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Review and meta-analysis of emerging technologies for tenderising red meat

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ABSTRACT

Tenderness is an important meat quality trait and the consumer is prepared to pay more for quality. The application of high pressure processing, shockwaves, ultrasound, pulsed electric field, *sous vide* cooking, exogenous enzyme addition and muscle stretching to pre- and post-rigor meat for tenderisation, are described in this review. These non-thermal, and thermal, innovative and new technologies can be used with varying success to cause physical disruption to muscle structure, enhanced proteolysis and ageing and muscle protein denaturation, solubilisation and gelation resulting in enhanced texture and juiciness. A meta-analysis has been conducted on the effect of selected technologies on post-mortem tenderisation. The meta-analysis results are presented and can assist in identifying the optimum application of these technologies for tenderisation of products from different muscles in the carcass and for different markets (food service, fresh product, export markets). In the future, a combination of new and innovative technologies will be ideally suited to deliver a range of desired textures for meat products.

EXECUTIVE SUMMARY

Tenderness is an important meat quality trait and the consumer is prepared to pay more for quality. A range of new and innovative technologies have potential for application in the meat industry in order to accelerate or improve the texture of meat. The application of high pressure processing, shockwaves, ultrasound, pulsed electric field, *sous vide* cooking, exogenous enzyme addition and muscle stretching to pre- and post-rigor meat for tenderisation, are described in this review. A meta-analysis has been conducted on the effect of selected technologies on post-mortem tenderisation. The meta-analysis results are presented and can assist in identifying the optimum application of these technologies for tenderisation of products from different muscles in the carcass and for different markets (food service, fresh product, export markets). In the future, a combination of new and innovative technologies will be ideally suited to deliver a range of desired textures for meat products.

Cooking - The principles underlying the structural, biochemical and texture changes during conventional cooking of meat are being increasingly understood. Conventional cooking of meat by application of heat affects the state of meat proteins, resulting in texture changes, structural changes and redistribution of water contained therein. For conventional cooking techniques, major improvements in meat tenderness have been achieved through selection of temperature/time/heating method based on the amount of connective tissue and an understanding of the mechanisms behind tenderization. More research is required to understand the varying effect of temperature on different muscles, as well as the absence of a continuous effect of time of cooking on the improvement of tenderness. Extensive knowledge of the effects of conventional cooking methods on meat texture needs to be extended to the assessment of new technologies.

Sous vide - *Sous vide* cooking is a recent development for chefs around the world, although some countries have been using *sous vide* for a number of years. *Sous vide* cooking gives a reliably tender and juicy meat product after extended cooking times. The challenge will be to optimise *sous vide* cooking for different muscles to ensure minimum cooking time/temperature combinations.

HPP - The application of HPP for meat tenderisation has been known since the 1970's but this has not been adopted by the meat industry. The number of high pressure processing vessels being installed around the world is accelerating. Meta-analysis was used to demonstrate that overall, HPP results in significant improvement in meat tenderness (WBSF decrease of 44 N), with the greatest improvements achieved with pre-rigor meat (WBSF decrease of 82 N) and significant improvements in post-rigor meat if the pressure was applied at 100-200 MPa (WBSF decrease of 49 N). Thus the application of HPP for tender ready-to-eat meat products and meal solutions shows promise, particularly if it is used to reduce the energy or time required for cooking methods such as *sous vide*. Further development of the HPP conditions for tenderising specific muscles is required as well as technological developments to ensure high target temperatures are reached during processing. There is a change shown in the colour of meat, as a consequence of the application of HPP. Thus HPP tends to be considered as a viable option for RTE food, but not for the 'fresh food' shelves, limiting its commercial application. There is an R&D opportunity to investigate the retention of fresh meat colour in HPP-treated muscle foods.

Shockwave - Detonation shockwave has also been around since the 1970's but the safer system for delivering shockwaves using electrical discharge under water has been developed more recently. Meta-analysis of all the available studies showed that detonation shockwaves significantly improve meat tenderness (reduce WBSF by 18 N) and the results are not as dramatic for electrical shockwave, but still significant (WBSF decrease of 5 N). Nevertheless, shock waves applied using electrical discharge show some promise for tenderisation and thus warrant further research.

Pulsed electric field (PEF) - Research on the application of high frequency PEF to meat for tenderisation is very new with promising results. The meta-analysis showed that the overall reduction in WBSF, when high intensity PEF is applied to cold-boned meat is 7 N, and there is no effect when applied to hot-boned (pre-rigor) meat. When PEF is applied to hot-boned meat, the effect can be toughening, or tenderisation, depending on the muscle. The application of PEF for meat tenderisation will most likely be optimised in the coming years and research will be needed to understand the tenderisation variation between muscles in response to the application of PEF.

Ultrasound (US) – Low and high intensity US application has some potential for improving the texture of post-rigor meat (decrease WBSF by 6 N), with the most promise appearing to be with the use of high intensity. However, for this to be a commercial viability, the acoustic conditions need to be thoroughly investigated in order to optimise the conditions for tenderisation.

Stretching - Smartstretch™/Smartshape™ and Pi-Vac® are new technologies which have been shown to result in tenderisation of beef, by about 10-15 N and lamb by a significant

20-50 N. The concept is not new as tenderstretch is also known to consistently yield more tender meat, but only in certain muscles in the carcass.

Enzymes - Enzymes from a variety of sources have shown promise for application to the tenderising of meat. Changes in flavour, odour or overly mushy can be problematic with some enzymes. In combination with other tenderising technologies, the application of enzymes to tenderise particularly collagenous or tough muscles shows promise.

Combination of technologies - Animal, genetic and processing factors are known to increase red meat toughness and different muscles in the carcass vary widely in their tenderness. There is potential for tenderisation technologies to be used to ameliorate the toughness in red meat resulting from genetic, production and processing practices. Furthermore the application of tenderisation technologies to pre- and post-rigor muscles, including muscles traditionally considered to be quite tough, should deliver real benefits to an industry that prides itself on quality, and is also rewarded for quality.

Use of WBSF - The majority of investigations reported in this review have used objective measures of tenderness, principally Warner-Bratzler shear force, although some have used texture profile analysis and trained sensory panels. As discussed in the introduction, tenderness is only one dimension of the consumers assessment of acceptability of a food product. Thus more data is needed on the effects of these interventions/cook methods on consumer sensory scores, particularly tenderness, flavour, juiciness and acceptability.

Future trends - High pressure processing, shockwave processing, ultrasound, pulsed electric field, *sous vide* cooking and Smartshape™ can be used with varying success to cause physical disruption to muscle structure, enhanced proteolysis and ageing and muscle protein denaturation, solubilisation and gelation resulting in enhanced texture and juiciness. The optimum application of these new and innovative technologies for tenderisation will need to be adjusted for different muscles in the carcass, different markets (food service, fresh product, export markets) and also target demographics. The consumer will likely become more discerning and demand a specific desired texture. The predictions are that the elderly, dentally-challenged consumers will demand a soft to mushy texture in their meat purchases while other consumers will desire other attributes such as firm texture and chefs will desire meat with some pre-purchase texture guarantee. A combination of new and innovative technologies will be ideally suited to deliver a range of desired textures for meat products in the future. Pre-rigor processing (hot-boning) of meat will allow meat to be delivered into Smartshape™ machines, packaged then delivered to a combination of PEF/US/shockwave treatments, plus or minus the addition of enzymes depending on the muscle, and then either into a cook process of *sous vide* or high pressure processing for case ready products or into a rapid chill process for immediate delivery to destination markets. The combination of systems used will ensure that the meat is safe, wholesome and 'tender' immediately after coming out of the factory. Processing of meat to final market- or case-ready product will occur in the room next to the hot-boning operation.

The implementation of these technologies in industry will be dependent on operators willingness to innovate, the capital and operating cost of the technologies and the cost-benefit. Previous research shows that the consumer will be willing to pay for the use of

innovation technologies for assured texture. The question may become, can the industry afford not to innovate, in order to grow their customer base?

1. Introduction

Tenderness is an important meat quality trait and the consumer is prepared to pay more for quality (Lyford *et al.* 2010). The producer, processor and retailer are often paid more for assuring quality and tenderness, an example being the Meat Standards Australia grading system for beef MLA (2016; Channon and Warner 2011). Payment for quality will increase in the future, as consumers of premium products in many countries become more discerning of quality. Animal and post-mortem factors influencing meat tenderness include the amount and solubility of connective tissue, presence of intramuscular fat (marbling), sarcomere shortening during rigor development, and post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koohmaraie and Geesink 2006; Warner *et al.* 2010). The callipyge gene in sheep, *bos indicus* cattle, the use of hormonal growth promotants and beta-agonists are all known to influence the tenderness of meat, principally through mechanisms via the protease system (Lean & Dunshea, 2014; Warner, Greenwood, Pethick, & Ferguson, 2010; Watson, 2008). The effect of heat applied during processing, for microbial inactivation, or preparation of ready-to-eat meals or in order to prepare meat for consumption are also of importance for meat tenderness. During heating, muscle proteins denature, solubilise and form gels, depending on the heating conditions applied, with effects on both meat texture and water-holding capacity, and important implications for sensory acceptability.

Texture and juiciness of meat defines a large part of the mouth-feel (Lawless and Heymann, 2010) and texture, juiciness and flavour together form the consumers' perception of 'overall quality'. So although this paper focusses on 'tenderness', the implicit effects of texture on the consumers' perception of overall quality, being both flavour, juiciness and texture, are important. Each piece of meat has a multi-scale, or hierarchical, nature in its' physical properties, with aspects of the scale being of differential importance in contribution to texture, depending on the inherent hierarchical structure of the muscle, including the contribution of connective tissue proteins, structural proteins within the muscle cell and degradation or disruption of these proteins. In addition meat tissue consists of numerous molecules which denature, interact and solubilise at different temperature and time scales.

Thus, acceptability of the texture of meat refers to the whole sensation of mouth-feel and is a consequence of (1) the endogenous tenderness traits of the muscle and applied exogenous tenderisation interventions prior to cooking, (2) the effect of cooking on the muscle protein denaturation, gelation and solubilisation and water loss, (3) the breakdown of the muscle structure in the mouth and (4) the interaction with other sensory components of juiciness, odour and taste. Juiciness is a result of the inherent moisture and fat content of the meat, and the release of fluids from the muscle structure during oral processing. Within the food industry, thermal processing is achieved by subjecting food to a temperature range of 60-100°C for a time duration of several minutes to longer duration, examples being high temperature-high hydrostatic pressure and *sous vide* cooking. Apart from a high energy requirement, which creates an impact on the cost of technologies and thus industry feasibility, thermal processing of food (such as high pressure processing) can lead to undesirable side effects such as nutritional loss or flavour issues (Knorr *et al.* 2002). Thus

there is also an interest to develop and use non-thermal treatments in food processing, such as ultrasonics, shockwave, pulsed electric field and pre-rigor stretching.

The action of heat on meat tissue is complex but it is the impact on the myofibrils (predominantly shrinkage through denaturation and coagulation of myosin), the sarcoplasmic proteins (aggregation and gel formation), the connective tissue (shrinkage and solubilisation) and water holding capacity that have the greatest impact on tenderness (Baldwin 2012) and the associated sensory parameter "juiciness" (James and Yang 2012). New developments in technology have led to the emergence of innovative processing methods which are being investigated for adoption by the meat processing industry. By manipulating processing and cooking parameters, such as time, temperature, atmosphere and pressure, the structural changes of meat during processing and cooking are influenced, with a resulting impact on tenderness (James and Yang, 2012). This paper will discuss the technologies that can be applied to improve meat texture and the importance, and manipulation, of cooking conditions in determining meat texture. Cooking has been conventionally understood as the application of heat to make the food more sensorially acceptable, safe and digestible. In the strictest sense, cooking is defined as the heating of meat to a sufficiently high temperature to denature proteins (Davey and Gilbert 1974). The denaturation of meat proteins is in turn reflected in changes in the sensory properties of meat. Cooking is not defined as a new or innovative technology, but is a necessary process applied prior to consumption, except for raw meat consumption such as Japanese sashimi. Cooking is explained in order to understand the importance and relevance for variations in texture and new methods for cooking (*sous vide*) are also described. The effects of cooking temperature on the changes in texture of meat depend on water-holding capacity, protein denaturation, solubilisation and gelation and determine the force required to shear the meat (in the mouth) and are described below.

Heating is associated with high temperature high pressure processing (HPP), with *sous vide* and long temperature long time cooking and can also be generated during the application of technologies such as PEF, US and shockwave, thus a consideration of the effects of cooking and heat on muscle structure and proteins is required and is included below. In order to understand the underlying mechanisms, the contribution of muscle structure and proteins to the changes in texture with the application of innovative and new technologies are presented.

2. Overview of meat tenderness

A brief overview of meat tenderness is presented in order to understand how different technologies may have their effect on muscle structure. This also helps to understand where there may be possible synergies between technologies. Tenderness is an important meat quality trait and the biological, structural and physiological mechanisms underlying meat tenderness have been extensively investigated (Dransfield & Jones, 1980; Harper, 1999; Koohmaraie, 1988; Tornberg, 1996). Essentially, meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koohmaraie & Geesink, 2006) and during heating/cooking the balance between myofibrillar toughening and solubilisation/gelation of proteins. Intramuscular fat and post-mortem

energy metabolism have effects on meat tenderness through these mechanisms (Harper, 1999; Hocquette et al., 2010; Nishimura, Hattori, & Takahashi, 1999; Tornberg, 1996; Thompson et al., 2006).

Interactions occur between these factors which can be difficult to separate (Harper, 1999) and each of these is influenced to a greater or lesser degree by genotype and the pre- or post-slaughter environment (Warner et al., 2010).

Effects of sarcomere length

Early work showed the pivotal effect of muscle sarcomere length on meat tenderness (Marsh & Leet, 1966). Cold shortening is a result of rapid temperature decline below 10-12 ^{o}C while the muscle is still pre-rigor, the consequence being sarcomere shortening and tough meat (Hwang, Devine, & Hopkins, 2003). Furthermore, heat-shortening is known to occur when muscles enter rigor at a high temperature (>30-35 ^{o}C) although the resulting toughness is though to be mediated through both protease inhibition and sarcomere shortening (Thompson et al., 2006).The effect of sarcomere length on meat toughness is predominantly mediated through the increased overlap of myofilaments (Dransfield & Rhodes, 1976) and the toughness comes from the myofibrillar structure (Lepetit, Grajales, & Favier, 2000). The amplitude of cold shortening, and thus toughening, is dependent on the muscle collagen content and the angle that perimysium collagen fibres make with muscle fibres, thus the toughening is strongly influenced by the cooking temperature (Rowe, 1974; Lepetit et al., 2000).

Any one muscle will of course not have uniform sarcomere lengths throughout as each muscle fibre goes into rigor at different times. At intermediate rates of temperature decline, minimal shortening of sarcomeres occurs at rigor (Marsh, Ringkob, Russell, Swartz, & Pagel, 1987b; Pike, Ringkob, Beekman, Koh, & Gerthoffer, 1993). If the temperature decline is too rapid and glycolysis is slow, cold shortening occurs resulting in a profound increase in toughness (Marsh et al., 1987b; Pike et al., 1993). If the temperature fall is too slow, and glycolysis is fast, heat-toughening (also called rigor-toughening, heat-shortening or rigor shortening) can occur with associated tougher meat, due to a failure of the tenderising processing during ageing (Dransfield, 1994; Marsh, Ringkob, Russell, Swartz, & Pagel, 1987a; Pike et al., 1993). Once rigor occurs, sarcomere length does not generally change with ageing (Koohmaraie, Doumit, & Wheeler, 1996), although there is a shrinkage in length upon heating (Purslow, Oiseth, Hughes, & Warner, 2016). Thus the normal pre-rigor restraint on muscles through tendons and attachments, which can be increased through the post-slaughter application of stretching processes such as tenderstretch (hanging by the aitch bone), can minimise the degree of sarcomere shortening and associated toughening.

Effects of proteolysis post-slaughter

The proteolysis contribution to meat tenderness is predominantly regulated by the protease levels in the muscle at slaughter, duration of post-rigor ageing and protease activity during ageing (Koohmaraie and Geesink, 2006). Access of proteases to myofibrillar protein substrate in cold-shortened beef semitendinosus is thought to be limited and partially explain increased toughness of cold-shortened meat (Weaver, Bowker, & Gerrard, 2008).

