# PROTEIN OXIDATION IN BEEF *m. triceps brachii* ROASTS DUE TO HIGH OXYGEN PACKAGING

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**Abstract – This study was conducted to evaluate high oxygen packaging (HiOx-MAP) on myofibrillar protein oxidation at different depths in beef *m. triceps brachii* roasts and the effects of dietary antioxidants as a control measure. Steers were fed corn-based finishing diets containing 0 or 30% wet distillers grains with or without AGRADO®PLUS antioxidant supplements. Roasts, aged 8 and 29 days, were packaged in HiOx-MAP and displayed for 6 days. Myofibrillar proteins were isolated from muscle strips (<4 g) cut both parallel and perpendicular to the muscle fibers from the outside to the inside of roasts and tested for protein oxidation. The rest of the steak was tested for tenderness. Protein oxidation (loss of free-thiols and accumulation of carbonyls and protein aggregates) was higher in the outer-most layer than subsequent inner layers in the roast ($P \leq 0.05$). Tenderness severely decreased in the outer-most later compared to the inner layers of the roast ($P \leq 0.05$). Moreover, longer aging greatly increased protein oxidation in beef compared to short-term aging ($P \leq 0.05$). Therefore, protein oxidation and toughening in beef due to HiOx-MAP happens at a descending gradient and is greatly influenced by the aging time. Also, it cannot be controlled by dietary antioxidants.**

**Key Words – aging, antioxidants, high oxygen packages.**

## I. INTRODUCTION

Modified atmosphere packaging systems with 80% oxygen and 20% carbon dioxide (HiOx-MAP) are widely used in fresh beef retail markets as it holds cherry-red color of beef for a longer time. However, Lund et al. [1] and Huff-Lonergan et al. [2] have reported that HiOx-MAP systems negatively affect organoleptic properties of retail meat, including tenderness, juiciness, and flavor. Lund et al. [1] showed that HiOx-MAP systems oxidize myofibrillar proteins and postmortem proteases by changing their molecular structures and forming intra/inter cross-links (di-sulfide or dityrosine). Therefore, oxidation impedes the proteolytic activity of proteases and increases formation of myofibrillar protein aggregates. Both outcomes, due to oxidation, reduce post-mortem tenderization process of beef and eventually make beef less tender. Estevez et al. [3] and Park et al. [4] showed that lipid-derived radicals and hydroperoxides, produced from lipid oxidation, rapidly promote protein oxidation in muscle food in HiOx-MAP; therefore, protein oxidation is correlated to lipid oxidation.

Therefore, we hypothesized that protein oxidation in beef could be controlled by feeding antioxidants. In addition, it would be important to study how other factors such as diet, postmortem aging time, thickness of the steak/roast, and muscle fiber orientation would influence the degree of protein oxidation in beef packaged in HiOx-MAP.

## II. MATERIALS AND METHODS

### *Feeding cattle*

Cross-bred (British × Continental) yearling steers (n = 483; initial BW = 427 kg ± 37 kg) were randomly assigned to one of four corn-based diets, containing 0, or 30% (DM basis) wet distillers grains plus solubles (WDGS) with (150 ppm) or without (0 ppm) AGRADO®PLUS (AG) antioxidant supplement for the last 160 d. After 48 h postmortem, USDA Choice beef chuck, shoulder clods (IMPS # 114; NAMP, [5]) 10 carcasses per dietary treatment were collected and wet-aged a total of 8 and 29 d at 0 ± 2ºC.

### *Fabrication, packaging, and retail display*

Beef clod hearts, *m. triceps brachii* (IMPS # 114E; NAMP, [5]) muscles from beef chuck, shoulder clods were removed. Clod heart roasts were prepared from the middle of the muscle after trimming off the ends. Roasts were packaged in high foam-barrier polypropylene trays with a gas mixture (80% $O_2$ and 20% $CO_2$), mechanically sealed with oxygen-impermeable

film and displayed under simulated retail conditions for 6 d.

