Available online at www.sciencedirect.com
ScienceDirect
Meat Science 77 (2007) 105–113
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages

Youling L. Xiong [a,b,*], Oliver E. Mullins [a], John F. Stika [c], Jie Chen [b], Sue P. Blanchard [a], William G. Moody [a]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[b] College of Food Science, Southern Yangtze University, Wuxi, Jiangsu 214036, China
[c] Certified Angus Beef Program, Wooster, OH 44691, USA

Received 8 March 2007; received in revised form 6 April 2007; accepted 11 April 2007

Abstract

Two experiments were conducted to investigate the influence of age of mature cows (2–4 yr, 6–8 yr, and 10–12 yr cows; $n = 6$ in each) on beef quality. In Experiment 1, *Longissimus dorsi* (LD) steaks were stored at 3 °C for 0–10 d. Steaks from more mature cows had an increased ($P < 0.05$) Warner-Bratzler shear force (WBSF) and a slower troponin-T post-mortem degradation. Storage reduced WBSF in all steak samples regardless of animal age. In Experiment 2, *Semitendinosus* (ST) and *Semimembranosus* (SM) patties were stored at 3 °C for 0–7 d simulating retail display. The rate of lipid oxidation during storage increased with animal age ($P < 0.05$) and was greater in ST than in SM patties. However, myoglobin oxidation was minimally affected by animal age. Thus, advanced maturation not only intensified cow meat toughness but also lowered its oxidative stability.

Keywords: Cow; Beef; Proteolysis; Tenderness; Lipid oxidation; Myoglobin

1. Introduction

Mature cows of advanced ages represent a significant source or meat for the beef industry. In 2005, mature cattle (mostly cows) accounted for 14.7% of the total beef cattle slaughtered in the US (USDA, 2007). Meat from mature cows is used mainly as ground beef. In order to enhance its economical value, efforts are needed to understand intrinsic factors that influence the quality attributes of beef from this particular group of cattle.

One such intrinsic factor is proteolysis, which produces muscle fiber fragmentation. Beef from physiologically mature carcasses is generally perceived to be less tender than that from young animals, for which the chemical nature of connective tissue collagen (i.e., type and degree of cross-linking) has been implicated (Shorthose & Harris, 1990; Lawrie, 1998). Furthermore, oxidative stability of meat may be affected by animal age. Lipid oxidation that takes place in post-mortem meat can be a main impediment to the successful marketing of cow meat. Likewise, discoloration resulting from myoglobin oxidation could negatively impact the utility of cow meat. Boccard et al. (1979) reported that increased chronological age in cattle resulted in a darker color of lean meat, but the post-mortem stability of the muscle pigments was not clear.

While little is known about the possible age influence on oxidative stability of beef from mature cows, or differences between their muscle types, biochemical studies on small animals or humans have demonstrated an increased susceptibility of muscle cells to oxidizing agents as age progresses (Stadtman, 2006). It may be speculated that age-related loss in the redox potential may occur in mature cows, and this loss could predispose post-mortem muscles

* Corresponding author. Address: Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA. Tel.: +1 859 257 3822; fax: +1 859 257 5318.
E-mail address: ylxiong@uky.edu (Y.L. Xiong).

0309-1740/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2007.04.012

from cows to a higher rate of oxidation. On the other hand, a limited number of investigations have been conducted to examine the possible effect of animal age on the post-mortem progression of proteolysis in relation to meat tenderness. Parrish, Selvig, Culler, and Zeece (1981), Koohmaraie, Kennick, Elgasim, and Anglemier (1984) and Huff-Lonergan, Parrish, and Robson (1995) observed a reduced rate in post-mortem myofibrillar protein degradation in muscle from physiologically mature (C50–E maturity) cattle when compared to muscle from young (A maturity) carcasses. The difference was implicated in the variation of beef tenderness from cattle of different physiological ages.

The lack of literature report on the potential differences in the oxidative stability and protein degradation in post-mortem muscles from mature cows of various ages, coupled with the abundant supply of mature cows as a meat source in the market, indicates a need to further explore factors that may be responsible for the overall quality of post-mortem meat. Thus, the objective of the present study was to investigate the influence of cow age on the above chemical and biochemical changes during post-mortem storage of meat.

2. Materials and methods

2.1. Experimental design and meat sample preparation

A total of 18 cows composed predominantly of Angus × Simmental genetics and absent of *Bos indicus* influence, purchased from local producers, were used in this study. These cattle represented three age groups (six in 2–4 yr; six in 6–8 yr; and six in 10–12 yr). Cows were placed on the same fescue pasture for a minimum of 2 months prior to slaughter to obtain a similar nutritional background.

In Experiment 1, nine cows with three from each of the age groups were humanely slaughtered at the USDA-inspected University of Kentucky abattoir using standard industry procedures. Each animal was used in an independent trial. After evisceration, carcasses were electrically stimulated with 550 V, 2.2 A, using a Boss electrical stunner (Model No. 1004D, Cincinnati Butcher Supply, Cincinnati, OH) as described elsewhere (Schaake et al., 1993). Carcasses were immediately split and chilled in a 3 °C cooler for 24 h. Carcass maturity, based on both lean color (*Longissimus* muscle between the 12th and 13th rib) and bone ossification, was determined using an A00–E100 maturity scale to establish physiological ages.

