearson and Young (1989) specified three factors are involved in cold shortening: 1) muscles are in pre rigor state with pH above *ca.* 6.0-6.2; 2) temperature is below approximately 10°C, and; 3) ATP is still available to allow muscle contraction. The combination of these conditions causes cold shortening to occur in muscles which means muscles will suffer intense interdigitation between the actin and myosin filaments forming the inextensible actomyosin and severe sarcomere shortening. The severe sarcomere shortening translates to a large increase in toughness and Marsh (1993) proposed that, if contraction approaches 35%, it is accompanied by a several fold increase in toughness. Cold shortening can be thought of as an irreversible muscle contraction, the effects of which cannot be improved by post mortem ageing (Dransfield, 1992).

One environmental factor which can influence the rate of temperature decline in a carcase is the amount of subcutaneous fat cover. This occurs because the fat acts as a layer of insulation against the temperature decline. Consequently, carcases which are leaner may be tougher simply because they suffered a greater degree of cold shortening. It is therefore imperative when conducting experiments on tenderness that confounding environmental influences such as this are avoided.

The increased toughness due to cold shortening has generally been attributed to increased myofibrillar toughness due to sarcomere shortening (eg. Marsh and Leet, 1966; Lee and Ashmore, 1985). However, other work (eg. Bouton *et al.*, 1973a; Harris, 1976) has demonstrated that changes in the connective tissue structure may also contribute to the toughness associated with cold shortening.

Cold shortening can be avoided by electrically stimulating carcases post-slaughter. Electrical stimulation causes the expeditious onset of rigor by speeding up the ATP turnover and glycolysis. Consequently, pH levels decline more rapidly and the carcase enters rigor within a relatively short time post slaughter. This means that carcases can be chilled normally without suffering any cold shortening as the pH is low and there is no energy left for muscle contraction.

During the course of rigor mortis the water holding capacity (WHC) of the meat is also affected. The WHC refers to the ability of the muscle structure to hold, or bind water such that it is retained within the meat. Differences in juiciness primarily relate to the ability of muscles to retain water (Judge *et al.*, 1989; Lawrie, 1991). The mechanisms and proteins involved in governing the WHC have been discussed by others (eg. Offer and Trinick, 1983; Offer and Knight, 1988; Pearson and Young, 1989; Lawrie, 1991). Offer and Trinick (1983) proposed that WHC was dependent on the swelling of myofibrils (as opposed to the protein binding suggested by others) which was associated with the contraction of the myofilament lattice. Consequently, the degree of fibre shortening which occurred during rigor would not only affect toughness, but also juiciness via its effect on the WHC of muscles.

Overall, the rate of glycolysis and pH and temperature decline are factors which can have a significant effect on meat tenderness. The most detrimental of these conditions is when cold shortening is induced as muscles enter rigor. High temperatures during rigor also appear detrimental to meat quality, but the likelihood of this in commercial practice is low. Muscles entering rigor between approximately 15°-20°C do not appear to suffer adversely and result in the most tender meat compared to other temperatures.

2.1.4 Raw beef colour

Although the colour of the raw beef does not influence tenderness or juiciness, it does influence the colour of cooked beef. The colour of cooked beef is very important in terms of consumer satisfaction and is discussed later (section 2.4.2.3.3). Therefore, a very brief outline of raw beef colour will be given here.

The colour of meat is essentially attributable to myoglobin which constitutes 80-90% of total pigment of muscle (Forrest *et al.*, 1975). However, the appearance of the meat surface depends on the quantity and chemical state of the pigments present and on the gross morphology of the muscle (Warner, 1989).

Lawrie (1991) described the structure of the myoglobin molecule as consisting of a haematin nucleus attached to a protein component of the globulin type. The haematin portion comprises a ring of four pyrrole nuclei coordinated with a central iron atom. The iron may exist in both reduced and oxidised forms and the ability to combine with oxygen is lost when the globin portion of the molecule is denatured as occurs with heat.

Myoglobin can exist in three forms, depending on it's oxidation state and this will have the major influence on the colour of raw meat. In its reduced form (ferrous), myoglobin is a purplish-red colour. Upon exposure to oxygen, such as with blooming, myoglobin is oxygenated (to the free binding site of the heme) to the bright red, oxymyoglobin form (Cross *et al.*, 1986). In raw meat, the undesirable brown colour is caused by the oxidation of the iron of the heme molecule from the ferrous to ferric state and the resulting compound is metmyoglobin. Forrest *et al.* (1975) stated that the brown colour of metmyoglobin is detectable when approximately 60% of myoglobin is in this metmyoglobin form. This undesirable metmyoglobin will persist when the meat has lost its reducing ability and the oxidised iron atom in the heme cannot be reduced (Cross *et al.*, 1986).

The appearance of meat colour will also be influenced by the pH of the meat. Warner (1989) explained that in meat of normal pH, muscle protein molecules tend to aggregate and the water between the molecules is squeezed out. White light reflections from between molecules are enhanced due to the high proportion of extracellular water, thus the cut surface is able to scatter light and has a bright appearance. However, in high pH meat (dark cutting meat), water is not squeezed out and the meat surface is likely to appear dark because the surface will scatter incident light less. Colour absorption is enhanced due to high proportion of intracellular water, thus giving a dull appearance.

When meat colour is assessed by humans, it is a matter of both visual perception and subjective evaluation. Consequently, assessments will vary considerably. The objective measurement of colour has been developed and devices such as the Minolta chroma meter for colour measurement have been developed. A description of colour, its measurements and the various colour spaces commonly used has been provided by Warner (1989) and Minolta (1994). A common system used for the measurement of meat colour is the L*, a*, b* system. This system measures colour as a combination of L* (lightness) values, a* (red/green) and b* (yellow/blue) values. Interpretation of colour requires the incorporation of all three values in a multivariate manner, they cannot be interpreted as three separate univariates.

2.2 Attributes influencing beef eating quality as measured by sensory evaluation

When consumers eat beef, they have an overall perception of the eating quality, or palatability of the beef. This overall perception determines their satisfaction and/or the desirability of repeating the experience. It is therefore important to maximise the eating quality experience for consumers. In order to do this, it is essential to identify and understand the attributes of beef which influence consumers' perceptions of eating quality.

There are three major attributes of beef which together comprise the eating quality evaluation; tenderness or texture, juiciness and flavour. It has long been established that of these three attributes, tenderness is by far the single most important attribute governing consumers' evaluation of eating quality (Gerrard, 1971; Bouton and Harris, 1972b; Brady and Hunecke, 1985; Dikeman, 1990; Monin and Ouali, 1991; SMART, 1994). Purslow (1991) stated that it is also possibly the least well understood and controlled parameter of meat quality.

This thesis is primarily focused on the tenderness attribute, however, juiciness is also an important component. Flavour is an extremely complex attribute and is beyond the scope of this thesis. Although not included in this thesis, its importance in terms of eating quality is by no means disregarded.

2.2.1 Tenderness

The terms tenderness and texture have been used synonymously although use of the term texture has usually implied greater complexity, and the definition of the term varies with its use. The evaluation of tenderness is primarily determined by two muscle components, the myofibrillar and connective tissue components. Harris (1976) also recognised that the physiological aspects of mastication must play some part in determining the human response and in affecting the consumers judgement of the toughness or tenderness of a piece of meat. Bailey (1964) provided a simplistic definition of tenderness as the psychological response to a physical-chemical stimuli caused by chewing.

The components of tenderness which can be considered in its evaluation during chewing include; the initial ease of penetration by the teeth on the first bite, the ease with which the meat breaks into meat fibres, the amount of resistance to chewing or the force and time required until meat is ready for swallowing (Cover *et al.*, 1962a,b,c,d; Harries *et al.*, 1972; Civille and Szczesniak, 1973; Dransfield *et al.*, 1984). Many consumer evaluations do not define tenderness, but simply allow it to be interpreted by individuals. Tenderness may be evaluated as a single attribute, or may be separated into categories such as myofibrillar tenderness, ease of fragmentation, connective tissue tenderness and overall tenderness. The specific method of evaluation will be dependent on the researcher.

The use of the term texture usually encompasses the myofibrillar and connective tissue components, but often includes characteristics which relate to the perceived muscle structure such as the coarseness, distribution and packing of the muscle fibre bundles and sometimes includes visual appraisal of the meat. Brandt *et al.* (1963) defined a texture profile as the organoleptic analysis of the meat texture complex of a food in terms of its mechanical, geometrical, fat and moisture characteristics, the degree of each present, and the order in which they appear from the first bite through complete mastication. Some characteristics used by previous workers (Brandt *et al.*, 1963; Brady and Hunecke, 1985) to evaluate texture include; chewiness, guminess, viscosity, grittiness, fibrousness, stringiness, strand separation, mealiness and coarseness.

A study by Harries *et al.* (1972) showed that when seven characteristics were used for texture evaluation, principal component analysis grouped them into two clusters. It was concluded that the two elements in the physiological appreciation of meat texture were measurable in terms of two attributes: 1) tenderness/toughness, and; 2) juiciness/dryness. Dransfield *et al.* (1984) also used principal component analysis and resolved the same two attributes after their panel originally evaluated nine characteristics.

The combination of the complexity and ambiguity of the term texture and the findings of two studies above led to the decision to use the single tenderness/toughness evaluation of this attribute in the experiments undertaken in this thesis.

2.2.2 Juiciness

In 1960, Weir (cited by Lawrie, 1985) outlined two organoleptic components in the juiciness of cooked meat ; 1) the release of water during the beginning of chewing, and 2) the more sustained release due to the stimulatory effect of fat on salivation. The latter of these would not appear to be an attribute of juiciness *per se*, rather a factor which influences consumer perception of juiciness. Judge *et al.* (1989) stated that the principal sources of juiciness, as detected by consumers, are intramuscular lipids and water. In combination with water, melted lipids constitute a broth that, when retained in meat, is released upon chewing and this broth may stimulate saliva flow and thus improve 'apparent' juiciness. Dikeman (1990) contended that the major contributor to sensory juiciness was the water remaining in the cooked meat.

Relatively less is known about juiciness than tenderness and the mechanisms involved in the regulation of juiciness. Monin and Ouali (1991) considered that the factors influencing water holding capacity would also affect juiciness to some extent. Water holding capacity is known to increase with increasing pH (eg. Bouton *et al.*, 1973a,b). However, Bouton *et al.* (1973a) also reported the water retained after cooking increased with pH, but the increase in expressible juice was small and not always significantly related to pH. They also reported panel juiciness scores showed no significant relationship with pH. Based on this work, it would appear that there are factors other than water holding capacity which influence juiciness. As the state of proteins of the myofibrils and connective tissue can affect the amount of moisture released from meat during the cooking process, it is also probable that juiciness is also influenced by such factors.

Although tenderness and juiciness have been theorised as two separate attributes, they may be evaluated similarly and their interdependence remains unclear. These attributes may be evaluated similarly for two reasons. First, the changes which occur in the meat structure may affect both tenderness and juiciness attributes similarly. Secondly, Shorthose and Harris (1991) proposed that a 'halo' effect tended to exist between subjective evaluations of tenderness and juiciness. That is, a piece of meat judged to be more tender would often be judged as juicy as well.

2.3 Methods of eating quality evaluation

As humans are the final arbiters of beef eating quality, it would be preferable to use human consumers for all eating quality evaluations. Unfortunately, however, there are a number of drawbacks (eg. cost, time) with such testing and over the years a number of mechanical devices have been developed for the objective measurement of eating quality attributes. The majority of these devices have been developed for the measurement of tenderness. Relatively less has been done in terms of developing an objective measurement of juiciness.

