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Meat Tenderness: Theory and Practice

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SUMMARY

The year 2003 marks the 43rd anniversary of the classic cold shortening experiment of Locker. These results showed that toughness of meat was closely related to fiber diameter and that the new industry practice of rapid chilling caused extensive muscle shortening, increased fiber diameter and tough meat. This observation led to both a fundamental understanding of meat quality and also improved technology such as electrical stimulation of carcasses. A second major finding in the mid-80s, that proteolysis of myofibril substrates by calpain explained many of the tenderness changes in muscle, can also be singled out as a classic result which advanced meat science research. In this review I will examine changes such as these and document what is clearly known and what has aided improvements in meat technology. The second part of the review will focus on applied technologies which improve tenderness. The review will focus on key references which advanced our understanding on the selected topics, and is not an exhaustive review of the literature. Most of the work cited is for beef, and in general applies to all meat sources.

Keywords

meat, tenderness, connective tissue, old shortening, calpain, cooking

I. WHAT CAUSES MEAT TENDERNESS

Role of Connective Tissue in Tenderness

The connective tissue of muscle is a minor component comprising approximately 1 to 4 % of dry weight in most muscle types, but with the critical functions of fiber adhesion, force transmission, tissue organization and an outer protective cover of epimysium. Connective tissue properties and composition have been previously reviewed (Bailey 1989; Greaser 1997; Harper 1997, 1999; McCormick 1994, 1999; Purslow 1994, 1999).

Collagen and muscle type: Before the cold shortening experiments of Locker (discussed below) it was generally believed that meat was tough due to connective tissue amount and properties. In 1937 Brady published that meat shear force relates to connective tissue organization, with large fiber bundles being associated with weak mechanical resistance. Brady also showed that fiber size does not vary extensively in different muscle types. These observations were extended by Ramsbottom (1945) who showed that muscle type differences in shear force are mostly due to collagen content as determined histologically. Force muscles such as the biceps have high collagen content, thick fascicles and are tough, whereas position muscles such as the psoas have low collagen content, thin fascicles and are tender. Strandine (1949) classified different muscle types based on perimysium organization and found a good relationship to tenderness. These classical observations explain

most of the role of collagen in meat quality – that collagen content and fascicle size explain muscle type differences of shear force.

Collagen content and solubility: A second role of collagen in tenderness is determined by content and solubility. In general for a given muscle type there is poor correlation of the amount of collagen and tenderness. The contribution of connective tissue to texture as measured by machine or sensory panel is much less than the myofiber contribution. Cross has estimated that about 12% of texture variability is related to connective tissue content (1973).

The two connective tissue properties most often measured are collagen content as hydroxyproline, and collagen solubility which can vary more than two fold in a given muscle type from animals of similar age, sex and nutrition (Culioli 1995). Many studies have shown that increasing animal age results in decreased collagen solubility and tenderness, with little or no change in total collagen content (Berge 1998; Boccard 1979; Cross 1973, 1984; Goll 1963; Harper 1999; Herring 1967; Shorthouse 1990; Vognarova 1968; Wilson 1954; Young 1993). Most of this work has been done on ruminants, but this is a general feature of connective tissues as reviewed (Bailey 1989; McCormick 1999). Numerous studies have shown that there is no measurable contribution of hydroxyproline content to instrument measures of texture variability in animals of the same age and muscle type (Culler 1978; Goll 1963; Herring 1967; McKeith 1985; Shorthouse 1990; Siedman 1987); therefore collagen solubility is the important parameter relating animal age to meat tenderness. An important consideration in examining the relative roles of myofibers and connective tissue is to eliminate the toughness due to cold shortening. Samples must be prepared so that the sarcomere lengths are all near resting length, and the meat must be aged to eliminate calpain mediated proteolysis as a variable. Using these conditions Wheeler (2000) found that all samples of pork meat with sarcomere lengths of 2.0µm or longer were tender, thus the texture of longissimus from pork of the same age animals seems to be independent of connective tissue content.