The rate and extent of post-mortem proteolysis by the calpain system explains the majority of the observed improvement in tenderness with ageing of meat (Koohmaraie, 1994; Taylor et al., 1995), although a role for cathepsins during prolonged ageing cannot be ruled out

(Moya, Flores, Aristoy, & Toldra, 2001; Moya, Flores, & Toldra, 2001; Toldrá, 2006). Furthermore, cathepsins may be active during heating, as they are known to be active up to temperatures of 55-60 °C (Purslow et al., 2016). The activity of these proteolytic enzymes is also regulated by the rate of pH and temperature decline during rigor development (Dransfield, 1994) and ionic strength (Ouali, 1984).

The effect of the calpain system on tenderisation post-slaughter relies on a balance between the rate of activation and activity, and the rate of inactivation or denaturation of the proteolytic enzymes (Dransfield, 1992; Dransfield, 1993; Dransfield, 1994; Dransfield, Etherington, & Taylor, 1992; Dransfield, Wakefield, & Parkman, 1992). The rate of activation and activity is reliant on the concentrations of calpain 1, calpain II and their inhibitor, calpastatin at slaughter and the conditions at slaughter (Koohmaraie, 1994).

Effects of connective tissue

The connective tissue contribution to meat tenderness is predominantly determined by the development of heat-stable cross-links as well as the total collagen content, and are predominantly established in the animal pre-slaughter (Harper, 1999) although there is some influence post-slaughter through sarcomere length (Lepetit et al., 2000).

Connective tissue is an integral component of muscle that transmits contractive forces from the myofibrils in the postnatal animal (McCormick, 1994). Connective tissue consists primarily of an extra-cellular matrix and is the predominant component of endomysium, perimysium and epimysium (Harper, 1999). Collagens are the major protein constituents of perimysial connective tissue (Dransfield, 1977), and as an animal matures, covalent cross-links between collagen fibrils become heat-stable and these links significantly increase meat toughness (McCormick, 1994).

Simplistically, the cross-links, along with myofibril shrinkage, determine the extent of tension generated during heating and the residual adhesion between muscle fibres and hence, the greater the number of cross-links, the tougher the meat (Bailey, 1985; Light, Champion, Voyle, & Bailey, 1985). It is therefore not the amount of collagen present, rather the degree of structural linkage in collagen that determines its contribution to meat tenderness (Bailey, 1985; Light et al., 1985). However as with all biology and meat science, there are always cases that do not fit our understanding. In Japanese black cattle, Nishimura et al. (1999) found that age-related increases in semitendinosus toughness were unrelated to collagen solubility or total collagen.

Connective tissue that is present within the muscle is macroscopically unaffected by slaughter per se or by rigor mortis (Harper, 1999). It is generally considered that connective tissue does not change or degrade post-slaughter although small post-slaughter changes during tenderstretch hanging may contribute to increased tenderness (Harper, 1999). As discussed above, sarcomere shortening influences the perimysial collagen fibrils and Lepetit et al. (2000) has shown that at cooking temperatures above 60 °C, the increased toughness of cold-shortened meat is derived from the myofibrillar as well as from the collagen components. The contribution of perimysial collagen in shortened muscles to the measured toughness, depends on the total collagen, the collagen thermal stability and on the cooking temperature.

Effects of glycogen metabolism

Post-mortem energy metabolism in muscle and in particular, glycolysis and rigor onset, are important for determining sarcomere length (Thompson et al., 2006). The pre-rigor metabolism and availability of substrate, combined with muscle temperature, in any muscle in a carcass will determine the shortening occurring at rigor and thus the post-rigor sarcomere length (Koohmaraie & Geesink, 2006). As discussed above, cold shortening and heat-toughening in muscles will influence the myofibrillar, the connective tissue and also the proteolytic contributions to meat tenderness. Thus it is evident that control of energy metabolism post-mortem is important for producing tender meat.

Meat tenderness varies not only with the rate of glycolysis and rigor onset post-slaughter, but also with the extent of glycolysis, classically identified through the ultimate pH (pHu) achieved in a muscle. The relationship between meat pHu and tenderness is quadratic, with a peak in toughness at pHu 6.1 (Purchas & Aungsupakorn, 1993). The improved tenderness as ultimate pH increases above 6.1 appears to be largely attributable to improvements in water-holding capacity and consequent decreases in cooking losses, and to the greater activity of proteases at pH values close to neutrality (Yu & Lee, 1986). The reasons for beef becoming tougher with intermediate elevations in pH to around 6.1 appear to be due to reduced sarcomere length (Purchas and Aungsupakorn, 1993).

Muscle metabolism pre-rigor in any muscle of a carcass can vary significantly with nutrition of the animal influencing pre-slaughter muscle glycogen levels, pre-slaughter stress influencing muscle metabolism at slaughter, muscle fibre type, post-slaughter electrical stimulation of the carcass and genetics (Channon, Payne, & Warner, 2000; Gardner, Kennedy, Milton, & Pethick, 1999; Martin, Gardner, Thompson, & Hopkins, 2006; Simmons et al., 2006; Thompson et al., 2006; Warner et al., 2006; Warner et al., 2007), to name a few. In theory, effective control of post-mortem pH and temperature decline can provide precise management of meat quality outcomes (Simmons et al., 2006) as well as allowing more precise measurement of heritability of traits of interest and variation between genotypes and breeds. In practice, this can be difficult to achieve under commercial conditions and variable responses are usually due to animal history rather than processing conditions (Simmons et al., 2006).

Changes in the texture of meat during heating

Objective measurements of Warner-Bratzler shear force (WBSF) and subjective sensory evaluations have identified two to three phases of changes in toughness and tenderness as a function of temperature. Figure 1 illustrates three phases of changes in texture, measured using WBSF, during heating of porcine *semitendinosus*; two phases of toughening between 40-50°C and between 60-80°C and a phase of tenderisation between 50-60°C (Christensen *et al.* 2000). Davey and Gilbert (1974) showed that the increase in toughness between 40 and 50°C was three to fourfold over the temperature range, and there was further doubling of toughness between 65 and 75°C in bovine *sternomandibularis* muscle. Over the temperature range 50-65°C, Davey and Gilbert (1974) did not observe any tenderisation but observed a 'plateau' (viz. no change) in tenderisation over this range. In the study of Martens, Stabursvik and Martens (1982), they related two phases of toughening (one below 53°C and the second between 63 and 73°C) to the denaturation of myofibrillar proteins and collagen (see following section for further discussion). Using sensory tenderness scores for porcine *longissimus* (Tornberg 2005), sensory chewing work for bovine *semimembranosus*

and *psoas major* (Machlik and Draudt 1963) and WBSF for bovine *semitendinosus* (Martens *et al.* 1982), other studies have similarly shown an increase in tenderness between the cooking temperatures 50 and 64°C and several demonstrated a maximum tenderness over the cooking temperature of 60-64°C (Machlik and Draudt 1963; Martens *et al.* 1982).

It is well known that species vary in the rate of muscle proteolysis post-mortem, and thus rates of meat tenderisation; pork>sheep>beef (Koohmaraie, Whipple, Kretchmar, Crouse and Mersmann 1991). Different muscles also vary in biochemical, structural, connective tissue and proteolytic activity (Rhee, Wheeler, Shackelford and Koohmaraie 2004; Wheeler, Shackelford and Koohmaraie 2000). Thus in spite of potential and actual variability in experimental/cooking conditions, species and muscles studied, all studies above consistently show at least two phases of texture change.

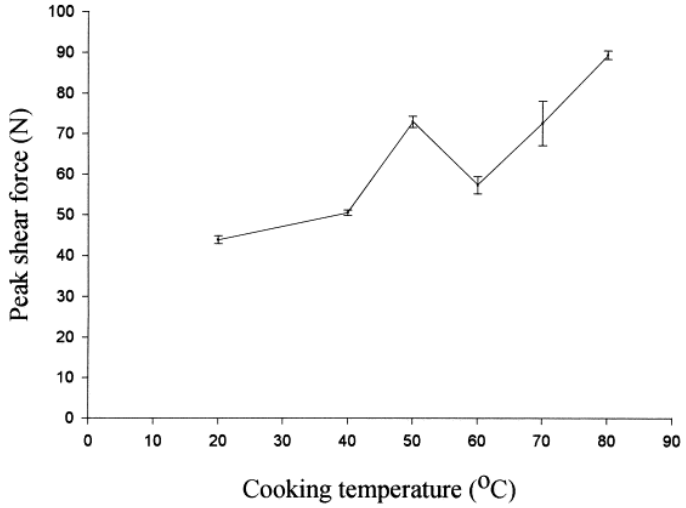


Figure 1 Changes in the peak shear force of pork semitendinosus *during cooking (Christensen et al. 2000).*

Structural and biochemical changes of meat during heating

Concomitant with the texture changes described in the previous section, heat induces transversal and longitudinal shrinkage of meat (Hostetler and Landmann 1968; Tornberg 2005; Hughes *et al.* 2014). The transverse shrinkage is reported to start when meat is heated between 35-45°C (Hostetler and Landmann 1968; Hearne *et al.* 1978; Bendall and Restall 1983; Palka and Daun 1999; Tornberg 2005) and to complete between 60 and 62°C (Hostetler and Landmann 1968; Hearne *et al.* 1978; Bendall and Restall 1983; Palka and Daun 1999). The longitudinal shrinkage reported as either sarcomere length or fibre length change starts between 55°C and 64°C (Hostetler and Landmann 1968; Hearne *et al.* 1978; Bendall and Restall 1983; Palka and Daun 1999) and completes at a maximum at around 90°C. Figure 2 illustrates transversal and longitudinal shrinkage of a single muscle fibre that is representative of the changes occurring in larger meat pieces.

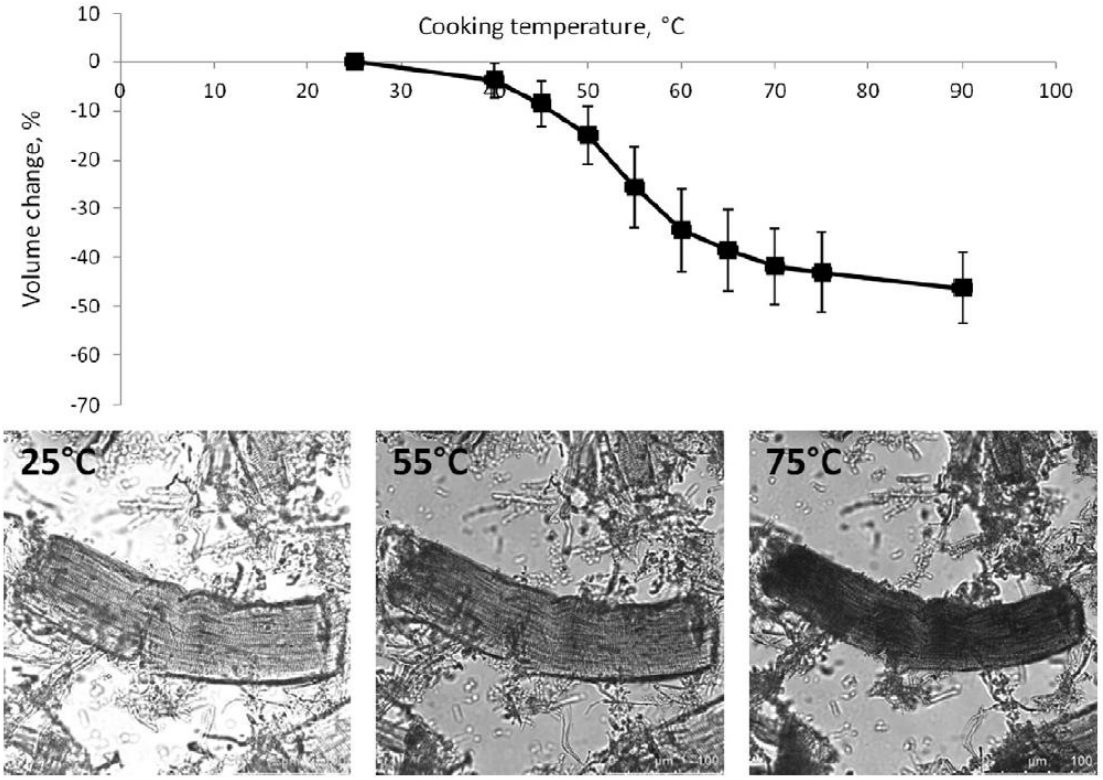


Figure 2 Changes in muscle fibre fragment volume with heating temperature of 14 days aged bovine semitendinosus and light microscopy images of one particular fibre fragment followed during heating, using a heating stage adaptor for a confocal microscope. The images show transversal and longitudinal shrinkage of a single muscle fiber. From (Hughes et al. 2014).

During extended duration of cooking, collagen can solubilise and increase meat tenderness (Laakkonen *et al.* 1970). Even when heat is applied at a constant rate, the distinct chemical nature of the different meat proteins does not result in a denaturation as a single rate process (Machlik and Draudt 1963). To understand the thermal behaviour of meat proteins, denaturation curves have been obtained by differential scanning calorimetry studies that measure the additional energy required for protein unfolding. Normally, three denaturation peaks appear on the differential scanning calorimetry thermograms of bovine, porcine or lamb muscles; the first ranging between 53 and 58°C, the second between 62 and 67°C and the third between 76 and 83°C (Findlay and Stanley 1984; Xiong *et al.* 1987; Bertram *et al.* 2006; Berhe *et al.* 2014). The first one is normally assigned to the denaturation of myosin, the second to sarcoplasmic proteins and collagen and the third to actin (Findlay and Stanley 1984; Xiong *et al.* 1987; Bertram *et al.* 2006; Berhe *et al.* 2014).

By undertaking simultaneous examinations of the biochemical and sensory changes during heating, relationships have been established between the denaturation of specific proteins and the texture changes and cooking loss at a defined temperature. The first rise in toughness, from 40-50^{o}C is attributed to either an increased concentration of collagen fibres in a cross-sectional area (Christensen *et al.* 2000) and/or to the denaturation of myosin (Bejerholm and Aaslyng 2004). The phase of tenderisation which is usually seen between 50-64^{o}C is thought to be due to partial denaturation, shrinkage and solubilisation of collagen

fibres (Christensen *et al.* 2000; Bejerholm and Aaslyng 2004). The final rise in toughness between 64-80°C is attributed to denaturation of myofibrillar proteins, particularly titin and actin (Christensen *et al.* 2000) and sarcoplasmic proteins (Christensen *et al.* 2000; Bejerholm and Aaslyng 2004). Actin denaturation occurs at temperatures of around 80-82°C relating it to the expulsion of water from the muscle, i.e. cooking loss (Bertram *et al.* 2006) although others show a denaturation temperature range for actin of 70-90°C (Bejerholm and Aaslyng 2004) or 68-78°C (Vilgis 2015). The discrepancies between studies highlights the complexity of the reactions and the need for further research.

Since the early years of meat science, the softening and disintegration of meat during cooking was considered to be solely due to the formation of gelatine from collagen at a temperature of around 63°C where cross linkages between the collagen molecules are separated (Hamm 1966). Similarly, the decrease of meat toughness at 50-60°C recently has been attributed to the solubilisation of the connective tissue (Christensen *et al.* 2000) or collagen shrinkage (Bejerholm and Aaslyng 2004). More recent papers question the role of collagen in short cooking times and propose that the changes in texture are dominated by myofibrillar and sarcoplasmic protein denaturation (Purslow et al. 2016)

Finally, although the sarcoplasmic proteins have received far less attention, there is some evidence for their role in texture and water loss changes during cooking. Tornberg (2005) suggests that the increase in tenderness over the cooking temperatures from 50 to 65°C is because of the formation of a gel of aggregated sarcoplasmic proteins. This gel glues the fibers and fibre bundles together, changing the viscoelasticity and making the meat more easily fractured by WBSF and by mastication in the mouth. The aggregation and gelation of sarcoplasmic proteins starts around 40°C and finishes around 60°C (Baldwin 2012), although the changes from a viscoelastic to elastic material, concomitant with tenderisation, only occur above 50°C (Tornberg 2005). Monin and Laborde (1985) and Wilson and van Laack (1999) have shown evidence for a role of sarcoplasmic proteins in water-holding capacity in fresh muscle. Liu, Arner, Puolanne and Ertbjerg (2016) recently have shown convincing evidence that the presence of sarcoplasmic proteins increases water-holding capacity and reduces water loss during cooking. They postulate that the sarcoplasmic proteins form a network around myofibrils that binds water, and describe it as a gel.