*Sample preparation and myofibrillar protein isolation*

After display, three 2.54 cm-thick steaks, from the outside to the inside (labeled as outer-most, middle, and inner-most steaks) perpendicular to muscle fibers, were removed, vacuum-packaged and stored at -20ºC. Myofibrillar proteins were isolated from muscles strips (<4 g) cut both parallel and perpendicular to the muscle fibers from each layer outside to inside of the same frozen steaks as shown in the Fig. 1. Myofibrillar protein isolation occurred according to the method described by Pietrzak et al. [6] with some modifications.

*Determination of protein oxidation*

Total carbonyls in myofibrillar proteins were measured spectrophotometrically at 370 nm after derivatized with 2,4-dinitrophenylhydrazine (DNPH). Free-thiol levels in myofibrillar proteins were quantified spectrophotometrically at 420 nm after reaction with 5,5'-dithiol-bis(2-nitobenzonic acid).

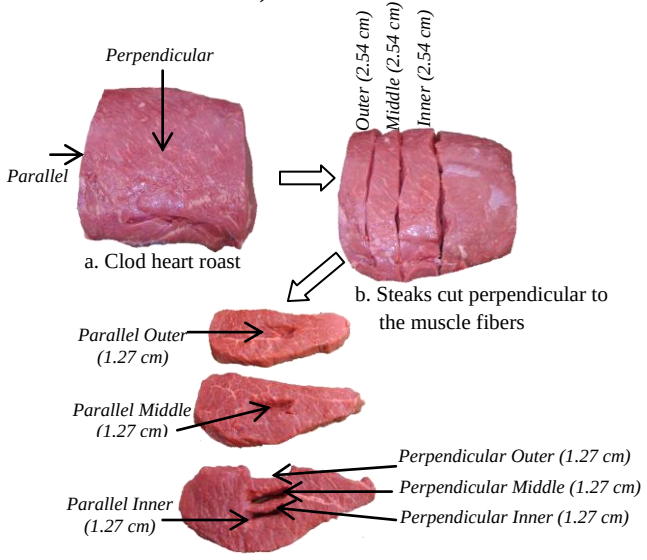


Figure 1. Diagram showing the location of steaks and muscle strips removed from the clod heart roast after retail display.

*Determination of cross-linked protein aggregates*

Myofibrillar protein-aggregates were separated by SDS-PAGE with a discontinuous Tris-HCL/glycine buffer system using 6% resolving and 4% separation gels under non-reducing conditions. Separation was performed at a constant voltage of 120 V and 80 mA current for 90 min. After staining and destaining, protein band intensities were measured at 700 nm using the Odyssey Infrared Imaging System. Band intensities were expressed as integrated intensities in K. pixels.

*Warner-Bratzler shear force (WBSF)*

Steaks were thawed at 4°C for 24 h and grilled at 71°C on a Hamilton Beach Indoor-Outdoor grill, turning over once at 35°C. After grilling, steaks were cooled at 4°C for 24 h. About 2 – 5 cores with 1.27 cm diameter were removed from each layer (as shown in the Fig. 1) of the steak parallel to the muscle fibers. Cores were sheared on a tabletop WBSF analyzer, attached with a triangular Warner-Bratzler shear attachment. An average of the peak shear force (kg) of each layer perpendicular or parallel to muscle fibers of each steak was used for statistical analysis.

*Statistical analysis*

Data were analyzed by ANOVA in the GLIMMIX procedure of SAS (version 9.2, Cary, NC., 2009) as a split-split-split-plot design with dietary treatments as the whole-plot treatment, aging period as the first split-plot treatment, muscle fiber orientation as the second split-plot treatment and depth in the roast as the third split-plot treatment. Separation of means was conducted using LSMEANS procedure with DIFF and SLICEDIFF options at $P \leq 0.05$. In addition, the CONTRAST statements in SAS were used to compare the effects of feeding Corn *vs.* WDGS, and No AG *vs.* AG.

## III. RESULTS AND DISCUSSION

More carbonyls, fewer free-thiols, and more protein aggregates indicate more protein oxidation of samples. Two-way interactions effects of aging time × layer position were significant for all the parameters, except WBSF values (Table 1). For both aging periods, the

outer-most layers had higher carbonyls, loss of free-thiols, and protein aggregations, compared to subsequent layers. Xiong [7] has reported that an increase in carbonyls and loss of free-thiols, due to protein oxidation, eventually increases myosin heavy chain cross-linking and forms myosin aggregates in muscle foods. This study also showed an increase in band intensities of cross-linked proteins decreased band intensities of intact myosin heavy chain proteins (Fig. 2).

