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Effect of aging technologies on some qualitative characteristics of Longissimus dorsi muscle of Marchigiana beef

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Abstract. In order to determine sensory preference and value of fresh beef steak differing in aging technique, this study evaluated two aging methods: dry and wet; a quality grade on physic-chemical traits of instrumental tenderness, color, and sensory properties of Longissimus dorsi beef muscle of Marchigiana bovine, correlated to calpain proteolytic activity too. Dry-aged loins had higher ($P < 0.0001$) weight loss than wet aged loins. Wet aged loins had higher ($P < 0.01$) L* values than dry aged loins. Warner-Blatzer shear force of steaks was not affected ($P > 0.05$) by aging method. We made a sensory panel evaluation too that showed no effect ($P > 0.05$) of aging method on myofibrillar tenderness, juiciness, connective tissue amount, overall tenderness or off flavor intensity.

Key words: beef, dry aging, wet aging, tenderness.

INTRODUCTION

Aging of fresh beef is essential to meet the high demands and expectations of an exceptional eating experience. Fresh meat is aged to enhance the palatability of the product, to increase the tenderness over time and to develop the flavors. Dry and wet aging are common aging techniques, dry aging is a process whereby beef carcasses, primals, and/or sub primals are stored – without protective packaging – at refrigeration temperatures for one to more weeks, while during wet aging meat is packaged in a sealed barrier film and held at a temperature above the freezing point of the product. The objective of our study was to quantify sensory differences between wet- and dry-aged strip loins and to determine the score that consumers place on their preferred product. The study was carried out on *Longissimus dorsi* muscles of 20 cattle of Marchigiana breed, 12 months-old, reared for 7 months in a wild state and for 5 months in a semi-wild state. Each loin was cut into half and randomly assigned to one of the two aging treatments. Loins were placed on wire racks, with the subcutaneous fat surface down. The yield, chemical composition, instrumental tenderness (Warner–Blatzer shear force) and color quality traits were determined on loin sections aged for 15 and 25 d at 2.2°C in a cooler with minimal air movement starting after 8 d post-mortem. Most important results showed that dry-aged loins had higher ($P < 0.001$) weight loss than wet aged loins. Moisture content of raw meat was significantly lower in dry aged group while was not different between treatment after cooking. Wet aged loins had higher ($P < 0.01$) L* values and lower red index (a* value) than dry aged loins. Warner–Blatzer shear force of steaks was not affected ($P > 0.05$) by aging method as well as sensory panel evaluation.

MATERIALS AND METHODS

The study was carried out on 20 cattle of Marchigiana breed, 12 months-old, raised for 7 months in a wild state and for 5 months in a semi-wild state.

Animals were treated according to the guidelines of the European Community on the treatment of experimental animals (Reg. CE 1/2005; directives 74/577/EEC; Law 439 2 August 1978). The slaughter house had EEC mark with reference to rules 852/853/854/2004; 2076/2005. The muscle used was Longissimus Dorsi (Ld). Each loin was cut into half and randomly assigned to one of the two aging treatments: (dry or wet). Loin sections allocated to wet aging (n = 10) were vacuum-packaged (8600-14EL, Cryovac Sealed Air Corporation, Duncan) in vacuum bags having O^2 permeability of 3–6 ml of O_2 m^{2-1} 24 h^{-1} at 4.4°C, atmospheric pressure, and 0% relative humidity; water vapor permeability of 0.5–0.6 g 64,516 $cm^{2\ -1}$ 24 h^{-1} at 37.8°C and 100% relative humidity. Loin sections destined for dry aging (n = 10) were aged unpackaged with direct exposure to air in the cooler. Muscle samples were withdrawn at different period (15-d and 25-d post-mortem) and by means of the Western Blot method, the μ-calpain, p-94 and calpastatin expressions were evaluated. Loin sections were aged from the time they were received at 8 d postmortem for 15 and 25 d at 2.2°C in a cooler with minimal air movement. Loins were placed on wire racks, with the subcutaneous fat surface down.