The thermal stability and decreased solubility with age is due to the extent of collagen cross-linking. In a series of articles Goll (1963, 1964a,b,c) measured the thermal and collagenase stability of beef collagen in animals from 40 days to 10 years of age. At 70°C in veal 42% of the collagen was soluble compared to 2% soluble at 10 years of age, and the thermal shrinkage temperature was 55°C for veal and 70°C at 10 years of age. Collagenase digested 21% of the collagen in veal and 10% in 10 year old animals. These results indicate that the cooked meat toughness due to age is directly associated with the change in collagen thermal stability.

Cross-links: Less clear is the role of cross-links in meat quality in animals of the same age. Stable cross-links do vary significantly by muscle type and by species. But the most tender muscle, the psoas, has high cross-link quantity (McCormick 1999) and is tender even at normal sarcomere lengths (Wheeler 1999). McCormick (1999) and Bailey (1989) have concluded that both high collagen content and cross-link formation are needed for connective tissue to contribute to toughness, such as occurs in the biceps. Several studies have failed to demonstrate a relationship between cross-link quantity and collagen solubility (Avery 1996; Berge 1998; Horgan 1991; Young 1993). In addition numerous studies (Avery 1996; Bailey 1989; Horgan 1991; Young 1993) failed to find a relationship between cross-links and shear force in animals of the same age. Avery measured five different types of cross-link so we can conclude that these types of cross-links are not related to shear force. This is supported by callipyge sheep having extremely tough meat but low cross-link content (Field 1996). Therefore, it is not yet clear which cross-links are responsible for the age related change in meat texture.

Background toughness: Reviews of the role of connective tissue in tenderness (McCormick 1994, 1999; Purslow 1994, 1999) have discussed the stability of connective tissue postmortem. There are minor structural changes with prolonged storage of meat but no degradation of collagen and decorin for at least the first two weeks, during which time most meat becomes tender. Therefore, the role of connective tissue is evident only when meat is cooked and collagen undergoes thermal shrinkage. The stability of collagen postmortem and the extensive degradation of the myofiber cytoskeleton show that collagen is responsible for the background toughness of meat or the ultimate toughness caused by non-degraded components.

Cold Shortening

Meat Science Revolution I: In 1960 Locker reported that rapid chilling of meat resulted in muscle contraction and tough meat (Locker 1960, 1963 and reviewed 1985). This fundamental observation led to many significant results in the 1960s showing that fiber diameter was closely related to meat toughness. Marsh reviewed in 1974 that postmortem contraction, including cold shortening, is due to the normal sliding filament contraction mechanism. Cold shortened meat can be as much as 3 times as tough as normal (Locker 1985) and fiber diameter can increase up to 2-fold with sarcomere shortening from 3.0 to 1.3 µm (Herring 1967), resulting in larger fibers which are more resistant to shear forces giving tougher meat. The relationship of cold shortening and sarcomere length to toughness was first demonstrated clearly by Herring (1965) who showed the direct relationship of sarcomere length to fiber diameter and toughness. This relationship explains that the shortening of sarcomeres with contraction is accompanied by an increase in fiber diameter. Larger fibers, when cooked, are tougher and thus meat is also tougher. Numerous studies have clearly supported that short sarcomere length and large fiber diameter, often due to cold shortening, are responsible for the initial toughness

of beef (Crouse 1991; Geesink 1995; Koohmaraie 1996a, 1996b; Lewis 1977; Tuma 1962).

Fiber size: That fiber size is the most important factor determining toughness of meat supported by several models including: 1) muscle from callipyge sheep at 24h postmortem is tougher than normal, has larger fiber size, normal sarcomere length, and less collagen (Koohmaraie1995); 2) animals injected with β-agonists also have increased toughness at day 1, larger fibers and little change in collagen content (Koohmaraie 1996c); 3) cold-shortened versus control muscle from the same animal is tougher and has increased fiber diameter (Locker 1985); 4) fiber size varies many fold in fish and correlates well with texture (Hatae 1990); 5) fiber size differences within and between muscles correlates with days 1 and 3 shear force (Crouse 1991; Lewis 1977; Tuma 1962); 6) double muscled cattle have small fibers (Fiems 1995) and more tender meat (tender by raw shear and sensory cooked but not by cooked shear, DeSmet 1998), and 7) meat is not tough when fibers do not shorten (Koohmaraie 1996b). Since some of these changes are independent of sarcomere length they show that fiber size **per se** is the common determining factor of toughness.