2.3.1 Objective measurements of tenderness

There have been a number of mechanical devices which have been developed to objectively measure the toughness attributable to the physical properties of the meat. Various devices have been developed which attempt to mimic the forces which occur during mastication, such as shearing, compression and separation (adhesion). There are a number of benefits associated with using mechanical devices for tenderness estimation. Using machines provides an objective measurement, the repeated use of a standard device gives reliability, repeatability and is relatively quick and easy.

Unfortunately these objective measurements are not able to incorporate into their measurement, other sensory attributes which influence consumers' assessment of beef tenderness. This is one of their limitations. They only provide an estimate of the physical toughness of the meat. Measuring the physical (and chemical) properties of meat will confer an understanding of the tenderness (and juiciness) of beef, but ultimately these measurements must relate to the way the meat behaves in the mouth when consumers eat it. The method of evaluation used in studies will be dependent on the aim of the experiment. The other difficulty with evaluating meat is that it is an extremely heterogenous product and often variation exists within a single muscle which makes multiple evaluation essential.

This brief review will cover those objective devices commonly referred to throughout this thesis. The discussion of other devices (eg. MIRINZ tenderometer) lie outside the scope of this thesis.

2.3.1.1 Warner Bratzler shear

The Warner Bratzler shear device (Bratzler, 1932) is most frequently used to objectively assess the tenderness of cooked meat (Shorthose and Harris, 1991), and a number of modified versions have been devised since the original was developed by Bratzler in 1932. This original device employed a vee-shaped blade which was drawn through a cylindrical core of cooked meat (Bratzler, 1932). A modified version was developed at the CSIRO Meat Research Laboratory at Cannon Hill (Shorthose and Harris, 1991) which has a square hole in the shear blade. The meat sample is cut parallel to the meat fibres, placed through the square hole in the shear blade and held in place by a sample holder. The blade then shears up through the sample, cutting across (perpendicular to) the meat fibres. Harris and Shorthose (1988) contended that this version is relatively simpler than the more conventional devices and ensures a constant shear blade-sample contact area.

The Warner Bratzler device attempts to measure the force required to shear through the meat fibres and hence mimic the shearing action which operates during mastication. Results obtained with the Warner Bratzler have indicated that peak force values relate more closely to the myofibrillar rather than connective tissue component of toughness (Bouton and Harris, 1972b). This is discussed by Harris and Shorthose (1988).

2.3.1.2 Instron compression

Developed at the CSIRO Meat Research Laboratory (Cannon Hill), the Instron compression method is very similar to the General Foods Texturometer described by Friedman *et al.* (1963). The Instron compression measures hardness as the force (in kilograms) required to drive a 0.63 cm diameter flat-ended plunger at 50mm/min, 0.8cm into a 1cm thick wedge sample of cooked meat where the meat fibres are parallel to the cut surface and perpendicular to the direction of plunger travel. The plunger is driven into the meat sample twice in the same location and the work done for each cycle is determined (Harris, 1976). Harris and Shorthose (1988) stated that the parameters measured are essentially those defined by Friedman *et al.* (1963), namely hardness, cohesiveness (the ratio of work done on the second penetration to the work done on the first penetration) and chewiness (hardness x cohesiveness). Friedman and his co-workers also included an 'elasticity factor' in their chewiness parameter but this not included

in the chewiness parameter determined by the Meat Research Laboratory Instron compression device (Harris and Shorthose, 1988).

Harris (1976) stated that the parameter used most frequently for comparison with other objective and subjective measurements has been that of chewiness. Bouton and Harris (1972b) conducted a variety of trials and reported that Instron compression measurements are considerably more sensitive to connective tissue differences between muscles than are Warner Bratzler shear values.

2.3.1.3 Tensile force

For tensile measurements, samples are gripped at both ends such that fracture does not occur at the grips, and stress is applied either parallel or perpendicular to fibres. Tensile strength tests applied both parallel and perpendicular to muscle fibres have been studied and a review of these methods is provided by Harris (1976).

Tensile stress measurements on cooked meat made perpendicular to the fibres are referred to as adhesion measurements (Harris, 1976). Although adhesion values are generally thought to be an index of connective tissue strength, they are also influenced by myofibrillar contraction state, suggesting that the spatial changes in connective tissue make a significant contribution to increased toughness (Harris, 1976).

2.3.2 Objective measurement of juiciness

An indication of muscle juiciness is usually provided by measures of water holding capacity, expressible juice and cooking loss. The water holding capacity of meat provides a measure of the water held within a muscle prior to cooking, and is affected by cooking. Harris and Shorthose (1988) discussed many methods which have been used to measure water holding capacity. The most common are the press methods, and others include sedimentation and various centrifuge methods.

Cooking loss or percentage moisture lost during cooking is often used as an indication of the moisture lost from the meat during the cooking process. However, this only gives a measure of the moisture lost during the cooking process, and not of the water actually contained within the meat after cooking.

The Akroyd high speed centrifugation method was used to determine how much expressible juice could be obtained from meat samples (Harris and Shorthose, 1988). Bouton *et al.* (1973a) reported that juiciness was more closely related to juice expressed during centrifuging than to cooking loss.

2.3.3 Sensory evaluation

The use of humans for the evaluation of meat is usually desirable because it provides a sensory, as opposed to an objective, evaluation of eating quality attributes. However, when untrained consumers are used for sensory evaluations of eating quality attributes, the increase in time and cost, and the possibility of results being influenced by irrelevant factors (eg. emotions, sickness) becomes a disadvantage of only using untrained consumers for evaluation. The disadvantages associated with untrained consumers has meant that many institutions have introduced trained taste panels for sensory analysis and these provide an intermediate between untrained consumers and objective measurements. The use of trained taste panels avoids the disadvantages associated with untrained consumers, whilst still providing a sensory evaluation of beef quality attributes.

The evaluation of cooked beef quality has usually been assessed by scoring tenderness, juiciness and flavour attributes, although the specific attributes evaluated would depend on the aim of the study. Of these attributes, tenderness has received the most attention because of the significantly greater importance it has in determining overall consumer satisfaction. The following discussion on the use of trained taste panels will focus on tenderness because it was the attribute of prime importance throughout the studies in this thesis.

2.3.3.1 Trained taste panel

A trained taste panel usually comprises a group of people who have undergone a selection and training process to improve their ability to evaluate the relevant sensory attributes with greater precision and repeatability. The selection process is conducted to identify those individuals who have above average ability to detect differences in tenderness (or flavour, for example) between samples. This group of people then undergo a period of training, the method and degree of which will depend on the nature of the experiments to be conducted and the

availability of resources (Martin, 1973). The panel is theoretically trained to ignore irrelevant external factors and essentially to provide a more objective evaluation of eating quality attributes, as interpreted by humans. The American Meat Science Association (AMSA) (1968) outlined the role of panellists and highlighted that the selection, training and monitoring of panellist performance required careful control and this was essential to the success of the panel.

The aims of the training phase have been described as two fold; first to minimise the variability of the individual's judgement and secondly, to enhance the individual's memory and sensitivity (Martin, 1973). The latter is important as it facilitates more precise subjective judgements and produces results that are more uniform from trial to trial (Martin, 1973). During training it is also important for panellists to learn to ignore personal preferences (Bennett *et al.*, 1956).

The benefits of using a trained panel (compared to untrained consumers) include reduced costs and time, minimisation of irrelevant influences and subjectivity and less variability within and between individual scores. Such benefits were observed by Bennett *et al.* (1956) who reported, that after training a group of inexperienced assessors over a three week period, their scores improved vastly in terms of greater uniformity and use of the scale. They were also able to separate preference from recognition and to become more objective in their scoring.

Some ambiguity surrounds the term 'trained' because of the different selection and training methods used to create a 'trained' taste panel. The use of a trained taste panel may simply involve a screening test, through to specialised groups who have undergone extensive training periods (eg. Cover *et al.*, 1962a; Amerine, 1965; Bouton *et al.*, 1975a; Brady and Hunecke, 1985; Poste *et al.*, 1993). The International Standards for sensory analysis and the AMSA (in cooperation with the National Live Stock and Meat Board) provide recommended guidelines for the selection and training procedures for the development of trained taste panels (International Standards, 1993; AMSA, 1995).

When trained panels have been used to evaluate an attribute such as tenderness, the scale and number of individual characteristics evaluated, has varied enormously. In 1963, Szczesniak classified characteristics of food evaluation and stated that textural characteristics could be grouped into three main categories: 1) mechanical; 2) geometrical and; 3) other characteristics. These characteristics were explained and about 10 attributes were listed for evaluation. Since then, some researchers have employed the use of the attributes suggested by Szczesniak, while others have specified attributes more specific to tenderness. The trained panels used by Brandt *et*

al. (1963) and Brady and Hunke (1985) evaluated nine separate quality attributes which were all characteristics of tenderness. Cover *et al.* (1962a) used six characteristics to evaluate beef texture. However, Harries *et al.* (1972) and Dransfield *et al.* (1984) asked their panellists to evaluate seven and nine characteristics respectively, but then, using principal component analysis, reduced them into essentially two independent dimensions; 1) tenderness/toughness, and; 2) juiciness/dryness. Harries *et al.* (1972) concluded that the physiological appreciation of the texture of meat was measurable in terms of these two key attributes. Many of the trained taste panels used in the USA (eg. Shackelford *et al.*, 1995) evaluate myofibrillar, connective tissue and overall tenderness separately.

Variability has also existed in the type of scale used for scoring, such as line or graphic scales, verbal scales, numerical or category scales and methods such as the chew count. There has been criticism of category scales, as Stone *et al.* (1974) pointed out that it cannot be assumed that each word phrase (or category) has the same meaning to each individual and the psychological interval between each category may not be equivalent. The chew count method can have variability introduced by individuals differing in their assessment of when the meat is ready for swallowing. Harrington and Pearson (1962) reported large variability in individual chew counts on similar samples and marked variation in the ability to discriminate unlike samples. The most common method of evaluation used now would probably be graphic line scoring with the extremes of the sensory attribute written at the ends of the lines.

Gillmore and Prescott (1993) argued that sensory data generated from trained panels are of limited usefulness in predicting consumer preferences, if the participating individuals are not representative of ordinary consumers. Even a small amount of training removes an individual from the realm of ordinary consumers. Because a taste panel has been trained to be objective, they should not be asked to give preference scores also as this then includes personal biases which they have been trained to ignore. This highlights the fact that trained taste panels and untrained consumers have different roles in sensory analysis depending on the objective of the experiment.

Trained taste panels can be employed when researchers want to detect whether there is a significant difference between samples, or treatments. It is usually assumed that if a trained taste panel does not detect a significant difference between samples, then neither will untrained consumers. Using a trained taste panel is also relatively quick and cheap compared to using untrained consumers when this type of experiment is being conducted.

Given the large range of variation which exists in the development of a trained taste panel, it would be interesting not only to investigate the effects of training on panellists evaluation, but also to investigate how the panel evaluates samples compared with untrained consumers.

2.3.3.2 Untrained consumers

The use of untrained consumers refers to the use of people with no formal training in meat quality evaluation. When untrained consumers are used for evaluation, they need to be selected randomly and to be representative of all consumers in the population under study. It is important to use untrained consumers when consumer preferences or desirability ratings are required.