Steak preparation

Five 3.00 cm-thick steaks were removed from the anterior end of all loin sections and randomly assigned to cooking temperatures (70°C) for Warner–Bratzler shear force determination and sensory analysis after the 15 and 25 day aging period. A sample was also taken from the most anterior end of the Ld for compositional analysis and pH. Steaks for sensory evaluation were frozen at −40°C until just before evaluations by a trained sensory panel. Each loin section was weighed before and after the assigned aging times (15 or 25 d). Weight loss percentage was calculated. After dry aging, loin sections were trimmed to remove dry and discolored portions. Wet aged loins were blotted by dry paper towels. They were calculated both percentage trim loss than combined loss.

pH, moisture, fat

Samples of Ld tissue, obtained before and after aging, were frozen in liquid nitrogen and pulverized in a warring blender. Ten grams of pulverized sample was added to 100 ml of distilled water and mixed for 30 s, and pH values were obtained with an accumet glass electrode attached to an accumet 210 pH meter (Model Hanna pH 210 microprocessor pH Meter, Italy). Moisture and fat content were determined using the CEM (CEM, Corporation; Mathews) SMART (moisture) and SMART Trac (fat) systems (AOAC PVM 1:2003; Keeton et al., 2003).

Instrumental and visual colour

Color measurements on the anterior Ld surfaces were taken after opening the sub-primal vacuum packages and allowing for 20 min of bloom time and prior to aging with a colorimeter equipped with a 2.54 cm orifice (Hunter Mini Scan XE, model 45/0-L). Color parameters were determined in triplicate with an instrument which uses the CIE L* a* b* color system, by measuring lightness (L*), redness (a*), and yellowness intensities (b*). Aperture of 8 mm, illuminant D65, and 10º standard observer were used.

Calibration was performed using a white standard plate (L* = 95.26, a* = 0.89, b* = 1.18). The colorimeter was calibrated daily against black and white tiles before steak measurements. After 15 and 25 d of aging, instrumental color of Ld muscles was determined by averaging five readings on each cut steak surface after blooming. Internal visual color was evaluated to the nearest 0.5 unit on a 6-point scale: 1, raw red centre, pink border, tan edge (medium rare); 2, reddish-pink centre, pink border, tan edge; 3, pinkish red centre, pink to light brown/tan to outer surface; 4, slightly pink centre, light brown to tan edge (medium); 5, tan/brown centre and edges, no evidence of pink; and 6, dry, brown throughout (well done; AMSA, 1991).

Shear force

Steaks were cooked in a forced-air convection oven (DFG-102 CH3; G.S. Blodgett Co., Arlington, VT) on trays to an internal temperature of 70°C. Internal temperature was monitored by using copper-constantan thermocouples (Omega Engineering, Stamford, CT) inserted into the geometric center of each steak and connected to a Doric temperature recorder (VAS Engineering). After cooking, steaks were over wrapped in polyvinyl chloride film and stored at 2°C for 24 h. Five round cores (1.27 cm diameter) were obtained from each strip steak, parallel to the long axis of the muscle fibers (AMSA, 1995). Each core was sheared once, perpendicular to muscle-fiber orientation, with a Warner–Bratzler shear force apparatus (Vnotch blade) connected to an Instron Universal Testing Machine (Model 4201; Instron, Corp., Canton, MA) with a 50 kg compression load cell operating at a crosshead speed of 250 mm/min. Shear-force steaks also were used to determine cooking loss as: cooking loss (%) = (raw weight − cooked weight) / (aw weight) x 100.

Sensory analysis

Sensory evaluation was essential in the assessment of consumer products by the use of the human senses (sight, smell, taste, touch, and hearing). The sensory analysis required the use of a panel of human evaluators, wherein test results were recorded based on their responses to the products under test. Particularly they evaluated tenderness and flavor intensity for the two aging processes. Steaks were broiled at 163°C to an internal core temperature of 70°C for sensory evaluation. Sensory analysis was conducted at the Department of Agriculture. Panelists (n = 6) were highly trained in descriptive sensory principles and methods. Panelists evaluated each parameter without collaboration and recorded individual evaluations on a 14-point scale, where 1 had the lowest intensity and 14 had the greatest. The testing room was a round-table panel room and had lighting, temperature, humidity, and noise controls designed according to the guidelines of A.S.T.M. (1986). Four of the cut pieces were placed randomly into plastic cups, kept warm by placing the cups on tiles heated to 121ºC, and presented to the panel within 5 min of cutting.

