ELSEVIER

Meat Science, Vol. 43, No. S, S193–S201, 1996

PII:S0309-1740(96)00065-4 0309-1740/96 $15.00+0.00

Biochemical Factors Regulating the Toughening and Tenderization Processes of Meat

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ABSTRACT

The purpose of this manuscript is to present a brief review of the bio- chemical basis for longissimus toughening and tenderization processes. Also, to explore the potential technologies that can be developed based on this knowledge to reduce variation in tenderness, thus, improving consumer acceptance of meat. Results suggest that after slaughter longissimus has low to intermediate shear force values (probably tender). Rigor development-induced changes increase its shear force. Maximum toughness is observed between 12 to 24 h post mortem. *The toughening process seems to occur equally in all carcasses. Post-mortem storage at refrigerated conditions tenderizes longissimus. Post-mortem tenderization is caused by enzymatic degradation of key myofibrillar and associated proteins. The function of these proteins is to maintain the structural integrity of myofibrils. Current data indicates that μ-calpain is responsible for degradation of these proteins. Unlike the toughening process, there exists a large variation in the rate and extent of tenderization which is responsible for variation in tenderness at the consumer level. Potential strategies for the control of the variation in meat tenderness are discussed. Copyright © 1996 Published by Elsevier Science Ltd*

INTRODUCTION

Solving the problem of inconsistent meat tenderness is a top priority of the meat industry. This requires a greater understanding of the processes that affect meat tenderness and, perhaps more importantly, the adoption of such information by the meat industry.

Eating satisfaction results from the interaction of tenderness, juiciness, and flavor. However, as outlined previously (Koohmaraie, 1995), the problem of consumer dissatisfaction will be solved only when we solve the problem of unacceptable variation in meat tenderness. The objectives of this manuscript are to briefly review the biochemical basis of meat tenderness, and to indicate the potential technologies for improving meat tenderness that could be developed using this information. This is not meant to be a comprehensive review of the meat tenderness literature.

The toughening phase

The variation in meat tenderness either exists at slaughter, is created during post-mortem storage, or is a combination of both. Certainly, meat preparation by consumers can also be

a considerable source of variation in meat tenderness; however, that is not a researchable problem, but a problem that can and should be addressed through consumer education.

In an attempt to determine the source(s) of variation in meat tenderness, we conducted an experiment which demonstrated that lamb longissimus has an intermediate shear force value immediately after slaughter (5.07 kg), toughens during the first 24 h (maximum toughness was achieved at 9 to 24 h; 8.66 kg), and then becomes tender during post-mortem storage at 4°C (3.10 kg). Because sarcomere length (SL) decreased (from at-death length of 2.24 μm to 24 h post-mortem length of 1.69 μm) as shear force increased, we concluded that sarcomere shortening during rigor development is the cause of lamb longissimus toughening from 0 to 24 h post-mortem (Wheeler & Koohmaraie, 1994).

To test the accuracy of our conclusion regarding the cause of meat toughening, we determined shear force values at various post-mortem times in the lamb longissimus that was not allowed to shorten (Koohmaraie *et al.*, 1996*b*). Results indicated that shear force value does not increase during rigor development when muscle is prevented from shortening. A conservative interpretation of this data is that post-mortem meat toughening is completely alleviated in the absence of shortening, but shortening is not necessarily the cause of toughening. A more direct interpretation is that since preventing sarcomere shortening prevented meat toughening, the toughening that occurs during the first 24 h of slaughter is the result of sarcomere shortening.

The conservative interpretation would suggest that factors other than sarcomere shortening are responsible for meat toughening. The question is what are these factors? A possible candidate could be the hypothesis, proposed by Goll *et al.* (1995), that toughening during the first 24 h post-mortem is caused by a change in the actin/myosin interaction from a weak-binding state to a strong binding state, and that this increase in toughness does not have to be, but may be accompanied by and exacerbated by shortening. The results of Koohmaraie *et al.* (1996*b*) do not support this aspect of the Goll et al, (1995) hypothesis, because no toughening occurred when shortening was prevented. The state of actin/myosin interaction hypothesis could explain the results of those studies that show no relationship between shortening and toughness when measured very early post-mortem (i.e., prior to tenderization).