Because of the natural heterogeneity between individuals, data from these tastings are more variable. This means that to obtain a meaningful result, a large number of samples need to be evaluated by large numbers of tasters.

For untrained consumer tastings, either line scoring or category preference ratings with relatively simple scales can be used for evaluations. Untrained consumers are usually less sensitive to differences in tenderness than those reported by objective measurements and trained sensory panels (Shorthose, 1993).

2.3.4 The relationship between objective measurements and sensory evaluations

In terms of assessing beef eating quality, sensory evaluations, which are conducted in a prescribed manner, are preferable to objective measurements. However, because of the advantages of time and cost of objective measurements, the latter are widely used. It is therefore important to investigate the relationship between objective measurements and sensory evaluations because objective measurements are of limited value if they do not relate to sensory evaluations. Obviously, objective measurements will not correlate one hundred percent with sensory evaluations as they are not able to incorporate the organoleptic component of eating quality which influences humans when they eat beef. The success of a mechanical device for measuring various components of tenderness depends on the closeness of its relation to human interpretation of tenderness (Cover *et al.*, 1962c).

The relationship between objective measurements and sensory evaluations has been variable and sometimes has shown to be dependent on the muscles used. Bouton *et al.* (1975a) demonstrated that over 75% of the variation in sensory toughness could be explained using Warner Bratzler shear values, Instron compression values and cooking loss measurements. They also noted that when samples of meat were presented so that panellists bit across the fibres, panel scores were more closely related to Warner Bratzler than Instron compression values, which implied that panellists were more sensitive to myofibrillar than connective tissue toughness in this situation. Similarly, when panellists bit between the muscle fibres, their scores were more affected by connective tissue toughness. Bouton *et al.* (1975a) stated that the portion of variance not explained in terms of these three parameters could be due to a number of causes: a) both instrumental and taste panel measurements being subject to sample variation; b) stress and strain patterns developed in the mouth not adequately represented by instrumental techniques employed, and; c) taste panel scoring being over simplified by assuming a linear tenderness scale.

Szczesniak (1963) reviewed early work which showed the correlation between Warner Bratzler shear values and sensory evaluations of beef ranged from -0.32 up to -0.94. Cover *et al.* (1962c) reported correlations of approximately -0.80 between shear values and three of the six attributes of *Longissimus thorcis et lumborum* (LT) samples evaluated by the panel. Using the *Biceps femoris* (BF), the correlation weakened to about -0.75 (Cover *et al.*, 1962c). Shackelford *et al.* (1995) reported the relationship between peak force and overall tenderness, within each muscle, ranged from non existent for *Gluteus medius* (GM) ($r = 0.00$) to strong for LT ($r = 0.73$). Such differences are not surprising in light of the fact that, if peak force measurements tended to be more indicative of myofibrillar toughness than connective tissue, then the toughness of muscles which have higher connective tissue content would override the myofibrillar component of toughness. Hence, a weaker relationship between high connective tissue muscles and objective measures of tenderness by devices which are primarily influenced by myofibrillar toughness.

Bouton *et al.* (1973b, 1975a,b) and Shackelford *et al.* (1995) also demonstrated that the relationship varied between muscles. Shackelford *et al.* (1995) reported Warner Bratzler shear force was able to detect that *Psoas major* (PM) and *Infraspinatus* (IS) were more tender than the other muscles, while the taste panel identified tenderness differences between more muscles (PM and IS more tender than *Triceps brachii* (TB) and LT which were more tender than *Semitenidosus* (ST), GM, and *Supraspinatus* (SU). *Biceps femoris* (BF), *Semimembranosus*

(SM) and *Quadriceps femoris* (QF) were the toughest). The differences in tenderness between muscles identified by the panel could not be detected with shear values. When peak force shear values were used in an equation to predict sensory tenderness ratings, they only explained 50% of the variation in overall tenderness. This result highlights the fact that factors other than those measured by shear values are involved in the sensory evaluation of tenderness.

Shorthose and Harris (1991) stated that the relationships tend to be S-shaped because of the reluctance of consumers to record extreme values. Consequently, this may also influence the relationships between objective measurements and sensory evaluations. Theoretically, this should not be as evident with trained taste panels scores. Another factor which will limit the strength of the objective-sensory relationship is the probable errors in the physical measurement(s), coupled with unavoidable variations of judgement both within and between individual panel members (Harris, 1976).

A common discrepancy between objective measurements and sensory evaluations is cooking method and serving temperature, both of which influence tenderness. The use of one cooking technique for samples for objective measurement and another for samples for sensory evaluation may create a cooking by method of assessment interaction which could weaken the strength of the relationship obtained. The samples used for objective measurements are usually evaluated cold, and often sensory evaluations are made on warm meat. Lawrie (1991) pointed out that the partial reversion of the collagen to gelatin transformation could partially explain Ledward and Lawrie's (1975) finding. Meat cooked to 80°C is tougher when measured at 20°C (room temperature) than 70°C (warm), and that cooked at 55°C shows no difference with temperature of assessment. The latter component of this finding indicates that the end point temperature to which samples are cooked may interact with serving temperature and influence tenderness measurements. It also implies that the muscle used, and hence its collagen content and interaction with internal cooking temperature, may also influence the results obtained.

Hovenier *et al.* (1993) proposed that low correlations between shear values and trained taste panel tenderness scores were due to low repeatabilities of panel judgements. These workers reported the repeatability of taste panel assessment was 0.53 and when two tasters scored one piece of meat, the repeatability was 0.08. With repeatabilities this low, it is not surprising that low correlations between objective and sensory evaluations have been reported. However, not only is there variation within humans, but meat is an extremely heterogenous product and variation exists within a single muscle, meaning the repeatability is affected.

The relationships determined between objective measurements and sensory evaluations have varied considerably and are likely to have been influenced by a variety of factors. The use of objective measurements to estimate both myofibrillar and connective tissue tenderness appear to provide an indication of the physical tenderness properties which relates to sensory evaluations, but still lack the ability to incorporate the organoleptic attributes sensed by humans.

2.4 Factors affecting tenderness and juiciness

There are many factors which can affect eating quality attributes, and they affect both the myofibrillar and connective tissue components, but in different ways. Generally the effects of post-slaughter treatments on tenderness (and juiciness) are more significant than the effects of pre-slaughter factors, with the exception of pre-slaughter stress.

2.4.1. Pre-slaughter factors

2.4.1.1 Breed

The effect of breed on eating quality attributes remains equivocal, however, breed differences in tenderness have been reported (eg. McKeith *et al.*, 1985; Cundiff *et al.*, 1988; Crouse *et al.*, 1989; DeRouen *et al.*, 1992; Johnson *et al.*, 1990a; Shackelford *et al.*, 1995). A large proportion of breed studies have investigated the effects on tenderness of *Bos indicus* versus *Bos taurus*. When *Bos indicus* breeds have not been involved, there has generally been extremely small effects of breed on tenderness. This review will focus on the effect of *Bos indicus* content on meat quality, in particular, tenderness.

Bos indicus cattle have been used extensively throughout northern Australia tropical and sub-tropical environment because of their natural adaptation to these conditions. However, an industry bias has developed against *Bos indicus* cattle based on the perception that they produce tougher meat.

It has been well documented that increasing *Bos indicus* content causes a decrease in tenderness, as measured objectively and by sensory evaluation (McKeith *et al.*, 1985; Cundiff *et al.*, 1988; Crouse *et al.*, 1989; Kohun *et al.*, 1990; DeRouen *et al.*, 1992; Johnson *et al.*, 1990a; Shackelford *et al.*, 1995). However, some workers did not find significant differences in

tenderness between *Bos indicus* and *Bos taurus* cattle as determined using objective measurements (Winer *et al.*, 1981; Christensen *et al.*, 1991) and untrained consumers (Rymill *et al.*, 1994).

Although the focus of breed differences has been on tenderness, juiciness evaluations have also been reported to decrease as *Bos indicus* content increased (Cundiff *et al.*, 1988; Crouse *et al.*, 1989; Kingston, 1989; Johnson *et al.*, 1990a). It is not known whether this decrease is attributable to the relationship between juiciness and tenderness, the differences in the myofibrillar and connective tissue components which affect tenderness also influence juiciness, or whether another unknown factor exists which influences juiciness in the meat from *Bos indicus* cattle. In contrast to these reports, Kohun *et al.* (1990) and Rymill *et al.* (1994) reported no difference in juiciness evaluations between breeds.

In attempts to identify the nature of the tenderness difference between breeds, many other factors have also been investigated and reported to differ between breeds which could contribute to the tenderness differences reported. The influence of carcase weight and carcase cooling has been demonstrated by Luckett *et al.* (1975) who reported a significant negative relationship of Warner Bratzler shear force with carcase weight. In Luckett and co-workers' study, *Bos indicus* carcases also had lower temperatures than *Bos taurus* carcases at one hour post-mortem and also had higher Warner Bratzler values. As well as a more rapid cooling in *Bos indicus* carcases, Wheeler *et al.* (1990a) and Shackelford *et al.* (1991) also reported a slower pH decline *Bos indicus* carcases. In both of the latter studies, tenderness was found to be lower in *Bos indicus* samples than *Bos taurus* samples The differences in pH and temperature decline between breeds may be a reflection of breed carcase differences, or as suggested by Shackelford *et al.* (1991) might be due to differences in metabolic rate between the two breed types. Contrary to these findings, Whipple *et al.* (1990a) reported no difference in temperature or pH decline between breeds.

Based on the studies discussed above, it is not known whether the differences in tenderness were attributable to intrinsic breed differences *per se*, or to the influence of extrinsic factors such as rate of carcase cooling. Effective electrical stimulation (see section 2.4.2.1) can be used to remove such environmental effects confounding tenderness results. When electrical stimulation has been applied to carcases post-slaughter, the magnitude of differences in tenderness between breeds has shown to be reduced considerably.

Results from Rymill *et al.* (1994) showed that in meat samples from carcases which had been electrically stimulated, Brahman percentage did not have a significant effect on either consumer evaluations of eating quality or laboratory measurements of toughness. Although not quite significant, there was a trend for Warner Bratzler initial yield values and instron compression values to increase as Brahman percentage increased.

Wheeler *et al.* (1990b) electrically stimulated one side of each carcase and observed an interaction between electrical stimulation and breed type. Without electrical stimulation, samples from Brahmans received lower myofibrillar and overall tenderness scores, higher Warner Bratzler and sensory (amount of) connective tissue scores. However, after stimulation the improvement in these scores was greater for Brahman samples than for samples from other breed types. After electrical stimulation, only a difference in Warner Bratzler values existed between breed types. These results indicate that tougher meat from Brahman cattle was not attributable to cold shortening (environmental influences), otherwise it would have been removed by electrical stimulation. The reduction in toughness in Brahmans post-stimulation would indicate that much of the toughness associated with them was attributable to the myofibrillar component. However, the trend for compression values to increase with Brahman percentage reported by Rymill and co-workers, and the improvement in sensory connective tissue scores reported by Wheeler *et al.* (1990b) in electrically stimulated carcases would indicate that the influence of connective tissue can not be ignored as also contributing to the differences in tenderness between breeds.

