

Proteomics of Meat Color

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INTRODUCTION

Meat color is an important quality trait influencing the purchase decisions of consumers at the point of sale. Maintaining the cherry-red color of fresh meat desirable to consumers is critical to retailing, and discoloration-induced quality deterioration leads to a \$1 billion annual revenue loss in the US meat industry. The redox chemistry of myoglobin and its interactions with other biomolecules in postmortem skeletal muscles are the major factors governing meat color stability. Several intrinsic (sarcolemmal proteins, lipid oxidation) and extrinsic (ligands) factors influence myoglobin chemistry, and elucidating the molecular basis of these interactions is critical to developing strategies to improve meat color stability.

High-throughput analytical tools in proteomics and mass spectrometry (MS) offer valuable means to examine the fundamental mechanisms of meat color biochemistry. While the predominant strategy for characterizing the proteome basis for meat tenderness and muscle-to-meat conversion is analyzing the whole-muscle proteome or its fractions, proteomic investigations into meat color have focused primarily on interactions between myoglobin and small biomolecules. The focus area in several of these investigations was lipid oxidation-induced meat discoloration. Nonetheless, MS and proteomics have been applied to explain the muscle specificity of beef color and to differentiate myoglobins.

LIPID OXIDATION-INDUCED MEAT DISCOLORATION

Investigations into the effect of lipid-soluble vitamin E on meat quality have indicated that lipid oxidation compromises fresh meat color stability (Faustman et al., 1989). Supplementing vitamin E in the finishing diets of beef cattle minimized rancidity and improved color stability by preventing the generation of reactive

lipid oxidation products, which promote myoglobin oxidation. The reactive products of lipid oxidation, which possess greater polarity than their parent compounds, easily diffuse from the lipid bilayer into the sarcoplasm, where they interact with water-soluble myoglobin. Mass spectrometric investigations into lipid oxidation-induced meat discoloration used 4-hydroxy-2-nonenal (HNE) as a model aldehyde because it is highly reactive to macromolecules and its biochemical interactions with proteins have been well documented in medical research.

Investigations by Faustman et al. (1999), one of the first studies in this area, examined adduct formation of HNE with equine oxymyoglobin and subsequent oxidation by using electrospray ionization (ESI)-MS. The results indicated that HNE exerted a prooxidant effect on myoglobin and formed adducts (mono-, di- or tri-) with myoglobin via Michael addition. This study suggested that adduction of HNE with myoglobin is responsible for oxidation of the heme protein. However, the specific sites of HNE adduction were not determined in this investigation.

Alderton et al. (2003) investigated HNE-induced redox instability in beef oxymyoglobin, and analyses of mass spectra revealed that HNE adduction in myoglobin was pH dependent. After 2 h of incubation at 37°C, while HNE formed mono-, di-, and tri-adducts at pH 7.4 (physiological pH), only mono- and di-adducts were observed at pH 5.6 (meat pH). Tandem MS and data mining identified 6 histidine residues (positions 24, 64, 93, 116, 119, and 152) in beef myoglobin adducted by HNE. Noticeably, the adducted residues included proximal (position 93) and distal (position 64) histidines, which coordinate the heme group. These authors concluded that HNE adduction at proximal and distal histidines could compromise the stability of the heme group and accelerate the oxidation of oxymyoglobin to metmyoglobin. At pH 5.6, nucleophilic histidines are protonated, which renders them less favorable candidates for HNE adduction than at pH 7.4. Although this study determined the specific sites of HNE adduction in beef oxymyoglobin, the possibility of preferential adduction of HNE to the 6 histidine residues was not addressed.

Yellowfin tuna is a muscle food with an intense red color, which undergoes rapid discoloration during retail display. The redox-destabilizing influence of HNE on yellowfin

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tuna oxymyoglobin was investigated to characterize the role of lipid oxidation in tuna color stability. HNE accelerated oxidation of tuna myoglobin, and the mono-HNE adduct of tuna myoglobin detected using MS was identified as the reason for enhanced myoglobin oxidation (Lee et al., 2003).

