INFLUENCE OF HIGH-OXYGEN AND LACTATE/PHOSPHATE ENHANCEMENT ON, PROTEOLYSIS, PROTEIN POLYMERIZATION, AND TENDERNESS OF POSTMORTEM BEEF MUSCLES

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Abstract— **Two consecutive experiments were conducted to determine the influecne of high-oxygen modified atmoshphere packaging (HiOx-MAP) and lactate/phosphate enhancement on biochemical processes involved with the development of tenderness of early postmortem beef cuts. In experiment 1, three different paired muscles (n = 10) - *longissimus lumborum* (LD), *semimembranosus* (SM), and *adductor* (AD) - were obtained at 24 h postmortem. Steaks (2.54-cm thick) were cut from each muscle, randomly assigned to either HiOx-MAP (80% O_2, 20% CO_2) or vacuum (VAC), and displayed for 9 d at 1°C. In experiment 2, at 24 h postmortem, three bovine muscles (LD, SM, AD; n=10, respectively) were enhanced (10% pump) with either lactate (2.5%)/phosphate (0.3%) solution or water, packaged in HiOx-MAP, stored 9 d 1°C, and then displayed for 7 d 1°C. For both experiment 1 and 2, SDS-PAGE, western blot (desmin, troponin-T, μ-calpain, and myosin heavy chain(MHC)), star probe, lipid oxdiation were determined on steaks at the begining and at the end of display. Sensory analysis was included in experiment 1. HiOx-MAP packaged beef steaks had a significant increase in lipid oxidation during display. The steaks in HiOx-MAP had significantly lower tenderness and juiciness, and higher off-flavor scores compared to steaks in VAC. HiOx-MAP did not affect ($P > 0.05$) μ-calpain autolysis and degradation of either troponin-T or desmin. SDS-PAGE, western blotting, and diagonal-PAGE revealed oxidative cross-linking of MHC in meat packaged in HiOx-MAP. The lactate/phosphate injection significantly decreased accumulation of lipid oxidation of all three bovine muscles throughout display period. The enhancement improved ($P < 0.05$) instrumental tenderness values of LD and SM. There were no significant differences found in desmin and troponin-T degradation, and cross-linked MHC between treatments. The results suggest that lactate/phosphate enhancement have beneficial effects on lipid oxidation stability, and instrumental tenderness development of beef cuts under a HiOx-MAP condition.**

Index Terms— **High-oxygen MAP, Lactate/phosphate, Oxidation, Tenderness**

I. INTRODUCTION

Modified atmosphere packaging systems with a high oxygen (80%) level are extensively practiced in retail meat markets because the bright red color of meat in this packaging system attracts consumers. However, high oxygen levels are likely to increase the incidence of oxidative changes in the meat, thus negatively affecting meat quality attributes. A high level of oxygen can result in protein polymerization, which subsequently decreases tenderness of meat (Lund, Lametsch, Hviid, Jensen & Skibsted, 2007). Besides protein polymerization, oxidative environment in early postmortem muscle may affect the rate of tenderization by negatively affecting proteolytic activity of μ-calpain (Rowe, Maddock, Lonergan & Huff-Lonergan, 2004).

Enhancement with a brine solution containing lactate and phosphate is commonly used in fresh meat as antimicrobial agent (Brewer, Rostogi, Argoudelis & Sprouls, 1995) and antioxidant (Kim, Keeton, Smith, Maxim, Yang & Savell, 2009). Therefore, it can be hypothesized that enhancement of early postmortem muscle with lactate/phosphate will promote an environment more conducive to postmortem proteolysis and tenderization of beef by minimizing protein polymerization and/or inactivation of μ-calpain inactivation under high-oxygen modified atmosphere packaging (HiOx-MAP) condition. The objectives of this experiment were to determine the influence of a HiOx-MAP system and lactate/phosphate enhancement on biochemical processes involved with the development of tenderness of early postmortem beef muscles.

