BIBLIOGRAPHIC REVIEW

BIOCHEMICAL ASPECTS OF MEAT TENDERNESS: A BRIEF REVIEW

ASPECTOS BIOQUÍMICOS DE LA TERNEZA DE LA CARNE. UNA REVISIÓN BREVE

Luciano*, F. B. , A. A. Anton and C. F. Rosa

Department of Food Science. Faculty of Agriculture & Food Sciences. University of Manitoba. Winnipeg, MB, Canada-R3T 2N2. *Corresponding author: umbitten@cc.umanitoba.ca

ADDITIONAL KEYWORDS

Tenderization. Proteolytic enzymes. Calpain. Apoptosis.

PALABRAS CLAVE ADICIONALES

Ablandamiento. Enzimas proteolíticos. Calpaína. Apoptosis.

SUMMARY

Meat tenderness appears as top rated issue to be solved concerning meat sensorial quality, which requires enhanced knowledge and further work to understand the processes involved. It is recognized that these processes are mainly enzymatic in nature and involve proteolytic systems. Two concepts based on enzymatic biochemistry during meat aging are currently in explaining meat tenderness. Firstly, some researchers postulate that meat tenderization is affected mainly or solely by µ-calpain, a proteinase responsible for myofibrilar protein degradation. Secondly, others have suggested a hypothesis that tenderization is a multienzymatic process corresponding to apoptosis, where µ-calpain is an important enzyme. This process is common to all cells when damaged, being associated with muscle cells after animal bleeding. The µ-calpain activity explains many, but not all pathways of tenderization and apoptosis appears be an attractive answer to explain the obscure processes within meat aging.

RESUMEN

La terneza es una de las más importantes características de la carne, en relación con su calidad sensorial, que demanda mejor conocimiento y esfuerzos adicionales para comprender los procesos implicados. Es admitido que esos procesos son principalmente de naturaleza enzimática e implican sistemas proteolíticos. Dos conceptos, basados en la bioquímica enzimática durante la maduración de la carne, se manejan habitualmente en la explicación de la terneza de la carne. En primer lugar, algunos investigadores postulan que el ablandamiento de la carne es afectado principal, o únicamente, por la µ-calpaína, una proteinasa responsable de la degradación de las proteínas miofibrilares. En segundo lugar, otros han sugerido la hipótesis de que el ablandamiento es un proceso multienzimático correspondiente a la apoptosis en la que la µ-calpaína es una enzima importante. Este proceso es común a todas las células dañadas, y se produce en las células musculares después de la sangría del animal. La actividad de la µ-calpaína explica muchos, pero no todos, los procesos del ablandamiento y la apoptosis, parece una respuesta atractiva para explicar el oscuro proceso de la maduración de la carne.

INTRODUCTION

Tenderness is one of or the most discussed features in meat. It is a real challenge for the scientific community and for the meat industry to achieve products with standardized and guaranteed tenderness, since these characteristics are exactly what consumers want in a meat product (Koohmaraie, 1995). The United States meat industry has identified solving the problem of inconsistent meat tenderness as a top priority. This requires a detailed understanding of the processes that affect meat tenderness and, perhaps more importantly, the utilization of such information by the meat industry (Koohmaraie, 1996).

Since the beginning of the 1990s, the North American meat industry has accelerated the adoption of new technologies to meet consumer expectations. Nowadays, we can find a product labeled as *guaranteed tender* such as marinades and case-ready products (Koohmaraie *et al*., 2002), and consumers are willing and able to pay more for these kinds of products (Feldkamp *et al*., 2005).

It is well recognized that the biochemical post-mortem processes are key-steps for meat tenderization (Herrera-Mendez *et al*., 2006). Actually, the tenderization process is unanimously known as an enzymatic process of proteolytic systems, where two strong current ways of thinking appear to offer the most probable explanations. Some think that calpains are the only proteases responsible for meat tenderization (Koohmaraie *et al*., 2002; Delgado *et al*., 2001), while others (Herrera-Mendez *et al*., 2006; Kemp *et al*., 2006) propose a multienzymatic process implicating calpains and other enzymes which function is less clear (proteasomes, caspases). This review will discuss the fundamental biochemical aspects of meat tenderization, as well as present both of the above-mentioned theories of meat tenderization.