On the other hand, there is substantial evidence that supports sarcomere shortening as the cause of meat toughening. The best evidence is the classic work of Locker & Hagyard (1963). Nevertheless, the literature is far from unanimous on the relationship between SL and meat tenderness. There are numerous examples to demonstrate that increased toughness is associated with decreasing SL (Herring *et al.*, 1965; Hostetler *et al.*, 1972; Bouton *et al.*, 1973; Davis *et al.*, 1979) and many examples that do not support such a relationship (Culler *et al.*, 1978; Parrish *et al.*, 1979; Smith *et al.*, 1979; Seideman *et al.*, 1987; Shackelford *et al.*, 1994; Koohmaraie *et al.*, 1995). The question is why does this disagreement exit? Marsh & Leet (1966) demonstrated a clear-cut relationship between sarcomere length and meat tenderness in excised tissue. In excised muscle, maximum toughness was shown to develop at about 40% shortening. With shortening more or less than 40%, the meat was more tender (Marsh & Leet, 1966). It is in the case of muscle attached to the skeleton that a different relationship has been reported. The best case is the report of Smulders *et al.* (1990). They examined the relationship between SL and tenderness in unaged and aged meat of 67 beef carcasses. They reported a strong relationship between SL and tenderness in unaged meat (48 h post-mortem). The $r = -0.50$ (based on shear force) was observed for all 67 animals. However, when they separated carcasses based on their pH value at 3 h post-mortem, an entirely different picture emerged. The correlation between shear force and SL in carcasses with pH value of 6.3 or greater was –0.84 in unaged meat and –0.80 in aged meat (i.e., between SL measured at 48 h post-mortem and shear force measured after 48 h or 14 d of aging). However, no relationship

was found between these traits in carcasses whose 3 h pH value was less than 6.3. These authors concluded that "Tenderness is very highly dependent on shortening in slow-glycolysing muscles, but it is completely independent in muscles of more rapid pH decline." They concluded that the tenderness of fast-glycolysing carcasses is due to more rapid aging. In my opinion, assuming that there exists a significant range in SL, the relationship between SL and meat tenderness is modified by the extent of post-mortem tenderization. The strongest relationship would then be expected when very little post-mortem tenderization has occurred (e.g., after one day post-mortem in lamb, r = –0.52 between shear force and SL; Table 1) and the weakest relationship is expected when extensive post-mortem tenderization has taken place (r = 0.12; Table 1).

Accepting the above premises, toughening during the first 24 h post-mortem can then be attributed to rigor-induced sarcomere shortening. I believe that this rigor-induced toughening occurs equally in all carcasses. This does not mean that there is no variation in SL within the longissimus of a given carcass. Rather, a greater proportion of the variation in sarcomere length is accounted for by within-animal variation than between-animal variation. In other words, due to a large variation in factors affecting rigor development, there exists a large variation in SL within a given longissimus. However, the overall mean SL for a given muscle from one carcass is probably the same as the mean of SL for another carcass and, thus, rigor-induced toughening is also the same. The shear force value at any given time is the balance between two opposing processes: SL shortening and tenderization (see below). However, because the events leading to tenderization begin before the SL shortening process is completed, it may be difficult to demonstrate that all longissimus reaches the same level of rigor-induced toughening. To conclusively demonstrate this point, one must examine these events in carcasses that do not undergo post-mortem tenderization. Lambs carrying the callipyge gene may prove to be a good model to test the accuracy of this hypothesis because post-mortem tenderization occurs to a very limited extent in these carcasses (Koohmaraie *et al.*, 1995, 1996*a*).