Proteomic tools have been used to elucidate the fundamental basis of the differential effect of vitamin E on lipid oxidation-induced discoloration in beef and pork. Previous research reported that feeding vitamin E improved the color stability and lipid stability of beef. However, in pork, whereas lipid oxidation was minimized by dietary vitamin E supplementation, a color-stabilizing effect was not observed (Phillips et al., 2001). Mass spectrometric analyses of beef and pork oxymyoglobins incubated with HNE at meat storage conditions indicated that the number of adducts was greater in beef oxymyoglobin than in pork oxymyoglobin. In addition, tandem MS determined 4 histidines (positions 36, 81, 88, and 152) adducted in beef oxymyoglobin compared with 2 histidines (positions 24 and 36) in pork oxymyoglobin (Suman et al., 2006). These findings, along with the fact that beef myoglobin contains 13 histidines compared with 9 in pork myoglobin, indicated that lipid oxidation-induced oxymyoglobin oxidation is more critical to color quality in beef than in pork. Further research (Suman et al., 2007) using mass spectrometric quantification of HNE-adducted myoglobin peptides reacted with isotope-labeled phenyl isocyanate indicated that while histidine 36 was preferentially adducted in pork oxymyoglobin, histidines 81, 88, and 93 were the predominant sites of HNE adduction in beef oxymyoglobin. Preferential HNE adduction at the proximal histidine (position 93), which is observed exclusively in beef, offers a partial explanation for why lipid oxidation-induced myoglobin oxidation appears more extensive in beef than in pork and why dietary vitamin E supplementation has only a limited effect on pork color.

Recent investigations have examined the molecular interactions between poultry (turkey and chicken) myoglobins and HNE, with the aim of explaining color defects in fresh poultry. Analyses of mass spectra suggested that both turkey and chicken myoglobins possess the same molecular mass (17,291 Da) and that HNE formed mono-adducts with the poultry myoglobins. Furthermore, tandem MS data indicated homogeneity in the fragmentation patterns of turkey and chicken myoglobins (Maheswarappa et al., 2009), indicating significant similarity in the sequences. A further study by Naveena et al. (2010) used tandem MS to identify the sites of HNE adduction in chicken myoglobin, and they observed that while histidines 64 and 93 were covalently adducted at pH 7.4 by HNE, at pH 5.6 only histidine 64 was adducted. The HNE adduction sites in turkey myoglobin were not determined because the amino acid sequence of turkey myoglobin was yet to be characterized. Noticeably, the

results of these investigations highlighted the criticality of lipid oxidation to poultry meat color quality.

In 2004, the US Food and Drug Administration approved the use of low levels (0.4%) of carbon monoxide in modified-atmosphere packaging for retailing red meats. Carboxymyoglobin, the pigment responsible for the stable cherry-red color of fresh meats in such packaging, thus became relevant to the meat industry. Because both oxymyoglobin and carboxymyoglobin have the same amino acid sequences, it is possible that lipid oxidation could affect the oxidation of carboxymyoglobin, as observed in oxymyoglobin. Joseph et al. (2009) used matrix-assisted laser desorption-ionization time-of-flight (**MALDI-TOF**) MS to explore this phenomenon, and the results provided evidence for HNE adduction in carboxymyoglobin in a pH- and temperature-dependent manner, similar to that observed in oxymyoglobin. Further studies, using tandem MS and data-mining tools, indicated that HNE adducted histidines at positions 24, 36, 48, 81, and 93 in carboxymyoglobin (Joseph et al., 2010a). The observations on lipid oxidation-induced carboxymyoglobin oxidation suggested the necessity of engineering a packaging-specific antioxidant approach to minimize the discoloration of meat retailed under modified-atmosphere packaging systems.

MEAT SPECIES IDENTIFICATION AND MYOGLOBIN CHARACTERIZATION

Adulteration of meat is a problem that has been encountered in meat inspection and hygiene in many countries. Expensive or preferred meats are adulterated with inferior or less expensive ones, and this practice is objectionable because of commercial, public health, and religious concerns. Different methods, from sensory evaluation to DNA-based assays, have been used to differentiate meat species, albeit with variable degrees of success. From this perspective, Taylor et al. (1993) demonstrated that ESI-MS could be used to measure the exact molecular mass of the globin polypeptide chain and thus differentiate heme pigments from farm animals such as pigs, cattle, sheep, and horses. Electrospray ionization-MS analyses of purified hemoglobin and myoglobin identified the ions corresponding to the molecular masses of the globin chains. In further studies (Ponce-Alquicira and Taylor, 2000), collision-induced dissociation was coupled with ESI-MS to successfully differentiate livestock myoglobins based on their unique fragmentation patterns and species-specific amino acid sequences.

