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Changes in proteasome activity during postmortem aging of bovine muscle

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TITOLO

Variazioni delle attività proteolitiche del muscolo bovino durante la conservazione post-mortem

KEY WORDS
Bovine, Protease, Meat tenderness

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Summary
Twelve cross-breed Limousine x Marchigiana beef steers approximately 250 kg live weight were slaughtered. Chilling took place at 2° C and carcasses were allowed to remain in the cooler for 21 days. A portion of the *Longissimus dorsi* (Ld) muscle was used for determined the activity of two lysosomal enzymes, (Cathepsin D and β-glucuronidase) at 1 hr post-mortem and after 4, 7, 14 and 21 days of aging. Shear force was also determined on the same muscle at each of the above mentioned sampling times. Tenderness improved after 7 days of aging but no significant differences was observed after 14 days. Cathepsin activity was significantly influenced from the aging but not the β-glucuronidase except for the samples from 1 hr vs 21 days ($P < 0.05$). More research is need to verify these findings.

Riassunto
Dodici manzi (Limousine x Marchigiana) di circa 250 kg di peso vivo sono stati macellati e le carcasse sono state refrigerate a 2 °C per 21 giorni. Una porzione del muscolo *Longissimus dorsi* (Ld) è stato utilizzata per determinare l'attività dei due enzimi lisosomiali, (catepsina D e β-glucuronidasi) a 1 hr post-mortem e dopo 4, 7, 14 e 21 giorni di conservazione. Lo sforzo di taglio (shear force) è stato determinato anche sullo stesso muscolo alle stesse epoche di conservazione di cui sopra. La tenerezza migliora dopo 7 giorni di conservazione, ma nessuna differenza significativa è stata osservata dopo 14 giorni. L'attività della catepsina era significativamente influenzata dal tempo di maturazione ma non la β-glucuronidasi tranne per i campioni osservati a 1 ora vs 21 giorni ($P <0.05$). Sono necessari ulteriori ricerche per verificare questi risultati.

Introduction

Meat structure in its simplest form is a collection of parallel fibers, the myofibrillar structure, bound together by a connective tissue network of a collagen fibers. Therefore, the application of a mechanical force to a piece of a cooked meat to determine tenderness is reflected by the amount of resistance to force exerted by both the

myofibrillar structure and connective tissue (1). Tenderness is know to be the main textural characteristics of meat and to be of prime importance for the consumers since it relates to both its price and acceptance as a food (2). During the post mortem ageing of muscle under chilled conditions, degradation of muscle proteins contributes to the rapid softening of flesh (3). In bovine muscle, rigor mortis occurs 24 h after the death of the animal and the acquisition of optimal tenderness requires at least 14 days (4, 5). The main myofibrillar proteolysis can be attributed to endogenous protease activity. Currently, two characterized proteolytic systems are known to hydrolyze myofibrillar proteins during post mortem storage of meat: calpains and cathepsins (6, 7). Most of the studies agree that there is a synergistic proteolytic action of calpains and cathepsins on key myofibrillar proteins, whereas the role of proteasome in post mortem tenderization needs to be further clarified (8). In order to understand the chemical changes that lead to the appearance of new biochemical products and the disappearance of many, a brief discussion of these changes and how they relate to tenderness will be considered. The aim of this study was to characterize and compare proteolytic activity during postmortem aging of bovine muscle. This better knowledge would help us to understand the ageing mechanisms in the meat.

Methods

Twelve cross-breed Limousine x Marchigiana beef steers were used in this study and the live weights ranged from 240 kg to 270 kg. The animals were slaughtered and dressed conventionally. Carcasses were held at 4°C and aged for 21 days. *Longissimus dorsi* (Ld) muscle sections from the ninth rib of the right side of each carcass were removed at 1hr post-mortem. After 4, 7, 14 and 21 days post-mortem, Ld samples were removed again from the same carcasses posterior to the proceeding sampling area after removing the dehydrated portion next to the previous sampling site. All samples were individually wrapped and frozen at -20°C for subsequent tenderness evaluation. Prior to cooking, Ld muscles were removed from the freezer and left overnight in a refrigerator to thaw. All samples were cooked in conventional oven and the meat was removed when the internal temperature reached 70°C. The cooked muscles were cooled for 3 hr at room temperature before tenderness determination were made using 3 cores. More samples of the Ld muscle were obtained from beef carcasses immediately after exsanguinations and subsequently thereafter at 4, 7, 14, and 21 days aging and were taken promptly to the laboratory to establish the changes in cathepsin D and β-glucuronidase activities during post mortem aging. Muscle tissues prepared for enzyme activity were separated into two different fractions according to Moeller et al (9). Cathepsin D activity was determined in both fractions that had been separated (sedimentable and unsedimentable) using urea-denatured hemoglobin as the substrate (10). The data were analyzed using the statistical analysis system (SAS) of Barr and Goodnight (11). The general linear model and least square means procedures were used. The Duncan's new multiple range test by Snedecor and Cochran (12) using the SAS was used to test for differences among treatment means.