If the greater toughness of *Bos indicus* cattle lies predominantly in the myofibres, then it could be influenced by the activity of endogenous enzymes post-mortem. Differences in the response to ageing between breeds has been reported. Johnson *et al.* (1990a) and Whipple *et al.* (1990a) found that the improvement in tenderness during ageing decreased as Brahman content increased. Wheeler *et al.* (1990a) reported that electrophoresis data indicated that *Bos taurus* meat was at a more advanced stage of proteolysis as early as day one post mortem than Brahman meat. Studies of proteolytic enzyme activity post-slaughter have shown calpastatin (inhibitor for calcium dependent proteases - see ageing section 2.4.2.2) levels to be higher in *Bos indicus* samples than *Bos taurus* samples (Johnson *et al.*, 1990b; Wheeler *et al.*, 1990a; Whipple *et al.*, 1990a) and consequently, greater toughness. In addition to reporting a difference in levels of calpastatin activity between breed types, Johnson *et al.* (1990b) found cathepsin B + L activity (see ageing section 2.4.2.2) had a negative association with Warner Bratzler shear values at day 10. However, Whipple *et al.* (1990a) reported no difference in cathepsin B or B + L activity

between breed types. In these studies, differences in ageing response were not substantial enough to fully explain tenderness differences among breed types, but do indicate that differences in tenderness among breed types may be partially explained by differences in proteolytic enzyme activity.

Dransfield (1992) proposed that the heterogeneity in ageing between breed types was due to a change in temperature coefficient or activation energy in *Bos indicus* cattle brought about by their adaptation to the heat. Dransfield (1992) proposed a model which indicated that a single change in the temperature coefficient could account for the toughening and was consistent with the known heat tolerance of these animals. However, no evidence of differences in the activation energies of enzymes was presented.

The majority of studies have investigated, and attributed tenderness differences between *Bos indicus* and *Bos taurus* cattle to, the myofibrillar component. However, some differences in connective tissue have also been reported. McKeith *et al.* (1985) and Crouse *et al.* (1989) reported that higher connective tissue toughness in aged *Bos indicus* meat contributed to greater toughness. The results of trained taste panel evaluations (Wheeler *et al.*, 1990b) found that Brahman meat had greater connective tissue toughness than other breed types. However, there have also been reports of no differences between breeds in sensory connective tissue scores (Johnson *et al.*, 1990a) and collagen amount, or solubility (Whipple *et al.*, 1990a).

It has been suggested by Shackelford *et al.* (1991) that the differences in fibre type may result in differences in tenderness between breeds by affecting collagen density. The perimysial connective tissue has also been reported to be thicker in meat from *Bos indicus* cattle than *Bos taurus* cattle (Norman, 1982, cited by Dikeman, 1990).

In an attempt to put the effects of breed into perspective in terms of consumer tenderness evaluations, in a study by Kingston (1989), it was concluded that in determining consumers' ratings of palatability, factors which exhibited statistical significance most frequently, and which also had the greatest impact were electrical stimulation and animal age. It should be noted, however, that in this study breed was very broadly defined and cattle were only classified visually prior to slaughter.

The muscle most commonly used by meat scientists is the LT and breed differences have most commonly been found when the LT has been used. An interesting observation was made by Shackelford *et al.* (1995) who reported that the level of significant differences between breeds was not constant across the various muscles tested. There were some muscles tested in

which the breed effect was not significant, whilst the differences between breeds were greatest in the LT muscle. The LT muscle has a very low connective tissue contribution to toughness and tenderness is therefore dominated by myofibre toughness. These results would indicate that there may be an inherent toughness in the myofibres of *Bos indicus* cattle which is emphasised in the LT. When electrical stimulation is employed, or other muscles are used, in which the myofibre contribution to toughness is relatively less, the breed differences are smaller and sometimes not detectable.

Based on the literature reviewed, it appears that there may be inherent differences in the myofibrillar, and to a lesser extent, in the connective tissue component and ageing mechanisms between *Bos indicus* and *Bos taurus* cattle which influences tenderness. However, given the results of Shackelford *et al.* (1995) and that breed has also been shown to interact with post-mortem treatments, it would appear that differences in tenderness between *Bos indicus* and *Bos taurus* cattle could often be overstated. This highlights the need to investigate how important the effect of breed is in terms of consumer tenderness evaluation, in particular, when compared with the effects of electrical stimulation and ageing.

2.4.1.2 Nutrition

Nutrition *per se*, appears unlikely to have direct effects on meat quality, but it may indirectly affect it via its influence on other factors such as growth, development and fat deposition (Wood, 1990). The most contentious issue about nutrition in recent times has been the difference in meat between quality pasture and grain feeding. Naturally it becomes extremely difficult to make meaningful comparisons between pasture and grain fed contempories because of the differences in growth rate and carcase composition.

Increasing time on feed, or the feeding of grain as opposed to pasture, generally has two effects. First, growth rate is increased and secondly, carcase fat is increased (Wood, 1990; Wood *et al.*, 1991; Aalhus *et al.*, 1992; May *et al.*, 1992; Wood, 1993). A difference in tenderness between pasture and grain fed animals could easily arise if there was a difference in carcase weight and/or fatness. The increase in carcase fat reported with grain feeding would provide greater protection against cold shortening, and hence grain fed animals would be more tender simply because they were fatter and suffered less cold shortening than leaner pasture fed animals.

Increased growth rate allows cattle to be turned off at an earlier age (Loxton *et al.*, 1991; Wood *et al.*, 1991; Loxton *et al.*, 1993; Wood, 1993). So if animals are being compared at a set weight, then the animals which grew slower (usually off pasture) would be older and the age related differences in collagen toughness might occur. Other theories have also been suggested in relation to the effects of increased growth rate on tenderness. Aberle *et al.* (1981) and Crouse *et al.* (1985) suggested a more rapid protein synthesis and turnover lead to a higher proportion of immature collagen causing greater tenderness in cattle on higher levels of nutrition. Koohmaraie (1992a) suggested that increased growth rate may also cause more rapid muscle deposition which in turn would influence the proteolytic capacity post mortem. In support of Koohmaraie's theory, Thomson *et al.* (1996) reported growth rate did affect the calpain system and shear force values. Animals which grew faster were more tender and had a lower ratio of calpastatin:CDP-I (see ageing section 2.4.2.2) than animals in which growth rate had been stable for eight weeks prior to slaughter. The latter results suggesting increased growth rate may cause greater enzyme activity post-mortem which effectively increases the rate of ageing, and hence, greater tenderness.

Low planes of nutrition have shown to increase the incidence of dark cutters (Shorthose and Harris, 1991). This would suggest that low levels of nutrition can reduce an animals' ability to cope with pre-slaughter stress and replenish muscle glycogen levels prior to slaughter. Consequently, these animals show a higher predisposition to suffering the effects of pre-slaughter stress.

Another proposal by Wood (1990) was that higher feeding levels promoted increased levels of marbling and this would increase eating quality. If higher levels of feeding, or grain feeding did result in greater tenderness in this situation, it could be confounded by the differences in marbling level, rather than being directly attributable to nutrition *per se*. Shorthose and Harris (1991) argued that increased marbling achieved with higher feeding levels, and increased tenderness was a reflection of greater carcase fat which effectively reduced the effects of cold shortening.

Differences in the concentrations of saturated and unsaturated fatty acids have been reported with pasture and grain feeding (eg. Wood, 1990). However, these differences are relatively small and would be extremely unlikely to influence tenderness scores.

Despite these different mechanisms which could affect tenderness, others (James *et al.*, 1991; Jeffery *et al.*, 1991a,b) have reported nutrition to have no effect on meat quality attributes.

In the study of James *et al.* (1991), all animals were slaughtered at a young age and had positive weight gains prior to slaughter. Similarly, the animals used by Jeffery *et al.* (1991a,b) were all gaining weight prior to slaughter, and the feeding period was short term. A overview of a number of studies by Loxton *et al.* (1993) reported grain feeding allowed animals to be turned off nine months earlier. Carcases were electrically stimulated and there was no difference in LT tenderness between feeding regimes, however, there were significantly lower connective tissue toughness in ST samples of animals which were slaughtered at the earlier age.

From these studies, it appears that differences in tenderness may be attributable to indirect effects of nutrition, rather than nutrition *per se*. However, the influence of nutrition on tenderness maybe emerging as important via its affect on growth rate prior to slaughter and ensuring animals are slaughtered at a relatively young age.

2.4.1.3 Animal Age

Increasing animal age is most well known for causing a decrease in tenderness because of its influence on the connective tissue structure. Other changes may also occur as animal age increases, but these are less well documented. Although connective tissue content has been reported to be greater in young animals (Bate-Smith, 1948; Wilson *et al.*, 1954, cited by Lawrie, 1991), it is the nature of collagen quality which becomes more important in older animals.

Increasing animal age has been reported to have a significant affect on decreasing tenderness (Bouton *et al.*, 1978; Robertson *et al.*, 1984; Cross *et al.*, 1985; Kingston, 1989; Shorthose and Harris, 1990; Loxton *et al.*, 1991; Loxton *et al.*, 1993; Wulf *et al.*, 1996). Loxton *et al.* (1991) also pointed out that the age of animals was a result of their nutritional history and growth rate. This emphasised the possibility of the effect of age confounding the effects of nutrition on tenderness. The decrease in tenderness with age was specifically related to an increase in connective tissue toughness (Robertson *et al.*, 1984; Cross *et al.*, 1985; Shorthose and Harris, 1990; Loxton *et al.*, 1993). In particular, Cross *et al.* (1985) reported the amount of soluble collagen decreased as aged increased. Kurth *et al.* (1992) reported the proportion of mature crosslinks increased by 30% from birth to maturity in cattle.

It is not really understood as to why there is an increase in mature crosslinks formed as an animal gets older, nor the rate at which this change occurs. However, so as to avoid increased toughness due to mature crosslinking in connective tissue, it is desirable to slaughter young animals.

2.4.1.4 Pre-slaughter stress

Pre-slaughter stress has long been known as a major cause of poor meat quality. In particular, it has been recognised as inducing high ultimate pH by depleting muscle glycogen stores (Lawrie, 1985). Lister *et al.* (1981) stated that there is an inverse relationship between amount of glycogen in muscle at death and the final pH and it is this association which is the direct cause of dark cutting meat and is attributable to pre-slaughter stress. Ultimate pH has been shown to have a curvilinear relationship with tenderness (Bouton *et al.*, 1973a) in which tenderness decreases as pH increases to about 6.0, and then there is a increase in tenderness as pH continues to increase above this point.

Pre-slaughter stress is a rather general term and defining what constitutes pre-slaughter stress, such that it has a negative effect on meat quality, can be quite difficult. Forrest *et al.* (1975) stated that stress is a general expression referring to the physiological adjustments (changes in heart rate, respiration rate, body temperature and blood pressure) which occur during the exposure of an animal to adverse conditions. However, Lister *et al.* (1981) contended that not only is pure physiological stress important, but an element of psychological stress also appears to be important in mediating the depletion of muscle glycogen. Stress can be imposed by a variety of factors; emotional excitement, fatigue, trauma, fear, shock, anoxia, and weather (Hedrick, 1968). During such experiences, a variety of hormones (epinephrine, norepinephrine, adrenal steroids, thyroid hormones) are involved in the adjustment of metabolism (Forrest *et al.*, 1975).

Forrest *et al.* (1975) outlined the following responses to stress in cattle. Hormones are released which place demand on the animals' energy reserve causing a depletion of muscle glycogen reserves. If stress is severe and the release of epinephrine causes the energy demand to exceed that available from aerobic metabolism, then the anaerobic pathway for glycogen breakdown will be favoured. This results in the production of lactic acid and if it cannot be removed from the muscles by the circulatory system prior to slaughter, then a condition known as pale, soft, exudative (PSE) meat results due to a very low pH. However, this condition is more common in pigs than cattle.