Calpain activity assay

For proteins extraction 300 mg of tissue samples from the muscle were homogenized using a Polytron (Brinkman Instruments, Westbury, NY) in 0.9 mL of post-rigor extraction buffer containing 100 mM Tris base, 10 mM EDTA, 0.05% 2 -mercaptoethanol, adjusted with HCl to pH 8.3 and a cocktail of protease inhibitors (Protease Inhibitor Cocktail Tablets; Roche). After centrifugation at 8.800 g for 30 min, the supernatant (containing soluble proteins) was collected. Immunoprecipitation was

carried out as follows: 100 µl of a 1:1 slurry of protein A-Sepharose beads was incubated for 1 h at 4°C with 5 µg of anti-µ-Calpain (DOMAIN IV) (SIGMA) or anti-m-Calpain (DOMAIN III/IV) mouse IgG1 (SIGMA). The beads were washed three times with lisys buffer (NaCl 150 mM, Hepes 20 mM, glycerol 10 %, triton 1%), and incubated overnight at 4°C with 100 µg of protein extract. Immunoprecipitated proteins were washed three times with 1 ml of lysis buffer (NaCl 150mM, Hepes 20mM, glycerol 10 %, Triton 1%). Cell lysates were used to detect calpain activity by means of the Calpain-Glo protease assay (Promega) that furnishes specific calpain luminogenic substrates (O'Brien et al. 2005). The Calpain-Glo Reagent was prepared as indicated in the Promega protocol. 25 µl of Calpain-Glo Reagent and 25 µl of sample were mixed and incubated for 30 min, and the luminescence was recorded with a luminometer (GloMax™20/20 Luminometry System GloMax™ 96 Microplate Luminometer).

Western blot analysis of µ-calpain, m-calpain and calpastatin

Protein samples were subjected to 10% SDS-PAGE according to the method of Laemmli (Laemmli 1970). In briefly, muscle extracts were incubated in buffer at 37°C for 10 min and fractionated by electrophoresis. The separated protein were electrophoretically transferred to a 0.22 µm nitrocellulose membranes (Hybond-C Super or Hybond-ECL) at 4°C overnight using a Bio-Rad Transfer Blot apparatus (30m Amp). Nonspecific sites were blocked with 5% skim milk in TTBS (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween-20) for 1 h at room temperature. The membranes were incubated for 2 h at room temperature and were sequentially diluted with 1:2500 of anti-rabbit, µ-, m-calpain or calpastatin. The membranes were washed and incubated for 1 h with 1:2000 dilution of secondary (AP-conjugated goat anti-rabbit IgG). After three time washings, antibody-reactive bands were visualized by the ECL detection system (Amersham). The blots were scanned using a Leica DM 1000 (Gene Gnome syngene Bio) imaging system.

Calibration Curve

We created a calibration curve for our results. The data-the percentage of the analyte and the relative response for each standard-were obtained using linear progression analysis.

Statistical design and analysis

The experimental was designed as a split-split-plot design with the incomplete assignment of the treatment combinations to the experimental units and the treatment was replaced six times. The whole plot treatment was quality grade and the sub-plot treatment was aging method (dry aging and wet aging). Data were analyzed using the Proc Mixed procedure of SAS (2009). The degree of freedom computation method was the Kenward-Roger (ddfm = kr) and the computation was included in the model statement. Means separated were significant for $P > 0.05$ using the PDIFF option. Least significant difference (LSD) for all significant factors were calculated and presented for ease of mean separation.