Low temperature – long time (LTLT) heating

Recently, LTLT cooking has attracted more attention for professional and private cooking (Becker *et al.* 2015). It is well known that for the tenderisation of the meat to happen, it is better to cook meat containing moderate to high levels of connective tissue for a longer time at the temperature where collagen solubilises rather than to cook it at high temperatures (Laakkonen *et al.* 1970). The final temperature and the time of cooking in the temperature range between 50°C and 60°C will decide if the tenderisation, as shown in Figure 1 as an intermediate phase between the two phases of meat toughening during cooking, is going to occur. Increasing the temperature of LTLT cooking from 48-53°C to 58°C leads to increased tenderness (WBSF in LD- ~37 N to 26 N; in ST ~52 N to 38 N), but also to an increased cooking loss and decreased juiciness in pork *longissimus dorsi* (Christensen *et al.* 2011). With beef *semitendinosus* from young bulls, the increase in tenderness with cooking is dependent on both temperature and time, with similar levels of tenderisation achieved for cooking at 58°C for 2.5 hrs and 43-58°C for 7.5 or 19.5 h, compared to 53°C for

2.5 hrs (WBSF; 43°C, 69 N vs 28-37 N for others) (Christensen *et al.* 2013). With LTLT cooking of pork and beef meat between 53 and 58°C, it was confirmed that increasing the heating time of meat over this temperature range increases the solubility of collagen and decreases the WBSF of *longissimus* and *semitendinosus* muscle, especially in muscles from older animals (Christensen *et al.* 2011; Christensen *et al.* 2013). Thus, the amount of connective tissue in the different muscles will influence the phases of toughening and tenderisation. Overall, it is important to distinguish if LTLT cooking is performed under vacuum (sous vide – see below), under alternating current (ohmic – see below) or with conventional cooking techniques to be able to interpret and document those studies.

3. Sous vide cooking

Sous vide (French for "under vacuum") is a cooking method in which raw food material is cooked inside vacuum pouches under a controlled environment (Roldán *et al.* 2015). The *sous-vide* cooking conditions for different types of meat used by chefs are quite different to those employed in traditional cooking methods or those adopted by the catering and food industries. Thus, common cooking conditions for beef, pork or lamb recommended by chefs involve LTLT *sous vide*, such as 58–63 °C for 10–48 h, while temperatures for catering and ready to eat meals very frequently reach 75–80 °C (García Segovia *et al.* 2007; Roldán *et al.* 2015). *Sous-vide* is typically performed either as cook-serve in which food is served immediately after cooking or as cook-chill in which cooked food is rapidly chilled to temperatures of 0-3°C (Baldwin 2012). The method of *sous vide* cooking has been widely adopted in top-end restaurants due to its comparatively easy handling as well as lower contamination risk. *Sous-vide* cooking can be performed in a water bath or a convection steam oven.

The effect of *sous vide* cooking on the storage stability of meat and the influence on shelf-life and hygiene reasons has been extensively investigated (Hansen *et al.* 1995; Hansen and Knöchel 1996; Schellekens 1996; Vaudagna *et al.* 2002). In comparison, the effect of *sous vide* cooking on meat texture is somewhat limited. As temperature and time are two of the essential variables in *sous vide* cooking, similar to conventional heating, some studies have examined the effect of these two parameters on the eating qualities of meat. As shown in Table 1, Figure 4and other papers, there is some variability across studies in the optimum time and temperature conditions during *sous vide* cooking for the maximum tenderisation response. Across these studies, the optimum conditions for *sous vide* ranged from the lowest temperature of 56 ^{o}C, for 12 hrs to the highest temperature of 70 ^{o}C, for 24 hrs The tenderisation response to *sous vide* cooking has been shown to depend on the connective tissue of the meat subjected to *sous vide*, as elegantly shown by Christensen et al. (2013). They showed that for *semitendinosus* muscle from young bovine, the shortest time of 2.5 hrs and lowest temperature of 53^{o}C for *sous vide* cooking was sufficient to produce the lowest WBSF values, equivalent values to higher temperatures (55, 58, 63^{o}C) and longer times (7.5 or 19.5 hrs) (Figure 3) (Christensen et al., 2013). In contrast, for ST muscle from cows, the longest time of 19.5 hrs and highest temperature of 63^{o}C was required for acceptable and lowest WBSF (Christensen et al., 2013).

Table 1: Summary of *sous vide* treatments of temperature (Temp.), time, age of animals (one study) and packaging (one study) across studies and effects on Warner-Bratzler peak shear force

Species and muscle	Treatment comparisons	Most tender (lowest peak shear force)	Reference
Bovine *semitendinosus*	Temp; 56, 58, 60°C Time; 3, 6, 9, 12 hr	56 °C for 12 hrs	(Mortensen, Frost, & Risbo, 2012)
Ovine *longissimus thoracis et lumborum*	Temp; 60, 70, 80°C Time; 6, 12, 24 hr	70 °C for 24 hrs	(Roldán, Antequera, Martín, Mayoral, & Ruiz, 2013)
Bovine *pectoralis profundus*	Temp: 60, 70, 80 °C Time; 15, 30, 45, 60 min Packaging : cook-vide, sous-vide, atmospheric	Relative to starting values; Sous-vide for 45-60 min at 80 °C	(García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007)
Bovine *semitendinosus*	Temp: 50, 55, 60, 65 °C Time; 90, 170, 250, 390 min	60-65 °C for 90-270 min	(Vaudagna et al., 2002)
Bovine *semitendinosus* **(see Figure 3)**	Temp: 53, 55, 58, 63 °C Time; 2.5, 7.5, 19.5 hrs Young bulls, cows	Young bulls; all tender except 2.5 hrs at 53 °C Cows; 19.5 hrs at 63 °C equivalent to young bull	(Christensen et al., 2013)

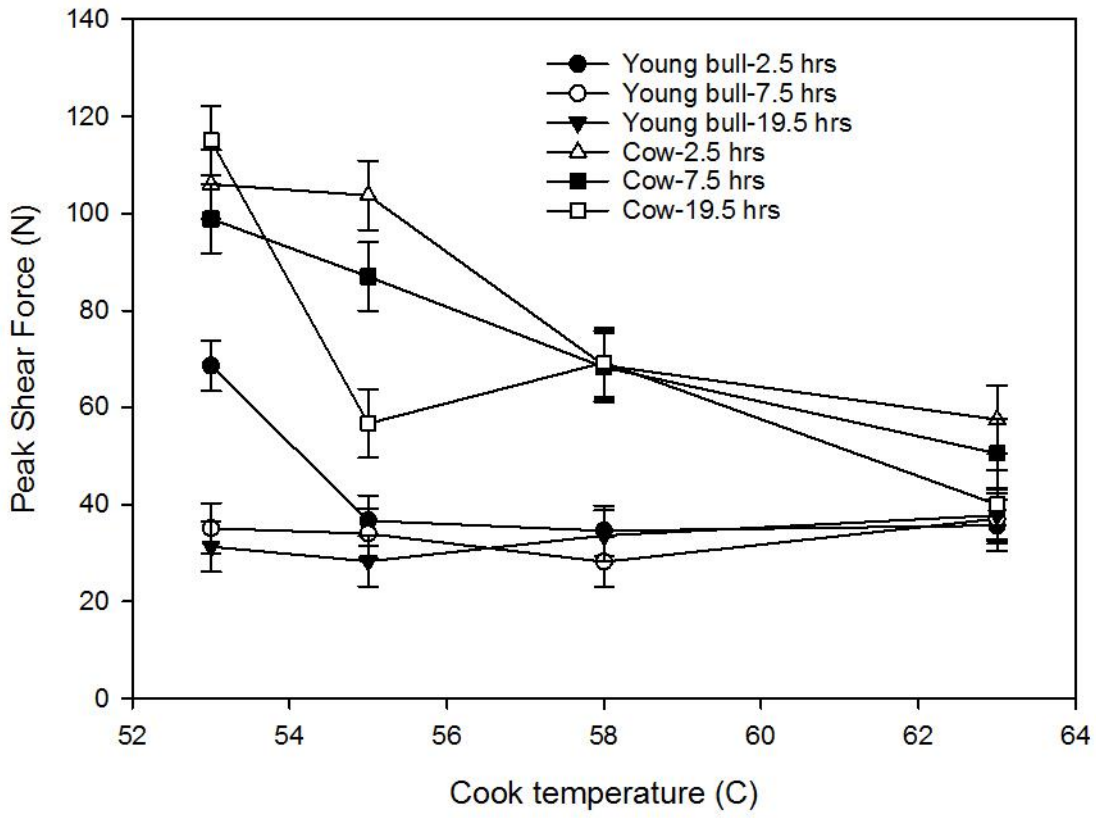


Figure 3 Peak shear force (N) as a function of cook temperature (53, 55, 58, 63 °C) and cook time (2.5, 7.5, 19.5 hrs) for bovine semitendinosus *from young bulls and cows. The values are least squares means and the standard error is shown. Derived from Christensen* et al. *(2013).*

It is worth noting that research on the biophysical and biochemical changes of individual meat proteins during vacuum cooking, and in relation to eating quality, is very limited. During *sous vide* cooking, the process of toughening that occurs during conventional heating between 60 and 80°C is reduced, most likely because of (1) the solubilisation of the connective tissue that leads to tenderisation in *sous vide* (García Segovia *et al.* 2007) (2) the change from a visoelastic to an elastic material and the aggregation of sarcoplasmic proteins which form a gel (Baldwin 2012; Tornberg 2005) and/or (3) the retention of water in the muscle structure at the low temperature used in sous vide cooking (Hughes, Oiseth, Purslow, and Warner 2014).

The role of connective tissue and actin denaturation in *sous vide* cooking has been demonstrated with the altered DSC profiles of *sous vide* cooked beef that showed a decrease in the enthalpy $\Delta H_{68°C}$ of collagen denaturation as well as a gradual decrease of $\Delta H_{73°C}$ that relates to the denaturation of actin (Christensen *et al.* 2013). Zielbauer *et al.* (2015) demonstrated a variety of DSC profiles for *sous vide* cooked pork *psoas major* as a function of different time-temperature combinations that showed alterations in the denaturation dynamics of proteins from a *sous vide* cooking system. The changes in collagen have been interpreted as increased solubilisation, while the changes in actin were considered as earlier denaturation as a result of proteolytic weakening of the structure (Christensen *et al.* 2013). In addition, the study of Okitani et al. (2009) examining actomyosin interaction showed that under vacuum cooking, actin is liberated from myosin at 65°C but not at 80°C in chicken, beef and pork, so proposing another mechanism in which toughening is reduced in low temperature *sous vide* cooking.

Finally, proteins denature across a range of temperatures and Vilgis (2015) asserts that protein can denature below their peak thermal denaturation temperature, if the samples are held at a lower temperature for a longer time. During *sous vide* cooking over the temperature range from 45-74^{o}C, although the water loss increases with time at a given temperature, the water loss at 45^{o}C is higher than at 51^{o}C, most likely because, although the myosin heads have denatured at 45^{o}C and can bind water, gel formation is too weak to hold the water in the structure (Zielbauer *et al.* 2015). Light meromyosin (LMM) is 80% denatured at 45 ^{o}C and is 100% denatured at 61^{o}C, whereas the respective temperatures for myosin heavy chain (MHC) are 53^{o}C and 61^{o}C (Vilgis 2015). Myosin heavy chain is essential for gel formation, whereas MLC plays no role (Samejima *et al.* 1981). These phenomena likely explain the results of Suriaatmaja and Lanier (2014), who found that tenderisation of beef *semitendinosus* by *sous vide* cooking only occurred above 51.5^{o}C and samples held at 50^{o}C for 48hrs did not tenderise.

Differential effects of varying temperatures on sarcoplasmic, myofibrillar and connective tissue proteins are well established (Tornberg 2005), but the influence of interactions between temperature, time and vacuum packaging in the effects of cooking on meat proteins, and tenderness are limited. Therefore, meat protein denaturation, structure and biochemical characteristics under *sous vide* cooking require further exploration for a better understanding of their contribution to the eating qualities of meat. In addition, the differences between sous vide and LTLT or HTST without vacuum-packing also need to be

carefully documented in future work in order to unravel and understand the underlying mechanisms.

4. High pressure processing (HPP)

High pressure processing (HPP) is a form of processing where a pressure is applied statically to a product, at or above 100 MPa by means of a liquid transmitter (Simonin *et al.* 2012). The pressure is isostatically and uniformly transmitted to the product (Norton & Sun, 2008). In theory, this pressure is transmitted almost instantaneously to the product, and is not dependent on the size and shape of the product, however, in commercial HPP processes, it does take time for the pressure to build up. HPP of food products, and of meat, has generally focussed on extending the shelf-life and improving food safety. The application of HPP for meat tenderisation was demonstrated a number of years ago (Bouton *et al.* 1977b), but its implementation in industry has been slow. HPP influences both the structure and function of proteins (Lee *et al.* 2007) and is known to modify only non-covalent bonds, thus not affecting small flavour molecules or vitamins. In contrast, heat affects hydrogen and covalent bonds, and thus results in both unfolding and irreversible denaturation of proteins. HPP can either be applied at ambient or low temperatures, or at high temperatures, with differential effects on meat proteins and texture. HPP can also either be applied to pre- or post-rigor meat, with quite different effects. The textural outcome of applying HPP to meat depends on the pressure applied, the temperature, the time, the muscle and the time post-mortem, with the consequent results varying from toughening to significant tenderisation. These variations are described below.

Application to pre-rigor meat

The application of HPP, at 100-225 MPa and 10-35°C, to pre-rigor meat has consistently been shown to improve the tenderness of beef, lamb and pork. There has also been a patent developed by Hormel foods on this process, for pork (Smit, Summerfield, & Cannon, 2010). The tenderisation associated with HPP of pre-rigor meat is proposed to be a result of either accelerated or arrested glycolysis.

Accelerated glycolysis, as measured by pH drop in muscles post-pressurisation (Macfarlane et al., 1973), is most likely a result of release of calcium during the application of pressure, and this is associated with severe contraction resulting in massive disruption to the myofibrillar structure and very tender meat (Bouton *et al.* 1977b) (Kennick and Elgasim 1981; Kennick et al, 1980; Macfarlane 1973). Where HPP was applied to pre-rigor meat and the result was accelerated glycolysis, the increase in tenderness is usually quite large (in the range of 30-80% improvement) across different muscles and species. Bouton *et al.* (1977b) applied HPP to pre-rigor beef *semintendinosus* (103 MPa, 35°C, 4 min) and if the muscle was not restrained prior to HPP application, the WBSF reduced from 188 N in the control to 38 N, a significant change from a highly unacceptable texture to a desirable and acceptable product. If the muscle was restrained prior to HPP application, the resulting WBSF was 71 N

and much tougher than the HPP-treated non-restrained muscle (Bouton *et al.* 1977b), providing evidence that severe shortening causes major disruption and tenderisation.

Arrested glycolysis during HPP treatment of pre-rigor meat is proposed to arise from the denaturation of glycolytic enzymes during the application of pressure, resulting in a higher ultimate pH in the muscle (Smit et al, 2010). Partial inhibition of glycolysis as a result of application of HPP to pre-rigor meat has only been demonstrated by Smit et al (2010) and Souza et al (2011). Souza et al (2011) applied HPP to pre-rigor pork carcasses (215 MPa, 33°C, 15 s) and measured the change in texture for the *longissimus, psoas major*, *triceps brachii* and *semimembranosus* muscles. Although HPP treatment significantly (P<0.01) increased the ultimate pH compared to the control in the *longissimus* (6.26 vs 5.78 respectively)*, semimembranosus* (6.48 vs 6.01) and *triceps brachii* (6.35 vs 6.08; P=0.04), a significant (P=0.03) decrease in WBSF was only seen for the *semimembranosus*, (21 vs 25 N; Souza et al, 2011). These results demonstrate the difficulty in inducing reliable tenderisation through the application of HPP to arrest glycolysis. It is well-known that there is considerable variation between muscles and carcasses in the stage of rigor, and thus muscle pH, at any defined time point in the pre-rigor period.