At 24 h post-mortem, the LD (13th rib through the 5th lumbar vertebra) from both sides of carcasses were removed, cut into 2.54-cm thick steaks, individually vacuum-packaged, and stored in a 3 °C cooler. Warner-Bratzler shear (WBSF) force and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analyses were performed with randomly selected steaks at 1, 3, and 10 d post-mortem.

In Experiment 2, another nine cows with three from each of the age groups were slaughtered and electrically stimulated as described in Experiment 1. Each animal was used in an independent trial. After chilling for 24 h, *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles from both sides of carcasses were removed and ground through a 4.5-cm orifice plate. Ground meat was formed into patties of approximately 100 g in weight and 2 cm in thickness. Patties were placed on Styrofoam plates and over-wrapped with oxygen permeable plastic film (Ziploc Hand-Wrap, Dow Chem. Co., Indianapolis, IN). The plates were placed on a 3 °C shelf under fluorescent lighting that simulated retail display. Patties were analyzed for lipid and myoglobin oxidation daily for a total of 7 d as described below.

2.2. Detection of proteolysis

The post-mortem proteolytic changes in cow muscle (LD) was monitored on myofibrils isolated from 1 d, 3 d, and 10 d most-mortem steaks. Myofibrils were extracted according to Goll, Young, and Stromer (1974) using a rigor buffer consisting of 0.1 M NaCl, 0.05 M Na_2HPO_4, 5 mM EDTA, and 1 mM NaN_3 (pH 7.0). To identify protein degradation, SDS–PAGE was performed using the procedure of Laemmli (1970), which was modified to adapt to a smaller electrophoretic apparatus, i.e., the SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). A 3% acrylamide stacking gel and a 10% acrylamide resolving gel were used. An equal amount of protein (15 μg) was loaded in each lane.

Protein bands were stained with Coomassie brilliant blue and identified by comparing the electrophoretic pattern with published literature (Porzio & Pearson, 1977). Molecular weights (MW) of unknown proteins were estimated from the regression line generated by plotting log MW of protein standards vs. their migration distance. The protein MW standard was a cocktail of eight individual marker proteins (MW 14–200 kDa) (Sigma Chemical Co., St. Louis, MO). Images of destained gels were captured with a digital camera, and the individual protein bands were quantitatively analyzed using the UN-SCAN-IT Gel™ digitizing software (Ver. 6.1, Silk Scientific Corp., Orem, UT). The number of pixels in each whole band were recorded and used to calculate the percent weight of the corresponding protein in the myofibril sample, assuming that the amount of pixels was equivalent to the mass of the protein.

2.3. Measurement of shear force

LD Steaks were cooked to an internal temperature of 70 °C on a Farberware Open Hearth electric broiler (Farberware, Inc., Bronx, NY). After cooling to 22 °C, six 1.27-cm-diameter cores from each cooked steak were removed parallel to the muscle fibers. Cores were sheared with a Warner-Bratzler shear device attached to an Instron

4301 universal testing machine (Instron Corp., Canton, MA) with a crosshead speed of 20 cm/min. The mean of the WBSF values from different sample cores was determined and used for statistical analysis.

2.4. Measurement of lipid oxidation

Lipid oxidation in ST and SM patties after specific storage times was measured before and after cooking using the thiobarbituric acid (TBA)-reaction method, and was expressed as TBA-reactive substances or TBARS (Sinnhuber & Yu, 1977; Wang & Xiong, 2005). For cooked samples, patties were cooked to an internal temperature of 70 °C on a Farberware Open Hearth electric broiler (Farberware, Inc., Bronx, NY) and then cooled to 22 °C prior to analysis. The TBARS values were calculated as mg of malonaldehyde/kg of muscle sample.

2.5. Measurement of surface color

The surface color of raw ST and SM patties stored for different days was measured with a Hunterlab colorimeter (Model D25-2; Hunter Associates Laboratories, Inc., Fairfax, VA). The instrument, with a type DZA halogen lamp light source and a 3.5-cm aperture, was calibrated using a Hunterlab calibration plate no. C2-13717 ($L^* = 68.6$, $a^* = 23.5$, and $b^* = 12.8$) every time before use. Values obtained from triplicate patties were averaged to obtain the mean for each color parameter.

2.6. Determination of pigments

Total myoglobin and metmyoglobin (MMb) in ST and SM raw patties were determined according to Krzywiki (1982) using a 40 mM phosphate extraction buffer (pH 6.8). The absorbance of the pigment solution was read at 572, 565, 545, and 525 nm in a UV–visible spectrophotometer (Model UV-160, Shimadzu Co. Ltd., Columbia, MD). Total myoglobin and percent MMb were calculated based on the following equations (Krzywiki, 1982):

$$\text{Total myoglobin(mmol/L)} = (-0.166R_1 + 0.086R_2 + 0.088R_3 + 0.099) \times A_{525}$$

$$\text{MMb}(\%) = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100\%$$

where R_1, R_2, and R_3 = absorbance ratios of A_{572}/A_{525}, A_{565}/A_{525}, and A_{545}/A_{525}, respectively. For the total myoglobin content, the values were converted to mg/kg meat based on the dilution and the molecular weight (16,950 Da) of myoglobin (Dobberstein & Schroeder, 1993).