II. MATERIALS AND METHODS

A. Raw materials and processing

Two consecutive experiments were conducted to determine the influence of HiOx-MAP and lactate/phosphate enhancement on protein polymerization, proteolysis, and tenderness of postmortem beef muscles. In experiment 1, ten of three different paired muscles (A-maturity; Low Choice) - *longissimus lumborum* (LD), *semimembranosus* (SM), and *adductor* (AD) - were obtained at 24 h postmortem. Steaks (2.54-cm thick) were cut perpendicular to the long axis of

each muscle and randomly assigned to either HiOx-MAP (80% O_2, 20% CO_2) or vacuum (VAC) for packaging by using Multivac C500 (Koch Supplies Inc., Kansas City, MO). Steaks were displayed for 9 d at 1°C under 2150 lux of fluorescent light. In experiment 2, ten of paired three muslces (LD, SM, and AD) were separated from each side of beef carcasses (n=10; A-maturity; Low Choice) at 24 h postmortem. One of two treatments [potassium lactate (PURASAL HiPure P, 60% potassium L-lactate/40% water; PURAC America, Inc., Lincolnshire, IL) + sodium tripolyphosphate (Brifisol®; BK Giulini Corp; Simi Valley, CA) or water injection enhancement] was randomly assigned to muscles from each side of each carcass. Enhancement was achieved using a multineedle injector (model N30, Wolftec, Inc., Werther, Germany; average 10% enhancement rate; final concentration of 2.5% lactate/ 0.3% phosphate) on the muscles immediately after the muscle separation. Muscles were cut into steaks (2.54 cm thick), placed in preformed trays, and packaged to HiOx-MAP. Packages were stored in the dark at 1 ºC for 8 d before display for 7 d at 1 °C under the display light. For both experiment 1 and 2, biochemical, quality, and sensory analyses (experiment 1 only) were determined on steaks at the begining and at the end of display.

B. pH determination

The pH measurements for each steak on d 1 (48 h postmortem) and d 9 (10 d postmortem) of display (experiment 1) and d 1 (48 h postmortem), d 9, and d 16 of storage and display (experiment 2) were measured by a glass penetration probe using a Hanna 9025 pH/ORP meter (Hanna Instruments, Woonsocket, RI).

C. Lipid oxidation

Lipid oxidation of steaks from each storage and display period (experiment 1 and 2) was determined by using the 2-thiobarbituric acid distillation method described by Tarladgis et al. (1960). The thiobarbituric acid-reactive substances (TBARS) value was determined (mg malonaldehyde per kg of sample) in duplicate per sample.

D. Gel electrophoresis and western blotting

Western blotting to determine µ-calpain autolysis, desmin, and troponin-T degradation for steaks from each storage and display time (experiment 1 and 2) was conducted using procedures described by Rowe et al (2004). SDS-PAGE, western blot for myosin light chain, and diagonal-PAGE (Winger, Taylor, Heazlewood, Day & Millar, 2007) were conducted to determine protein polymerization of samples under non-reducing conditions including 0.5 mM *N*-ethylmaleimide, the thiol blocking agent, to prevent disulfides from forming after sample preparation. Gel spots from the diagonal-PAGE were excised, and sent to the Proteomics facility at Iowa State University for MALDI-TOF MS/MS identification (Stewart, 1999).

E. Sensory evaluation and star probe analysis

A trained sensory panel (n = 6) evaluated sensory characteristics (Lonergan et al., 2007) of steaks on d 9 of display (experiment 1). Sensory traits including tenderness, chewiness, juiciness, beef flavor, and off-flavor were evaluated for cooked steaks (internal temperature of 71 ºC) using a 15-cm line scale (1 = not tender, chewy, juicy, low beef flavor; 0 = no off-flavor; 15 = very tender, chewy, juicy, high beef flavor, high off-flavor).

Star probe of steak samples on each storage and display period (experiment 1 and 2) was measured (Lonergan et al., 2007). Steaks were cooked on clamshell grills to an internal temperature of 71 ºC, which was monitored using thermocouples (Omega Engineering, Inc. Stamford, CT). Each steak was compressed three times with a circular, five-pointed star probe attached to an Instron Universal Testing Machine (Model 1122, Instron, Norwood, MA) and the average of the three values was calculated to determine the overall value.

F. Statisctical analysis

Data were analyzed by using the Mixed Model procedure of SAS for ANOVA (SAS, 2007). Type-3 tests of fixed effects for muscle, treatment, display time, and their interaction, and random effects for animal and animal by treatment were determined. Least squares means for all traits of interest were separated using least significant differences generated by the PDIFF option.

