Differential alkylation of myoglobin by 4-hydroxy-2-nonenal in high and normal pH beef

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Objective: Previous studies have characterized the role of secondary lipid oxidation products, such as 4-hydroxy-2-nonenal (HNE), in myoglobin redox stability. Alkylation of histidine residues by HNE accelerated oxidation and compromised redox stability of myoglobin. Recently in situ HNE alkylation has been documented in histidine and lysine residues of myoglobin from postmortem beef muscles at typical meat pH (5.6). Although in vitro studies using model systems have demonstrated increased HNE alkylation in myoglobin at high pH than at typical meat pH, no studies have determined HNE alkylation in myoglobin at high pH in situ. Therefore, the overall goal of this study was to determine the extent and biochemistry of HNE alkylation in myoglobin from high- pH beef compared with normal-pH beef.

Materials and Methods: Eight (n = 8) normal-pH and high-pH dark-cutting beef carcasses (24 h postmortem) were selected from a commercial beef processor. The normal-pH and high-pH beef loins (longissimus lumborum muscles) were selected based on the pH values to determine HNE alkylation in myoglobin. Steaks fabricated from both normal-pH and high-pH loins were placed in a Styrofoam tray, aerobically packaged in polyvinyl chloride overwrap, and randomly assigned to refrigerated retail display for days 0 and 3. Surface color was measured using a HunterLab MiniScan spectrophotometer on day 0 and day 3. Steak samples were col- lected on day 0 and day 3 to determine HNE alkylation in myoglobin. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis was used to separate myoglobin from other sarcoplasmic proteins. The protein band (17 kDa) representing myoglobin in the gels was excised and subjected to liquid chromatography-electrospray ionization-tandem mass spectrometry. Mass spectrometric data were analyzed using Proteome Discoverer for identification of HNE alkylation at histidine and lysine residues in beef myoglobin.

Results: The average pH values of normal and high-pH loins were 5.4 and 6.8, respectively. Dark-cutting high-pH steaks (L^* values = 32.8) exhibited lower ($P < 0.05$) lightness than normal-pH steaks (L^* values = 48.1). The redness of normal-pH steaks decreased with storage time, while no loss in redness was noticed in dark-cutting high-pH steaks. Tandem mass spectrometry identified HNE alkylation at multiple histidine and lysine residues in beef myoglobin. While 12 residues (4 lysine and 8 histidines) were alkylated in myoglobin from normal-pH beef on day 0, only 9 residues (6 lysine and 3 histidines) were modified in myoglobin from high-pH dark-cutting on day 0. On the other hand, 11 sites (4 lysine and 7 histidines) were modified in normal-pH beef on day 3 and 18 resi- dues (8 lysine and 10 histidines) were alkylated in high-pH darkcutting beef on day 3. Alkylation of distal histidine (position 64) was observed in dark-cutting samples on day 0 and day 3, whereas this was present only on day 0 in normal-pH samples. Proximal histidine (position 93) was alkylated in day 0 dark-cutting samples and day 3 normal-pH samples.

Conclusion: Differential alkylation by HNE was observed in myoglobin from normal-pH and high-pH dark-cutting beef samples. Greater number of alkylated sites in dark-cutting beef on day 3 could be attributed to its greater pH, which makes lysine and histi- dine in myoglobin more susceptible to nucleophilic attack by HNE than at pH 5.6. High muscle pH (closer to physiological pH) protects myoglobin against oxidation and enhances metmyoglobin reduction. This protective effect of high-pH may have contribut- ed to the lack of HNE-induced discoloration in dark-cutting beef. Further studies are required to elucidate the mechanistic bases of HNE alkylation of myoglobin in dark-cutting beef.

Key words: Post-translational modification, Beef color, 4-hydroxy-2-nonenal, Lipid oxidation, Proteomics