Calpain Proteolysis

Meat Science Revolution II: Having determined that fiber contraction and fiber size determined toughness, the next revolution in meat science required understanding causes of tenderization of meat. It had been observed in the 1960s that there seemed to be little protein degradation and solubility in meat (Davey 1966), but the biochemical techniques available limited precise characterization of protein changes. The first big advancement, and one of the most significant in meat science, was to show that tenderization is due to proteolysis and the protease responsible is μ-calpain (Koohmaraie 1996).

Role of calpain: In 1972 the group of Goll in Iowa (Busch 1972) purified calpain from muscle for the first time. In 1977 this same group provided the first evidence that calpains are the major protease responsible for fiber degradation and meat tenderness (Olson 1977). This work explained for the first time the biochemistry of meat aging, and has been reviewed (Goll 1990). Subsequent work, especially in the lab of Koohmaraie, has shown that μ-calpain is the form of calpain which causes degradation of myofibril associated proteins postmortem (Koohmaraie 1986). Comparative studies of several proteases showed that lysosomal enzymes are not related to major tenderness events (Koohmaraie 1988). Correlation analysis by the same group showed that calpastatin, the endogenous calpain inhibitor, most closely relates to shear force (Koohmaraie 1988). There are several recent reviews of the calpain system and postmortem enzyme activity in meat (Geesink 2000; Goll 1995; Hopkins 2002; Koohmaraie 1996) so this information will not be discussed in detail.

That calpains are the protease responsible for tenderization is supported by several key observations: 1) that the cytoskeletal substrates which are degraded in meat are also calpain substrates (Taylor 1995), 2) cathepsins degrade most muscle proteins including actin and myosin, and these are not degraded postmortem (Roncales 1995), 3) calpain is activated by calcium which increases markedly postmortem, and is active at pH and temperatures which occur in stored meat, albeit at reduced levels of activity (Koohmaraie 1986), 4) inhibition of calpain activity postmortem by marination of meat with calpain inhibitors or calcium chelators prevents ageing (Koohmaraie 1988; Uytterhaegen 1995), 5) activation of calpain by marination of meat in calcium buffers greatly enhances tenderization (Koohmaraie 1988), 6) variation of calpain activity postmortem correlates well with variation of tenderness (Koohmaraie 1996), 7) callipyge sheep have very tough meat and very low postmortem calpain activity but are normal in other postmortem changes such as activity of other proteases, protein solubility, pH etc. (Koohmaraie 1995). Collectively these arguments are as close to proof of calpain as the tenderness protease as proof can be in such a complex system as meat tenderization. In fact it is known that the rate limiting factor is actually calpastatin activity and inhibition of postmortem -calpain. A very important recent finding is that mice which are transgenic for over-expression of calpastatin have almost no calpain activity postmortem and lack proteolysis of key substrates such as troponin T and vinculin (M Koohmaraie, USDA, personal communication). Therefore, in the absence of calpain activity and the presence of all other muscle factors there is no meat tenderness.

The above cited calpain reviews present convincing evidence that the postmortem protease activity associated with meat tenderness is due to μ-calpain activation by calcium. Recent studies have also examined a potential role for muscle specific calpain 3 in meat tenderness (Ilian 2001; Ilian 2003). Using antibodies specific to calpain 3 it is evident that there is autolysis postmortem which correlates with the kinetics of tenderization. However, due to the rapid degradation of calpain 3 during purification we do not yet know its activity against know meat substrates such as cytoskeletal proteins. Certainly the potential role of calpain 3 in meat quality deserves further study.