Application to post-rigor meat at a low temperature

The application of HPP to post-rigor meat at low or ambient temperatures has shown quite variable effects on texture and tenderness, depending on the time, temperature and pressure used. Application of high pressure at ambient or low temperature (0-25^{o}C) usually results either in no change in tenderness, or an increase in toughness in beef, pork, lamb, alligator and poultry meat (Gimenez *et al.* 2015, Jung 2000a, 2000b, Duranton *et al.* 2012; Kim *et al*. 2007, Grossi *et al.* 2014; Hong *et al.* 2005; Hong *et al.* 2012; Kruk *et al.* 2011; Macfarlane *et al.* 1981; Ma and Ledward 2004; Zamri *et al.* 2006; McArdle *et al.* 2011; McArdle *et al.* 2013). Only five out of 17 references (see the review of Sikes and Warner 2016) showed any evidence for tenderisation of meat when HPP is applied at ambient or low temperatures (Suzuki *et al.* 1992; Shenkova *et al.* 2007; Kim *et al.* 2007; Canto *et al*. 2012; Ichinoseki *et al.* 2006). In these five studies, the tenderisation was highly dependent on the muscle, pressure, temperature and time applied. The change in WBSF values in beef *longissimus thoracis et lumborum* was from about 58 to 38 N (estimated from graph) when HPP was applied at 100 MPa for 10 min, with no tenderisation evident for higher pressures of 200-300 MPa (Schenková *et al.* 2007). Ichinoseki *et al.* (2006) showed a reduction in WBSF values from 58 to 45 N when HPP was applied at 100-500 MPa, at 8^{o}C for 10 min.

Application to post-rigor meat at a high temperature

Application of high pressure at higher than ambient temperatures (>25^{o}C), generally results in tenderisation. Generally, optimum tenderisation is evident for 100-200 MPa and applied at temperatures of 60-70°C (Ma & Ledward, 2004; McArdle, Marcos, Mullen, & Kerry, 2013; Rusman, Gerelt, Tamamoto, & Nishiumi, 2007) as shown in Figures 4 and 5. Across the thirteen studies reviewed by Sikes and Warner (2016), all studies showed tenderisation of 30-80%, through measurements of hardness or WBSF, when the pressure applied was in the range 150-400 MPa and the temperature was above 50-60^{o}C for beef, lamb, pork and chicken, across a range of muscles (Bouton *et al.* 1977a; Bouton *et al.* 1977b; Ratcliff *et al.* 1977; Bouton *et al.* 1980; Beilken *et al.* 1990; Ma and Ledward 2004; Rusman *et al.* 2007;

Sikes *et al.* 2010; McArdle *et al.* 2011; McArdle *et al.* 2013; Sikes and Tume 2014). Those studies where higher pressures were used showed that pressures above 400 MPa resulted in toughening of meat, relative to lower pressures (Ma and Ledward 2004; Zamri *et al.* 2006; McArdle *et al.* 2013). Sikes *et al.* (2010) and Sikes and Tume (2014) showed that HPP treatment at 200 MPa for 20 min of beef *sternomandibularis*, *semimembranosus* and *biceps femoris* reduced the WBSF from about 110-130 N down to about 50 N (estimated from graphs).

Mechanisms of tenderisation resulting from HPP, and other cosiderations

Meat tenderisation is generally associated with increases in protein solubility and HPP treatment appears to induce tenderisation through an increasing protein solubility, through depolymerisation of proteins (Sun and Holley, 2010). Under HPP treatment, noncovalent interactions between proteins are destabilized and a small degree of unfolding occurs, with subsequent formation of hydrophobic and disulphide bonds after pressure release (Sun and Holley 2010). The changes in proteins are usually reversible below 300 MPa, but irreversible above 300 MPa (Sun and Holley 2010). When pressure is applied at ambient temperature, there appears to be little to no change to the connective tissue (background toughness) because collagen, the main protein, is stabilised by hydrogen bonds (Gekko and Koga 1983). The increased hardness or toughness of post-rigor meat subjected to HPP at ambient temperatures may also be due to a greater integrity in the myofibrillar structure (Jung *et al.* 2000a). Generally, the HPP treatment of fresh meat at high temperatures results in greater tenderness after cooking, whereas similar meat without HPP treatment becomes tougher after cooking. The increase in tenderness of high temperature-HPP is variously attributed to accelerated proteolysis (Ma and Ledward 2004), increased fracturing of myofibrillar proteins and muscle structure due to greater stability of collagen (Sikes *et al.*, 2010), increased protein solubilisation (Sun and Holley 2010), reduced water loss from the myofibrillar structure (Hughes *et al.*, 2014) or combinations of these, depending on the conditions applied.

As shown in Figure 6, there is a shown in the colour of meat, as a consequence of the application of HPP. Thus when HPP is applied at 10^{o}C, pressures above 100 MPa resulted in subtle to obvious changes in the visual appearance and the colorimetric readings. Thus HPP tends to be considered as a viable option for RTE food, but not for the 'fresh food' shelves, limiting its commercial application. There is an R&D opportunity to investigate the retention of fresh meat colour in HPP-treated muscle foods.

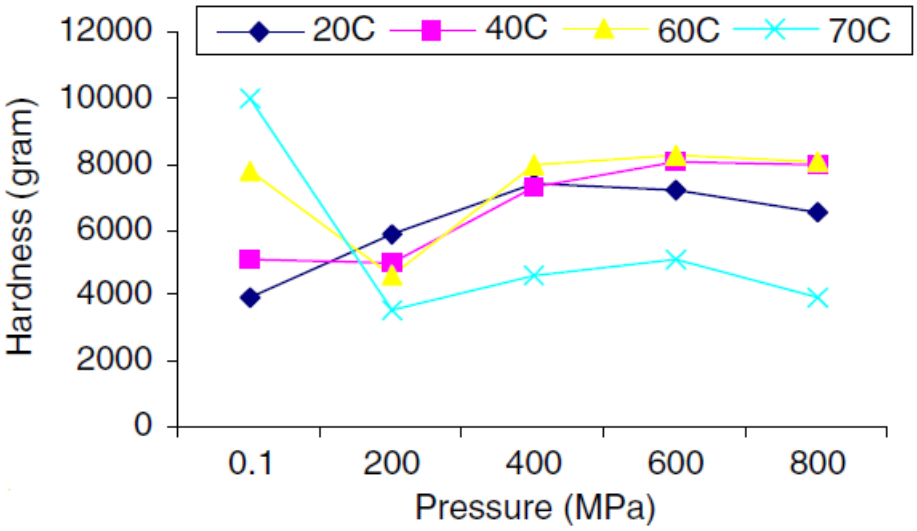


Figure 4 Effect of pressure (0.1 to 800 MPa) and temperature (20-70°C) on the hardness (g) of post-rigor beef longissimus *treated with high pressure processing for 20 min. (Ma and Ledward 2004).*

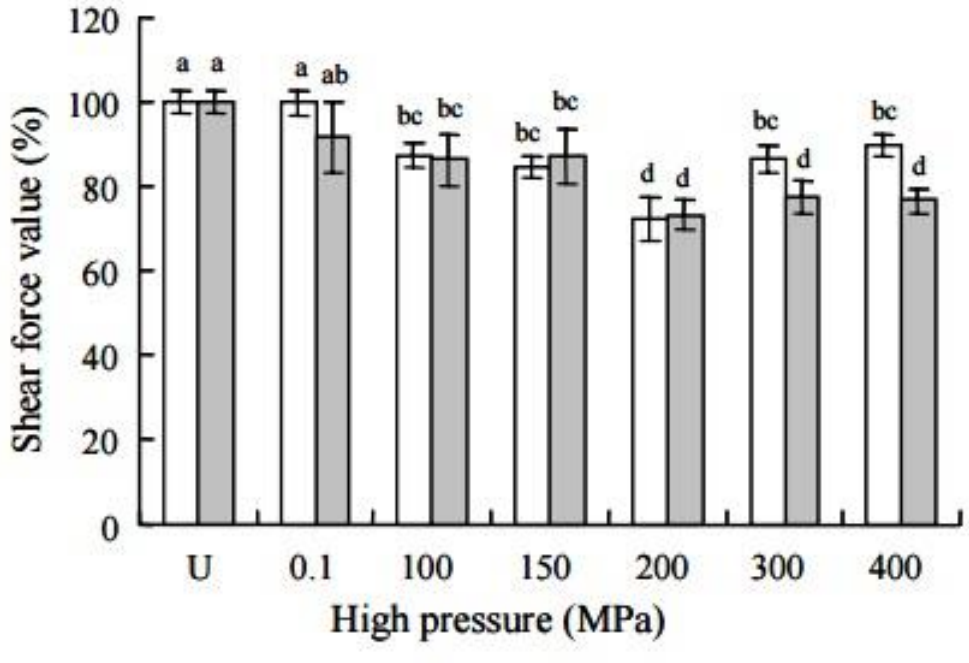
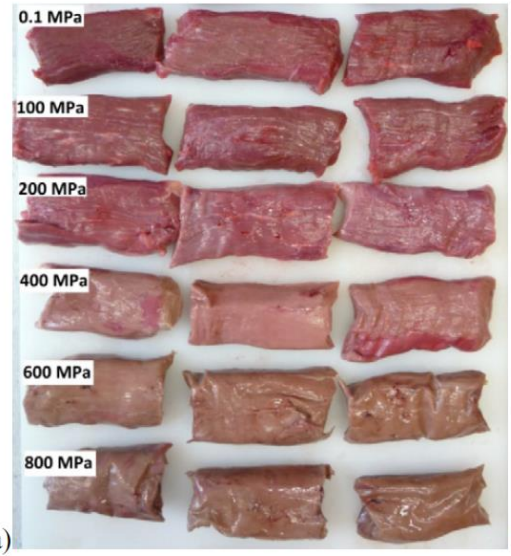


Figure 5 Effect of pressure and temperature on the percent change in shear force value of bovine skeletal muscle, relative to untreated control muscle. Means with different superscripts are different from each other (P<0.01). U= untreated, □ pressure-heated at 30°C, ■ pressure-treated at 60°C. From (Rusman et al. 2007).

(a)



(b)

Pressure (MPa)	L*	a*	b*
0.1	37.4	17.5	2.9
100	36.9	20.5	5.2
200	45.4	16.8	5.7
400	49.5	15.5	9.9
600	44.0	15.0	9.8
800	42.6	14.6	9.3
FPr	<0.001	0.031	<0.001
s.e.d	1.2	1.6	1.0

Figure 6 (a) Visual and (b) colorimetric changes in post-rigor beef sternomandibularis muscle after high pressure processing (HPP) treatment conducted at 10 °C for 5 min, at 0.1, 100, 200, 400, 600 or 800 MPa (n = 3 per pressure). Increases in pressure above 100 MPa are associated with an increased lightness (L) and yellowness (b*) and a reduction in redness (a*) as measured, using a Minolta chromameter (CR-300), light source C, 15 mm aperture, 3 transverse measurements/sample (~20 °C). From* (Hughes, Oiseth, Purslow, & Warner, 2014).

5. Shockwaves

Shockwaves, or hydrodynamic pressure processing, involve the application of high pressure waves up to 1 GPa in fractions of milliseconds (Bolumar *et al.* 2013) which propagates through a fluid (typically water). The shockwaves can be generated by piezoelectric, electromagnetic, electro-thermal or electro-detonation methods (Bolumar *et al.* 2013). The two methods that have been trialled for meat tenderisation are; (a) chemical, by detonating explosives underwater, first described by Long (1993) and shown to tenderise beef meat (Solomon *et al.* 1997) and (b) electrical discharge underwater, which was first described in (Long 2000) and shown to tenderise poultry meat (Claus *et al.* 2001b, 2001a). The electrical discharge system can either be via discharging a high voltage arc across a gap between two electrodes in water (Long and Ayers, 2001) or via a sparker system (Bowker et al 2011). Details of the principles of the design of the equipment and various configurations for both chemical detonation and electrical discharge are described in Solomon *et al.* (1997; 2006) and Bolumar *et al.* (2013), respectively.

The principal of hydrodynamic shock waves is that they travel through water and also anything that is an acoustical match for water (Claus *et al.* 2001b). Thus, as meat is 75% water, it is an acoustical match for water and when shockwaves are applied to muscle, the shock wave reflects off any object that is not an acoustical match (Solomon *et al.* 1997), resulting in ultrastructural damage to the muscle (Zuckerman *et al.* 2013) and proteolytic enzyme release (Bowker *et al.* 2008).

Explosive shockwaves

The early work on the application of a detonation (100 g of explosives) shock wave to beef muscle showed a major effect on the texture of very tough beef, the result being very tender meat in most cases (Solomon *et al.* 1997). The muscles tested varied in connective tissue content as well as protease activity and the WBSF value was reduced from 78-129 N in controls to 28-57 N in treated muscles, a 55-66% reduction (Solomon *et al.* 1997). Detonation shock waves applied to beef *semimembranosus* after 7 days ageing resulted in a reduction in WBSF by 20 N, from 62 to 39 N (Zuckerman *et al.* 2013). This is a significant reduction, as it was induced in a muscle that had already undergone ageing and also is known to be tough due to low protease activity and high amounts of connective tissue. Furthermore, the reduction in WBSF to below 40 N is significant. as many researchers use this as a cutoff for acceptability of tenderness for consumers. Furthermore, after 21 days of ageing post-treatment, shockwave-treated *semimembranosus* was still more tender than controls (Zuckerman *et al.* 2013). In contrast, turkey muscle treated with electrical shockwave at 3 days post-mortem showed very little tenderisation, although the muscle was already very tender prior to treatment (Claus *et al.* 2001b).

Previously, application of a detonation shock wave to pork loin has shown small levels of meat tenderisation, as measured by WBSF (32.4 N, 26.9 N; $P<0.01$), although a trained panel could not pick up any differences in tenderness (Moeller *et al.* 1999). The largest effects of detonation shock waves on tenderisation have been seen in lamb *longissimus* (57-60 N for control and 19-43 N for shockwaved muscles) (Solomon *et al.* 1998), beef *longissimus*, *semimembranosus*, *biceps femoris*, *semitendinosus* (see above; Solomon *et al.* 1997) and poultry *pectoralis* (60 N for control and 43 N for treated) (Meek *et al.* 2000). The biggest reduction in toughness with detonation shockwaves is seen with the highest amount of charge [for comparisons, see (Solomon *et al.* 1997; Meek *et al.* 2000)] and does vary with both muscle and species. As discussed above, low to zero levels of tenderisation have been seen for pork *longissimus* and turkey p*ectoralis* as a result of detonation shockwaves (Moeller *et al.* 1999; Bowker *et al.* 2010), most likely because the muscles being treated were not considered tough. In the case of turkey p*ectoralis,* the control samples had a WBSF of 25 N and after detonation shockwaves, the WBSF was 29 N, and not different to control (Bowker *et al.* 2010). A WBSF of 25 N is very tender and acceptable to consumers.

Electrical shockwaves

Recent research using commercial units/prototypes has shown less promise for tenderising pork compared to beef using electrical discharge shockwaves. Applying electrical discharge shock waves to hot- or cold-boned poultry *pectoralis* has been shown to result in tenderisation by 10-20 N (Claus *et al.* 2001b, 2001a). Pork *semimembranosus* and *semitendinosus* showed no change in tenderness in response to 2 pulses of electrical discharge shock wave from a commercial prototype (Bolumar *et al.* 2013). In contrast, using the same unit, beef ST and LL showed reductions in WBSF from 84 to 63 N and 48 to 39 N, respectively (Bolumar *et al.* 2013). The reason for the difference between species is unclear, as the pork muscles were not particularly tender (43 N for SM; 70 N for ST). For electrical shockwaves, the reductions in toughness are generally about 10-30% across beef, pork, turkey and chicken (Bolumar *et al.* 2013) and have not shown the dramatic tenderisation seen for detonation shockwaves. For electrical shockwaves, increased tenderisation results from repeating the pulses and increasing the energy setting (Bowker *et al.* 2011; Bolumar *et*

al. 2013). Due to concerns with the use of explosives, the technology for tenderising meat using shockwaves now centres around electrical discharge. The use of electrical discharge to apply the shockwaves allows continuous operation, rather than a 'batch' system, which is a characteristic of the shockwave systems utilising detonation. Recent research testing industrial-scale equipment for applying electrical shockwaves has shown no effect on pork *semimembranosus* and *semitendinosus* tenderness and a moderate reduction in beef *longissimus* and *semitendinosus* (84 to 63 N for *longissimus* and 48 to 39 N for *semitendinosus*) (Bolumar *et al.* 2013).