The MALDI-TOF MS has been used to characterize myoglobins from exotic and minor meat species based on the molecular mass of intact protein ions. Joseph et al. (2010b) reported that the molecular mass of turkey myoglobin is approximately 350 Da greater than that of beef myoglobin, whereas studies by Suman et al. (2010) documented that the molecular mass of emu myoglobin is 400 to 450 Da greater than those of meat-producing livestock species such as cattle, buffalo, sheep, goats,

and pigs. These researchers concluded that the molecular mass of myoglobins from mammalian and avian meat species are significantly different in mass spectra. Mass spectrometric analyses revealed that the molecular masses of beef and bison myoglobins are the same, and automated Edman degradation revealed that the 2 ruminant species share 100% similarity in the amino acid sequence of myoglobins (Joseph et al., 2010c).

LACTATE-MYOGLOBIN INTERACTIONS

Lactate is a nonmeat ingredient used to improve meat color stability. Giardina et al. (1996) argued that the direct interaction or adduction between lactate and amino acids in myoglobin is responsible for the lactate-modulated redox stability in myoglobin. Recently, using MALDI-TOF MS, Mancini et al. (2010) investigated the possibility of lactate adduction in myoglobin to elucidate the nature of direct interactions between lactate and myoglobin. Analyses of mass spectra revealed that lactate did not form covalent adducts with myoglobin.

SARCOPLASMIC PROTEOME AND MEAT COLOR

Although most investigations on proteome basis of meat color have focused on myoglobin chemistry, a few recent studies have attempted to characterize the role of the sarcoplasmic proteome in meat color stability. Sayd et al. (2006) examined the influence of sarcoplasmic proteome components on pork color stability. Sarcoplasmic proteomes of semimembranosus muscles of 2 groups of animals [light- and dark-colored meat, based on L^* (lightness) values at 36 h postmortem] were analyzed using 2-dimensional electrophoresis, and the results revealed that 22 proteins were differentially expressed. While dark pork had an over-abundance of mitochondrial enzymes, hemoglobin, and chaperones, light pork demonstrated an over-expression of glycolytic enzymes. The authors concluded that the observed differences in the sarcoplasmic proteome could affect metabolic pathways in skeletal muscles postmortem, and thus influence adenosine triphosphate depletion, pH decline, and protein denaturation, factors known to affect meat color.

In a recent study, Joseph et al. (2011) characterized the sarcoplasmic proteomes of beef color-stable (longissimus lumborum; LL) and color-labile (psoas major; PM) muscles by using 2-dimensional gel electrophoresis and tandem MS, with the objective of correlating the differential abundance of proteome components with meat color traits. The LL and PM muscles (at 24 h postmortem) were fabricated into 2.54-cm steaks and subjected to refrigerated retail display under aerobic packaging. Instrumental color and biochemical parameters governing meat color were analyzed on d 0, 5, and 9, whereas samples for the proteomic assay were collected on d 0. In comparison with PM, the LL steaks demonstrated greater ($P < 0.05$) a^* (redness) values and metmyoglobin-reducing

activity and had less ($P < 0.05$) surface discoloration and lipid oxidation, indicating superior color stability. Tandem MS identified 16 differentially abundant proteins, which included antioxidant and chaperone proteins and enzymes in energy metabolism. Proteins demonstrating a positive correlation with a^* values (aldose reductase, creatine kinase, and β -enolase) and surface color stability (peroxiredoxin-2, dihydropteridine reductase, and heat shock protein-27 kDa) were over-expressed in LL, whereas proteins abundant in PM (mitochondrial aconitase) exhibited a negative correlation with a^* values. These results indicated that the greater color stability of beef LL compared with PM could be attributed to the over-expression of antioxidant proteins. Differential abundance of the enzymes involved in energy metabolism suggested possible differences in the way metabolites (lactate, succinate, and pyruvate) are utilized by postmortem muscles and pointed to the necessity of engineering muscle-specific antioxidant, packaging, and enhancement strategies to improve beef color. Furthermore, the findings indicated that the aforementioned proteins may be used as potential biomarkers for beef color quality.

CONCLUSIONS

Analytical tools in MS and proteomics have been successfully used in characterizing the biochemical basis of meat color. Future research should address the relationship between the static genome and dynamic proteome (antemortem as well as postmortem) governing the muscle- and species-dependent color stability of skeletal muscles to enable members of the livestock industry to engineer novel strategies to supply high-quality meat to consumers.