TENDERIZATION PROCESS

The three factors that determine meat tenderness are background toughness, the toughening phase and the tenderization phase. While the toughening and tenderization phases take place during the post-mortem storage period, background toughness exists at the time of slaughter and does not change during the storage period (Koohmaraie and Geesink, 2006).

The background toughness of meat is defined as *the resistance to shearing of the unshortened muscle* (Marsh and Leet, 1966), and variation in the background toughness is due to the connective tissue component of muscle. In particular, the organization of the perimysium appears to affect the background toughness, since a general correlation between the perimysium and the tenderness of muscles has been found for both chicken and beef (Strandine, Koonz and Ramsbottom, 1949).

The toughening phase is caused by sarcomere shortening during rigor mortis development (Koohmaraie, Doumit and Wheeler, 1996). It was shown that there is a strong negative relationship between sarcomere length and meat toughness, where shorter

sarcomeres (less than 2 μm) result in tougher meat (Wheeler, Shackelford and Koohmaraie, 2000).

While the toughening phase is similar in all carcasses under similar processing conditions, the tenderization phase is highly variable. There is a large variation in both rate and extent of post-mortem tenderization of meat, and these result in the inconsistency of meat tenderness which are found at the consumer level (Koohmaraie and Geesink, 2006). The tenderization process begins just after slaughter. A measure of tenderness is the subjective consumer appreciation of meat's texture. On the other hand, an objective way to measure tenderness is the force required to shear a standardized piece of meat, with low shear values being desirable. During tenderization, proteolysis affects all muscle proteins, including connective tissue (Hwang *et al*., 2003), and it is now well established that post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins is responsible for this process (Koohmaraie and Geesink, 2006).

Some non-enzymatic aspects also influence the meat tenderization process, such as temperature, pH and Ca^{2+} concentration (Takahashi, 1996). Usually, meat aging is done at low temperatures and some studies showed that a freeze-thaw-refrigeration cycle can prevent a high rate of sarcomere shortening leading to a more tender meat (Bowling *et al*., 1987). Low temperature also decreases the glycolytic process by lowering enzymatic activity reducing the pH fall (Joseph, 1996). A low pH can cause inhibition of proteolytic enzymes activity, denaturation of myofibrilar proteins and excessive shortening - and consequently lead to toughness and loss of water-holding capacity (Khan and Cohen, 1977).

The sarcoplasmic calcium ion concentration increases ultimately to 0.2 mM during tenderization due to the loss of the ability of sarcoplasmic reticulum and mitochondria to accumulate calcium ions. This concentration is about 2,000 times that in resting skeletal muscle (Takahashi, 1996). While calcium is necessary for muscle contraction, it is an activator of proteolytic enzymes, and many authors have shown that calcium injection as carbonate enhances the tenderization process (Pringle *et al*., 1999).

ENZYMATIC TENDERIZATION

Breakage of myofibrilar and cytoskeletal proteins result from enzymatic proteolytic system activation. This degradation includes troponin-I, troponin-T, desmin, vinculin, meta-vinculin, dystrophin, nebulin and titin (Koohmaraie, 1996). Three major cytoskeletal structures are degraded when meat is tenderized: Z- to Z-line attachments by intermediate filaments, Z- and M-line attachments to the sarcolemma by costameric proteins and the elastic filament protein titin (Taylor *et al*., 1995).

Three proteolytic systems present in muscle have been investigated for their possible role in post-mortem proteolysis and tenderization: the calpain system, the lysosomal cathepsins and the multicatalytic proteinase complex (MCP) (Koohmaraie and Geesink, 2006). The cathepsins were

the first enzymatic system considered in the studies focusing on the mechanisms of meat tenderisation. Later, calpains received much more attention than cathepsins mainly because of their ability to alter the Z-line density, a modification often observed post-mortem, even if this change is not correlated with tenderness (Taylor *et al*., 1995). More recently, many sets of the evidence showed the potential role of the 20S proteasome in the tenderization process. Experiments based on different approaches reported results clearly showing the contribution of 20S proteasome onto the tenderization of stored meat (Lamare *et al*., 2002; Sentandreu *et al*., 2002; Thomas *et al*., 2004).