Cytoskeleton as the key substrate: Identification of calpain as the key protease was accompanied by a variety of studies showing that myofiber degradation is the key event in tenderness. One of the first observations was by Takahashi in 1967 showing that tenderness was accompanied by myofiber breaks. Davey in 1969 provided some of the first micrographs clearly showing that myofiber breaks were both between myofibrils and near the Z-line. The most consistently reported ultrastructural change associated with tenderness is breaks in the I band (Abbot 1977; Davey 1970; Dutson 1974; Ho 1996; Roncales 1995; Taylor 1995). These breaks occur at the junction of the I and Z bands in a region without any particular composition, meaning that the actin and gap filaments of the I band are continuous

from the A to Z band. It has been shown in a variety of studies that this change is associated with tenderness, and is observed in all species and muscle types. The observations are very similar for pork (Abbot 1977; Dutson 1974), bovine (Davey 1970; Ho 1996; Taylor 1995), ovine (Taylor 1998) and chicken (Sayre 1970). The contribution of this structural change to tenderness measures is significant as shown by the measure of myofibril fragmentation. Gothard in 1965 and Takahashi in 1967 were the first to report that myofibrils break in the I band when meat is tender. This index, the MFI index, was developed by Davey (1969), and subsequently associated with shear force by Olson (1977). Davey also hypothesized that the myofibril breaks required calcium release from the sarcoplasmic reticulum. Since then the MFI has been shown to be a predictor of tenderness (Whipple 1990), and associated with both shear force and degradation of specific substrates (Taylor 1995). The fragmentation of myofibrils in the MFI test also occurs at the I/Z junction, just as in postmortem meat (Taylor 1995).

The protein substrates which are degraded when myofibrils break are the cytoskeletal proteins. This subject has been reviewed by Bendall (1992), Greaser (1991), Goll (1995), Robson (1997) and Taylor (1995). These reviews give detailed descriptions of cytoskeletal proteins and their degradation postmortem. Young was the first to examine the degradation of the intermediate filament protein desmin and elastic filament protein titin in meat (1980). Three major cytoskeletal structures are degraded when meat is tender: Z- to Z-line attachments by intermediate filaments, Z- and M-line to sarcolemma attachments by costameric proteins, and degradation of the intra-myofibrillar elastic filaments composed of titin. Z- to Z-line attachment is mostly by the protein desmin which is a good postmortem substrate for calpain, as are all cytoskeletal proteins degraded in meat. These connections and their breaks in meat were first described by Davey (1969, 1976). That this is important to ultimate tenderness is shown by the model of callipyge sheep which do not have tender meat for several weeks postmortem, desmin is not degraded (Koohmaraie 1995), and the myofibril lateral attachments are intact (Taylor 1998). Locker (1977) was the first to report changes in titin filaments in meat, and subsequent work shows that this correlates to meat tenderness (Ho 1996; Koohmaraie 1995; Taylor 1995). Titin and desmin are probably the key substrates which determine meat tenderness. The third myofiber associated structure which is related with tenderness is myofibril attachment to the sarcolemma at costameres (Taylor 1995). A consistently reported change in meat is sarcolemma detachment (Abbot 1977; Sayre 1970; Taylor 1995), and the rate of degradation of the costameric protein vinculin correlates with shear force (Koohmaraie 1995; Taylor 1995). Vinculin is also slowly degraded in the tough callipyge sheep meat (Koohmaraie 1995); however, the detachment of sarcolemma in callipyge is not different from controls at day 14 when the meat is tough (Taylor 1998). This indicates that sarcolemma detachment is important to the early changes in tenderness, but not a limiting factor for ultimate tenderness variability.

Animal Models

Several animal models with unique aspects of tenderness have contributed greatly to our understanding. One is the double muscled cattle which have existed for at least 200 years and have very tender meat especially at low cooking temperatures. A series of experiments by Boccard and colleagues at INRA demonstrated that double muscled cattle were tender due to less collagen and also a change in perimysium organization (Boccard 1981). Double muscled cattle have less collagen (Boccard 1981; Steen 1997; Uyterrhagen 1994), increased collagen solubility (Steen 1997; Uyterrhagen 1994), low raw meat texture (Boccard 1981; Bouton 1982; DeSmet 1998), but cooked texture similar to normal (DeSmet 1998; Steen 1997; Uyterrhagen 1994). This suggests that collagen content explains the tenderness of these animals. In contrast, double muscled cattle tend to have decreased postmortem proteolysis due to changes in calpain/calpastatin activity (Uyterrhagen 1994). In addition they also have less lipid which is curious because the gene product myostatin which is mutated in these animals has a role in muscle differentiation (Kambadur 1997), but no known direct effect on connective and adipose tissue development.