2.7. Statistical analysis

Each animal within the same age group was treated as a replicate. Data from the three independent (replicated) trials were analyzed using the General Linear Procedure of the Statistix 7.0 software package (Analytical Software, Inc., Tallahassee, FL) for microcomputers. Two-way analysis of variance (ANOVA) was performed to determine the significance of the effect of animal age and muscle post-mortem storage time. The ANOVA tables obtained were further analyzed for the comparison of means by Least Significant Difference procedures.

3. Results and discussion

3.1. Tenderness and proteolysis

The LD steaks from 10 yr to 12 yr cows were less tender, i.e., having a higher mean WBSF ($P < 0.05$), than steaks from the other two age groups (Fig. 1). The 6–8 yr group steaks also tended to be less tender than the 2–4 yr group steaks. The result agreed with previous findings that LD muscle from E maturity carcasses (old) was tougher compared to either C or D maturity carcasses (younger) (Hilton et al., 1998; Smith et al., 1982). When the mean WBSF values of steaks across the storage time were compared, it was clear that tenderness progressively improved ($P < 0.01$) during post-mortem storage (Fig. 1), a physicochemical process that occurs in meat during post-mortem storage at refrigerator temperatures regardless of the animal age (Geesink & Koohmaraie, 1999; Goll et al., 1983; Kong, Diao, & Xiong, 2006; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004).

Because proteases are widely implicated in the tenderization of meat during post-mortem storage, SDS–PAGE was performed to identify different proteolytic patterns that might explain the age-related variations in meat tenderness as well as the post-mortem storage effect. Several discernable proteolytic events occurred in all LD muscle samples

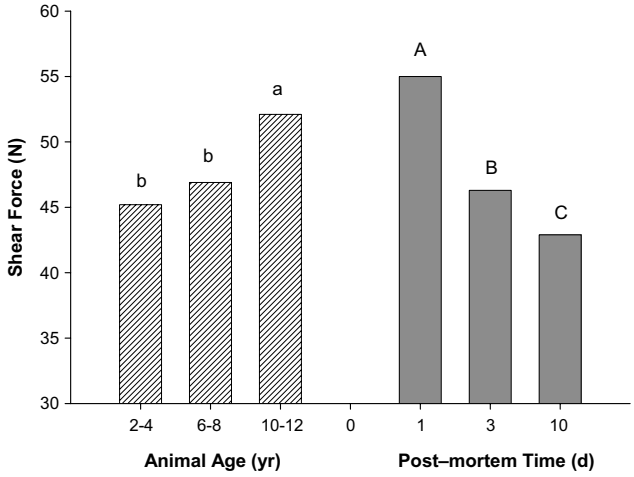

Fig. 1. Effects of animal age and post-mortem storage (3 °C) time on the WBSF of *Longissimus* muscle from cows of different ages. The standard errors of treatment means are indicated. Means in the same treatment group (age or storage time) with different letters differ significantly ($P < 0.05$).

during storage, with the progressive degradation of troponin-T and the concomitant appearance of a 33-kDa new polypeptide (“band Y”) being the most noticeable changes (Fig. 2). The progression of proteolysis into 10 d post-mortem also revealed a new protein band (designated as “band X”) between troponin-T and tropomyosin, presumably originating from troponin-T. All these proteolytic changes were consistent with previous literature reports (Huff-Lonergan et al., 1995; Koohmaraie et al., 1984; Xiong, Moody, Blanchard, Liu, & Burris, 1996). The results suggest that the improved tenderness of LD muscle during post-mortem storage (Fig. 1) was a result of proteolytic fragmentation of myofibrils.

Despite the similar proteolytic patterns exhibited by all the age groups, the proteolysis appeared to have proceeded at a faster rate and to a slightly greater extent in the 2–4 yr age group samples, which was revealed by SDS–PAGE (Fig. 2) and indicated by digital image analysis (Fig. 3). For example, by day 3, the troponin-T band was less intense but the 33 kDa band was more noticeable for the 2–4 yr age group steaks compared to samples from 6 to 8 and 10 to 12 yr age groups. By day 10, both the band “X” polypeptide and the 33 kDa component (band “Y”) became rather salient in the 2–4 yr age group samples (Fig. 3). The total digital pixels for protein “X” band in the 2–4 yr, 6–8 yr, and 10–12 yr age samples (10 d) were, respectively, 10,450, 7733, and 5748, which represented a 100:74:55 ratio. The more pronounced proteolysis in the 2–4 yr age group muscle would result in more rapid and extensive disruption of the myofibrils, thus, explaining the age effect on LD muscle tenderness observed in the present study.

Several muscle endogenous enzyme systems have been implicated in post-mortem tenderization of meat, including cathepsins, calpains, and proteosomes (Dutaud et al., 2006; Goll et al., 1983; Rowe et al., 2004). A large body of evidence links improved beef tenderization during post-mortem storage to calpain-mediated degradation of key myofibrillar proteins responsible for the integrity of the overall muscle cell cytoskeleton (Christensen, Young, Lawson, Larsen, & Purslow, 2004; Geesink & Koohmaraie, 1999; Rhee, Ryu, & Kim, 2006). Huff-Lonergan et al. (1995) postulated that LD samples from old cattle probably contained higher calpastatin activity that inhibited calpain-mediated protein degradation in post-mortem beef. Recent studies on aging have demonstrated that protein oxidation was an integral part of the physiological aging process (Rizvi, Jha, & Maurya, 2006; Stadtman, 2006), and in muscle cells, calpain was one of the most susceptible enzymes affected by oxidation (Guttmann, Elce, Bell, Isbell, & Johnson, 1997). It has been shown that when exposed to an oxidizing environment, m-calpain was readily inactivated, resulting in a reduced tenderization of post-mortem beef muscle (Rowe et al., 2004). When all these factors are put together, it can be hypothesized that muscle from older cows probably had reduced calpain activity due to greater oxidative stress and therefore, exhibited increased toughness, when compared with younger cattle.