If an animal suffers glycogen depletion, but no lactic acid build up, then the reduced glycogen limits the anaerobic glycolysis post mortem and hence, restricts the amount of lactic acid produced, resulting in a high ultimate pH. Severe muscle glycogen depletion which results in an ultimate pH greater than approximately 6.0, is referred to as dark cutting, or dark, firm, dry

(DFD) meat. Dark cutting meat is very dark in colour (not bright red) and although this meat is often acceptably tender (where pH>6.5), its unacceptability to consumers is reduced because of its extremely dark colour and reduced shelf life. The cost of discounting meat which is too dark is estimated at more than $30 million a year (Shorthose, 1992). The effects of pre-slaughter stress cannot be elevated by electrical stimulation, probably because not enough glycogen is present in the muscle to allow the normal processes required for lowering pH (Shorthose and Harris, 1991).

Given that pre-slaughter stress causes adverse effects on meat quality, it is desirable to minimise stress. The effects of transport, weather, unfamiliar surroundings, mixing with unfamiliar animals, human exposure can all cause pre-slaughter stress, yet it is extremely difficult to determine and measure what constitutes a stressful encounter or experience. Howard and Lawrie (1956), cited by Lawrie (1991), found it most difficult to deplete the glycogen reserves in cattle even when pre-slaughter exercise and fasting for 14 days were combined. Yet such depletion occurred, without fasting, if enforced exercise took place immediately after train travel. Carr *et al.* (1973), cited by Lister *et al.* (1981), fasted steers up to three days which decreased glycogen, but did not affect ultimate pH. However, mixing groups of unfamiliar stock may have a profound effect on their metabolism and result in reduced muscle glycogen (Lister *et al.*, 1981).

The susceptibility to pre-slaughter stress has been demonstrated to be muscle fibre type dependent. For example, Dransfield (1992) reported slow red muscles have a higher sensitivity to transport stress and increased ultimate pH. Lacourt and Tarrant (1985), stated that mixing stress causes a greater depletion from fast fibres than from slow, whereas depletion by adrenalin injection occurs more severely from slow fibres. These results indicate an interaction between the type of stress and muscle fibre type on muscle glycogen depletion. Naturally, the effect of stress on muscle glycogen levels would also vary with animal.

Despite the difficulties in quantifying pre-slaughter stress, it is obviously preferable to minimise any possible sources of pre-slaughter stress such that animal's muscle glycogen levels are high prior to slaughter, resulting in a low ultimate pH.

2.4.1.5 Intramuscular fat

The influence of intramuscular fat, or marbling, on the palatability of cooked beef remains equivocal. Savell and Cross (1986) argued that marbling has been associated with

increased palatability of meat and increased fat content in lean has been proposed to provide more 'insurance' against heat induced toughening of meat, and hence, improve palatability.

The definition of marbling, in the strictest sense, refers only to that fat which appears visible to the unaided eye on cut meat surfaces (Blumer, 1963). Intramuscular fat or lipid, however, includes the visible fat and also non visible microscopic lipid deposits within various cells of muscle. The amount of lipid extracted from the lean will depend on the technique used and may, for example, include phospholipids from cell membranes. The relationship between AUS-MEAT marbling scores (for example) and intramuscular lipid is approximately 50% (D. Ferguson, unpublished data.) because the visual assessment of marbling does not include non visible fats.

Tenderness and juiciness are pre-eminent factors in the sensory evaluation of meat texture which Goutefongea and Dumont (1990) claimed increased with increasing meat marbling and that fat should not be reduced to too low a level since it has been commonly observed that nutritional benefits gained through extensive defatting are more than outweighed by a correlated loss in palatability. Based on palatability research in the USA, Savell and Cross (1986) concluded that the minimum fat level required for acceptable palatability of broiling cuts is 3%, on an uncooked basis. However, other countries have reported minimum levels of 1.6% (Wood, 1990). A variety of theories have been proposed regarding the mechanisms associated with increased marbling causing increased tenderness (Savell and Cross, 1986) and juiciness (Weir, 1960; Smith and Carpenter, 1974).

Despite these theories being proposed, previous reviews (Blumer, 1963; Parrish, 1974; Dikeman, 1987; Smith *et al.*, 1988; Mersmann, 1990; Tatum *et al.*, 1992; Jones and Tatum, 1994) indicate that it is difficult to demonstrate a strong association of intramuscular fat with beef palatability and at best, that marbling has generally shown to have a low to moderate relationship with palatability and only accounts for 5-15% of the observed variation in palatability attributes.

Tatum *et al.* (1992) and Smith *et al.* (1988) reported marbling to account for 5% and 7% of the observed variation in sensory tenderness and juiciness scores. Smith *et al.* (1988) stated that the limited range of marbling (slight to moderate) was not expected to influence palatability and Tatum *et al.* (1982) reported that the magnitude of the regression coefficients suggested that large differences in marbling would be required to effect a detectable change in palatability. McBee and Wiles (1967) reported that tenderness and juiciness increased with increased

marbling but there had to be an increase of at least 3 (USDA) marbling grades to achieve a significant difference in tenderness and juiciness scores.

As the concentration of intramuscular fat is generally believed to increase as carcase weight and fatness increase (eg. Wood, 1990), this would mean that, without electrical stimulation, results may have been confounded by cold shortening. Shorthose and Harris (1991) believed that the increased tenderness associated with increased marbling was a reflection of greater carcase fat which effectively reduced the effects of cold shortening.

Since Smith and Carpenter (1974) claimed that marbling acted as insurance against the detrimental effects of extended cooking, many studies have been conducted to investigate the nature of this relationship. Luchak *et al.* (1991) reported an interaction between Choice and Select grade samples (which basically differ in marbling) and degree of doneness. Gardner *et al.* (1996) also stated that marbling level appeared to serve as an "insulator" in that steaks with more marbling could be cooked to an increased degree of doneness while maintaining acceptable tenderness values. Results of these studies were difficult to interpret because in the former, no level of significance was provided for the interaction, and in the latter, the marbling x internal temperature graph did not show consistent trends to support the theory. In contrast, however, Parrish *et al.* (1973a) and Akinwunmi *et al.* (1993) reported marbling to have no effect on tenderness and juiciness and their data did not provide support for the interaction between marbling and degree of doneness.

In the studies which investigated the relationship between intramuscular lipid and internal temperature, palatability scores decreased significantly as internal temperature increased (Parrish *et al.*, 1973a; Luchak *et al.*, 1991. Akinwunmi *et al.*, 1993). In these studies, it was concluded that internal temperature was a much more important factor in palatability than marbling (Luchak *et al.*, 1991) and that the degree of marbling and its interaction with cooking temperature have essentially no effect on palatability attributes (Parrish *et al.*, 1973a; Akinwunmi *et al.*, 1993).

An interesting observation emerging from studies was that leaner steaks required longer cook time than steaks high in fat when cooked to a set internal temperature (Heldman, 1975; Cross, 1977; Berry and Leddy, 1990) and had a more well done appearance (Berry and Leddy, 1990). Irtimer *et al.* (1967) reported meat with low levels of fat heated most rapidly in early stages and least rapidly in late stages of cooking. They stated that heat transfer through fat is slow until the temperature rises sufficiently to melt the fat. The liquid fat is a much more efficient conductor of heat than is solid fat. Hill *et al.* (1967), cited by Cross (1977), measured

thermal conductivity and found that values were substantially lower for beef fat than for lean, indicating that heat transferred faster through the fat. Irtimer *et al.* (1967) postulated that the increased in rate of temperature rise in high fat meat was associated with reduced moisture loss by the time the meat reached the end point temperature. They suggested that higher degrees of marbling may act as heat conductors during broiling, resulting in higher internal temperature before the appearance of advanced stages in cooked meat degree of doneness. Consequently, the effect of increased marbling on palatability may be more through its effect on reducing cooking time.

Tenderness and juiciness may be influenced by the observation of Moulton and Lewis (1940), cited by Pearson and Young (1989), who showed that water and fat contents of muscle are inversely related. This could mean that meat with a high fat content contains relatively less water which can be lost during cooking, when combined with a shorter cook time may result in increased tenderness and juiciness compared to low fat meat. In addition, Saffle and Bratzler (1959), cited by Lawrie (1991) stated that muscles with a high intramuscular fat content also tended to have high water holding capacity. Consequently, although there would be relatively less moisture in high fat meat, it would be more tightly held within the meat and this would probably decrease the moisture lost during cooking. However, further research is required to substantiate these theories.

Wood (1990) found that tissues from carcases of low levels of fat contain relatively high concentrations of saturated fatty acids and low concentrations of unsaturated fatty acids leading to a higher melting point of extracted lipid (Bensadonn and Reid, 1965; Leat, 1975; Wood, 1984). Consequently, if leaner meat contained more lipid of higher melting point, then higher temperatures would be required to melt the fat, and longer cook times would be required for leaner meat. This would result in tougher and drier meat. Whether or not the differences in the concentrations of saturated and unsaturated fatty acids commonly found in meat are large enough to effectively cause such a difference is not known, but would seem unlikely.

Juiciness and tenderness may be enhanced by the melting of the fat which is released as juice in the liquid state. It may then spread out from the meat or be recaptured by the fibrous protein network in the lean (Goutefongea & Dumont, 1990). Depending on the cooking method used, fat may or may not infiltrate into the lean portion. This would indicate that the cooking technique used in studies investigating the effect of marbling may influence results obtained.

The data in the literature reviewed would indicate that lipid content could possibly influence cooking time, and there may be a slight trend for increased tenderness and juiciness as

intramuscular fat increase, but often large increases in intramuscular fat are required for a difference to be detected. In terms of factors which can influence consumer evaluation of palatability attributes, marbling appears to play a relatively minor role.

In Australia consumers actively discriminate against marbling in raw beef, they prefer to buy lean steaks with little marbling (Hearnshaw *et al.*, 1992). Given this consumer preference, there is a need for research to be conducted to investigate whether Australian consumers detect the interaction between marbling and degree of doneness, preferably with meat from electrically stimulated carcases so as to eliminate any possibility of cold shortening confounding the results.

2.4.2 Post-slaughter factors

2.4.2.1 Electrical stimulation

The beneficial effects of using electrical stimulation on beef carcases to improve tenderness have been well documented (Harsham and Detherage, 1951, cited by Bendall, 1980; Chrystall and Hagyard, 1976; Davey *et al.*, 1976; Bendall, 1978; Savell *et al.*, 1978). Electrical stimulation of carcases causes an expeditious onset of rigor by speeding up the ATP turnover and glycolysis. Consequently, pH levels decline more rapidly and the carcase enters rigor within a relatively short time post slaughter. This means that carcases can be chilled quite rapidly without suffering the adverse effects of cold shortening as the pH is low and there is no energy left for muscle contraction. Initially, the accelerated onset of rigor and avoidance of cold shortening was thought to be the principal mode of action resulting in increased tenderness. However, Harris and Shorthose (1988) stated that the tenderising effect has been attributed to a number of causes (as well as the avoidance of cold shortening); an acceleration of the ageing process, increased activity of acid proteases, physical disruption of the myofibrillar structure and alterations in the thermal stability of collagen. The nature of the mechanism(s) responsible for the increase in tenderness in electrically stimulated meat are yet to be fully understood (Aalhus *et al.*, 1992).