It has been reported that proteolytic systems must fulfill requirements to be considered involved in postmortem proteolysis in meat (Goll *et al*., 1983). First, the proteases must have access to the substrates, and secondly, they must be able to reproduce the proteolysis pattern observed after post-mortem storage of meat. Incubation of myofibrillar proteins with cathepsins results in different degradation patterns than those that occur during post-mortem storage of muscle, and it is doubtful that cathepsins are released from the lysosomes in post-mortem muscle. It has also been postulated that a significant role for MCP can be excluded, since myofibrils are very poor substrates for this protease system (Koohmaraie, 1992). Moreover, the degradation pattern of myofibrillar proteins by MCP does not mimic the degradation pattern observed in post-mortem muscle (Taylor *et al*., 1995). This leaves the calpain system or potentially another, not yet investigated, proteolytic system responsible for post-mortem proteolysis of key myofibrillar proteins and the resultant meat tenderization (Geesink *et al*., 2006).

Calpains are calcium-activated proteases consisting of at least three proteases, µ-calpain, m-calpain and skeletal muscle-specific calpain, p94 or calpain 3, and an inhibitor of µ- and m-calpain, calpastatin (Koohmaraie and Geesink, 2006). Calpains' importance during tenderization is verified in many studies such as production of the same proteolytic pattern observed in post-mortem muscle when calpains are incubated with myofibrils (Geesink and Koohmaraie, 1999). It was also found that injection of calcium (calpain activator), in muscles accelerates post-mortem proteolysis and tenderization (Wheeler, Crouse and Koohmaraie, 1992), whereas infusion or injection of muscles with calpain inhibitors inhibits postmortem proteolysis and tenderization. Moreover, the greatly reduced rate and extent of post-mortem proteolysis and tenderization in callipyge lamb can be attributed to elevated levels of calpastatin in these animals, and therefore calpain ihibition (Geesink and Koohmaraie, 1999). Overexpression of calpastatin in transgenic mice also resulted in a large reduction in post-mortem proteolysis of muscle proteins (Geesink *et al*., 2006).

According to Koohmaraie and Geesink (2006), among calpains, µ-calpain appears as the only enzyme that fills all the requirements to work effectively on post-mortem proteolysis.

These conclusions come from a study showing that post-mortem proteolysis was largely inhibited in μ-calpain knockout mice (Kent *et al*., 2004).

APOPTOSIS THEORY

Other researchers agree that the process of meat tenderization results from the synergistic action of several endogenous enzymatic systems, even if the major peptidases of concern are not identified yet. They propose that the meat aging process can be explained by programmed cell death or apoptosis (Herrera-Mendez, *et al*., 2006).

Apoptosis is a physiological mechanism occurring in living organisms that eliminates excessive, damaged or potentially dangerous cells from an organism without damaging surrounding cells. This process is necessary for both the normal development of a multicellular organism during embryogenesis and the maintenance of tissue homeostasis in adults (Dirks and Leeuwenburgh, 2005). Cell death occurs in an ordered manner, mediated by a particular group of cysteine peptidases called caspases (Green, 2005). Among this group of enzymes, there are the caspases involved in apoptosis initiation (caspases 8-10), characterized by large prodomains often containing essential areas for their interactions with other proteins, regulating the cell death beginning. Moreover, there are the effectors caspases that disrupt the cell when activated (caspases 3, 6 and 7) (Fuentes-Prior and Salvesen, 2004).

In meat animals, whatever the animal species and whatever the technology of stunning used, the last phase of slaughter is bleeding. Consequently, all cells and tissues will irreversibly be deprived of nutrients and oxygen. Under these very harmful environmental conditions, muscle cells will have no alternative but initiate apoptosis. Under similar conditions, such cell behavior is currently observed in living organisms (Herrera-Mendez *et al*., 2006).

There are some features that are common in both apoptosis and meat tenderization. Maybe, it is not only coincidence, but meat tenderization is strongly correlated with apoptotic processes, such as inversion of membrane polarity (Martin *et al*., 1995). As mentioned above, injection of calcium in meat accelerates the process of tenderization. The action of calcium is generally attributed to an activation of calpains, the calcium-dependent peptidases. Because of the numerous roles of this cation in cell signalling pathways, other potential functions of calcium have received only little attention from meat scientists. However, if it is considered that, after slaughter, cells have no other alternative but engage towards suicide or apoptosis, we have to reconsider some of these functions. Calcium is indeed a crucial effector for triggering and controlling apoptosis (Herrera-Mendez *et al*., 2006). In post-mortem muscle, calcium concentration increases gradually in the cytoplasm during rigor mortis onset while the sarcoplasmic reticulum is emptied of its contents (Vignon, Beaulaton and Ouali, 1989). It is known that this cation

is a central element of the apoptotic process, inducing swelling and extensive alteration of mitochondria and the release of cytochrome c together with other proapoptotic proteins. This process ends by the activation of caspase 9, which in turn will activate the effectors caspases (Herrera-Mendez *et al*., 2006).

