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Thermal Stability of Beef Myoglobin is Compromised by Reactive Lipid Oxidation Products

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Objectives

Brown color of cooked meat is the result of heat-induced denaturation of myoglobin (Mb). The denaturation temperature of Mb is governed by its redox state in raw meat; metmyoglobin (MMb) undergoes denaturation at a lower temperature than oxymyoglobin (OMb) and deoxymyoglobin (DMb). The secondary products of lipid oxidation, such as the 4-hydroxy-2-nonenal (HNE), compromise Mb redox stability and can thus impact cooked meat color. While previous investigations extensively studied lipid oxidation-induced Mb redox instability, studies are yet to be undertaken to examine the relationship between lipid oxidation and Mb thermal stability. Therefore, the objective of the present study was to investigate the direct influence of lipid oxidation (using HNE as a model aldehyde) on thermal stability of beef Mb at typical meat conditions.

Materials and Methods

Beef Mb was purified, and OMb was incubated with HNE (0.15 mM Mb + 1.0 mM HNE) at pH 5.6 and 4°C for 21 d. The controls consisted of OMb plus a volume of ethanol used to deliver HNE to treatments. The samples were scanned spectrophotometrically from 650 to 500 nm on d 0, 7, 14, and 21, and MMb formation was calculated. The Mb samples were removed on d 0, 7, 14, and 21, and were digested with trypsin. The tryptic peptides were analyzed using liquid chromatography tandem-mass spectrometry (LC-MS/MS) for detecting HNE adduction sites. The thermal stability of Mb in the presence of HNE was assessed on d 0, 7, 14, and 21 by determining the percentage myoglobin denaturation (PMD) at 71°C in a water bath for 10 min. The experiment was replicated three times (n = 3). The effects of HNE on Mb redox and thermal stabilities during incubation were evaluated using the mixed procedure of SAS. The differences among means were detected at the 5% level using the least significant difference (LSD) test.

Results

While MMb formation increased ($P < 0.05$) during the storage in both control and HNE-treated samples, the oxidation was higher ($P < 0.05$) in HNE-treated samples. The PMD values increased ($P < 0.05$) in both treatments during the storage, and the HNE-treated samples exhibited greater ($P < 0.05$) PMD than the controls throughout the storage. Additionally, the PMD difference between HNE-treated and control samples increased over time. The LC-MS/MS analyses indicated that the number of histidines adducted by HNE increased with storage. HNE adducted four histidines (positions 24, 36, 93, and 152) on d 7, whereas five (positions 24, 36, 64, 93, and 152) and six (positions 24, 36, 64, 93, 113, and 152) residues were adducted on d 14 and 21, respectively.

Conclusion

The mass spectrometric data indicated that thermal stability of beef Mb was compromised by reactive lipid oxidation products. The HNE adduction at the distal histidine (position 64), which is critical to heme stability, observed on d 14 and 21 as well as the increased number of histidines adducted by HNE on d 14 and 21 could be the possible reasons for the increased PMD on

these time points. The adduction of HNE to histidines can alter the heme protein's tertiary structure and thus exposes the heme to oxidation, thereby accelerating the formation of MMb, which is more susceptible to thermal denaturation than the ferrous Mb redox forms.