On the other hand, a comparative study showed that meat toughness was better correlated with, thus, could be

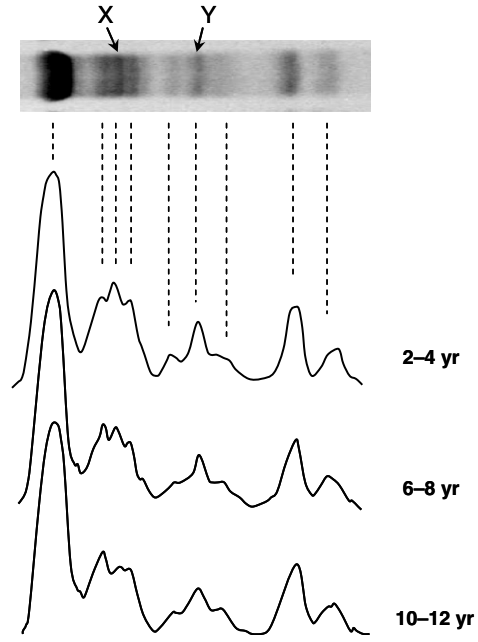

Fig. 3. Densitometric scan of an SDS–PAGE gel portion (22–45 kDa) of myofibrils isolated from 10-d post-mortem *Longissimus* muscle.

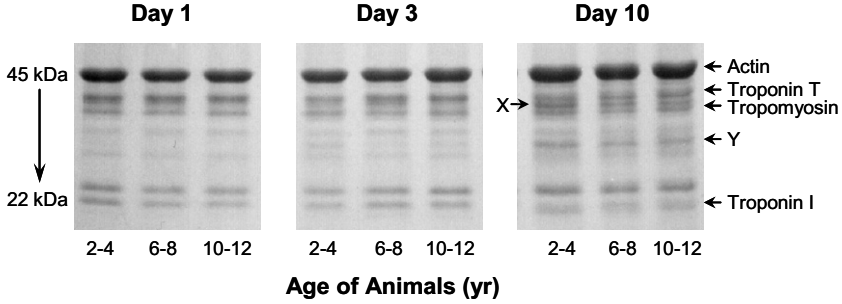

Fig. 2. SDS–PAGE of myofibrils isolated from post-mortem *Longissimus* muscle of cows from different age groups. Muscle samples were stored at 3 °C for various days. Shown in the graph is the portion of the gel (22–45 kDa) that exhibited most proteolytic changes. An equal amount of protein (15 μg) was loaded to each lane.

more reliably predicted by, muscle serine peptidase inhibitors than by calpastatin or cysteine peptidase inhibitors (Zamora et al., 2005). Moreover, using fragmentation structural analysis, Dutaud et al. (2006) found that 20S proteasome could reproduce the type of sequential, ultrastructural changes normally occurring in post-mortem myofibrils, suggesting that this protease superfamily may also be intimately involved in the aging process of meat. Further research is needed to identify the exact enzyme systems involved in the tenderization of mature cow meat seen in the present study.

3.2. Oxidative stability

3.2.1. Color and pigments

There were no significant differences between the 2–4, 6–8, and 10–12 yr age groups for raw ST or raw SM patties before storage or during storage for all the surface color parameters (L^*, a^*, and b^*), although numerically, the L^*-values of raw patties of the 10–12 yr age group tended to be slightly lower (but nonsignificant) and appeared darker when compared with the younger age groups. Hence, the respective colorimetric values were pooled to obtain the means. Overall, there was no consistent ($P > 0.05$) change in the L^*-value during storage, but a^*- and b^*-values decreased ($P > 0.05$) after 7 d for both ST and SM samples (Table 1). The most noticeable decrease in the a^*-value was seen between day 0 and day 1, indicating a significant loss of red color in the early stage of storage.

Comparison of ST and SM patties showed that the former had consistently greater lightness (L^*) but lower redness (a^*) ($P > 0.05$) than the latter. It can be suggested that the color difference was a result of unequal distributions of specific fiber types. Kirchofer, Calkins, and Gwartney (2002) reported 24.3%, 26.0%, and 49.7%, respectively, of β-red, α-red, and α-white fibers in beef ST, compared to 26.3%, 28.6%, and 45.1% in beef SM. However, total myoglobin content (3.15 ± 0.55 mg/g muscle) was similar ($P > 0.05$) between ST and SM samples, and it was not influenced ($P > 0.05$) by animal age (results not shown). Hence, the slightly less red (a^*-value) in ST muscle must be due to a lower oxygenation of myoglobin when compared with SM muscle.

