INFLUENCE OF OXIDATIVE DAMAGE TO PROTEINS ON BEEF TENDERNESS

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I. INTRODUCTION

Meat tenderization is a consequence of enzymatic degradation of key myofibrillar and cytoskeletal proteins. The degree of weakening of myofibrillar structures directly affects ultimate meat tenderness, with modification of several key proteins during the ageing period. Research has shown that weakening of muscle cells in postmortem conditions is highly dependent on the content and activity of endogenous proteases within skeletal muscle, including calpains, cathepsins, proteasomes, and caspases [1,2]. Mechanisms involved in the post-rigor tenderizing phase have been deeply studied by meat scientists along decades. However, very little research has explored the effects of oxidative damage to proteins on beef tenderness. Any variation in the oxidative status of animals can induce the formation of reactive oxygen species (ROS), which can lead to DNA, lipid and protein damage in muscle tissue and eventually change the chemical and physical structure of meat [3]. Perhaps the formation of ROS postmortem and their effects on cell structure and proteins could provide partial answers to still unexplained variability of beef tenderness. Therefore, a better understanding of how protein carbonylation affect postmortem tenderization could aid the development of new biomarkers for beef tenderness. In this context, the objective of this study was to determine the relationship between carbonylation of proteins and beef tenderness.

II. MATERIALS AND METHODS

Sixty-four finishing crossbred steers were fed a corn based diet for 134 days. At harvest, 24 Choice carcasses were selected and strip loins were collected. Loins sections were trimmed of subcutaneous fat, and fabricated into steaks (2.54 cm thickness) for Warner-Bratzler shear force (WBSF) measurements. A second set of 24 steaks was vacuum packaged and frozen (-80°C) for proteomic analysis. Based on the WBSF values of the 24 samples evaluated, three contrasting meat tenderness groups were selected for proteomic analysis: tender meat (3.95 ± 0.35 kg; n = 5), intermediate meat (5.27 ± 0.71 kg; n = 5), and tough meat (7.66 ± 0.77 kg; n = 5).The 15 selected samples were subjected to proteomic analysis. Proteins were separated via two-dimensional electrophoresis with hydrazide fluorophore derivatization. Pooled protein composed of the fifteen samples was labeled with cyanine dyes 3 (Cy3-Hz) and individual samples were labeled with cyanine dyes 5 (Cy5-Hz). First-dimension separation was performed with a Protean IEF Cell (BioRad). The second dimension was resolved on a 12% polyacrylamide gel along with a piece of filter paper containing 5 µL of a molecular weight standard (10 -180 kDa). The gels were scanned in the Typhoon FLA-9500 and the images were aligned, normalized, and analyzed using Progenesis SameSpots version 4.5. Intergel matching of the Cy3 pool was performed across all gels for comparative cross gel statistical analysis of all spots based on normalized spot volumes, enabling the detection of differentially abundant/expressed or oxidized spots between tender, intermediate and tough meat ($P < 0.05$). Protein identification was done using an LTQ XL™ Linear Ion Trap Mass Spectrometer. Degree of proteolysis was measured via immunoblotting to quantify troponin-T degradation.

III. RESULTS AND DISCUSSION

The concentration of 31 of the 220 spots detected by 2D-DIGE gel image analysis differed significantly ($P < 0.05$) in the comparative analysis between the tender, intermediate, and tough meat groups. Proteins

showing differential carbonylation are grouped into specific classes: structural proteins, oxidative stress proteins, heat shock, glycolytic proteins, and nucleotide metabolism. Alpha-actin (ACTA1), Beta-actin (ACTB) and Myosin light chain (MYL1, MYL3, MYL6B and MYLPF), Desmin (DES), Troponin T (TNNT1 and TNNT3), Troponin I (TNNI2) and Tropomyosin (TPM2) had increased oxidative damage for beef samples in the tender group when compared to the intermediate or the tough group ($P < 0.05$), suggesting that oxidation of structural proteins may contribute to beef tenderness. Peroxiredoxin 1 (PRDX1), peroxiredoxin 2 (PRDX2), and protein/nucleic acid deglycase DJ-1 (PARK7) had increased oxidative damage in the tender group when compared to the intermediate and tough groups ($P < 0.05$). This could explain the increased myofibrillar proteolysis observed in the tender group, as these proteins are protective agents against ROS. Differences in oxidative damage among tenderness groups was also found for other antioxidants enzymes. Superoxide dismutases (SODs) SOD1, had increased carbonylation in the tender group when compared to the other groups ($P < 0.05$), while SOD2 and carbonic anhydrase 3 (CA3) had decreased carbonylation in the tender group when compared to the intermediate and the tough group ($P < 0.05$). Heat shock proteins (Hsp) Hsp20 (HSPB1 and, CRYAB) had increased oxidative damage in the tender group when compared to the other groups ($P < 0.05$), while Hsp20 (HSPB6) had decreased oxidative damage in the intermediate and tough group ($P < 0.05$). These results suggest that a decrease in oxidative damage of Hsp70, HSPB1 and, CRYAB in tough meat may prevent myofibrillar proteolysis, which thereby contributes negatively to beef tenderness. An enzyme of glycolytic metabolism, pyruvate kinase (PKM2), had decreased oxidative damage in the intermediate and tough group when compared to the tender group ($P < 0.05$). On the other hand, others enzymes of glycolytic metabolism such as phosphoglycerate mutase (PGAM2), triosephosphate isomerase (TPI1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and beta-enolase (ENO3) had decreased oxidative damage in tender meat when compared to tough meat ($P < 0.05$), indicating that a decrease in oxidative damage of these glycolytic metabolism enzymes may favors to beef tenderness. The oxidative metabolism enzyme creatine kinase M-type (CKM) had decreased oxidative damage in tender and intermediate meat, while the nucleotide metabolism enzyme adenylate kinase isoenzyme 1 AK1 had increased oxidative damage in the tender and intermediate meat when compared to tough meat ($P < 0.05$). Steaks from the tender and intermediate groups had greater troponin-T degradation than steaks from the tough group ($P = 0.001$). These results provide important insights into possible biomarkers of meat tenderization.

IV. CONCLUSION

Our results suggest that oxidation of proteins of different metabolic pathways due to increased ROS activity postmortem can affect beef tenderness. Tender meat was shown to be associated with an increase of oxidative damage to antioxidant enzymes, heat shock proteins and structure proteins, as well as being associated with a decrease in oxidative damage to metabolic enzymes. The lower oxidative damage on oxidative stress proteins and heat shock proteins observed on intermediate and tough meat may allow the protection of structural proteins against proteolysis postmortem.

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REFERENCES

1. Alfadda, A. & Sallam, R. M. (2012). Reactive oxygen species in health and disease. J. Biomed. Biotech. 2012:1-14.
2. Koohmarie, M. 1994. Muscle proteinases and meat aging. Meat Sci. 36:93-104.
3. Ouali A., Herrera-Mendez, H. C., Coulis, G., Becila, S., Boudjellal, A., Aubry L. & Sentandreu, A. M. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Sci 2006; 74: 44-58.