A very instructive model is the callipyge sheep which have very tough meat for several weeks postmortem because of a two to three fold increase in calpastatin (Koohmaraie 1995). Most other parameters in these animals are normal including collagen quantity, collagen cross-links, ultimate pH, calcium mobilization, and lysosomal enzymes. The postmortem structural changes in muscle from callipyge sheep are the same as seen in normal animals but occur at a slower rate (Taylor 1998). Callipyge sheep show muscle hypertrophy (Koohmaraie 1995) due to a mutation in chromosome 18 (Freking 1999). This model emphasizes the important role of the calpain/calpastatin system in meat quality.

In a comparison of longissimus from young animals Koohmaraie (1991) did not find a difference in collagen content and solubility comparing cattle, sheep and pig indicating that longissimus has similar properties across species. Breed differences of texture can be related to connective tissue properties with **Bos indicus** breeds having higher collagen content, low collagen solubility and tough sensory texture (Crouse 1985; Shackelford 1994). However, in a comparison of **Bos indicus** and **taurus** crosses Whipple (1990) did not find significant difference in collagen content or solubility which may indicate that only the pure strains of **Bos indicus** have a significant connective tissue contribution to texture. However, **Bos indicus** cattle do have an increased calpastatin activity postmortem which does vary with percentage of **Bos indicus** in cross breeds and explains the toughness of this meat (Wheeler 1990; Whipple 1990b).

Most studies which have compared **Bos taurus** breeds do not find large breed differences in tenderness (Campo 2000; Shackelford 1994; Strydom 2000; Wegner

2000) and often the sire effect is stronger than breed. The USDA has compared many breeds and cross breeds for meat and carcass quality (see Wheeler 2001 and references therein). Therefore, in general only extreme animal models such as double muscle, callipyge and the **Bos indicus** breed show consistent breed effects on tenderness.

Tenderness Changes due to Cooking

In addition to the biological factors discussed above, cooking of course also has a significant role in tenderness, with meat toughening due to increasing cooking temperatures. Both collagen and myofibers shrink and lose water when cooked, the relative role of each depending on cooking temperature and meat composition. Ramsbottom (1945) summarized cooking effects softening of fat and connective tissue and hardening of fibers, depending on the temperature and the duration of heating. Connective tissue makes the largest contribution to raw meat texture and myofibers to cooked texture (Bouton 1975; Rhodes 1974). Cooking temperatures of 60°C to 70°C cause gelation of most of the perimysium, as reviewed by Offer (1989, 1992), but fibers are stable at these temperatures. Structural changes tend to vary by study with some reports of beef perimysium gelation at 70°C and above (Pohlman 1997) and others at 60 to 63°C (Leander 1980). Christiansen has verified, using isolated fibers and small muscle samples, that perimysium changes its thermal mechanical properties at 50 to 60°C, before myofibers toughen. These changes are mostly due to melting of collagen since the endomysium and elastin (Rowe 1989) are heat stable to 100°C. In a study of connective tissue properties at three different cooking temperatures Dransfield (1977) has shown that the connective tissue contribution to meat texture declines as temperature increases.

That thermal stability is the major property which determines the connective tissue role in cooked meat texture is clear from a number of studies and has been reviewed (Bailey 1989; McCormick 1999; Purslow 1994). Less clear, and requiring further study, is how the variability of structure and composition effect the structural changes. It has been shown (Offer 1998, 1989) that in cooked meat the perimysium changes are highly variable as are its thickness. An important property of collagen is that it can shrink to ¼ of normal length with heating to 70°C (Light 1985). If meat is not aged for long periods cooked perimysium retains much of its mechanical properties and can be stretched and relaxed (Offer 1989). These properties indicate that shrinkage of connective tissue can contribute significantly to cooked meat toughening (as reviewed by Bailey 1989; Davey 1983; Offer 1989). At temperatures above 70°C there is also shrinkage and hardening of fibers as shown clearly using single fiber assays (Christensen 2000). These studies support the previous data that cooking induced effects on meat tenderness occur in three phases: an initial toughening from 40-50°C apparently due to water loss and protein gelation, tenderization at 50-60°C as connective tissue melts, then further toughening above 60°C as fibers harden. Davey (1975) has shown that the difference in raw and cooked shear force measures can be as much as five-fold.