Moreover, acidification of muscle decreases protein charges and increases their hydrophobicity, thereby reducing water retention. This is confirmed by the very high correlation observed between the increase in extracellular space and muscle pH (Guignot, Vignon and Monin, 1993). The only point which remained unexplained was the early increase in extracellular space starting immediately after slaughter, while pH was still very close to neutrality. Events associated with cellular death provide an explanation since a cell entering in apoptosis is dissociated from others and "shrinks". The consequence will be a reduction in intracellular space and a parallel increase in extracellular space (Herrera-Mendez *et al*., 2006).

During stress, cells prepare their defense as quickly as possible. Among available means, the most described is the synthesis of various protective proteins known as Heat Shock Proteins (HSPs). They have an anti-apoptotic activity (Beere, 2005), slowing down the cellular death process. When animals are stressed before or during slaughter, their meat does not follow a satisfactory aging process that can be result not only from decreased pH, but also from apoptosis inhibition. If meat tenderization is reconsidered through introduction of programmed cell death, the first active peptidases after animal bleeding would be undoubtedly caspases. These peptidases are in a better position than others to alter cellular structures since this is their primary function in vivo. It is worthy to note that the implication of caspases can explain the often reported assertion that the first hours following slaughter are essential for the satisfactory meat aging process (Herrera-Mendez *et al*., 2006).

CONCLUSION

Tenderness in meat is a well-known issue to be solved by the scientific community and industry. Nowadays, meat tenderization process is primarily explained by two different theories. One explains this process as a result of μ-calpain action throughout the muscle cell. The other characterizes tenderization as a more complex procedure, dependent on many enzymatic processes, among them μ-calpain activity, called apoptosis. Apoptosis is known as programmed cell death that occurs in all kinds of tissues, including muscles. There are many more studies showing the importance of μ-calpain than apoptotic activities during meat aging. However, there are still unclear gaps of understanding in the tenderization process. Many recognized apoptotic steps coincide with tenderization pathways, and maybe, with further studies, can explain these obscure gaps on meat aging.

REFERENCES

Beere, H.M. 2005. Death versus survival: Functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J. Clin. Invest.,* 115: 2633–2639.

Bowling, R.A., T.R. Dutson, G.C. Smith and J.W. Savell.1987. Effects of cryogenic chilling on beef carcass grade, shrinkage and palatability characteristics. *Meat Sci.,* 21: 67.

Delgado, E.F., G.H. Geesink, J.A. Marchello, D.E. Goll and M. Koohmaraie. 2001. The calpain system in three muscles of normal and callipyge sheep. *J. Anim. Sci.*, 79: 398-412.

Dirks, A.J. and C. Leeuwenburgh. 2005. The role of apoptosis in age-related skeletal muscle atrophy. *Sports Med.,* 35: 473-483.

Feldkamp, T.J., T.C. Schroeder and J.L. Lusk. 2005. Determining consumer valuation of differentiated beef steak quality attributes. *J. Muscle Foods.,* 16: 1-15.

Fuentes-Prior, P. and G.S. Salvesen. 2004. The protein structures that shape caspase-activity, specificity, activation, and inhibition. *Biochem. J.,* 384: 201-232.

Geesink, G.H. and M. Koohmaraie. 1999. Effect of calpastatin on degradation of myofibrillar proteins by l-calpain under postmortem conditions. *J. Anim. Sci.*, 77: 2685-2692.

Geesink, G.H., S. Kuchay, A.H. Chishti and M. Koohmaraie. 2006. Micro-calpain is essential for postmortem proteolysis of muscle proteins. *J. Anim. Sci.*, 84: 2834-2840.

Goll, D.E., Y. Otsuka, P.A. Nagainis, J.D. Shannon, S.K. Sathe and M. Muguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.,* 7: 137-177.

Green, D.R. 2005. Apoptotic pathways: ten minutes to dead. *Cell,* 121: 671–674.

Guignot, F., X. Vignon and G. Monin. 1993. Post mortem evolution of yofilament spacing and extracellular space in veal muscle. *Meat Sci.,* 33: 333-347.