Mode of action

There are some indications that shockwaves disrupt the collagen fibril network of the endomysium (Zuckerman *et al.* 2013). Previous research has shown thatdetonation shockwaves applied to beef *longissimus* cause fragmentation in the I-band and that the jagged edges of thin filaments next to the Z-line imply physical disruption of myofibrillar proteins rather than proteolysis (Zuckerman and Solomon 1998). Others have also shown physical disruption to the muscle fibres and tissues, particularly to the myofibrils and the Z-line (Claus *et al.* 2002; Bolumar *et al.* 2014). The degradation of troponin-T (TnT) to a 30 kD protein is widely used as an indicator of tenderisation due to proteolysis (Huff-Lonergan *et al.* 1996). Bowker *et al.* (2008) used SDS-PAGE and Western blotting to show that shockwave treatment increased the tenderisation of beef *longissimus* and enhanced the accumulation of 30kD TnT degradation products, indicating enhanced proteolysis. Bolumar *et al.* (2014) did not show any effect of shockwave treatment of beef *longissimus* on myofibrillar proteins but as Western blotting was not used, the sensitivity of their assay may have been compromised.

6. Pulsed Electric Field (PEF)

Pulsed electric field (PEF) is one of the novel non-thermal technologies being investigated by the food industry. PEF is a treatment of food involving generation of an electrical field between two electrodes using direct current voltage pulses for periods of time ranging from microseconds to milliseconds. PEF results in modification of food products due to a change in permeability of cellular membranes and, subsequently, the cellular content of the food. The theory behind PEF and its applications in food plants and biomaterial processing have been examined in depth in several books and review papers (Raso-Pueyo and Heinz 2006; Toepfl *et al.* 2006; Vorobiev and Lebovka 2008; Donsi *et al.* 2010; Puertolas *et al.* 2012). The potential of PEF to improve food qualities has been investigated (Fryer and Versteeg 2008; Luengo *et al.* 2013; Wiktor *et al.* 2015) and the use of PEF as a post-mortem treatment to enhance qualities of muscle food has also been recently investigated. Due to the fact that PEF is an emerging technology in food processing, the amount of research on the potential of PEF in meat is limited in comparison with processing of other food types. However recent studies on the effect of PEF on different beef muscle types have shown promising results. As tenderness is arguably the most important attribute of meat, most of the research in this field has focussed on the effect of PEF treatment on meat tenderisation.

Application to post-rigor (cold-boned) meat

Application of different strengths of PEF treatments has shown intriguing results. Application of a low field strength PEF treatment (1.4 KV.cm^{-1}, 10 Hz) to post-rigor beef *longissimus lumborum* at different stages of ageing demonstrated that PEF could reduce toughness of meat (WBSF reduced from 88 N to 74 N), in addition to the tenderising effect of 10 day ageing (Arroyo *et al.* (2015). High intensity PEF (10 kV.cm^{-1}, 90 Hz) applied to cold-boned *longissimus lumborum* also resulted in a reduction of ~25 N (57 N to 35 N) in 21 day aged meat (Suwandy *et al.* 2015c, 2015d). The effect of PEF on tenderisation varies with the voltage and frequency of the applied PEF. Application of 10 kV.cm^{-1} and 90 Hz PEF to cold-boned beef *longissimus lumborum* produced the greatest tenderisation (WBSF reduced from 61-64 N to 49-50 N after 21 days of ageing) relative to 5 kV.cm^{-1} and 20 or 50 Hz PEF (Bekhit *et al.* 2014; Suwandy *et al.* 2015a). In addition, when the high intensity PEF (10 kV.cm^{-1}, 90 Hz) is repeatedly applied, the WBSF value decreased by an average of 2.5 N with each application, even in 1 day aged meat (61 N to 51 N) (Suwandy *et al.* 2015d). Interestingly, proteolysis analysis using SDS-PAGE and Western blotting indicated that, while degradation of troponin-T and desmin were associated with a reduction in WBSF of meat cuts treated once with PEF, the absence of degradation of these two proteins in samples treated with PEF two and three times suggested another mechanism, such as physical disruption of myofibrils, is involved in the tenderisation process (Suwandy *et al.* 2015d). Although PEF is theoretically a non-thermal technique, high intensity PEF treatment of meat has been shown to induce heating in meat samples, as demonstrated in various studies (Bekhit *et al.* 2014; Suwandy *et al.* 2015d, 2015c). The muscle temperature was elevated by 4-8^{o}C after PEF was applied at high intensity (10kV.cm^{-1}, 90Hz) to hot-boned beef *longissimus* and *semimbranosus* cuts (Suwandy *et al.* 2015d). This elevated temperature could very easily cause protein denaturation and possibly be responsible for the absence of troponin-T and desmin degradation in meat samples subjected to repeated PEF treatment (Suwandy *et al.* 2015d).

It is well-known that muscle types differ in morphology, fibre type composition, intrinsic myofibrillar proteomic composition and metabolic properties, resulting in differing meat qualities (Tornberg 2005). The effect of PEF treatment in different muscle types was examined under different PEF intensities, and repetition of treatment coupled with varying ageing durations. A difference in WBSF reduction was observed between beef cold-boned *longissimus lumborum, Semimembranosus* and Semitendinosus muscles treated with different PEF voltages and frequencies (O'Dowd *et al.* 2013; Bekhit *et al.* 2014; Suwandy *et al.* 2015a).

Application to pre-rigor (hot-boned) meat

The effect of PEF on meat qualities of hot-boned muscles has also recently been examined and has been found to have differential effects on different muscles. Application of PEF at 5-10 kV.cm^{-1} tended to cause an increase in the toughness of the *longissimus lumborum* with increasing PEF frequency (20, 50, 90 Hz) whereas the *semimembranosus* tended to show a decrease in WBSF (by about 25 N at each time point) with an increase in PEF frequency (Suwandy *et al.* 2015b). Bekhit *et al.* (2016) also showed an increase in the toughness (increased from 60 to 74 N) of hot-boned beef *longissimus lumborum* with repeated high strength PEF treatment (10 kV.cm^{-1} and 90 Hz) whereas the *semimembranosus* was more

tender after repeated PEF treatment. The effect of PEF on the water holding capacity of beef was found to differ between the two muscle types (Suwandy *et al.* 2015b; Bekhit *et al.* 2016), which may have contributed to the differences in tenderisation.

Mode of action

Cellular membranes become more permeable as a result of the application of pulsed electrical field to biological tissues. Thus it is postulated that the treatment of pre- or post-rigor meat with PEF prior to ageing may activate proteases and release calcium and possibly cathepsins, causing accelerated glycolysis (in pre-rigor meat) and enhanced tenderisation. The limited research on the effect of PEF in muscle foods shows some potential for the application of PEF for tenderisation as a post-mortem technology to improve meat qualities. However the variable effect on meat texture between different muscles needs to be resolved and further research is required to understand the mechanism by which PEF affects muscle structure. In addition, it appears that the design and application of different intensities and repetitions of PEF treatment in order to achieve desirable sensory attributes in different meat cuts needs further consideration.

7. Ultrasonics (US)

Ultrasound uses sound waves as a form of energy, with frequencies greater than the upper limit of the human hearing range, i.e. above 20 kHz. With high US intensities, small bubbles can implode, in what is known as cavitation, causing physical weakening of structures. Ultrasound applications in the food industry are categorised as either low power (low intensity) or high power (high intensity) ultrasound (Jayasooriya *et al.* 2004; Awad *et al.* 2012). Low power ultrasound is low energy and uses frequencies higher than 100 kHz at intensities below 1 W/cm^2. High power ultrasound operates with frequencies between 20 and 500 kHz at intensities higher than 1 W/cm^2. In the food industry, ultrasound is used for the analysis of the composition of food products (low power ultrasound) and for the modification of components (high power ultrasound), which has beneficial effects on quality parameters of the final product. In meat processing, ultrasonic applications range from cleaning and sterilisation, freezing and thawing, microbial inactivation, and altering biophysical properties which impact quality (Alarcon-Rojo *et al.* 2015), but the focus here is the use of ultrasound for modifying meat texture. Contrasting outcomes have been reported on the effectiveness of ultrasound for the tenderisation of meat and these are discussed below.

Low intensity US applied to post-rigor meat

Low intensity ultrasound baths (30 – 47 kHz, 0.29 – 0.62 W/cm^2) were used to apply ultrasound to post-rigor beef *M. semitendinosus* and *M. biceps femoris* steaks (Lyng *et al.* 1997) and it was shown that there was no effect on tenderness, as measured by a Volodkevich bite test (a shear test that simulates the action of incisor teeth), with a peak load bite force of approximately 105 N/cm^2 for all samples after 1 day ageing. Similarly, there was no tenderisation, as measured by Volodkovitch bite force, when low power ultrasound (20 kHz, 62 W/cm^2) was applied to beef *M. longissimus thoracis et lumborum* and *M. semimembranosus* (peak load bite force of 95 – 100 N/cm^2 after 1 day ageing) (Lyng *et al.* 1998a) and lamb *M. longissimus thoracis et lumborum* (peak load bite force after 1 day

ageing of approximately 60 N/cm^2) (Lyng *et al.* 1998b). Similarly, beef *M. semitendinosus* sonicated at 4°C for up to 24 min in a low intensity ultrasound bath (20 Hz, 1.55 W/cm^2) did not result in any change in the texture of cooked muscle; 53 N WBSF for the control sample compared to 46 N for the ultrasound treated sample (Pohlman *et al.* 1997). More recently, Sikes *et al.* (2014) also reported no effect on the texture of beef *M. longissimus dorsi* when high frequency ultrasound (600 kHz) was applied for 10 min at 10°C, with a WBSF of approximately 70 N for all samples tested.

High intensity US applied to post-rigor meat

Several studies have reported positive effects on texture (i.e. tenderisation) with the application of ultrasound to post-rigor meat. High intensity, low frequency ultrasound (1000 W, 26 kHz) applied for 2 and 4 min resulted in more tender beef *M. semitendinosus* compared to untreated muscle (Smith *et al.* 1991), with a change in WBSF from about 43 N to 36 N for 2 min exposure and 40 N to 33 N for 4 min exposure. Jayasooriya *et al.* (2007) applied high power, low frequency ultrasound (12 W/cm^2, 24 kHz) for up to 240 s to beef *M. longissimus thoracis et lumborum* and *M. semitendinosus* and reported increased tenderness at 0 days ageing (82 N to 68 N WBSF). Chang *et al.* (2009) also applied high power, low frequency ultrasound (1500 W, 40 kHz) at ambient temperature to beef *M. semitendinosus* and found that hardness (as measured by texture profile analysis) of the uncooked muscle was decreased from 55 N to 10 N with 10 min exposure. Scanning electron microscopy images also showed that ultrasound resulted in disordering of the collagen fibres. Different combinations of acoustic conditions have also been reported to increase the tenderness of beef muscles. Low intensity and low frequency ultrasound (2 W cm^2, 45 kHz) applied for 2 min at 4°C reduced the WBSF of beef *M. semimembranosus* (75 N to 65 N) after 2 days storage (Dolatowski *et al.* 2007; Stadnik and Dolatowski 2011). The application of low frequency ultrasound has also been reported to increase tenderness in other species, such as chicken (Dickens *et al.* 1991; Xiong *et al.* 2012), squid (Hu *et al.* 2014) and cobia (fish) (Chang and Wong 2012).

Application to pre-rigor meat

Ultrasound applied to pre-rigor muscle is hypothesised to damage membranes, thus potentially (1) increasing the release of calcium from the organelles, and therefore accelerating glycolysis and post-mortem muscle metabolism and (2) increasing the release of cathepsins from lysosomes. The two studies applying high intensity, high frequency ultrasound (2.0 – 2.6 MHz) to pre-rigor beef *M. semimembranosus* and *M. sternomandibularis* (tongue root) showed no effect on tenderisation or texture (Got *et al.* 1999; Sikes *et al.* 2014). Sikes *et al.* (2014) showed some evidence of an effect on metabolism, as measured by a calculated exhaustion factor measurement which suggested an increase in the glycolytic rate, even though there was no effect on tenderisation. They suggested that ultrasound could be a potential technology for treating dark, firm, dry (DFD) meat by modifying the atypical metabolic processes that occur to produce DFD meat.

Mode of action and future

It has been suggested that tenderisation of post-rigor meat using ultrasound is either by (a) physical disruption of the tissue caused by cavitation (Jayasooriya *et al.* 2004) and/or by (b) release and activation of enzymes (Roncales *et al.* 1993) or by altering the metabolism in pre-rigor meat by the release of calcium (Got *et al.* 1999). Ultrasound application has some

potential for improving the texture of post-rigor meat, with the most promise appearing to be with the use of high frequency. However, for this to be a commercial viability, the acoustic conditions need to be thoroughly investigated in order to optimise the conditions for tenderisation.

8. Smartstretch™/Smartshape™ and Pi-Vac®

It is well-established that the degree of meat toughness is highly correlated with muscle contraction during rigor mortis (Locker 1960). Thus the application of stretching to muscle is well-known to produce more tender meat, mainly through the prevention of pre-rigor muscle shortening. The development of methods such as 'tenderstretch', 'tendercut' (Sorheim and Hildrum 2002) and 'super stretch' (Kim *et al.* 2014; Warner *et al.* 2014) were developed for this purpose. These methods were applied to a carcass, which limited the number of muscles stretched. Recent developments of Pi-Vac® (Sorheim and Hildrum 2002) and Smartstretch™ (Taylor *et al.* 2012; Toohey *et al.* 2012d; Toohey *et al.* 2012e, 2012f; Taylor *et al.* 2013; Toohey *et al.* 2013b) involve not only stretching, but also shaping. These technologies are applied to hot-boned muscle and have shown significant improvements in meat tenderness. Tenderstretch and tendercut are briefly reviewed and more detail is given of the newer technologies Smartshape™/Smartstretch™ and Pi-Vac®.

Tenderstretch

Tenderstretching is a method in which the pre-rigor carcass or side is hung at the pelvis (either through the aitch bone obturator foramen or through the pelvic ligament), as opposed to the traditional *Achilles* tendon suspension (Sorheim and Hildrum 2002). As pelvic suspension results in an increase in tension on the major leg and loin muscles before the muscles pass through rigor, this method has been shown to result in longer sarcomeres and reduce WBSF of beef and sheep *M. semimembranosus* and *M. longissimus lumborum* (Hostetler *et al.* 1970; Bouton and Harris 1972; Bouton *et al.* 1973; Smulders *et al.* 1992; Sørheim *et al.* 2001). Apart from an increase in sarcomere length resulting in a reduction in overlap between myosin and actin, pelvic suspension has been suggested to lead to a rapid degradation of structural proteins, particularly those at the junction of the Z disk and intermyofibre filaments (Thompson 2002), and disruption and tearing in the muscle structure. A variation of this method is called Tendercut, where specific cuts are made into the skeleton in order to ensure targeted muscles are stretched (Sorheim and Hildrum 2002).

Pi-Vac® and Smartstretch™

Recent developments of new methods such as Pi-Vac® and Smartstretch™ combine both stretching and shaping and are applied to meat removed from the carcass. Unlike tenderstretch and tendercut, in which some muscles are restricted from stretching in intact carcasses, these new technologies are applied to hot-boned muscle and in the case of hot-boned muscle, have shown significant improvements in meat tenderness. Pi-Vac involves an initial wrapping technique which is applied via stretching of an extendable elastic sleeve by pressure inside a packaging chamber. Following insertion of muscles into the packaging chamber, the pressure is released, which results in the elastic sleeve preventing muscle contraction through restriction in expansion in the diameter of the cut. Pi-Vac® applied to hot-boned beef muscles chilled at different temperatures showed an increase in sarcomere

length (Hildrum *et al.* 2000; O'Sullivan *et al.* 2003) and allowed rapid chilling of hot-boned *M. longissimus* without compromising tenderness (Hildrum *et al.* 2002). Similar to Pi-Vac®, a more recent stretching and shaping technique named Smartstretch™ was developed and patented (Smartstretch™/Smartshape™) to increase tenderness of hot-boned meat cuts (Pitt and Daly 2012). Smartstretch™ employs a flexible sleeve placed in an airtight chamber and when meat inserted into the flexible sleeve is compressed, it prevents any expansion in the diameter of the product by forces perpendicular to muscle fibres. Pre-rigor beef *longissimus lumborum* and *gluteus medius* showed a reduction of 12-14 N in WBSF with the application of Smartstretch™ treatment and the application to sheep *semimembranosus* caused significant tenderisation with a reduction in WBSF of 22-49 N (Taylor *et al.* 2012; Toohey *et al.* 2012a, 2012c, 2012b; Taylor *et al.* 2013). Interestingly, Smartstretch™ treatment of both beef and sheep meat resulted in juicier non-aged meat cuts (Toohey *et al.* 2012a, 2012c) and stretching has been shown to increase the water-holding capacity of meat (Warner *et al.* 2014). These results demonstrate potential improvement of eating qualities of hot-boned primals by Smartstretch™ in accelerated meat production. In addition, application of Pi-Vac® and Smartstretch™ treatments to hot-boned primals offers advantages over whole carcass stretching such as a reduction in chiller space and energy requirements, and accelerating meat processing and serving portion size and shape control for consumers.

