# New Insights into Heme Proteins-Mediated Lipid Oxidation in Meat: Mechanisms and Inhibition

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#### I. INTRODUCTION

Lipid oxidation leading to quality deterioration is a critical issue and a hot topic of research in meat processing and storage [1]. This study, utilizing a multidisciplinary approach that includes model methodologies, molecular chemistry, structural biology, and computational simulations, provides new insights into the dynamic molecular mechanisms of lipid oxidation in meat and develops strategies for its control.

## II. MATERIALS AND METHODS

Hemoglobins (Hbs) were prepared from blood using heparin anticoagulant (120 Units/mL) as described previously [2]. Washed muscle from fresh pig, cod, and turkey was prepared as described previously [3]. Muscle structures were examined, and images were taken in a JEM-1011 electron microscope (JEOL, Japan). The covalent binding between quercetin and turkey Hb was determined by ESI-MS as described previously [4]. Quercetin and its quinone form were docked to structure of turkey Hb tetramer using AutoDock vina program. The crystal structure of Hb was retrieved from the protein data bank. Experiments in this study were repeated at least three times.

## III. RESULTS AND DISCUSSION

Uncover the molecular mechanisms of heme protein-mediated lipid oxidation in meat: Research has established heme proteins as the principal pro-oxidants in muscle foods [5]. Yet, the critical step associated with lipid oxidation remains unclear due to the concurrent occurrence of several processes, including heme protein autooxidation, ferryl radical formation, hemin release, heme protein crosslinking, and iron release. In this study, we used various models as well as real meat to identify the key steps of heme proteins-meditated lipid oxidation and found that the oxidation and dynamic dissociation of heme iron porphyrin molecules are central molecular drivers of lipid oxidation in meat (Figure 1).



Figure 1. Key processes of heme proteins-induced lipid oxidation in meat products

The role of phospholipids in meat products: From a conventional viewpoint, the amount of phospholipids and the elevated unsaturation of the acyl chains of phospholipids play important roles regarding onset of lipid oxidation in muscle [1]. However, in our study, we observed that adding myoglobin (Mb) increased lipid oxidation in washed cod muscle (WCM) but not in washed pig muscle (WPM) (Figure 2A). Added phospholipids with polyenoic indexes of 282 and 24 activated Mb as an oxidant similarly in WPM (Figure 2B) [6]. The differing microstructure of WCM e.g. more exposed fat cells or membrane of muscle cells compared to the "denseness" or "wrapped" structure of WPM, may have contributed to the better ability of Mb to facilitate lipid oxidation in the WCM.



Figure 2. Oxidation performance of phospholipids in cod and pig under the condition of myoglobin (Mb) as a pro-oxidant, along with their muscle structures [6]. Replicates per treatment was n = 3. Means and standard deviations are shown. (A) washed pig muscle and washed cod muscle treated with Mb; (B) washed pig muscle and treated with lipids (NL: Neutral lipids, FFA: Free fatty acids, PL: Phospholipids) and Mb during 2 °C storage.

The antioxidant effect of polyphenols on Hb-mediated lipid oxidation: Numerous studies have demonstrated that free radical scavenging and metal chelation could be the key factors responsible for the antioxidative activities of flavonols. In present study, we found a novel mechanism by which polyphenols with  $\pi$ -conjugated structures, covalently bind to hemoglobin at specific sites near the iron porphyrin, such as the  $\alpha$  chain Cys(H15) site (Figure 3). This interaction inhibits the oxidation and dissociation of iron porphyrin molecules, thereby diminishing their pro-oxidative activity [7].





#### IV. CONCLUSION

The oxidation and dynamic dissociation of heme iron porphyrin molecules are central molecular drivers of lipid oxidation in meat. Second, the muscle microstructure and the accessibility of heme proteins to phospholipids that are the dominant factors influencing lipid oxidation. Third, the study reveals a novel mechanism where polyphenols covalently bind to hemoglobin near the iron porphyrin, inhibiting oxidation and dissociation of iron porphyrin molecules and reducing their pro-oxidative activity.

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