What is Not Important

The above sections have focused on the structural and proteolytic changes causing meat tenderness. It is also important to mention what is not involved in tenderness, especially events which continue to be discussed. The first issue herein has already been discussed: that tenderness is by calpain degradation of cytoskeletal proteins, and is not protein solubilization. The other issues relate to rigor development.

Actin/myosin interaction: The extent of actomyosin binding and the resolution of rigor have been discussed as important factors in tenderness. The hypothesis is that rigor bonds **per se,** and their resolution postmortem, contribute to meat tenderization. Since the discovery of the sliding filament mechanism of muscle contraction, independently by Hugh Huxley (1954) and AF Huxley (1954), meat scientists have examined the strength of the actomyosin complex and its role in tenderness. During postmortem rigor development actin binds to myosin when ATP levels fall below 1μM (Reedy 1965). In 1993 Swartz reported that for sarcomere lengths less that 2.7μm the amount of actin and myosin binding is the same. For sarcomere lengths from 1.8 to 3.0μm shear force is quite similar (Davey 1975; Herring 1967; Marsh 1974; Swartz 1993; Wheeler 1999). However, at sarcomere lengths of less than 1.8μm the shear force increases greatly showing that there is no relation of quantity of rigor bonds to shear force. Swartz concludes that fiber diameter is the key parameter relating sarcomere length to shear force not actomyosin overlap. Davey (1976) in fact demonstrated that aging of muscle occurred even for sarcomere lengths of 4.2μm at which there is no actomyosin overlap or binding. In a recent study Hopkins (2000) has shown that there is no correlation of either sarcomere length or shear force to actomyosin dissociation. Collectively these studies show that there is no relationship of the extent of actomyosin binding to tenderness.

"Resolution" of rigor: The second issue is whether loss of actomyosin binding has a role in tenderness. Goll has reviewed some of the evidence showing that the actomyosin complex changes with storage time postmortem (1995). The conclusions depend on both species and techniques so several conclusions can be drawn. Thus, the post-rigor actomyosin complex is more soluble than at rigor in rabbit (Fujimaki 1965) but not in lamb (Hopkins 2000) or chicken (Hay 1972; Wolfe 1976). Herring (1969) found that actomyosin solubility increased post-rigor in beef but did not correlate to variation of tenderness. In comparative studies Wolfe was able to reproduce the results of Hay but not Fuijimaki, and concluded that there is no weakening of actomyosin binding post-rigor in rabbit or chicken. More extreme than loss of binding strength would be a true

resolution of rigor. This subject has been reviewed by Bendall (1973) and strong evidence was provided that there is no resolution of the rigor complex with aging of meat. Rigor by definition is loss of extensibility of muscle. Goll (1967, 1968), Jungk (1967), and Bendall show that this occurs with rigor development and that the meat remains inextensible post-rigor. Bendall shows that out to nine days postmortem there is no loss of extensibility of muscle so no "resolution" of the rigor complex. There is a change in the elasticity of muscle with long postmortem storage times which is most likely due to proteolysis of the myofibrillar structure (Bendall 1973; Goll 1968). Davey (1976) has provided evidence that aged meat is not extensible after 30h, the elasticity changes being due to I band breaks. Bendall (1973) states that for there to be loss of rigor there must be a "miraculous" regeneration of ATP to cause loss of actin-myosin binding, but there is no source of ATP post-rigor.

In summary, and as discussed in other sections of this review, the loss of extensibility post-rigor and the tenderness of meat is due to protease degradation of myofibril structures by calpain causing fragmentation of the sarcomeres and tenderness.