RESULTS AND DISCUSSION

The Marchigiana loins had higher weight loss during dry aging than wet 14.55% and 2.80 %, respectively (Table 1). The higher moisture content ($P < 0.0001$) (70.53%

for wet *vs* 56.64% for dry) before cooking might explain the higher eight loss due to evaporation and/or purge associated with Marchigiana bovine. In the study by Sitz, Calkins, Feuz, Umberger, and Eskridge (2006), they found that dry-aged steaks had lower moisture content than wet-aged steaks. Prime loins supporting our findings that lower intramuscular fat and higher moisture content resulted in greater weight loss during aging. Warren and Kastner (1992) also reported higher weight loss with dry aging as compared with wet aging. Similarly, Ahnströmet et al. (2006) concluded that dry aging resulted in higher weight loss than SB aging for steaks aged for 21 d. Aging method affected trim loss (Table 1), in fact it was highly significant ($P < 0.0001$). Dry method resulted in much higher ($P < 0.0001$) trim loss (23.05%) than wet aging (3.51%). This excessive trim loss required much more labour than for wet aging. Similarly Li et al., (2013) found higher ageing loss, trim loss, and total ageing and trim loss of meat aged in dry ageing bags compared to that aged in vacuum because there was a higher moisture loss when using the dry ageing bag compared to vacuum ageing. There was aging method effect for the combined loss (Table 1). Dry losses were higher than wet aging (34.98% for dry *vs* 6.23% for wet, Table 1). De Geer et al. (2009) reported similar combined losses for dry and SB aged strip or shell loin steaks

Table 1. Percentage value for physical and chemical parameters in relation to aging method and end point temperature

Trait	Aging method			End point temperature		
	Dry	Wet	*P*-value	61.8	70	*P*-value
Weight loss (boneless loins)	14.55	2.80	< 0.0001			
Trim loss % (boneless loins)	23.05	3.51	< 0.0001			
Combined loss	34.98	6.23	< 0.0001			
Moisture % (raw steaks)	56.64	70.53	< 0.0001			
Moisture % (cooked steaks)	53.06	53.20	0.65	63.24	61.0	< 0.0001
Fat %(raw steaks)	4.43	3.42	0.04			
Fat % (cooked steaks)	5.36	6.41	0.04	6.34	6.51	<0.31
WBSN (steaks)	29.75	29.80	0.99	27.54	31.10	< 0.0001
Cooking loss % (steaks)	17.73	19.23	0.01	17.70	22.98	< 0.0001
Visual color	2.80	2.78	0.24	2.15	4.53	< 0.0001

Prior to aging, Marchigiana loins had mean pH values of 5.60. The pH of dry aged loins was 5.66, which was higher ($Pb < 0.01$) than wet aged (5.49) loins. A faster, earlier decline in pH can cause detrimental effects on meat quality through the earlier reduction in activity in u-calpain combined with a relatively high calpastatin level resulting in tougher meat (Hwang and Thompson, 2001). Wet aged loins had higher moisture content (Table 1) than dry aging. This result was in accordance with that of Juárez et al., (2011) that in their study on pork found that dry-aged meat had lower ($P < 0.001$) moisture content when compared with wet aged meat. After cooking, there was no difference ($P > 0.05$) in moisture content due to aging method. As expected, higher end point temperature resulted in lower ($P < 0.0001$) moisture content (Table 1). Quality grade × aging method interaction was significant ($P < 0.05$) for L* values, but not for a* or b* values (Table 2). Aging method × end point temperature interaction was significant ($P < 0.05$) for a* and b* values. The decrease in a* values (less red) with the increased end-point temperature was not significant ($P < 0.04$) in dry aged steaks like it was in wet aged steaks (Table 2). The same decreasing trend in b* values with the higher end point temperature was also observed (Table 2).

In general, there was less difference in L*, a*, and b* between for dry aging and wet aging.

Table 2. Colo0ur profile of meat

Trait	Marchigiana Dry	Marchigiana Wet	Aging method and end point temperature, ºC				
			Dry (61.8)	Dry (70)	Wet (61.8)	Wet (70)	*P*-value
L*	55.44	56.24	53.75	54.23	54.53	57.24	0.26
a*	13.00	12.34	12.87	11.13	13.65	9.87	0.04
b*	19.01	19.56	18.4	17.24	20.72	16.45	0.03

L*– lightness, a*– redness, b*– yellowness intensities.