Results and discussion

Cathepsin D and β-glucoronidase activity in both "free" and "bound" fractions are presented in table 1 and 2, respectively. Cathepsin D activity obtained in "free" and "bound" forms increased significantly ($P < .05$) from 1 hr to 21 days post-mortem. The largest increase in cathepsin D occurred af-

Tabella 1 - Cathepsin D activity during post-mortem conditioning in two centrifugal fractions of Bovine *L. dorsi* muscle

	"Free" Enzyme Fraction[1]	"Bound" Enzyme Fraction[1]
Post-mortem		
Time		
1 hr	65.25 ±2.95a	14.44 ±2.30a
4d	105.31 ±3.00b	87.81 ±2.18b
7d	127.38 ±3.15c	101.05 ±2.34c
14d	156.10 ±2.87d	110.38 ±2.35d
21d	150.89 ±3.15d	121.75 ±2.87e

[1] = Enzyme activity defined as ug tyrosine equivalent over one hour at pH 3.8
a, b, c, d, e = Means in the same column bearing different superscripts differ significantly (P<0.05)

Tabella 2 - β-glucuronidase activity during post-mortem conditioning in two fractions of Bovine *L. dorsi*

	Unsedimentable "Free" Fraction[1]	Sedimentable "Bound" Fraction[1]
Post-mortem		
Time		
1 hr	3.20±.15a	3.95±.48a
4d	6.25±.62bc	8.02±.38b
7d	6.15±.31b	7.80±.51b
14d	6.93±.74bc	8.20±.62b
21d	7.98±.68c	9.10±.65c

[1] Enzyme activity defined as nm of 4-methylumbelliferyl released in 20 min. at pH 5.0
a, b, c, = Means in the same column bearing different superscripts differ significantly (P<0.05)

Tabella 3 - Effect of aging time and dietary treatment on tenderness (shear value) of *Longissimus* muscle

Post-mortem	
Time	
1 hr	7.21±.38a
4d	4.10±.30b
7d	3.31±.37c
14d	2.61±.30d
21d	2.50±.41d

Shear value = kg/1.27 cm. Core.
a, b, c, d = Means in the same column bearing different superscripts differ significantly (P<0.05)

ter 4 days of aging. Aging appeared to have a marked effect on enzyme activity especially during the first few days of aging period since the distribution pattern of cathepsin D between free and bound fractions remained unchanged after 4 days. Aging in addition to the release of cathepsin D also may have had an activating effect on the enzyme which remained "bound". Changes in β-glucoronidase activity was not as large as in cathepsin D. A very close ratio of "free" and "bound" forms indicated that aging did not release this enzyme as readily as cathepsin D. Balasuhramaniam and Deiss (13) and Sawant, Desai and Tappel (14) have proposed unequal binding of the lysosomal enzyme, which probably explains the difference found in enzymes activity in this study. β-glucoronidase activity, however, was significantly ($P < 0.05$) higher in muscle aged for up to 4 days. The results indicated that these enzymes had undergone some activation during conditioning which agrees with Suzuki and Cassens (15) who reported an increase in cathepsin D activity of rabbit muscle during a 7 day storage period. Dutson and Lawrie (16) found an increase in β-glucoronidase activity when aging beef longissimus muscle for up to 14 days. According to de Duve (1963) lysosomes are characterized by the structure-linked latency of their enzymes due to the existence of

lipoprotein membrane restricting the accessibility of their contain hydrolyses to an external substrate (17): This delimiting membrane has been shown repeatedly to rupture under various conditions. This occurs on the death of a cell thus releasing the lysosomal contents. The process of disruption of lysosomes is very complex and no conclusive explanation has been given. The results in this study agree with almost all studies on post-mortem tenderization which shows that the major increase in meat tenderness occurs during the first 72 to 96 hr of aging (16,18,19). This increase parallels the significant reduction in toughness. Many researches attribute this reduction in toughness to changes in myofibril content as a result of this enzyme action. Martin and Whitaker (1968) reported that cathepsin D in combination with other cathepsin can hydrolyze actomyosin and produce tender meat. The increase in enzyme activity during aging found in this study probably reflects a lower resistance of the lysosome to homogenization. The increase in susceptibility to homogenization during the conditioning period could be due to loss of integrity of the lysosomal membrane resulting from either the reduction in pH and/or change in permeability. By comparing the graphs for the two enzymes studied (Figure 1 and 2), it can be seen that cathepsin D and β-glu-