II. TECHNOLOGY WHICH IMPROVES TENDERNESS

The above review of factors contributing to tenderness of meat has led to many developments which aid in improving meat quality. Only several of these have been widely adopted by the meat industry, and the following sections are limited to those that have been implemented or should be tested further. The following quote from Koohmaraie (1996) remains the main question concerning this topic: "It is apparent that there are a variety of methodologies to eliminate the inconsistency of meat tenderness at the consumer level. The question that needs to be addressed is: Why are these technologies not adopted by the industry? It is, perhaps, far more urgent to answer this question rather than it is to develop more technologies." The following sections are a partial list of what has been tried and adopted. Much of the evidence is anecdotal but well known and of importance. For example, it is probably true that meat quality is higher in countries which have a large export market. They must be competitive in a global market and tend to have more uniform quality than domestic industries. It is also apparent that when industry takes the initiative to create their own branded mark of quality by adopting a number of quality control practices it works. Restaurants with branded marks tend to have better meat, at least to my experience. This type of information is hard to document and no large international surveys of this sort seem to have been done.

Technology in use

Consumer surveys and quality management: The starting point in controlling tenderness should be a complete survey of what the consumer desires. Certainly all meat eaters prefer tenderness but in some regions a fresh flavor is also very desirable and long storage a negative factor. An example of an effective survey system is the national beef quality audit of the in the USA (Morgan 1991) which identified variability of tenderness as the major quality problem of beef. Surveys have identified desirable and acceptable levels of tenderness, type of cuts preferred, and the price consumers are willing to pay for quality (Boleman 1997). After it is determined exactly what the consumer wants the most practical approach is to implement a total quality management system to increase tenderness. Such a system has been tested and proven successful a short period after implementation (Tatum 1999). Critical control points which were managed included feeding and watering before slaughter, electrical stimulation, storage time and temperature etc.

Storage time: The most effective means to ensure tender meat is long storage at refrigerated temperatures. Although this is obvious and extremely effective it is still common that meat is stored for 4 or 5 days postmortem at which time at least half of it will be unacceptably tough. None of the technologies discussed below can replace the need for proper storage time and conditions. For beef the minimum storage time is 14 days, at which time about 80% of meat will be tender. If there is more than 33% **Bos indicus** in the breed then prolonged storage will improve tenderness to acceptable levels (Wheeler 1990).

Electrical stimulation: Cold shortening was identified above as a revolution in meat science. The technology of electrical stimulation of carcasses was developed to decrease the effects of carcass chilling and improve tenderness. There are a variety of methods used as detailed in a book which reviews of this subject (Pearson 1985). Electrical stimulation improves tenderness and also helps with bleeding and hide removal. In virtually all studies in which electrical stimulation is done correctly it improves tenderness and it should be used routinely – it remains the best technology to ensure quality. The tenderizing effect is due to more rapid onset of rigor and thus prevention of cold shortening (reviewed by Bendall 1980), and also structural damage of myofibrils, at least at certain currents (Dutson 1977; Ho 1996). The structural changes include stretching of sarcomeres, tears and contraction bands. It has been used extensively in most large abattoirs, in countries with a large export industry, and to create quality branded meat. It is still not used in small abattoirs and old abattoirs which lack the space for safe implementation of this technology.

Carcass hanging: Suspension of carcasses is normally done by the Achilles tendon. Hostetler (1972) first reported that suspension by the aitch bone stretched the leg muscles, thereby increasing sarcomeres length and decreasing fiber diameter, resulting in more tender

meat. This technique is widely adopted in some countries (Thompson 2002) because it is a very inexpensive and effective way to increase tenderness of hindquarter steaks. Forequarter steaks tend to not be effected because they are not stretched by this technique. This technique has not been developed in cases where the increased storage space required for pelvic suspension is not available, or it is estimated to be too expensive relative to the gain in profit. Since this is clearly beneficial for hindquarter cuts it is a useful technique when quality products have a higher price such as is the case for branded marks.

Herring (1965) found that laying carcasses on their side also improved tenderness but this technique is not practical. Severing the backbone at the 12th thoracic vertebrae, a process termed tendercut (Claus 1997), stretches the longissimus and several other muscles thereby improving tenderness. In comparison to aitch bone suspension (Thompson 2002) tendercut is more difficult to apply and improves fewer muscles in the hindquarter.

Animal breeds: As discussed above the breed effect is significant for extreme animals such as double muscled and also cross breeds with a lot of **Bos indicus**. This knowledge has led to development of specific cross breeds which are both heat tolerant and also have acceptable meat palatability. For example the Afrikaner breeds are well adapted to the local environment and have quality similar to continental breeds (Strydom 2000). In Australia where 40% of cattle have some **Bos indicus** inheritance it is very important to identify breeds and prolong storage if necessary. In this case total quality management has proven effective (Thompson 2002) to identify the conditions necessary to ensure tenderness, especially proper storage time.