Most of the pigments in fresh, raw patties were deoxymyoglobin (interior) and oxymyoglobin (surface) that imparted the bright red color with a relatively high a^*-value. During the simulated retail display under fluorescent light, a large proportion of the pigments was converted to metmyoglobin, with the most rapid conversion occurring during the first day (Fig. 4). By day 7, metmyoglobin accounted for almost 70% of the total pigment. Animal age was not a factor in the metmyoglobin production except for the ST patties that contained a slightly greater ($P > 0.05$) amount of metmyoglobin in the 10–12 yr age group on day 5 than in the 2–4 yr and 4–6 yr groups. The results followed the trend of decreasing a^*-

Table 1
Hunter calorimetric values of raw ground beef patties from *Semitendinosus* and *Semimembranosus* muscles of cull cows during simulated retail display at 3 °C

Sample	Color parameter	Storage time							Average
		0	1	2	3	4	5	6	7	
Semitendinosus	L^*-value	38.87^{a} (1.45)	36.87^{a} (1.55)	37.15^{a} (1.74)	37.70^{a} (1.84)	37.62^{a} (2.19)	38.06^{a} (2.18)	37.77^{a} (1.96)	37.89^{a} (1.96)	37.74^{x}
	a^*-value	24.66^{a} (1.42)	21.24^{b} (1.11)	19.32^{c} (0.98)	18.11^{d} (0.88)	17.22^{d} (1.15)	15.57^{e} (1.04)	15.20^{e} (1.09)	14.96^{e} (1.19)	18.29^{y}
	b^*-value	13.48^{a} (0.33)	11.65^{b} (0.29)	11.19^{bc} (0.40)	10.60^{c} (0.33)	10.37^{c} (0.50)	10.38^{c} (0.33)	10.37^{c} (0.50)	10.61^{c} (0.47)	11.08^{x}
Semimembranosus	L^*-value	34.76^{a} (1.35)	32.49^{b} (1.85)	32.24^{b} (2.11)	32.27^{b} (2.22)	32.64^{b} (1.89)	32.62^{b} (1.82)	33.20^{ab} (1.71)	33.16^{ab} (1.68)	32.92^{y}
	a^*-value	26.00^{a} (1.80)	22.51^{b} (1.29)	21.28^{c} (1.22)	20.38^{cd} (1.25)	19.69^{de} (1.21)	18.86^{ef} (1.11)	17.83^{f} (0.86)	17.90^{f} (0.94)	20.56^{x}
	b^*-value	12.40^{a} (0.60)	10.64^{b} (0.41)	10.13^{bc} (0.32)	9.72^{c} (0.39)	9.47^{c} (0.42)	9.52^{c} (0.51)	9.32^{c} (0.43)	9.44^{c} (0.50)	10.08^{y}

$^{a–f}$ Means in the same row without a common superscript letter differ significantly ($P > 0.05$). Values in parentheses are standard errors of the respective means.
^{x,y} Indicate significant difference ($P > 0.05$) between the two muscle types for the same color parameters shown in the 'Average' column.

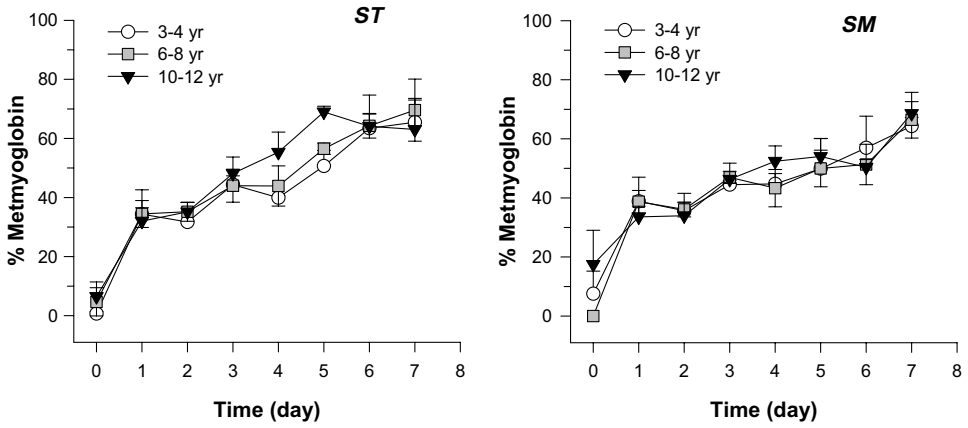

Fig. 4. Metmyoglobin content in raw beef patties stored at 3 °C for various days. Patties were prepared from *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of cows from different age groups.

values during storage (Table 1). Demos and Mandigo (1996) studied the discoloration of ground beef stored at refrigerator temperature in an oxygen-permeable packaging system and showed that by day 7, approximately 65% of myoglobin was changed to metmyoglobin. Similar results were reported by van den Oord and Wesdorp (1971), who observed that percent oxymyoglobin dropped to about 50% by day 7 during storage (5 °C), and the reduction was positively correlated with the undesirable color ratings by a sensory panel.

It was noteworthy that although both the a^*-value reduction and the metmyoglobin formation occurred most rapidly during the first 24 h, the latter was more dramatic. This can be explained because samples for pigment extraction were whole meat patties (blended) while only the surface color of the patties was measured by the colorimeter. The color of the patties, and thus the pigment composition, were not consistent throughout the whole patties, i.e., red on the surface (up to 3–4 mm deep) and purplish/brown in the interior after 1 d (preliminary observation). The reduced partial pressure of oxygen inside the meat patties facilitated myoglobin oxidation.