The tenderization phase

Sometime after death, an opposing process called tenderization begins and will continue for some time *post mortem*. To maximize the benefits of post-mortem storage on meat tenderness, beef should be stored for 10-14 d, lamb for 7-10 d, and pork for 5 d. Unlike the toughening phase, the tenderization phase does not occur equally in all animals. In fact, it is well documented that there is a large variation in the rate and extent of post-mortem tenderization (for review see Koohmaraie, 1992*a*, *b*; 1995). It is this variability in the tenderization process that results in inconsistency in meat tenderness at the consumer level. To solve this problem, we must identify the reasons for the variability in the rate and

TABLE 1
Effect of Length of Postmortem Storage on the Correlation Between Sarcomere Length (SL) and Lamb Shear Force

Time postmortem	*n*	*SL mean, μm*	*SL range, μm*	*Shear force mean, kg*	*Shear force range, kg*	*r*
1 d	30	1.70	1.43-1.89	7.85	3.88-12.90	–0.52
3 d	19	1.72	1.59-1.86	4.61	3.22- 6.36	– .31
14 d	20	1.83	1.52-2.26	2.79	1.72- 4.60	.12

Based on data from Wheeler and Koohmaraie, 1994; Wheeler, Doumit, and Koohmaraie, unpublished.

extent of post-mortem tenderization so that the tenderization process can be manipulated to equalize it between carcasses and/or develop the necessary technology to identify those carcasses that will not respond to post-mortem tenderization. Without this information, we will continue to have inconsistency in meat tenderness at the consumer level, and branded product (these may exist in countries other than the U.S.) and, thus, value-based marketing will not be possible.

Mechanisms of post-mortem tenderization

The mechanisms of meat tenderization during storage of carcasses at refrigerated temperatures has been researched by various laboratories (for review see Penny, 1980; Davey, 1983; Goll, 1991; Goll *et al.*, 1983, 1995; Greaser, 1986; Koohmaraie, 1988, 1992*a*, 1994, 1995; Koohmaraie *et al.*, 1995; Ouali, 1990, 1992; Taylor *et al.*, 1995*a*). Current evidence suggests that proteolysis of key myofibrillar and associated proteins is the cause of meat tenderization. These proteins are involved in inter- (e.g., desmin and vinculin) and intra-myofibril (e.g., titin, nebulin, and possibly troponin-T) linkages or linking myofibrils to the sarcolemma by costameres (e.g., vinculin, dystrophin), and the attachment of muscle cells to the basal lamina (e.g., laminin, fibronectin and the newly described 550 kDa protein [Hattori *et al.*, 1995]). The function of these proteins is to maintain the structural integrity of myofibrils (for review see Price, 1991). Degradation of these proteins would, therefore, cause weakening of myofibrils and, thus, tenderization. Although the list of these proteins may change over the years, I believe the principle will stand the test of time; that is, proteolysis of key myofibrillar and associated proteins is responsible for post-mortem tenderization.

There has been considerable debate about the specific proteases responsible for these changes. A protease must meet certain criteria to be considered a possible candidate for involvement in post-mortem tenderization (Koohmaraie, 1988). Goll *et al.* (1983) provided the logic for the first criteria which is that the protease must be endogenous to skeletal muscle cell. Secondly, the protease must have the ability to reproduce post-mortem changes in myofibrils in an in-vitro setting under optimum conditions. Finally, the protease must have access to myofibrils in tissue. If a protease does not have these characteristics, it can not be considered as a candidate in the post-mortem tenderization process. Likewise, if a protease meets these criteria, it would be impossible to exclude its possible involvement in the tenderization process. Of all the potential candidates (Koohmaraie, 1988, 1992*a*, *b*, 1994), calpains are the only proteases that meet all of the above requirements. Based on the results of numerous experiments reported by different laboratories, it can be concluded that proteolysis of key myofibrillar proteins by μ-calpain is the underlying mechanism of meat tenderization that occurs during storage of meat at refrigerated temperatures. There is much evidence in support of proteolysis causing tenderization and that it is mediated by calpains (for review see Penny, 1980;Goll, 1991; Goll *et al.*, 1983, 1995; Koohmaraie, 1988, 1992*a*, 1994, 1995; Koohmaraie *et al.*, 1995; Ouali, 1990, 1992; Taylor *et al.*, 1995*a*).

