INFLUENCE OF 4-OXO-2-NONENAL ON MYOGLOBIN REDOX INSTABILITY

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I. INTRODUCTION

The products of lipid peroxidation, such as alpha-beta-unsaturated aldehyde 4-hydroxy-2-nonenal (4-HNE), is a reactive substance towards the proteins that influence meat quality. 4-HNE is a stable lipid hydroperoxides that cause extensive cellular damage and oxidative pathology in meat. Glycolytic enzymes such as lactate dehydrogenase (LDH), has been shown to be highly reactive with 4-HNE and become less functional. While 4-HNE has been studied extensively in model food systems, research documenting influence of 4-oxo-2-nonenal (4-ONE) affecting LDH activity and its influence on myoglobin (Mb) redox stability and meat quality deterioration has never been reported. We hypothesized that reactive aldehyde, 4-ONE induces Mb redox instability and oxidative pathology to moieties of glycolytic enzymes (LDH) and mitochondrial functions. Research in human medicine has shown 4-ONE to be "both cytotoxic and protein reactive than 4-HNE" (De Lin 2005). While structurally similar, 4-ONE is more reactive towards thiols and amine groups than 4-HNE. Both of these functional groups impart form and function to both structural and catalytic proteins. Given the lack of data on 4-ONE in meat quality research, the objectives of this study were 1) to characterize 4-ONE's effects on lactate dehydrogenase activity, Mb, and mitochondrial ultrastructure and 2) to compare these effects to the more well-known 4-HNE. Results showed that 4-ONE inhibited LDH activity at both pHs 5.6 and 7.4. Electron microscopy observations revealed evidence of induced severe vacuolization and rupturing of mitochondrial membranes after incubation with 4-ONE at pH 5.6 and 7.4. The findings of this study support the contention that 4-ONE inhibits LDH activity and caused extensive damage to mitochondrial functions and induced Mb redox instability.

II. MATERIALS AND METHODS

Active LDH (Type III; 20 units) were incubated with 4-ONE or 4-HNE or an equal volume of ethanol (positive control) to deliver the aldehyde in either pH 5.6 or 7.4 in phosphate buffer (100 mM) for 72 hours according to methods of A. Purohit *et. al*., (2014). The reaction solution contained 50 mM pyruvate and 0.2 mM NADH, with NADH added last to initiate the reaction. Reaction progress was monitored with a UV-VIS spectrophotometer by recording a decrease in absorbance at 340 nm. For pyruvate trapping, 50 units of glutamate pyruvate transaminase were added to the reaction to sequester pyruvate by conversion to alanine. Completion of chemical reaction was monitored with a Shimadzu UV-Vis Spectrophotometer by recording an increase in absorbance at 340 nm, indicating NADH formation (Bergmeyer 1974). Mitochondrial ultrastructure study was performed to assess 4-ONE abilities to damage mitochondrial structural integrity. Mb (0.15 mM) was incubated with either 4-ONE or 4-HNE and diluted with 50% methanol (v/v) and 0.1% (v/v) formic acid to a final concentration of 0.0375 mM for injection into a Waters Micromass quadrupole Time-of-flight mass spectrometer. The 800-3000 Dalton range was scanned for multiply charged peaks and MaxEnt 1Analysis software was used to deconvolute the spectra obtained. For mitochondrial electron microscopic experiment, mitochondria, were processed according to briefly modified methods of R. Ramanathan 2012. Images were captured using a JEOL JEM1011 a 100kV transmission electron microscope, with a magnification of 10,000×.

III. RESULTS AND DISCUSSION

A significant loss in LDH activity was observed after incubation with 4-ONE as compared with 4-HNE and control. The reaction (NAD + Lactate = NADH + Pyruvate) at pH 5.6 (Figure 1A) and pH 7.4 (Figure 1B) was inhibited ($P<0.05$) by 4-ONE (at all concentrations) examined in this study. The inhibitory effect of 4-ONE on LDH activity was far greater than 4-HNE with a steady decrease in specific activity. A 4 mM concentration of 4-ONE eliminated LDH activity almost entirely. Mass spectrometry results confirmed the covalent modification of reduced Mb by 4-ONE. Figure 2 shows the gradual oxidation of Mb at pH 5.6 at 4°C. Differences between