3.2.2. Lipid oxidation

The analysis of TBARS in patties indicated pronounced differences in the rate and extent of lipid oxidation between the three age groups of cows as well as between ST and SM muscles during refrigerated retail display. Raw patties of both ST and SM from 10 to 12 yr cows were most susceptible to lipid oxidation, followed by the 6–8 yr age group, and the 2–4 yr group was least susceptible (Fig. 5). On average (ST and SM from all ages combined), the TBARS values of raw samples increased from 3.87 mg/kg on day 0 to 11.42 mg/kg after 7 d ($P > 0.05$).

Proximate analysis in a preliminary experiment showed no significant difference in total lipid between all samples, i.e., 5.59%, 5.44%, and 5.03% for ST, and 5.15%, 5.57%, and 5.37% for SM from 2 to 4 yr, 6 to 8 yr, and 10 to 12 yr cows, respectively. Hence, the animal age-associated variation in lipid oxidation as well as the difference between muscle types cannot be attributed to the lipid content. It has been shown that in humans, the total plasma antioxidants decreased with age (Rizvi et al., 2006). While there is no literature report on age-related changes in redox potential of post-mortem meat, it may be suggested that

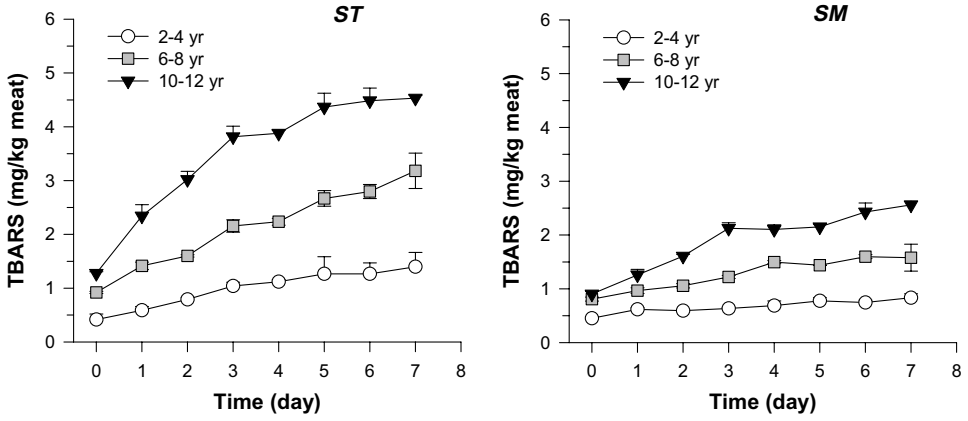

Fig. 5. TBARS content in raw beef patties stored at 3 °C for various days. Patties were prepared from *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of cows from different age groups.

the endogenous antioxidants could be lower in the 10–12 yr cow muscle than in muscle from younger groups, thus, enhancing the oxidative susceptibility of lipids. The rate and extent of TBARS generation were greater ($P > 0.05$) for ST than for SM. It is plausible that the ST muscle was deficient in endogenous antioxidants when compared with the SM muscle. Cooking slightly increased the TBARS production. The amount of TBARS in these stored patties after cooking increased by an average of about 10% when compared with uncooked patties (Fig. 6). Membrane damage, the release of prooxidative heme, and the heat input would all promote lipid oxidation (Grunwald & Richards, 2006; Monahan, Crackel, Gray, Bukley, & Morrissey, 1993).

The significant animal age effects on muscle lipid oxidation but not on metmyoglobin formation, and the notable difference between muscle types in their lipid stability but not in their color stability, suggest that lipid and pigment oxidation in these muscle samples were probably controlled by different mechanisms, one of which was physical barriers. With coarse grinding, some of the cells were disrupted, irrespective of origin of the muscles, resulting in the release of myoglobin. These myoglobin molecules were readily oxidized and were responsible for the rapid production of metmyoglobin observed in the first 24 h of retail display. Subsequent oxidation was slow because the remaining myoglobin molecules, which would still be confined in the cell, were relatively inaccessible to molecular oxygen for all muscle samples. On the other hand, lipid oxidation occurred mostly outside the cell or at the membrane interface, in which case the concentration and type of oxidants or antioxidants, which may vary among animal age groups, became crucial factors.

Indeed, within the same muscle type or animal age group, lipid oxidation (TBARS) was significantly correlated with metmyoglobin production (Fig. 7), suggesting that the same oxidation initiators may be involved. Monahan, Asghar, Gray, Buckley, and Morrissey (1994) noted that oxidation of myoglobin preceded oxidation of muscle lipids in pork chops stored at 4 °C. However, the opposite was observed by the same research lab for beef muscle homogenates that were treated with oxidizing agents ($FeCl_3$/ascorbate) (O'Grady, Monahan, & Brunton, 2001). The conflicting results reported by these researchers