Figure 1 - Cathepsin D activity during post-mortem conditioning in two centrifugal fractions of Bovine *L. dorsi* muscle

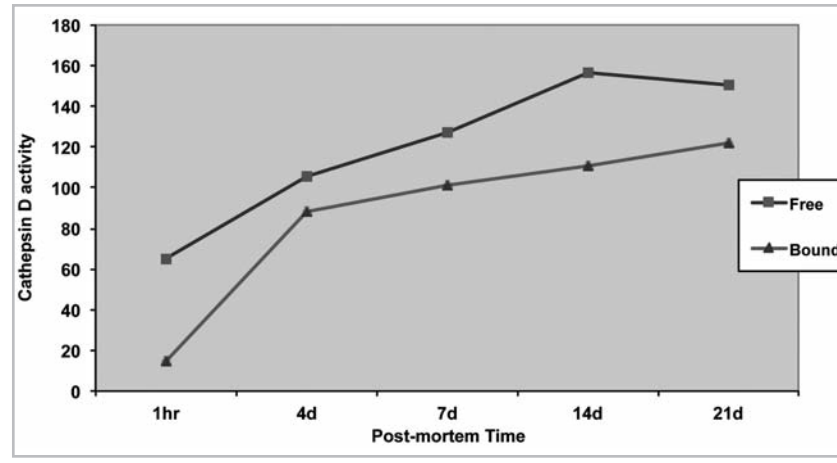


Figure 2 - β-glucuronidase activity during post-mortem conditioning in two fractions of Bovine *L. dorsi*

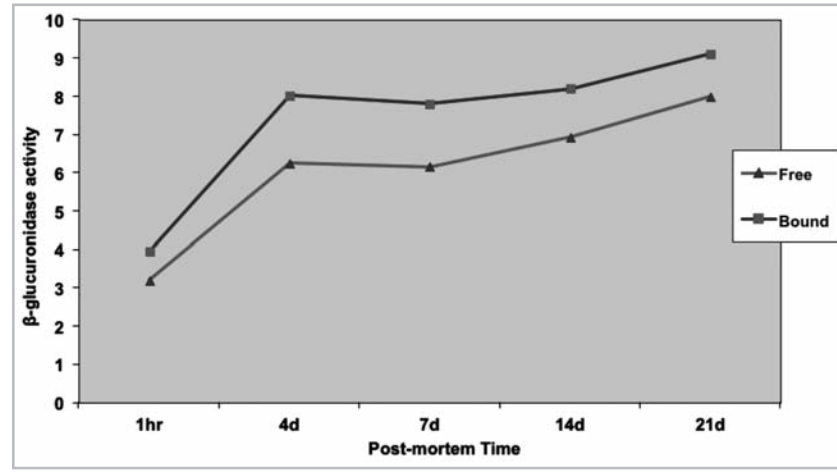
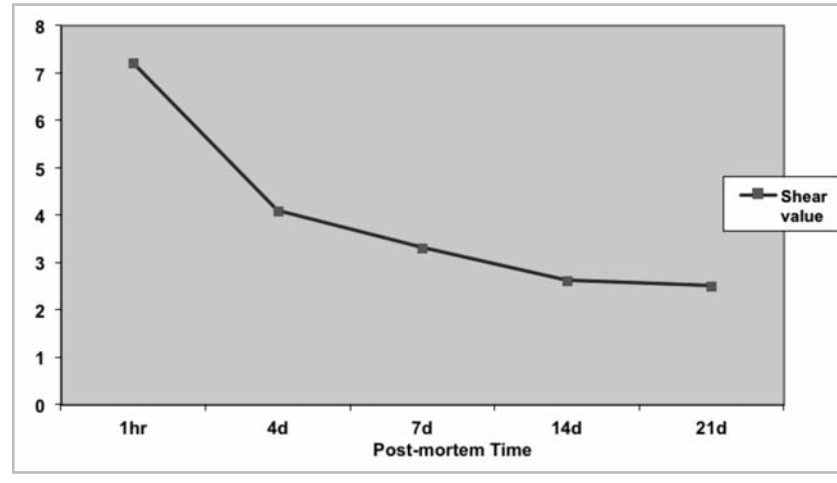


Figure 3 - Effect of aging time and dietary treatment on tenderness (shear value) of *Longissimus* muscle



coronidase activity in both free and bound form are in close agreement with the tenderness of the muscle. We can conclude that it was quite unlikely that any single enzyme functioned alone to degrade miofibrils and myofibrillar protein. Rather, it was accomplished by synergistic action of several different enzymes.

Conclusions

This study was undertaken to determine the effects of aging on the activity of two lysosomal enzymes (cathepsin D an β-glucoronidase). The main results obtained were that enzymes activity increased significantly during aging and that paralleled the improvement in tenderness.

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