Combined with other technologies

Increasingly, research into improving eating quality of meat is exploring the combinatory effect of more than one tenderising method. Several studies have investigated the potential of a combination of electrical stimulation and ageing coupled with muscle tenderstretching and Pi-Vac® and reported mixed results on different meat cuts (Sørheim and Hildrum 2002; Troy 2006). More recently, a study of Toohey et al. (2013a) showed that medium voltage electrical stimulation neither improved nor inhibited the tenderising effect of Smartstretch™ in sheep *M. semimembranosus*. It is noteworthy that the degree of muscle contraction varies with muscle types (Locker 1960), reflecting underlying differences in glycolysis, temperature and pH of meat at the time of rigor. It is therefore important for research on meat stretching and shaping to be performed in targeted primals in order to ensure consistent end-results in commercial settings.

9. Exogenous tenderising proteases

By-products of the food industry are a popular source of enzymes for the purpose of fortifying food products. Proteases are enzymes which performs protein catabolism by hydrolysing peptide bonds. Proteases produced for the food industry include those from papaya latex, pineapple fruit and stem, kiwifruit, ginger rhizome, some microbes, such as *Aspergillus oryzae*, *Bacillus subtilis*, *Bacillus subtilis var. amyloliquefaciens* and *Bacillus licheniformis* and some marine organisms. Many of these commercially available proteases have been approved as 'generally recognised as safe' (GRAS) by the US Food and Drug Administration (Payne, 2009). As these proteases are produced from naturally occurred sources, their toxicity is less of a concern for consumers. These exogenous proteases have been shown to be capable of hydrolysing meat proteins, thus facilitate tenderization of meat.

Plant proteases

Papain (EC 3.4.22.2) is one of the most studied and effective meat tenderizing agents (Ashie, Sorensen, & Nielsen, 2002; Schenkova et al., 2007). The proteolytic activity of papain has been shown to be present over a comparatively wide range of pH (5.0-8.0) and temperature (above 65°C) (Smith & Hong-Shum, 2011). Papain is also effective at hydrolyzing heat denatured collagen and therefore is a valuable tenderizer for meat cuts from older animals (Wilson, Young, Coolbear, & Daniel, 1992). Papain has been shown to be deactivated by oxidizing agents or temperatures above 90^{o}C (Gomes, Sumner, & Ledward, 1997). With the application of pressure there is a subsequent decrease in papain enzyme activity, which is thought to be due to the oxidation of the thiolate ion at the active site to either SO_2 or SO_3. This effect is particularly significant at elevated pressure (800 kPa) and temperature (60^{o}C) (Gomes et al., 1997). Inactivation of papain as a result of oxidation in prolonged storage at 4^{o}C can be partially reversed by thiol agents such as cysteine and sodium metabisulfite. Application of papain to meat resulted in hydrolysis of muscle proteins including connective tissue, however, juiciness and flavor of meat can be negatively affected (Ashie et al., 2002; Gerelt, Ikeuchi, & Suzuki, 2000; Ha, Bekhit, Carne, & Hopkins, 2012; Ionescu, Aprodu, & Pascaru, 2008). In addition, papain has been shown to over-tenderize, leading to a “mushy” texture of meat and therefore limits its commercial use (Han, Morton, Bekhit, & Sedcole, 2009; Schenkova et al., 2007).

Bromelains, extracted from stem (EC 3.4.22.32) and fruit (EC 3.4.22.33) of the pineapple plant, are well-studied members of the papain family. Crude extracts of stem bromelain (EC 3.4.22.32) contain a mixture of other cysteine endopeptidases. The optimal conditions for bromelains are at pH 6.0-8.5 and within a temperature range of 50-60°C. Although similar in structure, fruit bromelain has a much higher proteolytic activity and a broader specificity in comparison to stem bromelain (Kim & Taub, 1991). The proteolytic activity of bromelain, determined by proteolysis of synthetic peptides at pH 5.0-7.0 and 50°C, is slightly less than that of papain (Smith & Hong-Shum, 2011). Evaluation of bromelains as meat tenderizers has shown promising results, including hydrolysis of several myofibril and connective tissue proteins and lowering WBSF of various beef muscles (Ionescu et al., 2008; Kang & Rice, 1970; Kim & Taub, 1991; Kolle, McKenna, & Savell, 2004; Sullivan & Calkins, 2010).

Ficin (EC 3.4.22.3) is another cysteine protease from the latex of *Ficus glabrata, Ficus anthelmintica* and *Ficus laurifolia*. Optimal activity of ficin is in pH range of 5.0-8.0 and temperature range of 45-55°C and cysteine and/or other reducing agents are necessary for activation (Kramer & Whitaker, 1964). Previous studies on ficin observed that the pH is dependent on the substrate concentration and has a half-life of 1.5 hours at 60°C (Kramer & Whitaker, 1964). Investigation of ficin as a meat tenderizer is relatively limited. Ficin was shown to be capable of hydrolysing myofibril and collagen proteins using a beef protein extract (Kang & Rice, 1970). In addition, beef inside rounds injected with 40 ppm ficin attracted a higher sensory score for tenderness, however, flavour and juiciness appeared to deteriorate in enzyme treated samples (Stefanek, Scanga, Belk, & Smith, 2002).

Another well-studied cysteine protease of the papain family is actinidin (EC 3.4.22.14) extracted from kiwifruit. Actinidin, although sharing a striking structural homology with papain, exhibited different substrate binding affinity (K_M) when examined with ester and amide substrates (Baker, Boland, Calder, & Hardman, 1980). Kiwifruit crude extracts were

shown to contain three major allergens including actinidin (Moller, Kayma, Steinhart, & Paschke, 1997; Pastorello et al., 1998). Meat tenderising studies demonstrated that actinidin is less active on collagen in comparison with papain (Han et al., 2009; Lewis & Luh, 1988), suggesting limitation in meat with higher connective tissue content. However, actinidin exhibited a more controlled tenderization action through hydrolysis of myofibril proteins which may offer advantage in reducing the mushy texture and off flavour observed in meat treated with papain (Ashie et al., 2002). Interestingly, kiwifruit juice appeared to activate meat m-calpain in enzyme-infused lamb as early as 0.5 h post mortem (Han et al., 2009) (Figure 7).

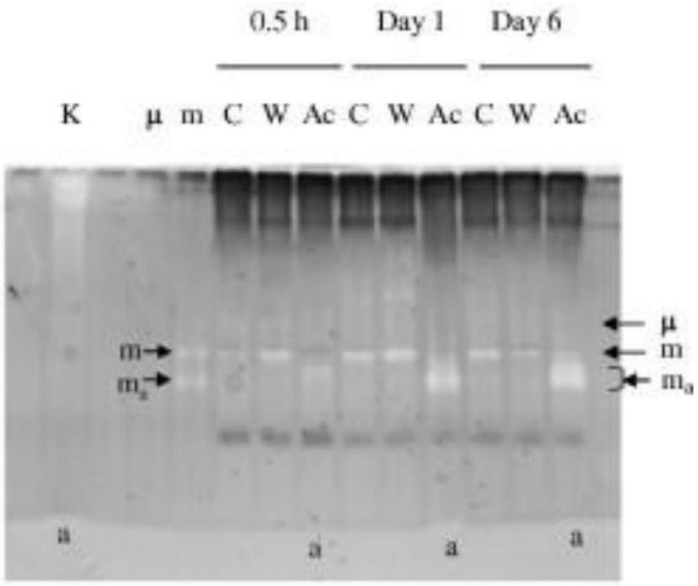


Figure 6 Casein zymography gels of sarcoplasmic proteins extracted from lamb Longissimus dorsi *muscle using various infusion treatments sampled after 0.5 h, 1 day and 6 days post-mortem. a = actinidin; K = kiwifruit juice, m = m-calpain, ma = m-calpain autolysed form, C = control, W = water infusion, Ac = kiwifruit juice infusion. Adapted from Han et al. (2009).*

Ginger proteases, also known as Zingibains, (EC 3.4.22.67) are sourced from ginger rhizome extract. Addition of reducing agents is required during extraction for the purpose of optimising protein yield and stabilising the proteases (Adulyatham & Owusu-Apenten, 2005; Qiao, Tong, Wei, Du, & Tang, 2009). Meat tenderization studies using ginger extracts observed an increase in water holding capacity, hydrolytic activity on both myofibril and connective tissue proteins and a reduction in WBSF of various beef, sheep, buffalo, camel and chicken muscles (Abdeldaiem & Ali, 2013; Bhaskar, Sachindra, Modi, Sakhare, & Mahendrakar, 2006; Lee, Sehnert, & Ashmore, 1986; Mendiratta, Anjaneyulu, Lakshmanan, Naveena, & Bisht, 2000; Naveena & Mendiratta, 2001; Sullivan & Calkins, 2010). Sensory results reported from meat tenderizing trials reported improvement in scores for tenderness, flavour, juiciness and overall acceptability (Bhaskar et al., 2006; Mendiratta et al., 2000; Naveena, Mendiratta, & Anjaneyulu, 2004; Pawar, Mule, & Machewad, 2007). However, Sullivan and Calkins (2010) found that beef treated with zingibain had a higher off-flavour rating than other plant proteases. This may be explained by differences in ginger

extract concentrations used and cultural differences in culinary familiarity with ginger flavour.

Microbial proteases

Proteases produced for the food industry can also come from bacterial and fungal sources (commonly refered to as microbial proteases). Microorganisms are fast to culture and produce different proteases depending on cultured environment. Thus, compared to plant proteases, those of microbial sources offer added advandtages in cost, time and the culture conditions can be modified for selective production of particular proteases.

Fungal proteases have been used for thousdands of years in oriental cultures for the production of fermented foods such as soy sauce, Miso soup and Natto. Its safety for consumption has been reviewed extensively (Barbesgaard, Heldt-Hansen, & Diderichsen, 1992; Domsch, Gams, & Anderson, 1980). A common source of fungal proteases is from the genus *Aspergillus* whose proteases have been grated the GRAS status by the US Food and Drug Administration. This fungus produces both acidic and neutral proteases, being those active at acidic and neutral pHs respectively. Fungal proteases have been shown to have a different hydrolytic specificity compared to those of plants, thus potentially reducing bitterness in food products caused by hydrophobic amino acids commonly associated with hydrolysates of plant proteases (Rao, Tanksale, Ghatge, & Deshpande, 1998; Saha & Hayashi, 2001). Meat tenderization capability of fungal proteases have been demonstrated in previous studies. Fungal proteases have been shown to hydrolyse both myofibril and connective tissue proteins (Ashie et al., 2002; Gerelt et al., 2000; Ha, Bekhit, Carne, & Hopkins, 2013b; Sullivan & Calkins, 2010), lower WBSF (Ashie et al., 2002; Sullivan & Calkins, 2010) and produce meat with higher sensory scores for tenderness than untreated beef and pork muscles (Gerelt et al., 2000; Kim et al., 2011; Sullivan & Calkins, 2010).

Unlike fungal proteases, those produced for the food industry from bacteria such as the *Bacillus* strains are predominantly neutral and alkaline proteases. While complete inactivation of papain requires relatively high temperature (≥90°C) (Dransfield & Etherington, 1981), bacterial proteases have a low thermotolerance and a high catalytic specificity towards meat proteins which are both advantageous for controlling tenderization. Additionally, bacterial proteases also have an moderate hydrolysis rate and specificity for hydrophobic residues which may result in a reduction of bitterness in the hydrolysate (Rao et al., 1998). In addition, the hydrolytic conditions of several collagenase and elastase from bacterial (and fungal) sources have been shown to be at 10-55°C and pH 4.0-6.0 that are suitable for meat tenderization (de Souza et al., 2015; Takagi et al., 1992; Yeh, Yang, & Tsai, 2002; Zhao et al., 2012). Proteases from several bacterial strains such as *Bacillus subtilis* and *Bacillus subtilis var. amyloliquefaciens* and *Bacillus licheniformis* have been granted GRAS status.

It is worth noting that, compared to proteases from plants, bacterial proteases generally shows a higher collagen proteolytic activity and a more selective myofibrillar portein hydrolytic activity (Foegeding & Larick, 1986; Ha et al., 2013b; Takagi et al., 1992; Yeh et al., 2002). Sensory evaluation of meat treated with bacterial proteases is limited in comparison with plant proteases. A recent study of Kim et al. (2011) showed that treatment of pork with

Bacillus polyfermenticus protease significantly improved sensorial tenderness score during 1 week storage at 4°C compared with non-treated meat.

Marine proteases

Apart from plant and microbial sources, proteases for the food industry can be extracted from marine sources such as microoragnisms, algae, polychaeta, crustaceans, fishes and invertbrates (Homaei, Sajedi, Sariri, Seyfzadeh, & Stevanato, 2010; Navarrete-del-Toro, Garcia-Carreno, Hernandez-Cortes, Molnar, & Graf, 2015; Oh, Kim, Kim, & Kim, 2000; Tsai, Lu, & Chuang, 1991) Tsai et al. 1991). A protease from Northern shrimp (*Pandalus borealis*) was demonstrated to decrease WBSF of beef cubes during incubation at 21°C for 1 h (Aoki, Ahsan, Matsuo, Hagiwara, & Watabe, 2004). A major potential problem with extracting proteases from fish, crustaceans and other marine sources for meat tenderization is that unless the extraction process is performed to an extensive extent, the flavour of meat can be negatively impacted by a 'fishy' flavour of the protease extracts. This may explain the limited number of published research articles on usage of this enzyme source in meat tenderization. A summary of exogenous proteases from plant and microbial sources, which have been used for the purpose of meat tenderization, can be found in Appendix 1.

10. Meta-analysis of effects of technologies on meat tenderisation

Data was collected and compiled from studies on HPP, shockwave, PEF, Ultrasound and Smart stretch. Table 2 describes the studies included and the data that was compiled prior to meta-analysis. Most data was from published studies, but a small amount was from unpublished data of Jim Claus (University of Wisconsin-Madison) and Aladdin Bekhit (Otago University, New Zealand). In the case of data where there was distinct groupings of treatment, and sufficient data was available for each grouping, the data was initially analysed across all the studies, and then was separated into the separate groupings. Thus HPP and PEF were separated into pre- and post- rigor and shockwave was separated into electrical and explosive.

Data from the *sous vide* studies was not able to be compiled for meta-analysis because the control treatment in each study was not always clear, and varied significantly. Data from the enzyme studies was also not able to be compiled as most experiments did not measure Warner-Bratzler shear force.

In Table 3, it is evident that all technologies subjected to meta-analysis showed a significant overall improvement in tenderness, as measured by the change in Warner-Bratzler shear force from a control. Overall, PEF resulted in a reduction in WBSF of 4.4 N, but when split into two groups, there was no effect of pre-rigor application of PEF ($P>0.05$) but the application of PEF to post-rigor meat resulted in a reduction of 7.1 N in WBSF. In the case of the technology shock-wave, the only technology which is approved for use commercially is electrical shock wave, and overall the reduction in WBSF across all studies is 5.2 N. This compares to a much larger reduction in WBSF with explosive shockwave of 17.7 N. The reduction in WBSF with ultrasound was similar to that achieved with PEF and was 6.0 N.

Excluding HPP, Smartstretch applied to pre-rigor meat achieved the greatest improvement in tenderness of 10.8 N.

Overall, HPP applied to meat resulted in the largest reduction in WBSF, of 43.5 N. By far the greatest effect was achieved with application of HPP to pre-rigor meat, with an 82.3 N reduction in WBSF. Application of HPP to post-rigor meat resulted in 36.8 N reduction in WBSF but if the HPP conditions are limited to 100-200 MPa, the reduction in WBSF increases to 49.2 N.