Basically, two types of electrical stimulation have been developed which are differentiated by the level of voltage and timing of application post-slaughter. Harris and Shorthose (1988) outlined that high voltage stimulation involves using up to 800V RMS (with a 350 V peak at 14.3 Hz), and low voltage stimulation uses less than 100V. For effective

stimulation, application of low and high voltage stimulation should occur within about five minutes and an hour of slaughter, respectively.

The accelerated glycolysis and onset of rigor caused by electrical stimulation has meant that studies have been conducted to investigate the pH decline post-stimulation. Chrystall and Devine (1978) reported that a two stage fall of muscle pH resulted from stimulation. The first stage induced a 0.5 to 0.7 pH unit drop in 120 seconds after stimulation, which represented a 100 to 150 fold increase in the rate of underlying biochemical reaction. The second stage occurred after the cessation of stimulation, and the rate was much slower, but was still almost twice as fast as in non stimulated muscle over the same pH range. Similar results were reported by Bendall (1980) who observed a pH fall from 7.1 to 6.3 within two minutes of stimulation, followed by a decline during the next three hours to an ultimate pH of 5.7. If temperature conditions imposed post-stimulation were going to have an effect on meat quality, Chrystall and Devine (1978) contended that it would be likely only to effect the second stage of pH decline.

Compared to non stimulated controls, high voltage stimulation has resulted in an increased rate of glycolysis and earlier onset of rigor (Pedersen *et al.*, 1993), more rapid pH fall (Aalhus *et al.*, 1992), higher cooking loss (Eikelenboom *et al.*, 1985), longer sarcomere length (George *et al.*, 1980) and physical myofibrillar damage (Savell *et al.*, 1978; Bouton *et al.*, 1980). In all of the cases cited, a concomitant improvement in tenderness was reported with high voltage stimulation.

When low voltage stimulation has been used, the effects on tenderness have varied and myofibrillar damage has not been shown to occur. Bouton *et al.* (1980), Fabiansson (1984), Eikelenboom *et al.* (1985), Hawrysh *et al.* (1987) and Koh *et al.* (1987) reported that low voltage stimulation accelerated the rate of pH fall and improved tenderness compared to non stimulated samples. Salm *et al.* (1981) and Olsson *et al.* (1994) reported higher tenderness levels in electrically stimulated meat, but sarcomere length (which is an indicator of cold shortening) did not differ between stimulated and control samples. Based on the latter results, it could be concluded that electrical stimulation appears to influence tenderisation by mechanisms other than simply preventing cold shortening.

It has not been established whether the physical myofibrillar damage reported with stimulation (Bouton *et al.*, 1980; Takahashi *et al.*, 1984) actually contributes to the increased tenderness of meat or whether it allows earlier release of proteolytic enzymes which improve tenderness due to an increased rate of ageing. Takahashi *et al.* (1984) suggested that the

rupturing of tissue allows proteolytic enzyme release and activation, hence the possibility of ageing contributing to increased tenderness. This would mean that the effects of ageing would be observed at an earlier time post-mortem in electrically stimulated samples than non stimulated samples.

Studies of calpain activity post stimulation (Duncastaing *et al.*, 1985; Koohmaraie *et al.*, 1987; Dransfield *et al.*, 1992; Geesink, 1993) have shown activity of both CDP-I and CDP-II (see ageing section 2.4.2.2), and then decrease in activity to occur at an earlier time than non stimulated samples. Koohmaraie *et al.* (1987) stated that the pattern was analogous to that observed during post mortem ageing of non stimulated beef, although it was at an accelerated rate in comparison. This rapid decline in calpain enzyme activity would mean that it would need to exert it's effects in a relatively short time period post stimulation. As changes in tenderness are observed subsequent to the decline in CDP-I activity, it is possible that the action of CDP-I is only the first step in the tenderisation process (Geesink, 1993), or that an action lag period operates subsequent to CDP-I action which could explain the delaying of changes observed in the increased tenderness.

Cathepsins (see ageing section 2.4.2.2) have also been reported to be released at a faster rate following low (Pommier *et al.*, 1987) and high (Geesink, 1993) voltage stimulation. As cathepsins display optimal activity in an acid environment (Geesink, 1993), the low pH at relatively higher temperatures post stimulation could increase cathepsin activity and enhance tenderness in a short time period post stimulation.

Very little evidence of electrical stimulation affecting connective tissue has been reported. As stimulation can cause myofibrillar damage, it may also damage the connective tissue structure. It is generally assumed that electrical stimulation does not affect collagen solubility (Geesink, 1993). Despite this, some workers (eg. Wheeler *et al.*, 1990b) have reported taste panel connective tissue toughness scores to decrease with stimulation.

The reported effects of stimulation on juiciness have been equivocal. Some workers have reported juiciness evaluations increased with electrical stimulation (Kostov *et al.*, 1987; Aalhus *et al.*, 1992; Olsson *et al.*, 1994), while others have reported no difference in juiciness scores (Hertzman *et al.*, 1993) and cooking loss (Fabiansson, 1984). Wheeler *et al.* (1990b) also reported that stimulation had no effect on cooking loss or cooking time, but that juiciness scores were lower in stimulated samples compared to non stimulated samples. Martin *et al.* (1983) reported a decline in water holding capacity in samples from stimulated samples which may

contribute to the decreased juiciness scores observed in some studies. On the other hand, the increased juiciness reported with stimulated samples may result from the 'halo' effect (suggested by Shorthose and Harris, 1988) between tenderness and juiciness.

Based on theoretical considerations, Powell *et al.* (1986) suggested that benefits to tenderness in muscles subjected to electrical stimulation will be:

- greatest in muscles free to shorten post mortem;
- greatest during the onset of rigor;
- small when chilling conditions employed do not result in deleterious post mortem shortening in unstimulated muscles, and;
- small or non-existent when the muscle has a high ultimate pH (ie. pH>5.9).

From the studies, it appears that electrical stimulation may not exert its influence solely through the avoidance of cold shortening, but through a number of mechanisms which collectively act to improve tenderness. The effects of stimulation also appear to be dependent on factors such as voltage, other pulse characteristics and the chilling regime imposed on carcases post stimulation. The effects of electrical stimulation and its interactions with other factors on tenderness still require further investigation to gain a better understanding of the mechanisms by which it affects meat quality.

2.4.2.2 Ageing

After a carcase has entered rigor and meat is in its toughest state (Dransfield and Rhodes, 1975), there gradual improvement in tenderness during meat storage (post-mortem) which is referred to as post mortem ageing, or conditioning. It is generally accepted that the tenderisation results from proteolysis by endogenous enzymes (Asghar and Bhatti, 1987; Dransfield, 1992).

The improvement in tenderness of samples from meat which has been aged has been well documented (eg. Eino and Stanley, 1973; Calkins and Seideman, 1988; Johnson *et al.*, 1990a,b; Whipple *et al.*, 1990a,b; Koohmaraie *et al.* 1991; Powell, 1991; Geesink *et al.*, 1992). The general conclusion from these reports has been that the longer meat is aged (between about 1 and 14 to 28 days), the greater the improvement in tenderness. The most dramatic, or the greatest changes in tenderness have been reported to occur in the first 4 days (Eino and Stanley, 1973), 6 days (Crouse *et al.*, 1991) and 7 days (Koohmaraie *et al.*, 1991). The general

conclusion has been that the majority of the improvement in tenderness arises from decreased myofibrillar toughness and that ageing has a minimal effect on the connective tissue component. The increase in tenderness achieved with ageing is influenced by factors such as the initial toughness of the meat, ultimate pH, the length of time and the temperature at which the meat is aged.

Results of changes in juiciness during ageing have not been so clear. Whipple *et al.* (1990b) reported a slight increase in juiciness with 14 days ageing in samples which had been exposed to a short period of high temperature immediately post mortem, but no difference in samples which had been chilled normally. In contrast to this, Powell (1991) reported juiciness scores decreased slightly after 21 days ageing, but in electrically stimulated samples, there was no difference in juiciness scores after ageing.

In terms of the rate of tenderisation which occurs during the ageing period, Dransfield (1992) stated that about 50% of the tenderisation occurred before 24 hours post-slaughter, after which tenderisation continued approximately exponentially with time. Of the factors which can influence the rate of ageing, temperature is the most important factor (Dransfield, 1992). This is because the kinetics of enzyme reactions are highly dependent on temperature (Asghar and Bhatti, 1987). Dransfield (1992) stated that modelling shows that the effect of different temperatures is 'additive' and the total amount of ageing is then the sum of the tenderisation which takes part in each of the time/temperature parts. Others (Davey and Gilbert, 1976; Dransfield *et al.*, 1981) have also shown that at a constant temperature, in the range 0° to 40°C, the rate of tenderisation increased approximately 2.5 fold for every 10°C rise in temperature.

Many structural changes have been reported to occur in the muscle during ageing, (eg. Davey and Gilbert, 1968; Gann and Merkel, 1978; Penny, 1980; Koohmaraie, 1992a; Geesink, 1993). The most commonly observed ultrastructural changes in muscle during ageing are breaks or tears in the I band area, or near the Z disks (Penny, 1980; Geesink, 1993; Taylor *et al.*, 1995). Although weakening in the Z disk and I band region coincides with improvement in tenderness during ageing, the exact sites of degradation and weakening responsible for the improvement in tenderness have not yet been identified.

Many changes in the myofibrillar structure have been reported during ageing, however, the endogenous enzymes responsible and their mechanisms regulating their action remain unclear. Koohmaraie (1992a) contended that of the three proteolytic enzyme systems proposed to be involved in post mortem proteolysis, the calpains (calcium dependent proteases) were the

primary proteolytic system responsible. The three systems were the lysosomal cathepsins, the calpains and the multicatalytic proteinase complex (MPC). The calpain system has certainly received the most research attention recently.

The calpain system is composed of at least three components; two separate calcium dependent proteases, one and two (CDP-I and CDP-II) and an inhibitor specific for them both (calpastatin). The calpains require Ca^{2+} and have a neutral pH optimum for activity (Calkins and Seideman, 1988). If the calcium ion concentrations are sufficient to activate calpains, calpastatin can inhibit proteolytic activity by forming a complex with calpains. Calpain activity is also reduced by autolysis which commences once the calpain is activated (Koohmaraie, 1992a).

A considerable amount of research has been conducted to investigate the calpain activities responsible for changes in tenderness, with a variety of hypotheses being proposed (eg. Koohmaraie *et al.*, 1988a,b; Ouali and Talmant, 1990; Wheeler *et al.*, 1990a; Dransfield, 1993; Geesink, 1993). In terms of changes in tenderness which occur with ageing, the rate of ageing and the amount of ageing appear to be related to the level of inhibitor (calpastatin) in the muscle. Ouali and Talmant (1990) concluded that the rate of ageing may be positively correlated to the calpain:calpastatin ratio and negatively to the calpastatin content. In support of this theory, Johnson *et al.* (1990b) reported no relationship between calpain levels and Warner Bratzler shear values, but found shear values were positively related to calpastatin levels at day one post-mortem. Hence, shear values increased as calpastatin levels increased, indicating that higher levels of inhibitor were related to increased toughness, presumably due to the inhibitor reducing the tenderisation by calpain activity. Work by George *et al.* (1991) led them to conclude that the relationship between calpastatin and CDP-I is both temperature and pH dependent. Despite these observations, Geesink (1993) showed that the rate of protein degradation and tenderisation do not parallel the changes in calpain activity which indicates that calpains alone cannot account for the tenderisation process.