III. RESULTS AND DISCUSSION

1. Experiment I

A. pH and lipid oxidation

Packaging type did not affect ($P > 0.05$) in pH of steaks at either display time. However, HiOx-MAP packaged beef steaks increased ($P < 0.05$) lipid oxidation during display. The AD in HiOx-MAP had the greatest increase of lipid oxidation (0.14 to 1.57 mg malonaldehyde/kg meat at d1 and 9 respectively) during display followed by SM (0.12 to 1.17) and LD (0.14 to 0.9) suggesting that these beef round muscles are more susceptible to oxidation than LD.

B. Sensory and star probe analysis

Steaks packed in HiOx-MAP had significantly lower tenderness and juiciness scores and higher off-flavor development compared to steaks in VAC (Figure 1). Particularly, the LD steaks packaged in HiOx-MAP had a lower ($P < 0.01$) tenderness scores (8.7 ± 0.7) and higher chewiness scores (6.1 ± 0.7) than the steaks packaged in VAC (11.3 ± 0.7; 3.7 ± 0.7 respectively). The star probe data confirmed the sensory tenderness evaluation (steaks from LD packaged in HiOx-MAP had higher ($P < 0.05$) star probe values (indicating less tender; 5.5 ± 0.2 kg) compared to the steaks in VAC (4.8 ± 0.2 kg)). In contrast, the steaks from SM and AD were not affected ($P > 0.05$) by packaging types, which is likely due to the high amount of connective tissue in those muscles.

C. μ-Calpain autolysis, proteolysis and protein polymerization

Packaging type did not affect ($P > 0.05$) μ-calpain autolysis and degradation of either troponin-T or desmin. Interestingly, the sensory and star probe data showed that the HiOx-MAP system decreased meat tenderness, although protein degradation and autolysis of μ-calpain were not influenced by the packaging type. Therefore, there must be some other biochemical explanation for the change in sensory quality. One explanation is that HiOx-MAP packaging resulted in protein polymerization. SDS-PAGE (Figure 2A) under non-reducing conditions revealed a high molecular weight band in protein samples prepared from the LD steaks at d 9 in HiOx-MAP, but not those packaged in VAC. Western blots (Figure 2B) confirmed that the cross-linking was associated with myosin heavy chain (MHC). The MHC western blot also revealed that the cross-linked MHC occurred at 1 d of HiOx-MAP storage, and became more pronounced at the end of the display (d 9) in HiOx-MAP. The diagonal-SDS-PAGE also confirmed the cross-linked MHC based on the MS identification of one major spot off the diagonal (spot number 3, Figure 3B), indicating that the protein in this spot had been cross-linked in the HiOx-MAP sample, but not the VAC sample (Figure 3A). Importantly, the spot migrating within the diagonal and a higher molecular weight than the MHC spot (spot 1; Figure 3B) was identified as titin. These results suggest that titin and myosin cross-link under the high oxygen atmosphere condition resulting in decreases tenderness and juiciness of beef steaks.

2. Experiment II

A. pH and lipid oxidation

The lactate/phosphate injection enhancement increased ($P < 0.05$) pH of beef steaks from all three different bovine muscles from average of 5.44 (water injected steaks) to 5.72 (lactate/phosphate injected steaks), which was probably due to the alkaline phosphate effect (Kim et al., 2009). Based on TBARS results, the lactate/phosphate injection treatment significantly minimized lipid oxidation of steaks from all three muscles during storage and display period under HiOx-MAP condition (Table 1).

B. Star probe analysis and western blot

The lactate/phosphate enhanced steaks of LD and SM had lower star probe values (indicating more tender) than the water-injected steaks (Table 1). However, the lactate/phosphate enhancement did not affect ($P > 0.05$) the star probe values of the AD. Western blotting assay data revealed that the injection enhancement of lactate/phosphate did not influence ($P > 0.05$) protein degradation of either troponin-T or desmin of beef cuts (Table 1). Further, western blotting assays for the cross-linked MHC formation showed no lactate/phosphate treatment effect ($P > 0.05$) on the protein polymerization of the beef steaks in HiOx-MAP (Table 1). Thus, it can be speculated that increased tenderness through the lactate/phosphate enhancement might result from a different mechanism, such as increased myofibrillar solubilization through weakening of the structrual integrity of myofibrils by elevated ionic strength and pH.