Technology developed

The following section discusses technologies which have been developed as prototype procedures or tested in commercial facilities, and have potential to improve tenderness and could be made cost effective.

Carcass classification: Sorting of carcasses for retail product yield has recently been tested in plants using an image analysis system developed at the USDA (Shackelford 1998). Similar systems are being tested in other countries. Coupling carcass classification with tenderness measures is a very important tool for the industry, especially if the cost is reasonable. Automated image analysis of carcass quality parameters in combination with tenderness classification by slice shear force (Shackelford 1999) allows calculations of retail product yield, subprimal cut weights, and gives a tenderness classification at 2 days postmortem which can be used to determine value of the product and storage time. The cost is estimated at approximately $4/carcass for automated classification of tenderness. This system has not yet been developed by industry, but is probably the most accurate technology available for classifying tenderness. It is more accurate than other systems currently being tested (Wheeler 2002).

Calcium infusion: Infusion of meat with calcium to activate calpain and increase tenderness is a procedure developed after it was shown that calpain activity explains muscle protein degradation post-mortem. The procedure initially involved marination of meat in calcium solutions, then infusion of the carcass via an artery (Koohmaraie 1989) and eventually the preferred technique of injecting cuts of meat (Koohmaraie 1998). Infusion can be done at any time post-mortem and does not cause over tenderization. It has been tried in commercial facilities but not yet adopted.

Freezing: Koohmaraie and colleagues determined that the cause of the extreme meat toughness in callipyge sheep was high postmortem calpastatin activity as discussed above. Because of the high carcass yield of these animals they are of potential commercial interest is the meat quality problems are solved. Calcium infusion does improve tenderness in callipyge steaks (Koohmaraie 1998), as does freezing. The freezing protocol involves immersion of the carcass in liquid nitrogen then storage at -2°C for 4d to prevent cold shortening induced toughness. The combination of freezing and calcium infusion produces meat of acceptable tenderness. This technology has not been implemented but is a means to ensure that all meat is uniformly tender.

QTL markers of tenderness: A very active area of current research is development of markers of tenderness quality. Burrow has reviewed (2001) the QTLs for bovine carcass and meat quality traits. Although very promising progress in tenderness marker identification has been slow and as of yet there is no consensus. Keele (1999) reported a tenderness loci on chromosome 15 that was not significant in other studies. Casas and colleagues have identified -calpain on chromosome 29 as a positional candidate for beef shear force (2000). Due to the complexity of the postmortem tenderization process and the many factors involved it is likely that many gene products act in concert to give ultimate tenderness, and successful marker use will require a panel of markers. There is not a tenderness gene, rather a tenderness process. This will probably be the most active area of meat science research in the next decade.

Cooking: None of the knowledge and technology discussed above is of any use if meat is not properly cooked. Cooking has been a controlled parameter in all studies which characterize meat quality and we know how to improve low quality steaks by cooking (Wheeler 1999). The knowledge base of cooking effects is very complete and was discussed above. It is also the parameter that can not be controlled so the approach is two-fold, to know what the consumer wants by doing surveys (Savel 1999), and to educate the consumer (Thompson 2002). An effective means is recommended cooking procedures included in the product packaging.

PERSPECTIVES

Sixty years of meat research has created a knowledge base explaining most of the factors which determine the ultimate tenderness of meat. For a given muscle type the initial toughness of meat is mostly due to fiber diameter which will increase if there is cold shortening. Between muscle type tenderness differences are largely due to connective tissue properties and also protease activity. The tenderness of meat is determined by postmortem calpain mediated proteolysis of cytoskeletal proteins. Knowledge lacking is the physiochemical factors which determine postmortem calpain activity. Technology has been developed which aids in improving meat quality. At this time it is more important to implement technology throughout the industry than to develop new technologies. The future will be aided greatly by functional genomics which will identify factors allowing improvement in the selection of animals for factors contributing to tenderness and selection against toughness.

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