Table 1. Two-way interaction effects of aging time × layer location on carbonyls, free-thiols and cross-linked proteins (carbonyls and free-thiols: $P < 0.0001$; protein aggregates: $P = 0.05$).

| Layer | Aging, d | | SEM[1] | *P* |
|---|---|---|---|---|
| | 8 | 29 | | |
| Carbonyls, nmole/mg | | | | |
| Outer | $2.38^{Ba}$ | $3.28^{Aa}$ | 0.10 | <.0001 |
| Middle | $1.82^{b}$ | $1.97^{b}$ | 0.10 | 0.13 |
| Inner | $1.56^{Bc}$ | $2.14^{Ab}$ | 0.10 | <.0001 |
| *P* | <.0001 | <.0001 | | |
| Free-Thiols, nmoles/mg | | | | |
| Outer | $60.14^{Aa}$ | $56.55^{Ba}$ | 0.10 | 0.0002 |
| Middle | $69.07^{Bb}$ | $71.33^{Ab}$ | 0.10 | 0.02 |
| Inner | $70.18^{b}$ | $70.79^{b}$ | 0.10 | 0.53 |
| *P* | <.0001 | <.0001 | | |
| Aggregation, k.pixcel | | | | |
| Outer | $4.91^{Ba}$ | $5.82^{Aa}$ | 0.42 | 0.03 |
| Middle | $3.24^{b}$ | $2.85^{b}$ | 0.42 | 0.35 |
| Inner | $2.91^{b}$ | $2.55^{b}$ | 0.42 | 0.40 |
| *P* | <.0001 | <.0001 | | |

[1]SEM = pooled standard error of means; [A-B] within a row, means lacking a common superscript were different at $P \leq 0.05$; [a-c] within same column and under same parameter, means lacking a common superscript were different at $P \leq 0.05$.

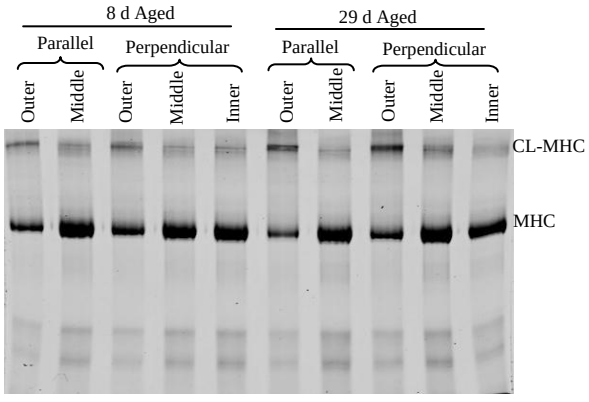


Figure 2. SDS-PAGE separation of myofibrillar proteins on 6% polyacrylamide gel. MHC: myosin heavy chains, CL-MHC: cross-linked MHC aggregates.

However, myofibrillar proteins isolated from middle and inner layers did not show many cross-linked myosin bands (Fig. 2), and had less carbonyls and higher free-thiols (Table 1). Therefore, outer most layers were more susceptible to protein oxidation than inner layers in the beef roast packaged in HiOx-MAP. In addition, carbonyls ($P < 0.0001$) and protein aggregates ($P = 0.03$) increased, and free-thiols ($P = 0.0002$) decreased extensively in the outer-most layers of beef aged longer (Table 1). Therefore, aging time also boosts protein oxidation.

Shear force increased to a greater extent in the outer-most layers, compared to the inner layers ($P < 0.0002$), and also parallel to the muscle fibers than perpendicular to the muscle fibers ($P = 0.02$; Table 2). Lund [1] and Huff-Lonergan [2] have reported that HiOx-MAP negatively affects meat eating quality by decreasing tenderness, juiciness, flavor, protein solubility and digestibility.