Another system believed to be involved in the tenderisation during ageing is the lysosomal cathepsins. Geesink (1993) stated that the cathepsins (B, D, H, L) belong to the lysosomal protease group and are normally enclosed in a membrane subcellular organelle (lysosome). Cathepsins require acidic pH for optima activity (Calkins and Seideman, 1988). The activity of cathepsins appears to be controlled by a number of protease inhibitors known as cystatins (Geesink, 1993).

In order for the cathepsins to become active, they first have to be released from the lysosomes and there is evidence of lysosomal membrane degradation during ageing (Penny, 1980; Kas *et al.*, 1993, cited by Barnier *et al.*, 1993; Barnier *et al.*, 1993). Barnier *et al.* (1993) reported an increase of free cathepsin B+L activity during ageing, with most of the increase occurring between days 1 and 3 post mortem (which is when the largest reduction in toughness is usually recorded). Calkins and Seideman (1988) and Johnson *et al.* (1990b) reported that changes in shear values during days 3 to 6 of ageing to be correlated with total activities of cathepsins B+L.

Although the increase in cathepsin activity has been observed to coincide with changes in shear values, Geesink (1993) proposed that their role in meat tenderisation is still questionable. Calkins and Seideman (1988) suggested that because of the pH optima of the calpains and cathepsins, the calpains could function early post mortem while pH is still high and cathepsins could then function best when the pH has dropped.

Little is known about the multicatalytic proteinase complex (MCP) system but Koohmaraie (1992b) contended that it may play a regulatory role in the post-mortem muscle proteolytic system. It can degrade multiple synthetic substrates as well as tropin-C and myosin light chains (Koohmaraie, 1992b). However, evidence for the MCP system to have any effect on tenderness during ageing is limited.

In comparison to the work conducted on the myofibrillar structure and enzymes involved in ageing, very little work into the changes which occur in the connective tissue structure during ageing has been reported. This is probably because of the general belief that ageing does not have a large effect on the connective tissue structure and it does not contribute much to the changes in tenderness observed. Asghar and Bhatti (1987) stated that although changes which occur in collagen have not yet been characterised and indirect evidence suggests that some alterations do occur in connective tissue which are reflected in the weakening of the collagen structure.

Evidence for changes in connective tissue during ageing have been reported. Lewis *et al.* (1991) found the non-solubilised part of perimysial collagen suffered a small degree of proteolytic damage during conditioning. Kruggel and Field (1971) reported intramuscular collagen was changed at the molecular level during ageing, and this was attributed to the cleavage of crosslinks between the polypeptide chains of the collagen molecule. Goll *et al.* (1964) suggested that weakening or rupture of cross linkages between collagen molecules,

resulting in increased collagen solubility could affect tenderness. Using electron microscopy, Nishimura *et al.* (1994) also observed structural changes in connective tissue during ageing.

The effects of ageing have been demonstrated to be extremely beneficial for improving tenderness. However, the exact nature of the changes which occur and the mechanisms responsible for these changes have not been totally elucidated. Because of this, it is difficult to quantify and control the actual increase in tenderness that will be achieved during the ageing of beef. Further research should enable such mechanisms to be identified and to better quantify the improvement in tenderness during ageing which will be detected by consumers.

2.4.2.3 Cooking

When meat is heated, its structure undergoes significant changes and these changes directly influence the palatability of the meat. The effect of heat on muscle structure and protein denaturation is extremely complex and not well understood. The changes in the muscle structure and protein denaturation are influenced by both cooking method and temperature (and hence, cooking time). However, the influence of the heating method will also interact with components of the meat, such as connective tissue content and pH.

The effects of cooking have shown to be extremely complex as highlighted in reviews by others (eg. Cross *et al.*, 1986; Harris and Shorthose, 1988; Judge *et al.*, 1989) and can be summarised as a toughening of meat fibres due to heat induced coagulation and shrinkage of the myofibrillar proteins and connective tissue. However, with slow, moist heating, tenderness increases as collagen is converted to gelatin (Harris and Shorthose, 1988). The changes which occur during the cooking process are the final factors which influence beef palatability. In terms of tenderness, the changes which occur in both myofibrillar and connective tissue structures are important because of their sometimes opposing effects. Beef is usually consumed after being cooked to an internal temperature between *ca.* 55°C (bleu) and 90°C (very well done).

2.4.2.3.1 The effect of heat on meat and its eating quality

When proteins of muscle are exposed to heat, they lose their native structure and undergo several changes in configuration (Judge *et al.*, 1989). Judge *et al.*(1989) stated that this change is initiated by denaturation of the proteins (alteration of structure due to non proteolytic changes). This may be followed by aggregation or clumping of protein molecules, referred to as

coagulation, the presence of which indicates loss of protein solubility. Protein coagulation is generally assumed to take place between 57°C and 75°C, but a specific temperature has not been established because muscle fibres consist of several different proteins, each with a different coagulation temperature (Cross *et al.*, 1986).

At the individual protein level, work has been done to investigate the temperatures at which myosin, actin, α-actinin, sarcoplasmic and regulatory proteins denature, coagulate and lose solubility (eg. Locker, 1956; Bendall, 1964; Hamm, 1966; Samejima *et al.*, 1969; Davey and Gilbert, 1974; Cheng, 1976; Cheng and Parrish, 1979; Leadner *et al.*, 1980; Stabursvik and Martens, 1980; Cross *et al.*, 1986; Resurreccion, 1994). However, discrepancies arose in the results reported for the different proteins and these were likely to have arisen from variation in experimental methodology employed between experiments, such as extraction techniques. Similar work has been conducted for connective tissue with Illing and Swan (1992) and Bendall and Restall (1983) specifying the individual shrinkage temperatures of the epi-, peri-, and endomysium.

Occurring concomitantly with the protein changes are structural changes in the myofibres and connective tissue which affect tenderness and juiciness. One of the most noticeable changes in muscle structure with heating is sarcomere shortening and fibre shrinkage which Cross *et al.* (1986) contended occurs when myofibrillar proteins are denatured and collagen shrinkage occurs, and is associated with the actual coagulation of the various proteins in the muscle.

Myofibrillar shortening has been reported to commence in isolated fibres between 40-50°C (Hegarty and Allen, 1972, cited by Cross *et al.*, 1986). However, for intact muscle, Davey and Gilbert (1974) stated that changes in fibre length did not appear to start until 60°C. According to Hamm (1966), between 50°-55°C a reorientation of the myofibrillar proteins also apparently occurs in parallel with the coagulation process.

During the cooking process, collagen fibrils have been reported to shrink to one third of their original length. This may occur at 50°C, but is usually complete in half of the fibrils at 61°-62°C (Judge *et al.*, 1989). Bendall and Restall (1983) proposed that with the stewing of beef, the epi-, peri- and endomysium started to shrink at 64°C with a considerable decrease in cross sectional area. Bendall and Restall (1983) and Sims and Bailey (1992) argued that collagen undergoes a shrinkage phase at about 60°-65°C.

Tenderness of cooked meat has been reported to reach a maximum between 55°-60°C (Harris and Shorthose, 1988). It has been debated whether this decrease in shear values was attributable to a decrease in connective tissue strength (Bouton and Harris, 1972a; Draudt, 1972; Beilken *et al.*, 1986) and/or myofibrillar strength (Bouton *et al.*, 1976b; Tornberg and Persson, 1988). The increased tenderness would appear to occur just prior to the shrinkage of myofibrillar and connective tissue.

It has been a common finding that shear force increases and sensory tenderness deteriorates as internal temperature increases from 50°C to 80° or 90°C (Hostetler and Landmann, 1968; Draudt, 1972; Bouton *et al.*, 1982; Robertson *et al.*, 1984; Belk *et al.*, 1991; Luchak *et al.*, 1991; Heaton *et al.*, 1991; Calkins *et al.*, 1995; Wulf *et al.*, 1996). The increased toughness associated with a higher internal temperature has generally been attributed to the myofibrillar component and sarcomere shortening (Dube *et al.*, 1972; Cross *et al.*, 1976; Bouton *et al.*, 1982; Resurreccion, 1994). In support of this, Bouton *et al.* (1981) and Beilken *et al.* (1986) reported initial yield values increased as internal temperature increased from 40°C to 80°C while peak force minus initial yield values decreased as temperature increased above 50°-55°C. The initial yield values reflect an increase in myofibrillar toughness and the peak force minus initial yield values indicate a decrease in connective tissue strength as temperature increased. However, the shrinkage of collagen fibres should not be disregarded as it has been reported to create tension on the muscle fibres and cause some moisture loss, along with a toughening effect (Davey and Gilbert, 1974; Sims and Bailey, 1992).

Sensory juiciness scores have also been reported to decrease (drier meat) as internal temperature increases above approximately 60°C (Rogers and Ritchey, 1969; Cross *et al.*, 1976; Belk *et al*, 1991; Luchak *et al.*, 1991). At the same time cooking loss has also been reported to increase. Meat containing 68-75% moisture in the raw state will contain approximately 70, 65 and 60% moisture after being dry heat roasted to 60°, 70° and 80°C, respectively (Cross *et al.*, 1986).

The expulsion of juice from the myofibrillar proteins has been attributed to the thermal shrinkage of both myofibrillar and connective tissue structures (Bouton *et al.*, 1976b). Bendall and Restall (1983) argued that the greatest amount of water expelled from meat during cooking was associated with collagen shrinkage. The moisture released during cooking has not only been attributed to the tightening of the muscle structure, but also to the reduction in the water holding capacity of the proteins, that occurs with protein denaturation (Resurreccion, 1994). Water

holding capacity has been shown to decrease with increased temperatures and also to be dependent on pH (Bouton *et al.*, 1973a; Harris, 1976).

After collagen shrinkage occurs, prolonged moist heating allows collagen fibres to swell and gradually denature. Continued heating causes further disruption of collagen and eventually collagen is solubilised to gelatin (Illing and Swan, 1992). The temperature at which collagen solubilises to gelatin has been found to occur at 65°C (Cross *et al.*, 1986) and 70°C (Illing and Swan, 1992). The solubilisation and conversion of collagen to gelatin has been reported to be essentially responsible for an increase in tenderness (Hamm, 1966; Bendall and Restall, 1983; Resurreccion, 1994).

The amount of collagen solubilised by heat has been reported to gradually increase as temperature increases from about 60°C to 93°C (Paul *et al.*, 1973; Forrest *et al.*, 1975; Judge *et al.*, 1989; Belk *et al.*, 1991; Lawrie, 1991). Sensory evaluations of amount of connective tissue have been reported to decrease as internal temperature increased from 60° to 90°C (Cross *et al.*, 1986) and from 68° to 78°C (Rogers and Ritchey, 1969).

Horgan *et al.* (1991) studied the effect of pH on the thermal transition temperature of collagen and reported that a lower pH corresponds to a lower thermal transition onset temperature. This result has two implications. First, studies investigating the effect of heating on collagen will be influenced by the pH of the meat. Secondly, the curvilinear trend observed between pH and tenderness reported by Bouton *et al.* (1973a) may have been influenced by this effect.

The effects of heating on collagen are also influenced by the relative portions of immature and mature crosslinks in the muscle because of their solubilities. Bouton *et al.* (1981) and Resurreccion (1994) reported that changes begin to occur at 50°C in young animals and 60°C in old animals. Even with slow, moist heating, the tenderness of meat from older animals will not undergo as much collagen solubilisation because of the higher content of heat resistant mature crosslinks. This will cause such meat to be considerably tougher than meat from younger animals.