Herrera-Mendez, C.H., S. Becila, A. Boudjellal and A. Ouali. 2006. Meat Aging: Reconsideration of the current concept. *Trends Food Sci. Tech.,* 17: 394-405.

Hwang, I.H., C.E. Devine and D.L. Hopkins. 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Sci.,* 65: 677-691.

Joseph, R.L. 1996. Very fast chilling of beef and tenderness - a report from an EU concerted action. *Meat Sci.*, 43: 217-227.

Kemp, C.M., R.G. Bardsley and T. Parr. 2006. Changes in caspase activity during the postmortem conditioning period and its relationship to shear force in porcine longissimus muscle. *J. Anim. Sci.*, 84: 2841-2846.

Kent, M.P., M.J. Spencer and M. Koohmaraie. 2004. Postmortem proteolysis is reduced in transgenic mice overexpressing calpastatin. *J. Anim. Sci.*, 82: 794-801.

Khan, A.W. and D.C. Cohen. 1977. Rapid estimation of muscle proteins in beef-vegetable protein mixtures. *J. Agric. Food Chem.*, 25: 236-238.

Koohmaraie, M. 1992. Ovine skeletal muscle multicatalytic proteinase complex (proteasome): purification, characterization, and comparison of its effect on myofibrils with m-calpain. *J. Anim. Sci.*,70: 3697-3708.

Koohmaraie, M. 1995. The biological basis of meat tenderness and potential genetic approaches for its control and prediction. *Proc. Rec. Meat Conf.,* 48: 69-75.

Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci.,* 43: 193-201.

Koohmaraie, M., M.E. Doumit and T.L. Wheeler. 1996. Meat toughening does not occur when rigor shortening is prevented. *J. Anim. Sci.*, 74: 2935-2942.

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

Koohmaraie, M. and G.H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci.,* 74: 34-43.

Lamare, M., R.G. Taylor, L. Farouta, Y. Briand and M. Briand. 2002 . Changes in proteasome activity during postmortem aging of bovine muscle. *Meat Sci.,* 61: 199-204.

Marsh, B.B. and N.G. Leet. 1966. Studies in meat tenderness. III. The effects of cold shortening on tenderness. *J. Food Sci.,* 31: 450-459.

Martin, S.J., C.P. Reutelingsperger, A.J. McGahon, J.A. Rader, R.C. van Schie and D.M. LaFace. 1995. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: Inhibition by overexpression of Bcl-2 and Abl. *J. Exp. Med.,* 182: 1545-1556.

Pringle, T.D., J.M. Harrelson, R.L. West, S.E. Williams and D.D. Johnson. 1999. Calcium-activated tenderization of strip loin, top sirloin, and top round steaks in diverse genotypes of cattle. *J. Anim. Sci.*,77: 3230-3237.

Sentandreu, M.A., G. Coulis and A. Ouali. 2002. Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends Food Sci. Tech.,* 13: 400-421.

Strandine, E.J., C.H. Koonz and J.M. Ramsbottom. 1949. A study of variations in muscles of beef and chicken. *J. Anim. Sci.*,8: 483-494.

Takahashi, K. 1996. Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. *Meat Sci.*, 43: 67-80.

Taylor, R.G., G.H. Geesink, V.F. Thompson, M. Koohmaraie and D.E. Goll. 1995. Is Z-disk degradation responsible for postmortem-tenderization?. *J. Anim. Sci.*, 21: 1351-1367.

Thomas, A.R., H. Gondoza, L.C. Hoffman, V. Oosthuizen, J. Ryno and A. Naude. 2004. The roles of the proteasome, and cathepsins B, L, H and D, in ostrich meat tenderisation. *Meat Sci.,* 67: 113-120.

Vignon, X., J. Beaulaton and A. Ouali. 1989. Ultrastructural localization of calcium in post-mortem bovine muscle: A cytochemical and X-ray microanalytical study. *Histochem. J.,* 21: 403-411.

Wheeler, T.L., J.D. Crouse and M. Koohmaraie. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. *J. Anim. Sci.*, 70: 3451-3457.

Wheeler, T.L., S.D. Shackelford and M. Koohmaraie. 2000. Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *J. Anim. Sci.*, 78: 958-965.

Recibido: 31-1-07. Aceptado: 17-05-07.