Previously, other studies have used meta-analysis to consider the effect of metabolic modifers, applied to cattle, on the tenderness of beef meat (Table 4). From a meta-analysis of 22 studies, hormonal growth promotants (HGP) have been found to increase the WBSF (toughness) of beef *longissimus* by 2.61 N (Table 4). This is a significant increase in toughness. In the MSA model for predicting eating quality, the meat from HGP-treated cattle has a 5 point reduction in CMQ4 score applied, to adjust for this effect. Thus HGP-treated meat has to be aged for longer to reach the same degree of tenderisation as meat from untreated cattle. The beta-agonists zilpaterol and ractopamine have been found, to increase the WBSF by 8.23 and 1.99 N respectively (across 47 and 17 studies respectively; see Table 4). These compounds are not presently registered for use in Australia and if they were to be registered and used, similar to large reductions in CMQ4 score would need to be included in the MSA model for predicting eating quality.

Other animal, genetic and processing factors known to increase red meat toughness include the callipyge gene in sheep, the Carwell sire in sheep, *bos indicus* genetics (Warner et al., 2010), pre-slaughter stress (Warner et al., 2007), absence of electrical stimulation (Warner et al., 2010) and heat-toughening in cattle (Warner, Thompson, et al., 2014), to name a few. Furthermore, different muscles in the carcass vary widely in their tenderness, depending on anatomical location and function which relates to sarcomere length, collagen content and cross-linking and protease content and post-mortem activity. For example, amongst eleven major muscles in the beef carcass, the sensory and WBSF tenderness ranges from very tender in the *psoas major* (sensory tenderness =7.4 out of 10 points; WBSF = 2.95 kg) to tough in the *supraspinatus* (sensory tenderness = 4.1 out of 10 points; WBSF = 4.95 kg) (Rhee et al., 2004).

There is potential for tenderisation technologies to be used to ameliorate the toughness in red meat resulting from genetic, production and processing practices. Furthermore the application of tenderisation technologies to pre- and post-rigor muscles, including muscles traditionally considered to be quite tough, should deliver real benefits to an industry that prides itself on quality, and is also rewarded for quality.

Table 2: Summary of studies, total number of individual results tests within studies, species and muscles tested and source references.

Technology – description of samples included	**No. of studies**	**No. of results within study**	**Species**	**Muscles**	**References**
PEF – overall	8	260	Beef and Turkey	*pectoralis major; longissimus lumborum; semitendinosus; semimembranosus*	Arroyo et al. (2015); A. E. D. Bekhit, van de Ven, Suwandy, Fahri, and Hopkins (2014); A. E. D. A. Bekhit, Suwandy, Carne, van de Ven, and Hopkins (2016); Faridnia, Bekhit, Niven, and Oey (2014); Faridnia et al. (2015); V. Suwandy, A. Carne, R. van de Ven, A. E. A. Bekhit, and D. L. Hopkins (2015a); V. Suwandy, A. Carne, R. van de Ven, A. E. D. Bekhit, and D. L. Hopkins (2015b, 2015c)
PEF – Pre-rigor	4	114	Beef and Turkey	*pectoralis major; longissimus lumborum; semimembranosus*	Arroyo et al. (2015); A. E. D. A. Bekhit et al. (2016); Suwandy et al. (2015a); Suwandy et al. (2015c)
PEF – Post-rigor	8	146	Beef	*longissimus lumborum; semitendinosus; semimembranosus*	Arroyo et al. (2015); A. E. D. Bekhit et al. (2014); A. E. D. A. Bekhit et al. (2016); Faridnia et al. (2014); Faridnia et al. (2015); Suwandy et al. (2015a); Suwandy et al. (2015b, 2015c)
Shockwave – overall	21	85	Beef; Pork; Turkey and Chicken	*longissimus lumborum; Top round; longissimus thoracis; Pectoralis major; biceps femoris; Semitendinosus; Semimembranosus;* rib-eye loin	Bolumar et al. (2014); Bowker, Callahan, and Solomon (2010); Bowker, Fahrenholz, Paroczay, Eastridge, and Solomon (2008); Bowker, Liu, et al. (2010); Bowker et al. (2011); Callahan, Berry, Solomon, and Liu (2006); Claus et al. (2001a, 2001b); Liu et al. (2006); Marriott, Wang, Solomon, and Moody (2001); Meek et al. (2000); Moeller et al. (1999); Schilling et al. (2002); Schilling, Marriott, Wang, and Solomon (2003); Solomon et al. (2008); Solomon, Long, and Eastridge (1997); Spanier, Berry, and Solomon (2000); Zuckerman, Berry, Eastridge, and Solomon (2002); Zuckerman, Bowker, Eastridge, and Solomon (2013); Zuckerman and Solomon (1998); James Claus, unpublished data.

Technology – description of samples included	No. of studies	No. of results within study	Species	Muscles	References
Shockwave – Electrical	4	34	Beef; Turkey and Chicken	*Longissimus lumborum; pectoralis major;*	Bolumar et al. (2014); Bowker et al. (2011); Claus et al. (2001b); ; James Claus, unpublished data.
Shockwave Explosive	17	53	Beef; Pork; Turkey and Chicken	*longissimus lumborum; top round; longissimus thoracis; pectoralis major; biceps femoris; semitendinosus; semimembranosus*;	Bowker, Callahan, et al. (2010); Bowker et al. (2008); Bowker, Liu, et al. (2010); Callahan et al. (2006); Claus et al. (2001a); Liu et al. (2006); Marriott et al. (2001); Meek et al. (2000); Moeller et al. (1999); Schilling et al. (2002); Schilling et al. (2003); Solomon et al. (2008); Solomon et al. (1997); Spanier et al. (2000); Zuckerman et al. (2002); Zuckerman et al. (2013); Zuckerman and Solomon (1998)
SmartStretch	6	12	Beef and Lamb	*longissimus lumborum; gluteus medius; semimembranosus*	Taylor, Toohey, van de Ven, and Hopkins (2012, 2013); E. Toohey, R. Van de Ven, J. Thompson, G. Geesink, and D. Hopkins (2012); E. S. Toohey, R. van de Ven, J. M. Thompson, G. H. Geesink, and D. L. Hopkins (2012a, 2012b); Toohey, van de Ven, Thompson, Geesink, and Hopkins (2013)
Ultrasound	6	50	Beef and Lamb	*longissimus lumborum; semitendinosus; semimembranosus; sternomandibularis; pectoralis major*	Jayasooriya, Torley, D'Arcy, and Bhandari (2007); Lyng, Allen, and McKenna (1998); Pohlman, Dikeman, and Zayas (1997); Pohlman, Dikeman, Zayas, and Unruh (1997); Sikes, Mawson, Stark, and Warner (2014); Stadnik and Dolatowski (2011)

Technology – description of samples included	No. of studies	No. of results within study	Species	Muscles	References
HPP – overall	19	204	Beef; Lamb and Pork	*longissimus lumborum; biceps femoris; semitendinosus; semimembranosus; pectoralis minor; pectoralis profundus; psoas major; adductor; glutius medius; triceps brachii*	Beilken, Macfarlane, and Jones (1990); Bouton, Ford, Harris, Macfarlane, and Oshea (1977); Bouton, Harris, and Macfarlane (1980); Bouton, Harris, Macfarlane, and O'shea (1977); Duranton, Simonin, Cheret, Guillou, and de Lamballerie (2012); Hong, Shim, Choi, and Min (2008); Jung, De Lamballerie-Anton, and Ghoul (2000); Jung, Ghoul, and de Lamballerie-Anton (2000); Ma and Ledward (2004); Macfarlane (1973); Macfarlane and Mckenzie (1986); Macfarlane, Mckenzie, and Turner (1986); Macfarlane, Mckenzie, Turner, and Jones (1981); McArdle, Marcos, Kerry, and Mullen (2011); McArdle, Marcos, Mullen, and Kerry (2013); Park, Ryu, Hong, and Min (2006); Ratcliff et al. (1977); Sikes and Tume (2014); Souza et al. (2011)
HPP – Pre-rigor	5	30	Beef; Lamb and Pork	*semitendinosus; psoas major; pectoralis minor; longissimus lumborum; biceps femoris; adductor; semimembranosus; glutius medius; triceps brachii*	Bouton, Ford, et al. (1977); Bouton et al. (1980); Bouton, Harris, et al. (1977); Macfarlane (1973); Souza et al. (2011)

Technology – description of samples included	No. of studies	No. of results within study	Species	Muscles	References
HPP – Post-rigor	18	174	Beef; Lamb and Pork	*longissimus lumborum; biceps femoris; semitendinosus; semimembranosus; pectoralis minor; pectoralis profundus; psoas major; adductor; glutius medius;*	Beilken et al. (1990); Bouton, Ford, et al. (1977); Bouton et al. (1980); Bouton, Harris, et al. (1977); Duranton et al. (2012); Hong et al. (2008); Jung, De Lamballerie-Anton, et al. (2000); Jung, Ghoul, et al. (2000); Ma and Ledward (2004); Macfarlane (1973); Macfarlane and Mckenzie (1986); Macfarlane et al. (1986); Macfarlane et al. (1981); McArdle et al. (2011); McArdle et al. (2013); Park et al. (2006); Ratcliff et al. (1977); Sikes and Tume (2014)
HPP – Post-rigor 100-200 MPa only	15	163	Beef; Lamb and Pork	*semitendinosus; semimembranosus; biceps femoris; deep pectroralis; longissimus lumborum; psoas major; adductor; glutius medius*	Beilken et al. (1990); Bouton, Ford, et al. (1977); Bouton et al. (1980); Bouton, Harris, et al. (1977); Hong et al. (2008); Jung, De Lamballerie-Anton, et al. (2000); Ma and Ledward (2004); Macfarlane (1973); Macfarlane and Mckenzie (1986); Macfarlane et al. (1986); Macfarlane et al. (1981); McArdle et al. (2013); Park et al. (2006); Ratcliff et al. (1977); Sikes and Tume (2014)
Sous-vide	8	65	Beef; Lamb and Pork	*Pectoralis; longissimus lumborum; semitendinosus*	Becker, Boulaaba, Pingen, Krischek, and Klein (2016); Becker, Boulaaba, Pingen, Rohner, and Klein (2015); Christensen et al. (2013); Garcia-Segovia, Andres-Bello, and Martinez-Monzo (2007); James and Yang (2012); Rinaldi et al. (2014); Roldan, Antequera, Martin, Mayoral, and Ruiz (2013); Vaudagna et al. (2002)

Table 3: Results of meta-analysis of the effect of technologies on meat tenderness. The technologies included are pulsed electric field (PEF), shock wave, Smartstretch™, Ultrasound and high pressure processing (HPP). The 'Effect' is the effect of application of a technology on the Warner-Bratzler shear force (WBSF, N) relative to untreated meat. The SED is the standard error of the difference in WBSF across all studies.

	Effect	SED	95% confidence interval	No. of studies/tests
PEF[1]				
- Overall	4.44***	0.75	2.96, 5.95	260
- Pre-rigor	0.522[ns]	0.63	-0.76, 1.88	114
- Post-rigor	7.10***	1.11	4.88, 9.28	154
Shock-wave				
- Overall	15.12***	1.993	11.17, 19.12	66
- Electrical	5.169***	0.932	3.33, 7.57	13
- Explosive	17.71***	2.145	12.91, 22.57	53
Smartstretch™[,4]	10.76***	3.537	3.203, 18.31	12
Ultrasound[1]	6.034***	1.388	3.246, 8.80	50
HPP – overall[1]	43.54***	4.14	35.38, 51.71	204
- Pre-rigor[2]	82.30***	10.759	60.64, 104.14	30
- Post-rigor[3]	36.82***	4.306	28.33, 45.31	174
- Post-rigor 100-200 MPa only	49.22***	4.869	39.61, 58.83	136

[1] Treatments applied to a mixture of pre- and post-rigor meat (time applied in excel worksheet).
[2] Treatments tested are limited to 100 PMA- 35 ^{o}C; 150 MPa- 60 ^{o}C; 200- 35 ^{o}C (HPP-temperature respectively)
[3] Treatments tested were across the range of pressures (HPP) 20, 55, 100, 150, 200, 300, 400, 520, 600 MPa and across the temperatures 15, 27.5, 35, 40, 45, 50, 55, 60, 70, 75, 80 ^{o}C.
[4] All treatments were applied to pre-rigor meat but some samples were assessed after 4, 8 or 14 days of ageing.
*** P<0.001; [ns] not significant, P>0.05.

Table 4: Summary of studies where a meta-analysis was conducted on the effects of beta-agonists (zilpaterol and ractopamine) and hormonal growth promotants (HGP's) on the change in Warner-Bratzler shear force (WBSF, kg) with treatment, relative to untreated cattle.

Treatment	Change in WBSF (kg)	SE/SD[+]	95% CI Number (N)	Reference
Zilpaterol*	0.840 (8.23 N)	SD=1.212	0.720- 0.960 N=47	(Lean & Dunshea, 2014)
Ractopamine*	0.203 (1.99 N)	SD=0.4293	0.122, 0.284 N=17	(Lean & Dunshea, 2014)
HGP's	0.266 (2.61 N)	SE = 0.034	0.199,0.334 N=22	(Watson, 2008)

*weighted mean difference and 95% confidence interval
[+] SE= standard error, SD = Standard deviation

11. Summary, conclusions

A range of new and innovative technologies have potential for application in the meat industry in order to accelerate or improve the texture of meat and there is variable understanding of how these technologies achieve their effect, highlighting the need for further research.

(i) The principles underlying the structural, biochemical and texture changes during conventional cooking of meat are being increasingly understood. Conventional cooking of meat by application of heat affects the state of meat proteins, resulting in texture changes, structural changes and redistribution of water contained therein. For conventional cooking techniques, major improvements in meat tenderness have been achieved through selection of temperature/time/heating method based on the amount of connective tissue and an understanding of the mechanisms behind tenderization. More research is required to understand the varying effect of temperature on different muscles, as well as the absence of a continuous effect of time of cooking on the improvement of tenderness. Extensive knowledge of the effects of conventional cooking methods on meat texture needs to be extended to the assessment of new technologies.

(ii) *Sous vide* cooking is a recent development for chefs around the world, although some countries have been using *sous vide* for a number of years. *Sous vide* cooking gives a reliably tender and juicy meat product after extended cooking times. The challenge will be to optimise *sous vide* cooking for different muscles to ensure minimum cooking time/temperature combinations.

(iii) The application of HPP for meat tenderisation has been known since the 1970's but this has not been adopted by the meat industry. The number of high pressure processing vessels being installed around the world is accelerating. Meta-analysis was used to demonstrate that overall, HPP results in significant improvement in meat tenderness (WBSF decrease of 44 N), with the greatest improvements achieved with pre-rigor meat (WBSF decrease of 82 N) and significant improvements in post-rigor meat if the pressure was applied at 100-200 MPa (WBSF decrease of 49 N). Thus the application of HPP for tender ready-to-eat meat products and meal solutions shows promise, particularly if it is used to reduce the energy or time required for cooking methods such as *sous vide*. Further development of the HPP conditions for tenderising specific muscles is required as well as technological developments to ensure high target temperatures are reached during processing. There is a change shown in the colour of meat, as a consequence of the application of HPP. Thus HPP tends to be considered as a viable option for RTE food, but not for the 'fresh food' shelves, limiting its commercial application. There is an R&D opportunity to investigate the retention of fresh meat colour in HPP-treated muscle foods.

(iv) Detonation shockwave has also been around since the 1970's but the safer system for delivering shockwaves using electrical discharge under water has been developed more recently. Meta-analysis of all the available studies showed that detonation shockwaves significantly improve meat tenderness (reduce WBSF by 18 N) and the results are not as dramatic for electrical shockwave, but still significant (WBSF decrease of 5 N). Nevertheless, shock waves applied using electrical discharge show some promise for tenderisation and thus warrant further research.

(v) Research on the application of high frequency PEF to meat for tenderisation is very new with promising results. The meta-analysis showed that the overall reduction in WBSF, when high intensity PEF is applied to cold-boned meat is 7 N, and there is no effect when applied to hot-boned (pre-rigor) meat. When PEF is applied to hot-boned meat, the effect can be toughening, or tenderisation, depending on the muscle. The application of PEF for meat tenderisation will most likely be optimised in the coming years and research will be needed to understand the tenderisation variation between muscles in response to the application of PEF.