In spite of overwhelming evidence in support of the calpain proteolytic system as the underlying mechanism of post-mortem proteolysis, there still exist some doubts. Some of these are legitimate and will have to be addressed before the calpain theory can become fully convincing. Some of these are based on the following: 1) μ-calpain is so rapidly inactivated that it can not account for tenderization beyond 24 to 48 h *post mortem*; 2) how could μ-calpain ever be active when muscle contains twice as much calpastatin as μ-calpain activity?; and 3) if μ-calpain is indeed involved in post-mortem proteolysis, why is m-calpain not degraded during post-mortem storage? Some of these questions can be answered easily by using existing data and others will require additional data. One of the arguments is that not enough μ-calpain is available to cause tenderization after 24 to 48 h

(i.e., the question #1 above). This argument is based on data that uses perhaps the least sensitive methodology to quantify μ-calpain activity (i.e., hydrolysis of casein and quantification of released polypeptides after TCA precipitation). Use of a more sensitive quantification method (e.g., radiolabeled casein) indicates that indeed skeletal muscles contain significant μ-calpain activity, even after extended storage at 4°C (about 5-10% remains after 14 d; Koohmaraie, Arbona and Whipple; unpublished data). Therefore, the inability to detect μ-calpain activity during post-mortem storage with such an insensitive method should not be used as the basis for drawing far reaching-conclusions. In fact, because μ-calpain autolysis and inactivation is an intermolecular process, it will not go to completion, and, therefore, μ-calpain will retain some of its activity even after extensive autolysis (Cottin *et al.*, 1986; Inomata *et al.*, 1988,; Edmunds *et al.*, 1991; Nishimura & Goll, 1991; Koohmaraie, 1992*a*). Unlike μ-calpain, m-calpain autolysis is an intramolecular process; extensive autolysis will result in complete inactivation of m-calpain (Inomata *et al.*, 1988, Cottin *et al.*, 1991; Edmunds *et al.*, 1991; Nishimura & Goll, 1991; Koohmaraie, 1992*a*). Therefore, once m-calpain is exposed to sufficient calcium it will undergo autolysis which results in its complete inactivation. Since even after extended storage all m-calpain activity can be recovered and because m-calpain is completely inactivated when sufficient calcium is present (infusion of carcasses with calcium chloride; Koohmaraie *et al.*, 1989), I conclude that μ-calpain and not m-calpain is responsible for meat tenderization.

Another frequently mentioned argument against calpain involvement in post-mortem tenderization is that muscle contains an excess of calpastatin relative to μ-calpain and, therefore, μ-calpain can never be active (i.e., the question #2 above). Firstly, this issue is species dependent. The ratio of calpastatin:μ-calpain is about 4:1, 2.5:1 and 1.5:1 in beef, lamb and pork muscle, respectively (Ouali & Talmant, 1990; Koohmaraie *et al.*, 1991). Secondly, an important clarification is needed. The data reported by Ouali & Talmant (1990) and Koohmaraie *et al.* (1991) and most, if not all, of the literature uses m-calpain to quantify calpastatin activity. This is a very important point, since it takes twice as much calpastatin to inhibit μ-calpain as to inhibit m-calpain (Koohmaraie, unpublished data). Therefore, the actual ratio of calpastatin:μ-calpain is only one-half of that mentioned above, i.e., about 2:1, 1.25:1, and 0.75:1 in beef, lamb. and pork muscle, respectively. The argument regarding excess calpastatin activity is not as significant as it first appears and, in fact, at most, the ratio is only 2:1 in beef. These ratios are consistent with the rate of post-mortem proteolysis and tenderization in these three species (Dransfield *et al.*, 1981; Etherington *et al.*, 1987; Koohmaraie *et al.*, 1991). We are in the process of determining the ability of the calpains to use each other as substrates to help answer question #3) above.