This review has highlighted that the ultimate tenderness of cooked beef will be influenced by the effect of heat on both the myofibrillar and connective tissue components of the meat. Generally, research has indicated that as internal temperature increases, meat becomes

tougher and drier, as determined by both shear values and sensory evaluations (Cross *et al.*, 1986). It would appear that as internal temperature increases above about 60°C, toughness and juiciness decline primarily due to shrinkage and tightening of the myofibrils and partly due to the collagen. Shorthose (1991) stated that when steaks (generally low connective tissue content) were cooked (by dry heat) methods, there is insufficient time for any connective tissue softening and so only myofibrillar toughening would occur. However, muscles which are high in connective tissue would become more tender when cooked by moisture heat for long periods (above *ca.* 65°C). This treatment softens connective tissue through its conversion of collagen to gelatin. Despite these seemingly simple generalisations, the effect of cooking on eating quality is quite complex and cannot be based solely on these generalisations.

2.4.2.3.2 The effect of rate and method of heating on tenderness and juiciness

Traditionally, the most beneficial method of cooking high quality cuts of beef (ie. low connective tissue content) has been to cook at high temperatures for a short period of time. However, evidence (Cover, 1937; Penfield and Meyer, 1975; Cross *et al.*, 1976) suggests that a slower rate of cooking for a longer period of time may enhance both tenderness and juiciness. The tenderisation effect of cooking meat for long periods at low temperatures has been attributed to collagen degradation or softening without extensive hardening of muscle fibres (Cross *et al.*, 1986).

Cover (1937), Penfield and Meyer (1975) and Cross *et al.* (1976) reported slower cooking at lower temperatures produced more tender meat than faster rates of heating. Greater amounts of collagen solubilisation (Penfield and Meyer, 1975) and less connective tissue contraction (Harris and Shorthose, 1988) were reported with slower rates of cooking.

When McDowell *et al.* (1982) cooked samples in a waterbath and in an oven (to 60°C), the waterbath samples were more tender than dry heat oven roasted samples. Differences in rate of heat penetration between the cooking methods was observed with waterbathed samples in the 55°-60°C temperature range for a time period twenty percent longer than oven roasted samples. Paul (1963), cited by McDowell *et al.* (1982), suggested the slower rate of heating in the 57°-60°C range may promote softening of connective tissue without hardening muscle fibres. Bramblett *et al.* (1959, 1964) also concluded that the length of holding time between 57° and 60°C was closely related to increased tenderness.

Another theory was postulated by Penfield and Meyer (1975), Buck *et al.* (1979) and Laakkonen *et al.* (1970b) that slower heat penetration may have allowed greater enzyme activity to be retained in meat, effectively increasing tenderness. The rate of ageing was measured by Davey and Gilbert (1976) which indicated that the rate increased exponentially up to 40°C, rose more slowly to a maximum at 60°C and then decreased sharply to approach zero at 75°C. Other work has claimed that the tenderisation by prolonged cooking was achieved by increased enzyme activity on the myofibrillar structure between the temperatures of 50°-60°C (Beilken *et al.*, 1986) and up to temperatures of 65°C (Davey and Niederer, 1977). The weakening of the connective tissue structure was then responsible for tenderisation at temperatures greater than 55°C (Beilken *et al.*, 1986) and above 70°C (Davey and Niederer, 1977). The weakening of connective tissue reported by Beilken *et al.* (1986) at 55°C appears slightly lower than the temperature reported for collagen solubilisation at about 65°C. This may have occurred because Beilken *et al.* (1986) included some very young animals in their study and therefore collagen would solubilise at a lower temperature than with older animals. However, it would appear that further research on these two theories is required.

Cross *et al.* (1979) roasted steaks in a oven at 175°C and broiled them at 275°C to an internal temperature of 70°C. The roasted steaks were evaluated juicier and suffered less cooking loss and had a longer cook time than the broiled steaks. Another study by Cross *et al.* (1976) reported samples cooked at 223°C received lower tenderness and juiciness scores than those cooked in an oven at 121°C and 177°C. Buck *et al.* (1979) cooked samples in an oven at 94°C and a waterbath at 60°C and reported waterbathed samples were more tender. The waterbathed samples heater faster initially but as internal temperature approached the waterbath temperature, the rate of heating slowed substantially. Samples cooked in the oven had a considerably faster heating rate as the internal temperature approached the set endpoint temperature. In contrast however, Bramblett and Veil (1964) stated that meat cooked quickly to a given internal temperature has a lower cooking loss and is more juicy than meat cooked slowly. When a variety of cooking methods are employed to provide different rates of cooking, the effects of cooking method and temperature are confounded. Given that these effects cannot be separated in the experiments above, it highlights the need for work to be conducted to compare the effect of different cooking temperatures within one cooking method and also to compare cooking methods at the same temperatures. This would enable the effects of cooking rate and method on eating quality to be assessed. Obviously the differences in cooking devices sometimes makes it difficult to compare different cooking methods at the same temperatures.

The relationship between cooking method and temperature, and tenderness and juiciness, has been demonstrated to be muscle dependent (Paul, 1974; McKeith *et al.*, 1975; Belk *et al.*, 1991; Anon, 1995). Belk *et al.* (1991) used eigenvectors to investigate the loadings of sensory evaluations and shear values on the first principal component (thought to be a measure of overall palatability). These loadings varied depending on the muscle analysed. This indicated that the influence of myofibrillar and connective tissue toughness and juiciness on overall palatability differed significantly between muscles. These findings may partially explain some of the differences in results reported on the effects of cooking and also indicates future research into the effects of cooking on eating quality needs to investigate specific interactions with muscle type.

Another source of variation between experiments which may influence results could be the temperature of the meat prior to cooking. Hostetler *et al.* (1982) and Berry and Leddy (1990) reported higher internal temperatures of meat (eg. 26 °C) prior to cooking reduced cooking time and significantly improved tenderness compared to meat with an lower temperature (eg. 2 °C).

The beneficial effects of slow cooking have been demonstrated on relatively large pieces of meat. It is not known whether such benefits would also be obtained when steaks were cooked at different rates. This review highlights the complexity and variation which surrounds the effects of heating on meat quality, the lack of understanding of the mechanisms of change which occur with heating and the specific changes in the contributions of myofibrillar and connective tissue components to tenderness and juiciness. It also demonstrates the need of further research required in this area.

2.4.2.3.3 Effect of heat on the colour of cooked beef

Degree of doneness of a cooked steak is one of the first quality attributes a consumer will assess and is often a source of customer complaint (Cox *et al.*, 1997). Consumers use the internal colour of cooked meat as a measure of degree of doneness and the importance of delivering beef to the degree of doneness requested by consumers, on their overall satisfaction, has been highlighted by Cox *et al.* (1997). However, as yet, the only way to assess the internal colour of meat is to cut it and this does not usually occur until the consumer has received their steak at the completion of cooking. It would be extremely beneficial if the internal colour of meat could be determined during the cooking process (without cutting the meat) to guarantee

consumer satisfaction. A relationship between colour changes during the heating process and internal temperature or cook time appears to be highly variable and not well defined.

During cooking, usually two types of reactions occur which can influence the colour of cooked meat. Judge *et al.* (1989) outlined that the external colour of cuts heated with dry heat results from a combination of surface dehydration and chemical structures at the surface. The amine groups of the amino acids of the muscle proteins react with available reducing sugars and undergoing a Maillard browning reaction. This reaction occurred at high temperatures (90°C) and creates the dark brown colour typical on roasts or steaks.

Other dramatic changes also occur within the chemical structure of meat during heating and it is usually those changes that occur in the interior of the meat which form the basis of doneness assessment. Cross *et al.* (1986) outlined that as meat is heated, the globin moiety of myoglobin becomes denatured, and thus, it's function of protecting the haem diminishes. In essence, what occurs is that all reduced myoglobin and oxymyoglobin molecules are converted to metmyoglobin which is brown in colour. This progressive denaturation of pigments results in the internal colour showing a progressive loss of redness with increasing temperature. Objective measurements of cooked colour reflect a loss of redness and increase in lightness as internal temperature increased (Lyon *et al.*, 1986; Bowers *et al.*, 1987; Hague *et al.*, 1994).

Myoglobin denaturation has been shown to be dependent on both cooking time and temperature (Bernofsky *et al.*, 1959, cited by Bowers *et al.*, 1987; Palombo and Wygards, 1990a,b). However, Toumy and Lechnir (1964), reported that meat samples still appeared rare after seven hours at 60°C and concluded that the colour of cooked meat depended on temperature rather than time of exposure. Martens *et al.* (1982) reported meat lightness increased with holding time (eg. 20 minutes as opposed to five minutes) in the 45°-63°C temperature range. Work by Cox *et al.* (1994) has shown that in a set cook system, the range in degree of doneness and internal temperature varied widely. When values were adjusted to the same temperature, doneness scores were significantly affected by pH, steak thickness and weight. Hamm (1960) stated that the relationship between percent myoglobin denaturation and internal temperature is sigmoidal in nature and pH dependent. Although the gradual loss of redness with increased cooking is well known, there is basically no understanding of the nature of the relationship, or even whether there is a relationship, between colour change and temperature and time of cooking (and other factors which may influence this relationship).

Studies have been conducted to investigate the relationship between internal colour as measured objectively and by sensory panels (eg. Lyon *et al.*, 1986; Bowers *et al.*, 1987). Lyon *et al.* (1986) reported panel colour/doneness scores were more highly correlated with internal temperature than objective values of meat colour. It was suggested that protein aggregation could be a source of masking red pigment and/or coprecipitation of myoglobin with other proteins, thereby changing surface light scatter characteristics which affect both visual and objective measurements of colour in cooked meat.

Unklesbay and Unklesbay (1994) reported that, under real restaurant situations, no relationship exists between specified degree of doneness and broiling times. Customer satisfaction was strictly based on the performance of the cook. Under the rigid conditions employed in their study, there was a strong possibility of obtaining steaks one degree higher or lower than the degree of doneness requested when they were cooked to set end point temperatures. The use of different cooking temperatures did not appear to enlarge the existent variation in colour. However, higher oven temperature broiling was reported to result in a broader band of more consistent cooked colour (Unklesbay and Unklesbay, 1994).

It is apparent that the mechanisms involved in the change of colour of red meat during heating are not well understood. The absence of a relationship between internal temperature and colour makes the aim of providing consistent colour for a given doneness virtually impossible. Even with set cooking conditions, colour will vary. Given that degree of doneness is of vital importance to consumers and that no guarantees in terms of colour can be provided, further work in this area is essential.

2.5 Literature review overview

Previous research has highlighted the wide variety of factors, and their interactions, which can influence beef eating quality. Many of the differences in the results obtained may be partly attributable to variations in techniques and conditions employed, and the complexity of mechanisms determining tenderness.

In terms of the evaluation of tenderness and juiciness, trained taste panels are widely employed, yet it is not known how taste panels evaluate samples in specific relation to consumers, or if training has a large effect on sensory evaluation. The ultimate tenderness and juiciness of beef is known to be highly influenced by the method of cooking and the rate and

extent of cooking. Consequently, this can affect the results obtained when investigating the effects of other parameters. An example of when cooking may influence studies of tenderness and juiciness results, is the situation in which the effects of intramuscular fat and degree of doneness are investigated.

Another area of research receiving constant investigation is the effect of *Bos indicus* content on tenderness and juiciness and its possible interaction with electrical stimulation and ageing. The effects of these factors are investigated in this thesis.