REFERENCES

- Alderton, A. L., C. Faustman, D. C. Liebler, and D. W. Hill. 2003. Induction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonenal. *Biochemistry* 42:4398–4405.
- Faustman, C., D. C. Liebler, T. D. McClure, and Q. Sun. 1999. α , β -Unsaturated aldehydes accelerate oxymyoglobin oxidation. *J. Agric. Food Chem.* 47:3140–3144.
- Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J. Food Sci.* 54:858–862.
- Giardina, B., P. Ascenzi, M. E. Clementi, G. De Sanctis, M. Rizzi, and M. Coletta. 1996. Functional modulation by lactate of myoglobin. A monomeric allosteric hemoprotein. *J. Biol. Chem.* 271:16999–17001.
- Joseph, P. 2011. Proteomics of color phenomena in red meats and poultry. Ph.D. Dissertation, University of Kentucky, Lexington, KY, USA.
- Joseph, P., S. P. Suman, and C. M. Beach. 2010a. Mass spectrometric identification of amino acid residues susceptible to aldehyde adduction in carboxymyoglobin. Abstr. 192-03 in Inst. Food Technol. Annu. Mtg., Chicago, IL.
- Joseph, P., S. P. Suman, S. Li, C. M. Beach, and J. R. Claus. 2010b. Mass spectrometric characterization and thermostability of turkey myoglobin. *Lebensm. Wiss. Technol. Food Sci. Technol.* 43:273–278.
- Joseph, P., S. P. Suman, S. Li, C. M. Beach, L. Steinke, and M. Fontaine.

- 2010c. Characterization of bison (*Bison bison*) myoglobin. *Meat Sci.* 84:71–78.
- Joseph, P., S. P. Suman, S. Li, J. R. Claus, M. Fontaine, and L. Steinke. 2011. Primary structure of turkey myoglobin. *Food Chem.* 129:175–178.
- Joseph, P., S. P. Suman, R. A. Mancini, and C. M. Beach. 2009. Mass spectrometric evidence for aldehyde adduction in carboxymyoglobin. *Meat Sci.* 83:339–344.
- Lee, S., S. T. Joo, A. L. Alderton, D. W. Hill, and C. Faustman. 2003. Oxymyoglobin and lipid oxidation in yellowfin tuna (*Thunnus albacares*) loins. *J. Food Sci.* 68:1664–1668.
- Maheswarappa, N. B., C. Faustman, N. Tatiyaborworntham, S. Yin, R. Ramanathan, and R. A. Mancini. 2009. Mass spectrometric characterization and redox instability of turkey and chicken myoglobins as induced by unsaturated aldehydes. *J. Agric. Food Chem.* 57:8668–8676.
- Mancini, R. A., S. P. Suman, M. K. R. Konda, R. Ramanathan, P. Joseph, and C. M. Beach. 2010. Mass spectrometric investigations on lactate adduction to equine myoglobin. *Meat Sci.* 85:363–367.
- Naveena, B. M., C. Faustman, N. Tatiyaborworntham, S. Yin, R. Ramanathan, and R. A. Mancini. 2010. Detection of 4-hydroxy-2-nonenal adducts of turkey and chicken myoglobins using mass spectrometry. *Food Chem.* 122:836–840.
- Phillips, A. L., C. Faustman, M. P. Lynch, K. E. Govoni, T. A. Hoagland, and S. A. Zinn. 2001. Effect of dietary α -tocopherol supplementation on color and lipid stability in pork. *Meat Sci.* 58:389–393.
- Ponce-Alquicira, E., and A. J. Taylor. 2000. Extraction and ESI-CID-MS/MS analysis of myoglobins from different meat species. *Food Chem.* 69:81–86.
- Sayd, T., M. Morzel, C. Chambon, M. Franck, P. Figwer, C. Larzul, P. L. Roy, G. Monin, P. Cherel, and E. Laville. 2006. Proteome analysis of the sarcoplasmic fraction of pig semimembranosus muscle: Implications on meat color development. *J. Agric. Food Chem.* 54:2732–2737.
- Suman, S. P., C. Faustman, S. L. Stamer, and D. C. Liebler. 2006. Redox instability induced by 4-hydroxy-2-nonenal in porcine and bovine myoglobins at pH 5.6 and 4°C. *J. Agric. Food Chem.* 54:3402–3408.
- Suman, S. P., C. Faustman, S. L. Stamer, and D. C. Liebler. 2007. Proteomics of lipid oxidation-induced oxidation in porcine and bovine oxymyoglobins. *Proteomics* 7:628–640.
- Suman, S. P., P. Joseph, S. Li, C. M. Beach, L. Steinke, and M. Fontaine. 2010. Amino acid sequence of myoglobin from emu (*Dromaius novaehollandiae*) skeletal muscle. *Meat Sci.* 86:623–628.
- Taylor, A. J., R. Linforth, O. Weir, T. Hutton, and B. Green. 1993. Potential of electrospray mass spectrometry for meat pigment identification. *Meat Sci.* 33:75–83.