At the endpoint temperature of 61.8ºC, dry aged steaks tended to have a higher early cooking rate than wet steaks. Dry aged steaks had a mean visual color score of 3.80 (more done appearance) while wet steaks had mean visual color scores of 2.78, but the difference was not significant ($P > 0.05$) (Table 1). The mean visual colour scores of steaks cooked to 61.8°C and 70°C were 2.15 and 4.53 ($P < 0.01$), respectively. As expected higher endpoint temperature resulted in higher degree of doneness. There was no aging method effect ($P > 0.05$) on warner bratzler shear force (WBSF) (Table 1). In contrast George-Evins et al., 2004 found that each increase in aging period resulted in lower ($P < 0.05$) WBSF values. In our study, WBSF increased ($P < 0.0001$) as endpoint temperature increased. Similarly, George Evins et al., (2004) in their study on beef found that WBSF values increased ($P > 0.05$) as endpoint temperature increased. In agreement, Lorenzen et al. (2003) found that WBSF for top sirloin steaks increased when cooking to higher in internal endpoint temperatures, too. Dry aging resulted in similar cooking loss ($P > 0.05$) as wet aging (Table 1). Similarly, Warren and Kastner (1992) reported that wet and dry aged strip loins had similar ($P > 0.05$) cooking losses. However, Laster et al. (2008) reported that dry aging caused less cooking loss than wet aging. A strong ($P < 0.0001$) endpoint temperature effect existed for cooking loss (Table 1). Cooking loss for steaks cooked to 70 °C was about 5% higher ($P < 0.0001$) than that for steaks cooked to 61.8°C (Table 1). Fang Liu et al., (2013) have reported increased cooking loss with increased endpoint temperature. Cooking induces the shrinkage of myofibrillar, sarcoplasmic proteins, and shrinkage and solubilization of the connective tissue, which decrease the water holding capacity of the meat (Tornberg 2005; García-Segovia et al. 2007). According to Tornberg et al., (1997), the denaturation of sarcoplasmic proteins started at 40ºC and terminated at 65ºC, which might contribute to the increase in the cooking losses. The only significant effect for myofibrillar tenderness was aging method x endpoint temperature. Dry aged steaks cooked to 70ºC had a mean myofibrillar tenderness score of 5.20, which was higher ($P < 0.05$) than that of wet aged steaks (Table 3). Quality grade and aging method did not affect ($P > 0.05$) juiciness but steaks cooked to 61.8°C were juicier ($P < 0.05$) than those cooked to 70°C (Table 3), which might be attributed to higher cooking losses with the higher endpoint temperature. Similarly, Sitz et al. (2006) reported no significant differences for flavor, juiciness, tenderness, and overall acceptability between dry-aged and wet-aged Choice strip loins. In our study, connective tissue amount, overall tenderness, flavor, and off flavor intensity were not affected ($P > 0.05$) by any treatment or treatment combinations (Table 3). Smith et al. (2008) similarly reported no differences in overall like scores between dry aged and wet aged steaks.

Table 3. Percentage value for physical and chemical parameters in relation to aging method and end point temperature.

Trait	Aging method			End point temperature (ºC)			Aging method x endpoint temperature (ºC)				
	Dry	Wet	*P*-v.	61.8	70	*P*-v.	Dry(61.8)	Dry(70)	Wet(61.8)	Wet(70)	*P*-v.
Myofibrillar tenderness	5.17	5.03	0.42	5.11	5.00	0.06	5.16	5.20	5.13	4.80	0.05
Beef flavor intensity	4.63	4.56	0.46	4.57	4.54	0.01	4.74	4.65	4.69	4.45	0.87
Juiciness	4.65	5.63	0.68	4.78	4.46	0.01	4.78	4.47	4.85	4.50	0.81
Connective tissue amount	5.86	5.70	0.31	5.74	5.84	0.22	5.75	5.99	5.65	5.78	0.29
Overall tenderness	5.18	4.99	0.44	5.10	5.04	0.32	5.19	5.20	5.09	4.89	0.45
Off flavor intensity	6.06	6.15	0.56	6.11	6.14	0.56	6.03	6.04	6.18	6.19	0.84