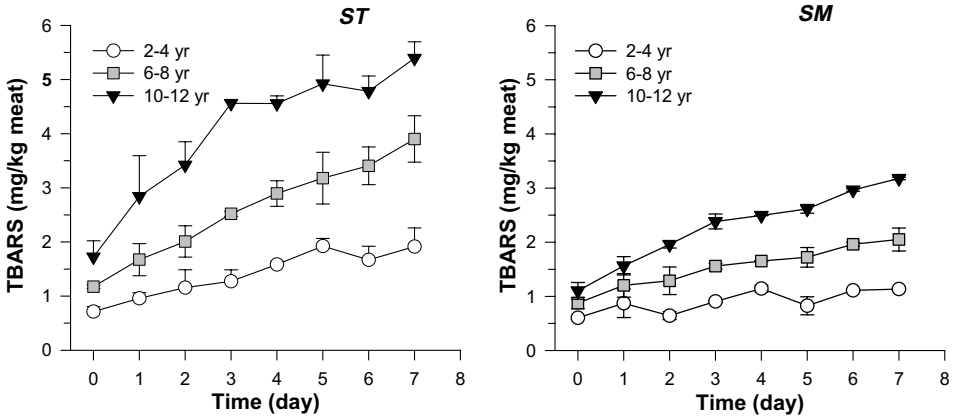

Fig. 6. TBARS content in cooked beef patties prepared from *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of cows from different age groups. The raw patties were stored at 3 °C for various days before cooking.

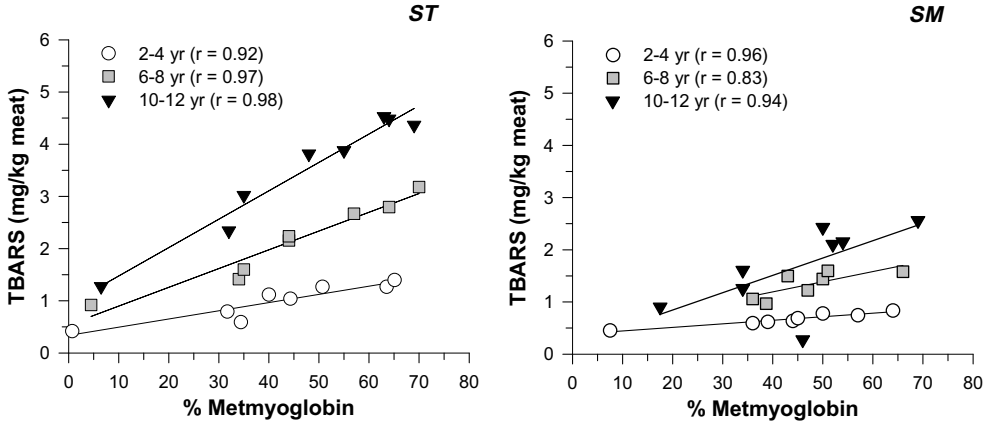

Fig. 7. Linear regression plots of TBARS versus % metmyoglobin for raw patties prepared from *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of cows from different age groups. The correlation coefficients (r) are indicated.

with our present findings (in which beef sample oxidation occurred under fluorescent light) indicated the specificity of lipid and myoglobin oxidation to the type of oxidation initiators.

4. Conclusions

The findings validated the hypothesis that oxidative stability of lipids and tenderness in post-mortem beef muscle decreased with the age of mature cows, and substantiated the general notion that the overall quality of beef is reduced with the age of cattle. The results also demonstrated that muscle type was a significant factor affecting the overall oxidative stability of beef from mature cows. Thus, meat processors should take into consideration the specific ages of cows when utilizing meat as fresh steaks or for further processing. For this particular group of beef cattle, while toughness is expected to become a lesser issue in ground cow meat, antioxidative strategies are needed in order to improve its oxidative stability and flavor.

References

Boccard, R. L., Naude, R. T., Cronje, D. E., Smit, M. C., Venter, H. J., & Rossouw, E. J. (1979). The influence of age, sex and breed of cattle on their muscle characteristics. *Meat Science, 3*, 261–280.

Christensen, M., Young, R. D., Lawson, M. A., Larsen, L. M., & Purslow, P. P. (2004). Effect of added μ-calpain and post-mortem storage on the mechanical properties of bovine single muscle fibers extended to fracture. *Meat Science, 66*, 105–112.

Demos, B. P., & Mandigo, R. W. (1996). Color of fresh, frozen and cooked ground beef patties manufactured from mechanically recovered neck bone lean. *Meat Science, 42*, 415–429.

Dobberstein, P., & Schroeder, E. (1993). Accurate mass determination of a high-molecular-weight protein using electrospray-ionization with a magnetic-sector instrument. *Rapid Communication in Mass Spectrometry, 7*, 861–864.

Dutaud, D., Aubry, L., Guignot, F., Vignon, X., Monin, G., & Ouali, A. (2006). Bovine muscle 20S proteasome. II: Contribution of the 20S proteasome to meat tenderization as revealed by an ultrastructural approach. *Meat Science, 74*, 337–344.

Geesink, G. H., & Koohmaraie, M. (1999). Effect of calpastatin on degradation of myofibrillar proteins by micro-calpain under post-mortem conditions. *Journal of Animal Science, 77*, 2685–2692.

Goll, D. E., Otsuka, Y., Nagainis, P. A., Shannon, J. D., Sathe, S. K., & Muguruma, M. (1983). Role of muscle proteases in maintenance of muscle integrity and mass. *Journal of Food Biochemistry, 7*, 137–177.

Goll, D. E., Young, R. B., & Stromer, M. H. (1974). Separation of subcellular organelles by differential and density gradient configuration. In *Proceedings of reciprocal meat conference* (Vol. 27, pp. 250–297).

Grunwald, E. W., & Richards, M. P. (2006). Studies with myoglobin variants indicate that released hemin is the primary promoter of lipid oxidation in washed fish muscle. *Journal of Agricultural and Food Chemistry, 54*, 4452–4460.