(vi) Ultrasound application has some potential for improving the texture of post-rigor meat, with the most promise appearing to be with the use of high frequency. However, for this to be a commercial viability, the acoustic conditions need to be thoroughly investigated in order to optimise the conditions for tenderisation.

(vii) Smartstretch™/Smartshape™ and Pi-Vac® are new technologies which have been shown to result in tenderisation of beef, by about 10-15 N and lamb by a significant 20-50 N. The concept is not new as tenderstretch is also known to consistently yield more tender meat, but only in certain muscles in the carcass.

(viii) Enzymes from a variety of sources have shown promise for application to the tenderising of meat. Changes in flavour, odour or overly mushy can be problematic with some enzymes. In combination with other tenderising technologies, the pplication of enzymes to tenderise particularly collagenous or tough muscles shows promise.

(ix) Combination of technologies. Animal, genetic and processing factors are known to increase red meat toughness and different muscles in the carcass vary widely in their tenderness. There is potential for tenderisation technologies to be used to ameliorate the toughness in red meat resulting from genetic, production and processing practices. Furthermore the application of tenderisation technologies to pre- and post-rigor muscles, including muscles traditionally considered to be quite tough, should deliver real benefits to an industry that prides itself on quality, and is also rewarded for quality.

The majority of investigations reported in this paper have used objective measures of tenderness, principally Warner-Bratzler shear force, although some have used texture profile analysis and trained sensory panels. As discussed in the introduction, tenderness is only one dimension of the consumers assessment of acceptability of a food product. Thus more is are needed on the effects of these interventions/cook methods on consumer sensory scores, particularly tenderness, flavour, juiciness and acceptability.

12. Future Trends

High pressure processing, shockwave processing, ultrasound, pulsed electric field, *sous vide* cooking and Smartshape™ can be used with varying success to cause physical disruption to muscle structure, enhanced proteolysis and ageing and muscle protein denaturation, solubilisation and gelation resulting in enhanced texture and juiciness. The optimum application of these new and innovative technologies for tenderisation will need to be adjusted for different muscles in the carcass, different markets (food service, fresh product, export markets) and also target demographics. The consumer will likely become more discerning and demand a specific desired texture. The predictions are that the elderly, dentally-challenged consumers will demand a soft to mushy texture in their meat purchases while other consumers will desire other attributes such as firm texture and chefs will desire meat with some pre-purchase texture guarantee. A combination of new and innovative technologies will be ideally suited to deliver a range of desired textures for meat products in the future. Pre-rigor processing (hot-boning) of meat will allow meat to be delivered into Smartshape™ machines, packaged then delivered to a combination of PEF/US/shockwave treatments, plus or minus the addition of enzymes depending on the muscle, and then either into a cook process of *sous vide* or high pressure processing for case ready products or into a rapid chill process for immediate delivery to destination markets. The combination of systems used will ensure that the meat is safe, wholesome and ‘tender’ immediately after coming out of the factory. Processing of meat to final market- or case- ready product will occur in the room next to the hot-boning operation.

The implementation of these technologies in industry will be dependent on operators willingness to innovate, the capital and operating cost of the technologies and the cost-benefit. Previous research shows that the consumer will be willing to pay for the use of innovation technologies for assured texture. The question may become, can the industry afford not to innovate, in order to grow their customer base?

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APPENDIX 1: A summary of exogenous plant and microbial proteases investigated for meat tenderization

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Actinidin	Beef	*Longissimus*	Marination at 37°C for 2h	10% reduction in WBSF. Degradation of sarcoplasmic proteins was detected by SDS-PAGE.	Aminlari, Shekarforoush, Gheisari, and Golestan (2009)
Actinidin	Lamb	*Longissimus* and leg chops	Pre-rigor infusion	Extensive tenderization after 12 hours post mortem and a slight further improvement after 3 weeks ageing in vacuum pack.	Bekhit, Han, Morton, and Sedcole (2007)
Actinidin	Pork	*Bicep femoris*	Injection of kiwifruit extract (0-11g/L)	Lower WBSF and shorten ageing time by up to 7 days. Degradation of myofibril and heat soluble collagen proteins. Protease-induced damage of endomysium surrounding isolated single muscle fibers shown by atomic force microscopy.	Christensen et al. (2009)
Actinidin	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myofibril and collagen type I proteins.	Ha et al. (2012)
Actinidin	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myofibril and connective tissue proteins	Ha, Bekhit, Carne, and Hopkins (2013a)
Actinidin	Lamb	*Longissimus* and hind leg cuts	Pre-rigor infusion and stored for 6 days	A significant decrease in WBSF which was attributed to significant degradation of myofibril proteins.	Han et al. (2009)
Actinidin	Pork	*Longissimus*	Injection of samples after various freeze-thaw cycles	A reduction in WBSF regardless of freeze-thaw cycles. Degradation of myofibril proteins shown by SDS-PAGE.	Liu, Xiong, and Rentfrow (2011)
Actinidin	Beef	Cattle *achilles* tendon	Incubation in buffer	Up to 3% of collagen was solubilised at neutral and acidic conditions. Collagen was degraded into α and β chains and several peptide fragments of various sizes shown by SDS-PAGE.	Wada, Hosaka, Nakazawa, Kobayashi, and Hasegawa (2004)
Bacterial *Bacillus subtilis* protease	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myofibril and connective tissue proteins	Ha et al. (2013b)
Bacterial *Baccilus subtilis* protease	Beef	*Semimembranosus*	Injection at different concentrations	Lower WBSF for higher concentration of protease without salt. Lower WBSF for both low and high concentration of protease with salt. Higher sensory scores only in samples injected with salt.	Pietrasik and Shand (2011)

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Bacterial *Bacillus subtilis* protease	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	A lower WBSF and higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)
Bacterial *Bacillus sp.* EL31410 elastase	Beef	Neck ligament; shoulder muscles	Marination at 4°C	Higher sensory score for tenderness. Selective myofibril degradation proteins after 48h in treated samples.	Qihe, Guoqing, Yingchun, and Hui (2006)
Bacterial proteases from various mutants of *Bacillus* YaB	Beef	Unspecified muscles	Marination at 10°C for 15h and cooked at at 70°C for 20 min	An increase in myofibril fragmentation index and soluble collagen content.	Yeh et al. (2002)
Bacterial *Clostridium histolyticum* collagenase	Beef	Meat protein extracts for activity; *Semitendinosus* for sensory and WBSF	Incubation with buffer or injection and cooked at 65°C.	Hydrolysis of insoluble collagen with maximal activity at 40-60°C. Little effect on myofibril proteins. No change in WBSF with meat environment or the lack of sensitivity in shear force evaluation attributed.	Foegeding and Larick (1986)
Bacterial *Pseudoalteromonas* sp. 9913 MCP-01	Beef	*Pectoral*	Marination at 4°C for 20h	A reduction in WBSF. Extensive hydrolysis of myosin. Disruption of connective tissue shown by electron microscopy.	Zhao et al. (2012)
Bacterial unspecified collagenase	Beef	Meat protein extracts	Incubation in buffer	Hydrolysis of proteins in both myofibril fractions.	Kang and Rice (1970)
Bromelain	Chicken	*Pectoralis major*	Blade-tenderized meat was marinated in 0.003% protease	A decrease in WBSF which was correlated with an increase in sensory score for tenderness compared to both blade-tenderized only and control samples	Devitre and Cunningham (1985)
Bromelain	Beef	*Pectoral*	Injection with various concentrations to 10% meat weight and meat cooked fast or slow modes	A reduction in WBSF in both fast and slow cooking was correlated with an increase in free amino acids and hydroxyproline. Decrease in WBSF was rapid in fast cooking and more gradual in slow cooking. A lower protease concentration is required in slow cooking.	Fogle, Plimpton, Ockerman, Jarenback, and Presson (1982)
Bromelain	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myosin but not connective tissue proteins.	Ha et al. (2012)
Bromelain	Beef	Unspecified muscles	Injection and kept at 65°C for various lengths of time	Limited hydrolysis of muscle proteins was detected. An increase in rigidity index (compression test) and higher hydroxyproline content.	Ionescu et al. (2008)
Bromelain	Beef	Meat protein extracts	Incubation in buffer	Hydrolysis of proteins in myofibril fractions.	Kang and Rice (1970)

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Bromelain	Beef and chicken	Unspecified muscles	Marination of meat cubes with powder pineapple peel extract	Decreasing firmness and hardness as measured by shear force with increasing protease concentration. Disruption of muscle structure shown by scanning electron microscopy and protein hydrolysis by SDS-PAGE.	Ketnawa and Rawdkuen (2011)
Bromelain	Beef	Round muscles	Marination of mince meat	Myosin was extensively degraded while actin was left intact. Bromelain also increased protein solubility	Kim and Taub (1991)
Bromelain	Beef	*Adductor; Gluteobiceps; Rectus femoris; Semimembranosus; Semitendinosus; Yastus lateralis*	Injection with 0.004% protease or buffer containing salt and phosphate (6.0% sodium chloride and 3.5% sodium tripolyphosphate)	A reduction in WBSF, although salt & phosphate injection was more effective in some cases.	Kolle et al. (2004)
Bromelain	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	Lower WBSF and increased insoluble collagen degradation with no difference between the two types of muscle. Higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)
Bromelain	Beef	*Pectoral*	Marination at 4°C for 20h	A reduction in WBSF. Disruption of connective tissue shown by electron microscopy.	Zhao et al. (2012)
Ficin	Chicken	*Pectoralis major*	Blade-tenderized meat was marinated in 0.002% protease	A decrease in WBSF which was correlated with an increase in sensory score for tenderness compared to both blade-tenderized only and control samples	Devitre and Cunningham (1985)
Ficin	Beef	Meat protein extracts	Incubation in buffer	Hydrolysis of proteins in myofibril fractions.	Kang and Rice (1970)
Ficin	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	Lower WBSF and an increase in sarcoplasmic and insoluble collagen protein degradation. Higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)
Fungal *Aspergillus oryzae* 400 protease	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	A lower WBSF and higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)
Fungal *Aspergillus oryzae* 31K protease	Beef	Meat protein extracts	Incubation in buffer	Gradual hydrolysis of myofibril and extensive hydrolysis of connective tissue proteins	Ha et al. (2013b)

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Fungal *Aspergillus oryzae* 60K protease	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myosin and gradual hydrolysis of other myofibril and connective tissue proteins	Ha et al. (2013b)
Fungal *Aspergillus oryzae* protease	Beef	*Semimembranosus*	Injection at different concentrations and stored at 4°C for 1, 7 and 14 days. Meat was roasted with moist or dry heat.	A reduction in WBSF with the protease concentration in the range 0.005-0.0015% with higher tenderness in moist cooked samples. 0.005% protease resulted in mushy and creamy texture. The protease was inactive during the vacuum pack storage for 14 days at 4°C.	Pietrasik and Shand (2006)
Fungal *Aspergillus oryzae* protease	Beef	*Semimembranosus*	Injection at different concentrations	Lower WBSF for higher concentration of protease without salt. Lower WBSF for both low and high concentration of protease with salt. Higher sensory scores only in samples injected with salt.	Pietrasik and Shand (2011)
Fungal *Aspergillus oryzae* concentrate protease	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	A lower WBSF and an increase in insoluble collagen and myofibril protein degradation. Higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)
Fungal *Aspergillus oryzae* Rhozyme P-11	Beef	Meat protein extracts	Incubation in buffer	Hydrolysis of proteins in both sarcoplasmic and myofibril fractions.	Kang and Rice (1970)
Papain	Beef	Top rounds and briskets; Collagen extract; Myofibril extract	Injection and tumbled marination Incubation in buffer Incubation in buffer	WBSF decreased with dose of papain to 0.01 AU/100g of meat. Mushy meat was reported for dose beyond 0.01 AU/100g of meat. Significant increase in hydroxyproline. Increased in myofibril protein solubility.	Ashie et al. (2002)
Papain	Chicken	*Pectoralis major*	Marination in 0.002% protease	A decrease in WBSF which was correlated with an increase in sensory score for tenderness.	Devitre and Cunningham (1985)
Papain	Beef	Pectoral	Injection with various concentrations to 10% meat weight and meat cooked fast or slow modes	A reduction in WBSF in both fast and slow cooking was correlated with an increase in free amino acids and hydroxyproline. WBSF decrease was rapid in both fast and slow cooking.	Fogle et al. (1982)

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Papain	Beef	Shoulder muscles	Marination at 4°C for 3h	A decrease in hardness, an increase in myofibril fragmentation index. Disruption of myofibril structure shown by transmission electron microscopy. Significant increase in sensory score for tenderness.	Gerelt et al. (2000)
Papain	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of myofibril but not connective tissue proteins.	Ha et al. (2012)
Papain	Beef	Unspecified muscles	Injection and kept at 65°C for various lengths of time	Limited hydrolysis of muscle proteins was detected. An increase in rigidity index (compression test) and higher hydroxyproline content.	Ionescu et al. (2008)
Papain	Beef	Meat protein extracts	Incubation in buffer	Hydrolysis of proteins in both sarcoplasmic and myofibril fractions.	Kang and Rice (1970)
Papain	Beef	Round muscles	Marination of mince meat	Indiscriminate hydrolysis of myosin and actin.	Kim and Taub (1991)
Papain	Beef	Neck ligament; shoulder muscles	Marination at 4°C	Higher sensory score for tenderness in protease treated samples. Rapid myofibril fragmentation after 24h in treated samples.	Qihe et al. (2006)
Papain	Beef	*Triceps brachii; Supraspinatus*	Injection and vacuum tumbling	A decrease in WBSF and increase in sensory scores for tenderness and connective tissue components. A significant increase in both soluble and insoluble collagen.	Sullivan and Calkins (2010)
Papain	Beef	Pectoral	Marination at 4°C for 20h	Disruption of connective tissue shown by electron microscopy.	Zhao et al. (2012)
Zingibain	Camel	Meat chunks	Marination with 0, 15, 30 or 45% v/w ginger extract	More reduction in WBSF with increasing dose of ginger extract. Increase in sarcoplasmic and myofibril protein solubility Sensory score for tenderness improved at all concentrations of ginger extract.	Abdeldaiem and Ali (2013)
Zingibain	Chicken	Breast and leg muscles	Marination at ambient temperature for 3h and grilled at 180-200°C for 25-30 min.	A significant reduction in WBSF. SDS-PAGE indicated protease-induced degradation of muscle proteins. Higher sensory score for tenderness.	Bhaskar et al. (2006)
Zingibain	Beef	Meat protein extracts	Incubation in buffer	Extensive hydrolysis of actin and connective tissue proteins	Ha et al. (2012)

Protease	Meat type	Muscle or Substrate	Treatment	Tenderization effects	Reference
Zingibain	Beef	*Longissimus; bicep femoris; semitendinosus*; *quadriceps femoris*	Marination	A reduction in WBSF at higher concentrations. Different concentrations are required to tenderize different muscles. Preferential degradation of filaments in the I-bands. Improvement in sensory score for tenderness in all muscles.	Lee et al. (1986)
Zingibain	Lamb	Meat chunks	Marination with different concentrations of protease	Higher collagen solubility and lower WBSF. Ginger extract at 3% was effective at improving sensory scores.	Mendiratta et al. (2000)
Zingibain	Chicken	Breast muscle	Marination at 4°C and cooked to an internal temperature of 70°C	A decrease in WBSF and extensive proteolysis of sarcoplasmic proteins. Improvement in collagen solubility, protein extractability, moisture and cooking yield.	Naveena and Mendiratta (2001)
Zingibain	Buffalo	*Biceps femoris*	Marination at 4°C for 48h	A reduction in WBSF An increase in collagen and muscle protein solubility with extensive proteolysis of muscle proteins shown by SDS-PAGE.	Naveena et al. (2004)
Zingibain	Goat	*Bicep femoris*	Marination at 4°C	Significant hydrolysis of sarcoplasmic and myofibril proteins Collagen solubility was significantly increased. Overall sensory scores for tenderness and acceptability were higher compared to sample treated with water.	Pawar et al. (2007)
Zingibain	Beef	*Triceps brachii; supraspinatus*	Injection and vacuum tumbling	A lower WBSF and an increase in insoluble collagen protein degradation. Higher sensory scores for tenderness and connective tissue components.	Sullivan and Calkins (2010)