In my opinion, current data indicates that calpains (and more specifically, μ-calpain) are the only proteases that are directly involved in the events leading to meat tenderization. Because myofibrils are a poor substrate for the multicatalytic protease complex (MCP) and because MCP does not degrade the same proteins that are degraded *post mortem* (Koohmaraie, 1992*c*; Taylor *et al.*, 1995*b*), MCP can not have a direct role in post-mortem proteolysis that results in meat tenderization. Also, we have ruled out a primary role for MCP in post-mortem calpastatin degradation (Doumit & Koohmaraie, 1996), but not a secondary role (further degradation of calpastatin fragments generated by calpain). Because MCP is not active at pH less than 7.0, it is doubtful if they play any role in post-mortem tissue (Tanaka *et al.*, 1988). With regard to lysosomal proteases, until it is clearly documented that they are released from lysosomes (in living muscle, lysosomal proteases are normally located in lysosomes and, presumably, have to be released to have access to myofibrils) and an adequate explanation is provided for lack of actin and myosin degradation (cathepsins degrade myosin and actin efficiently, but neither are degraded

during post-mortem storage), no role, primary or secondary, can be assigned to these proteases.

Strategies for eliminating inconsistency in tenderness

Based on the information presented, strategies for enhancing meat tenderness might include steps to prevent/minimize the toughening phase or accelerate/enhance the tenderization phase.

One method of preventing the development of toughness is to freeze carcasses immediately after slaughter and then store them at subfreezing temperatures to prevent thaw-shortening when carcasses are thawed. Koohmaraie *et al.* (1996*b*) demonstrated that meat does not toughen when sarcomere shortening is prevented. The principal source of error in cooking of prerigor meat is excision- and heat-induced shortening. Locker (1985) stated that "Tenderness relates to cooked meat, but cooking in a prerigor state involves such a dramatic modification of the myofibrils that the resultant material is sheer artifact." Thus, to prevent excision- and heat-induced shortening, we developed and used a novel approach to measure tenderness of meat having prerigor sarcomere lengths (Wheeler & Koohmaraie, 1994). The protocol involved clamping of muscle sections while on the carcass, excision, brief storage at -30°C, unclamping and storing muscle sections at –5°C for 8 d. At this temperature, glycolysis will proceed and ATP will be depleted (Moran, 1930; Smith, 1930; Marsh & Thompson, 1958); thus, shortening does not occur during thawing. At present, we do not fully understand the biochemistry of muscle stored at –5°C, but it appears to be different than that of muscle stored at or below –10°C. Davey & Gilbert (1976) demonstrated that during storage at -10°C glycolysis does not occur, whereas, the ATP concentration declines due to enzymatic hydrolysis to levels insufficient to cause contraction during thawing. Smith (1930) detected accumulation of lactic acid in frog muscle at -5°C, but not at -10°C. Davey & Garnett (1980) reported that rapid freezing of carcasses and subsequent extended storage at –10°C (> 10 d), removes the hazards of toughness development from cold- and thaw-shortening. Bowling *et al.* (1987) reported that compared to carcasses stored according to conventional protocol, longissimus from carcasses stored at –70°C for 5 h, then at + 16°C for 4 h, and then at 1°C for 15 h had longer sarcomere lengths (2.0 vs. 1.93 μm), less shear force (4.26 vs. 5.03 kg), and higher sensory panel tenderness rating (5.3 vs. 4.69). With the current stage of knowledge, it would seem that it is practical to use this technology. A potential protocol to prevent the development of toughness would include the following steps: 1) electrically stimulate carcasses immediately after slaughter (to reduce ATP concentration, thereby reducing frozen storage time necessary to deplete ATP), 2) pass carcasses through a freeze tunnel for a period of time to be determined (time necessary to freeze SL in prerigor state; the goal should be conditions to produce SL of not less than 2.2 μm), 3) store at –5°C (rather than –10°C to speed up ATP depletion) for a period of time to be determined (time necessary to drop pH to < 5.8; thus, no thaw-shortening should occur), and 4) fabricate and distribute. Therefore, if practical, the commercialization of this technology should consistently result in production of tender meat.