IV. CONCLUSION

Results from these experiments demonstrated that HiOx-MAP can create oxidative conditions that are detrimental to improvement in tenderness, juiciness, and flavor of fresh meat with storage. It appears that decreased tenderness and juiciness of beef steaks packaged in HiOx-MAP resulted from protein oxidation and concomitant protein polymerization of intermolecular cross-links between myosin and possibly titin. The present study also determined that the lactate/phosphate injection enhancement of early postmortem beef cuts had beneficial effects on lipid oxidation stability and instrumental tenderness values of beef steaks packaged in the HiOx-MAP. The proteolysis and formation of protein polymerization of fresh meat was not affected by the lactate/phosphate treatment.

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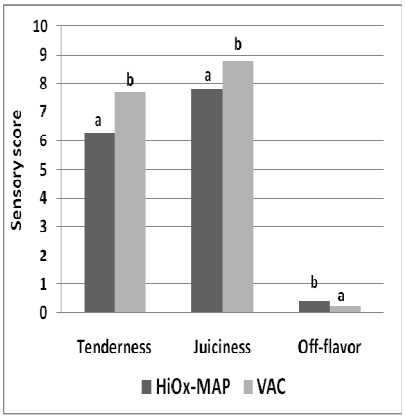

Figure 1. Sensory attributes of beef steaks packaged in either HiOx-MAP or VAC, and displayed for 9 d at 1 ºC.
[ab] Least squares means that do not have a common superscript differ ($p < 0.05$).

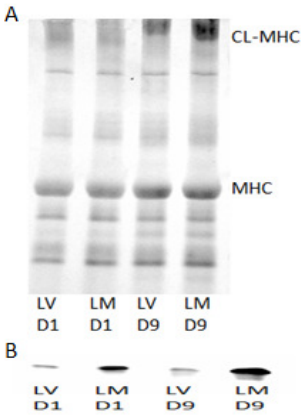

Figure 2. [A]. SDS-PAGE of longissimus muscle steaks packaged in HiOx-MAP (LM) or VAC (LV); CL-MHC = cross-linked myosin heavy chain. [B]. Western blot of myosin heavy chain (MHC) for longissimus muscle steaks in HiOx-MAP or VAC, and displayed for 1 d and 9 d at 1 ºC.

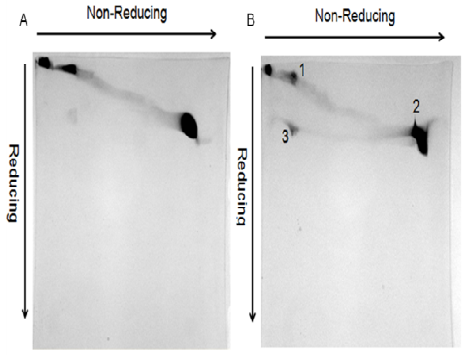

Figure 3. Separation of a cross-linked protein polymerization by oxidant/reductant 2-D diagonal-PAGE of longissimus muscle steaks packaged in either VAC (A) or HiOx-MAP (B), and displayed 9 d at 1 ºC. Spot 1 = Titin; Spot 2 = Myosin heavy chain; Spot 3 = Myosin heavy chain.

Table 1. Least squares means for desmin and troponin-T degradation products, cross-linked myosin heavy chain (MHC), lipid oxidation (TBARS), and star probe analyses of bovine *longissimus lumborum* (LD), *semimembranosus* (SM), and *adductor* (AD) steaks injection enhanced with water (WAT) or lactate/STP (LAC) packaged in HiOx-MAP.

	Desmin[A]		Troponin-T[B]		Cross-linked MHC[C]		TBARS[D]		Star probe (kg)	
	WAT	LAC	WAT	LAC	WAT	LAC	WAT	LAC	WAT	LAC
LD	0.64	0.70	0.53	0.67	1.51	1.38	3.52[a]	0.44[b]	6.00[a]	4.00[b]
SM	0.69	0.66	0.54	0.58	1.51	1.43	4.88[a]	1.69[b]	6.63[a]	5.59[b]
AD	0.96	0.85	0.64	0.47	2.25	1.96	4.64[a]	0.63[b]	6.32[a]	6.08[a]
SEM	0.13	0.13	0.12	0.12	0.22	0.22	0.35	0.35	0.23	0.23

[ABC] Unitless ratio value.
[D] mg malonaldehyde/ kg of sample.
Means in a row with different superscripts (a-b) bewteen muscles within each trait are different ($P < 0.05$).