However, carbonyls increased ($P = 0.0002$) more in the outer-most layer perpendicular to the muscle fibers than the parallel. Other parameters did not show any significant interaction effects with muscle fiber orientation for protein oxidation.

Table 2. Two-way interaction effects of muscle fiber direction × layer location on [1]Warner-Bratzler shear force and carbonyls (WBSF: $P = 0.015$; Carbonyls: $P = 0.030$).

| Layer | Muscle fiber direction | | SEM[1] | *P* |
|---|---|---|---|---|
| | Parallel | Perpendicular | | |
| WBSF[1], kg | | | | |
| Outer | $5.39^{Aa}$ | $5.04^{Ba}$ | 0.15 | 0.02 |
| Middle | $4.20^{b}$ | $4.47^{b}$ | 0.15 | 0.07 |
| Inner | $4.25^{b}$ | $4.25^{b}$ | 0.15 | 1.00 |
| *P* | <.0001 | 0.0002 | | |
| Carbonyls, moles/mg | | | | |
| Outer | $2.65^{Ba}$ | $3.01^{Aa}$ | 0.10 | .0002 |
| Middle | $1.81^{b}$ | $1.97^{b}$ | 0.10 | 0.10 |
| Inner | $1.84^{b}$ | $1.84^{b}$ | 0.10 | 1.00 |
| *P* | <.0001 | <.0001 | | |

[1]SEM = pooled standard error of means; [A-B] within a row, means lacking a common superscript were different at $P \leq 0.05$; [a-b] within same column under same parameter, means lacking a common superscript were different at $P \leq 0.05$.

Table 3 shows that feeding WDGS diets increased ($P$ = 0.02) tenderness in 8 d aged steaks, compared to corn diets. However, after 29 d of aging, steaks from WDGS diets became less tender ($P$ = 0.04) than steaks from cattle fed corn. A plausible reason would be due to the occurrence of excessive oxidation in longer aged steaks due to WDGS diets.

Table 3. Effects of diets on [1]Warner-Bratzler shear force, free-thiols, and protein aggregates (WBSF: diet × aging time, $P$ = 0.006; free-thiols: diet, $P$ = 0.052; protein aggregates: diet, $P$ = 0.006).

| | Diets | | | | SEM[4] | *P* | Contrasts, *P* | |
|---|---|---|---|---|---|---|---|---|
| | No AG[2] (0 ppm) | | AG (150 ppm) | | | | | |
| | Corn | 30% WDGS[3] | Corn | 30% WDGS | | | Corn *vs.* WDGS | No AG *vs.* AG |
| WBSF[1], kg | | | | | | | | |
| 8 d aging | 4.60[A] | 4.65[A] | 4.87[A] | 4.21[Ba] | 0.25 | 0.003 | 0.02 | 0.52 |
| 29 d aging | 4.44 | 4.74 | 4.55 | 4.73[b] | 0.25 | 0.26 | 0.04 | 0.26 |
| *P* | 0.37 | 0.62 | 0.07 | 0.004 | | | | |
| Free-thiols, (nmoles/mg) | 65.70[B] | 67.68[A] | 65.96[B] | 66.05[B] | 1.58 | 0.05 | 0.06 | 0.22 |
| Aggregates (k.pixels) | 4.37[A] | 3.40[B] | 3.38[B] | 3.71[AB] | 0.49 | 0.006 | 0.19 | 0.16 |

[2]AG:AGRADO®PLUS; [3]WDGS:wet distillers grains plus solubles; [4]SEM = pooled standard error of means; [A-B] within a row, means lacking a common superscript were different at $P \leq 0.05$; [a-b] within same column and under same parameter, means lacking a common superscript were different at $P \leq 0.05$.

However, protein oxidation, measured by all protein oxidation parameters did not significantly increase due to WDGS feeding. Also, feeding AG, antioxidant supplements, did not significantly affect protein oxidation or shear force values of beef clod hearts.

## IV. CONCLUSION

Protein oxidation due to high oxygen modified atmosphere packages occurs at a descending gradient in beef and degree of protein oxidation is positively affected by aging time. Also, protein oxidation cannot be controlled by feeding AGRADO®PLUS antioxidants.

## ACKNOWLEDGEMENTS

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