Another method which is also based on preventing development of toughness is variation in the way carcasses are hung during rigor development. Based on his observations on the relationship between contraction and toughness, Locker (1960) stated that "it should be possible to improve the quality of the longissimus for example by hanging the carcass in such a way that this muscle is stretched and prevented from shortening." Others demonstrated a marked improvement in longissimus tenderness on longissimus when carcasses were laid horizontally or hung from the pelvis (Herring *et al.*, 1965; Hostetler *et al.*, 1972; Bouton *et al.*, 1973).

TABLE 2
Effect of Length of Postmortem Storage on Beef Shear Force Values

		7 Days aging			*14 Days aging*			*Correlation*
Breed	*n*	*Mean*	*Range*	*% > 6 kg*	*Mean*	*Range*	*% > 6 kg*	*Day 7 to day 14*
Angus	102	5.11	2.57- 9.30	22	4.05	2.48-9.04	5	.58
Tuli	158	5.71	2.94-12.38	34	4.58	2.33-9.24	8	.65
Hereford	106	5.67	2.37-11.91	31	4.74	2.41-8.30	12	.66
Belgian Blue	144	5.82	2.52-10.57	42	4.82	2.64-8.41	14	.61
Boran	138	6.58	3.15-11.79	55	5.14	2.84-11.25	26	.76
Brahman	119	7.30	3.43-12.50	63	6.05	2.66-11.03	34	.80
All breeds	767	5.95	2.37-12.50	42	4.86	2.33-11.25	17	.72

Wheeler, T. L., L. V. Cundiff, M. Koohmaraie, S. D. Shackelford, and K. E. Gregory. 1996. Characterization of biological types of cattle (Cycle V): Carcass traits and longissimus palatability (In preparation).

One could take advantage of our knowledge of the mechanisms of post-mortem meat tenderization and manipulate the systems involved to accelerate/enhance the tenderization process, such as Calcium-Activated Tenderization (Koohmaraie *et al.*, 1988, 1989, 1990; Koohmaraie & Shackelford, 1991; Wheeler *et al.*, 1991, 1992, 1993, 1994; Kerth *et al.*, 1995; Lansdell *et al.*, 1995; Miller *et al.*, 1995*a*, *b*; Wulf *et al.*, 1996). In spite of its well documented effectiveness, there is no evidence of its use by the industry.

The simplest and best documented method of improving, but not eliminating, the inconsistency of meat tenderness is to ensure that meat is not consumed without adequate aging (Table 2). To maximize consistency in tenderness, beef, lamb, and pork should be aged for 10-14, 7-10, and 5 d, respectively.

If none of the above technologies are adopted, then the surest method of ensuring consistency of meat tenderness is to classify carcasses based on tenderness. We have developed a tenderness-based classification method for beef that can operate at the chain speed of 400 carcasses per h. The system is essentially an automated version of shear force measurement that only takes about 10 min. to perform (Shackelford *et al.*, 1996).

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.

ACKNOWLEDGEMENTS

I am grateful to Drs. M. E. Doumit, D. E. Goll, M. L. Greaser, S. D. Shackelford, G. R. Siragusa, P. A. Speck, and T. L. Wheeler for their review and constructive criticism of this manuscript and to M. M. Bierman for secretarial assistance.

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