Guttmann, R. P., Elce, J. S., Bell, P. D., Isbell, J. C., & Johnson, G. V. (1997). Oxidation inhibits substrate proteolysis by calpain I but not autolysis. *Journal of Biological Chemistry, 272*, 2005–2012.

Hilton, G. G., Tatum, J. D., Williams, S. E., Belk, K. E., Williams, F. L., Wise, J. W., et al. (1998). An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows. *Journal of Animal Science, 76*, 2094–2103.

Huff-Lonergan, E., Parrish, F. C., Jr., & Robson, R. M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science, 73*, 1064–1073.

Kirchofer, K. S., Calkins, C. R., & Gwartney, B. L. (2002). Fiber-type composition of muscles of the beef chuck and round. *Journal of Animal Science, 80*, 2872–2878.

Kong, B. H., Diao, X. P., & Xiong, Y. L. (2006). Postmortem calcium chloride injection alters ultrastructure and improves tenderness of mature Chinese yellow cattle *Longissimus* muscle. *Journal of Food Science, 71*, C124–C129.

Koohmaraie, M., Kennick, W. H., Elgasim, E. A., & Anglemier, A. F. (1984). Effects of postmortem storage on muscle protein degradation: Analysis by SDS–polyacrylamide gel electrophoresis. *Journal of Food Science, 49*, 292–293.

Krzywiki, K. (1982). The determination of heme pigments in meat. *Meat Science, 7*, 29–36.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature, 227*, 680–685.

Lawrie, R. A. (1998). *Meat science* (6th ed.). Cambridge, UK: Woodhead Publishing, Ltd., p. 336.

Monahan, F. J., Asghar, A., Gray, J. I., Buckley, J. D., & Morrissey, P. A. (1994). Effect of oxidized dietary lipid and vitamin E on the colour stability of pork chops. *Meat Science, 37*, 205–215.

Monahan, F. J., Crackel, R. L., Gray, J. I., Bukley, D. L., & Morrissey, P. A. (1993). Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. *Meat Science, 34*, 95–106.

O'Grady, M. N., Monahan, F. J., & Brunton, N. P. (2001). Oxymyoglobin oxidation and lipid oxidation in bovine muscle – Mechanistic studies. *Journal of Food Science, 66*, 386–392.

Parrish, F. C., Jr., Selvig, C. J., Culler, R. D., & Zeece, M. G. (1981). CAF activity, calcium concentration, and the 30,000-Da component of tough and tender bovine longissimus muscle. *Journal of Food Science, 46*, 308–309, 311.

Porzio, M. A., & Pearson, A. M. (1977). Improved resolution of myofibrillar proteins with sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Biochimistry and Biophysics Acta, 490*, 27–34.

Rhee, M. S., Ryu, Y. C., & Kim, B. C. (2006). Post-mortem metabolic rate and calpain system activities on beef longissimus tenderness classifications. *Bioscience, Biotechnology and Biochemistry, 70*, 1166–1172.

Rizvi, S. I., Jha, R., & Maurya, P. K. (2006). Erythrocyte plasma membrana redox system in human aging. *Rejuvenation Research, 9*, 470–474.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of m-calpain. *Journal of Animal Science, 82*, 3254–3266.

Schaake, S. L., Skelley, G. C., Halpin, E., Grimes, L. W., Brown, R. B., Cross, D. L., et al. (1993). Carcass and meat sensory traits for steers finished on fescue and clover, summer forage, or for different periods in drylot. *Journal of Animal Science, 71*, 3199–3205.

Shorthose, W. R., & Harris, P. V. (1990). Effect of animal age on the tenderness of selected beef muscles. *Journal of Food Science, 55*, 1–8.

Sinnhuber, R. O., & Yu, T. C. (1977). The 2-thiobarbituric acid reaction, an objective measure of the oxidative deterioration occurring in fats and oils. *Journal of Japanese Oil Chemist Society, 26*, 259–267.

Smith, G. C., Cross, H. R., Carpenter, Z. L., Murphey, C. E., Savell, J. W., Abraham, H. C., et al. (1982). Relationship of USDA maturity group to palatability of cooked beef. *Journal of Food Science, 47*, 1100–1107.

Stadtman, E. R. (2006). Protein oxidation and aging. *Free Radical Research, 40*, 1250–1258.

USDA (2007). *USDA-NASS agricultural statistics*. <http://www.nass.usda.gov/index.asp>.

van den Oord, A. H. A., & Wesdorp, J. J. (1971). Color rating and pigment composition of beef. *Journal of Food Technology, 6*, 15–20.

Wang, L. L., & Xiong, Y. L. (2005). Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *Journal of Agricultural and Food Chemistry, 53*, 9186–9192.

Xiong, Y. L., Moody, W. G., Blanchard, S. P., Liu, G., & Burris, W. R. (1996). Postmortem proteolytic and organoleptic changes in hot-boned muscle from grass- and grain-fed and zeranol-implanted cattle. *Food Research International, 29*, 27–34.

Zamora, F., Aubry, L., Sayd, T., Lepetit, J., Lebert, A., Sentandreu, M. A., et al. (2005). Serine peptidase inhibitors, the best predictor of beef ageing amongst a large set of quantitative variables. *Meat Science, 71*, 730–742.