the treatments and control were significant (P<0.05). At pH 5.6, differences between Mb oxidation in the presence of 4-ONE and 4-HNE were noticeable. The data reported here confirms that the redox destabilizing effect of 4-ONE is comparable to that of 4-HNE. Figure 3 (panels A and B) shows control mitochondria, incubated in 50 mM phosphate buffer at pH 7.4 or 5.6 respectively, with a volume of ethanol equivalent to what was used to deliver the 4-ONE or 4-HNE. Mitochondrial membranes are mostly intact, with minimal or no outer membrane disruption. Panels C and D of figure 3 show mitochondria that were incubated with 0.4 mM 4-ONE, at pH 7.4 or 5.6 respectively. Significant alterations in mitochondrial morphology are observed. Panels E and F of figure 3.6 show mitochondria incubated with 0.4 mM 4-HNE at 7.4 or 5.6 respectively. These samples exhibit significant disruption of mitochondrial ultrastructure, confirming that these chemicals are capable of effecting organelles as well as enzymes and pigments in meat. The deterioration of mitochondria further contributes to the deleterious effects of the compounds investigated in this study.

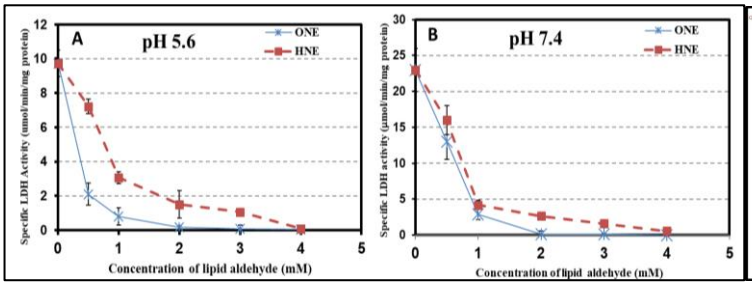

Figure 1A and 1B Effect of 4-ONE and 4-HNE on LDH activity at pH 5.6

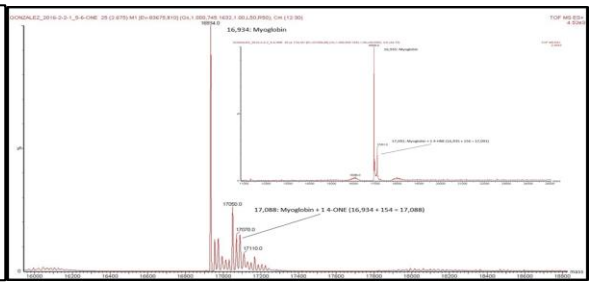

Figure 2 Mass spectrum of Mb with 1mM 4-ONE at pH 5.6

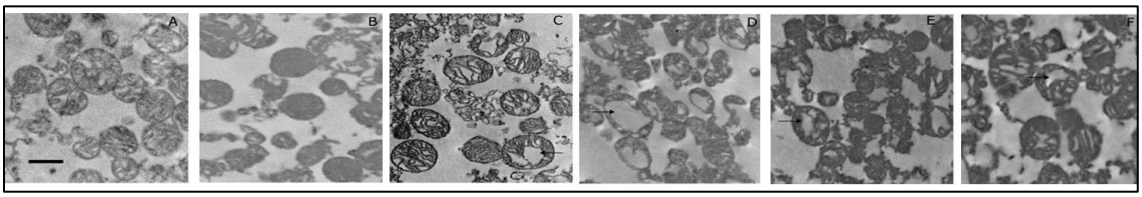

Figure 3 Electron micrographs of mitochondria incubated in a) pH 7.4 buffer, b) pH 5.6 buffer, c) pH 7.4 buffer with 0.4 mM 4-ONE, d) pH 5.6 buffer with 0.4 mM 4-ONE, e) pH 7.4 buffer with 0.4 mM 4-HNE, and f) pH 5.6 buffer with 4-HNE. The scale bar in panel A corresponds to 500 nm and is the same for panels A-F. Magnification = 10,000x. Arrows in panels C-F indicate mitochondrial vacuolization.

IV. CONCLUSION

This study evaluated influence of 4-ONE on LDH activity and Mb redox instability. Biochemical reactions of 4-ONE with Mb showed covalent modification of Mb. Influence of 4-ONE on Mb redox stability was more pronounced than 4-HNE at both pHs (5.6 and 7.4). Mass spectrometric study showed covalent adduct formation of Mb redox reactions with 4-ONE and 4-HNE. Reactions of 4-ONE with Md redox status revealed more complex pattern of spectrometric signals showing adducts formation with 4-ONE. Electron microscopic study revealed 4-ONE induced severe vacuolization and rupturing of mitochondrial outer membrane. Biochemical reaction of LDH, Mb redox states, and 4-ONE appear interlinked and the oxidation of one of either LDH or Mb redox forms leads chemical species that exacerbate deterioration of meat color and overall quality. Further study is in progress to determine the influence of species specific 4-ONE influence on Mb redox instability under different packaging and storage environment.

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