The calpain system has been extensively studied over the past few decades. Nevertheless, the mechanisms that control the calpain activity in postmortem muscle have not been fully elucidated (Lindahl et al., 2010). An important characteristic of calpains is that they autolyze once activated, ultimately leading to loss of activity (Goll et al 2003). In bovine muscle the extractable activity of μ-calpain has been reported to decline rapidly during ageing, whereas the activity of m-calpain is more stable (Ducastaing et al., 1985 and Koohmaraie et al., 1987). Western Analysis was performed to determine μ-calpain, p-94 and calpastatin content in the Longissimus dorsi samples (Figs 1, 2). Calpain and calpastatin content were determined in the same samples that were evaluated for calpain and calpastatin activity. Our results by western blot analysis indicated that autolysis of the large subunit of μ-calpain was, already, detected in the standard sample, which was an independent at-death bovine muscle. During the period analyzed, in the muscle there was a gradual decline in the intensity of the 80 kDa band of μ-calpain while the intensity of the 60 kDa band, presumably the product of μ-calpain autolysis, increased throughout the post-mortem storage period. The expression of p94 remained the same in the muscle during the period considered, the calpastatin, at 15d post mortem, presented a slight decline.

The rate of calpastatin degradation and inactivation is related to the rate of proteolysis and tenderization observed in meat (Geesink and Koohmaraie 1999; Lonergan et al. 2001). The analysis of bioluminometer showed that the aging system significantly affected the activity on μ-calpain. Infact it was lower in dry aging system than wet aging. Extended aging from 7 to 15 days decreased μ-calpain activity. Particularly for dry aging approximatively 45% of at-death μ-calpain activity remains at mortem; 80% of at death μ-calpain activity remains after 7d of post mortem storage and approximatively 65% of at death μ-calpain activity remains after 15d of post mortem storage. For wet aging method 50% of at-death μ-calpain activity remains at mortem; 100% of at death μ-calpain activity remains after 7d of post mortem storage and approximatively 85% of at death μ-calpain activity remains after 15d of post mortem storage. Activity of p94 doesn't change during post mortem storage in both methods (100%). Activity of calpastatin changes during post mortem storage and there wasn't significantly differences between two methods: approximatively 100% of at death

calpastatin remains at mortem; 70% of at death remains after 7d of post mortem storage and it was absent after 15d of post mortem storage.

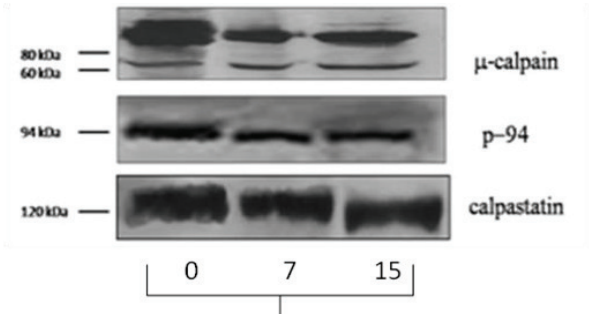

Figure 1. Western blot analysis of μ-calpain, p-94 and calpastatin on Longissimus Dorsi muscle.

CONCLUSIONS

In conclusion our results showed that in the muscle object of study: μ-calpain expression was in inverse relation to the calpastatin expression; μ-calpain expression decreased in the time interval considered; μ-calpain activity increased at 7 days post mortem and decreased at 15 days post-mortem; the variability in tenderness after 15 days cannot be attributed to p-94; in particular there was much more differences for μ-calpain activity in the two aging methods while the calpastatin and p-94 followed the same trend.

Both aging methods used in our study resulted in similar palatability. However, D aging resulted in greater weight and trim loss (≥ 36%) and required extensive labor in trimming. This data are in accordance with the minor μ-calpain activity showed. A trained sensory panel revealed few, if any, differences among dry and vacuum aging. Dry aging beef process beyond the traditional aging period give to meat taste and mouth that feel optimize. Therefore, wet aging should be the preferred method of aging beef for most of the industry. We made this study in order to determine the score that consumers place on the two aging methods. A greater percentage of consumers favored wet-aged samples, indicating that high quality beef can be wet aged with desirable palatability results.

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