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EFFECT OF MITOCHONDRIA ON BEEF COLOR STABILITY VIA MYOGLOBIN AFFINITY FOR OXYGEN

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I. INTRODUCTION

Growing concerns over future protein shortages have driven efforts to develop alternative protein sources and enhance the sustainability of the meat industry. A major challenge in meat production is preventing discoloration, which leads to significant food waste. In this context, Ramanathan et al. [1] highlighted that reducing meat discoloration could have substantial economic and environmental benefits. Discoloration occurs due to the oxidation of myoglobin (Mb), a protein responsible for oxygen storage in muscles. Several factors affect Mb oxidation, including temperature, oxygen levels, pH, Mb reductase activity, and light exposure [2], with oxygen levels being the most influential. When the partial pressure of oxygen decreases, Mb supplies oxygen to the mitochondria, where respiration occurs. However, when the bond between Mb and oxygen becomes weak, oxygen molecules exchange more rapidly, thereby accelerating the oxidation rate of Mb [3]. This suggests that the oxygen affinity of Mb determines the oxidation rate under different oxygen concentrations. Despite this, the effect of oxygen affinity on the oxidation rate of meat is not well understood. Waga et al. [4] demonstrated that mitochondria affect the oxygen affinity of Mb in beef and that factors like pH and aging further influence this relationship. These findings suggest that mitochondrial conditions could regulate the oxygen concentration levels that promote beef oxidation. To investigate this, we examined whether mitochondria influence the color stability of beef by altering the oxygen affinity of Mb.

II. MATERIALS AND METHODS

Effect of mitochondria on Mb affinity for oxygen: Mitochondrial fractions were obtained from bovine *biceps femoris* muscle. Oxymyoglobin (OxyMb) was prepared by reducing equine Mb (SERVA Electrophoresis, Heidelberg, Germany) with sodium hydrosulfite, followed by desalting. The affinity of Mb for oxygen ($MbAfO_2$) was assessed by determining the oxygen concentration at which the deoxygenation ratio reached 50%, referred to as the partial pressure at 50% saturation (P_{50}) [4]. This evaluation was conducted using a solution of OxyMb with mitochondria, either with or without the addition of sodium succinate.

Measurement of oxygen levels and oxygen consumption rate: A non-contact oxygen analyzer (OXY-1ST, PreSens, Regensburg, Germany) was used to measure oxygen concentration. Solution samples were tested in quartz cells, or 5 mL vials with sensor chips affixed to the bottom of these containers [4]. For meat samples, the sensor tip was placed inside sealed bags. The oxygen consumption rate was quantified as the rate at which oxygen was depleted in the container per hour ($-\Delta O_2\%/h$).

Effect of tartrate on beef oxidation rate at different oxygen concentrations: To assess the impact of tartrate on oxidation, 10 grams of beef, either supplemented with 10 mmol/L tartrate or untreated as a control, were sealed in bags after a nitrogen purge. The purge duration was adjusted to vary oxygen levels. Meat samples were spread on a thin high-density polyethylene (HDPE) sheet (oxygen transmission rate > 2,000 mL m^{-2} d^{-1} atm^{-1}) to a thickness of approximately 5 mm, stretched over a hollow frame. Oxygen concentration in the bags and the percentage of metmyoglobin (MetMb %) were measured after 24 h of storage in a refrigerator at 4 °C. The MetMb (%) was calculated using the formula described by Tang et al. [5].

Statistical analysis: At least two independent trials were conducted for each test to determine the mean and standard error unless otherwise stated. A significance threshold of $P < 0.05$ was used. A two-factor analysis of variance (ANOVA) was performed to examine the interaction between mitochondria and tartrate on P_{50} values. Differences relative to the control were evaluated using Dunnett's test. Additionally, an analysis of covariance (ANCOVA) was applied to assess the effect of tartrate on beef oxidation, with sodium tartrate as a fixed effect and oxygen concentration as a covariate.

III. RESULTS AND DISCUSSION

Fig. 1 illustrates the effect of tartrate on $MbAfO_2$. The two-factor ANOVA revealed that tartrate and mitochondria interacted with $MbAfO_2$ ($P = 0.016$). As is evident from Fig. 1, the presence of mitochondria significantly decreased

$MbAfO_2$ ($P < 0.05$). However, the addition of tartrate reversed this effect, increasing $MbAfO_2$ to levels similar to those found in the absence of mitochondria. Notably, tartrate did not affect $MbAfO_2$ in samples without mitochondria. These findings indicate that tartrate mitigates the mitochondria-induced reduction in $MbAfO_2$.

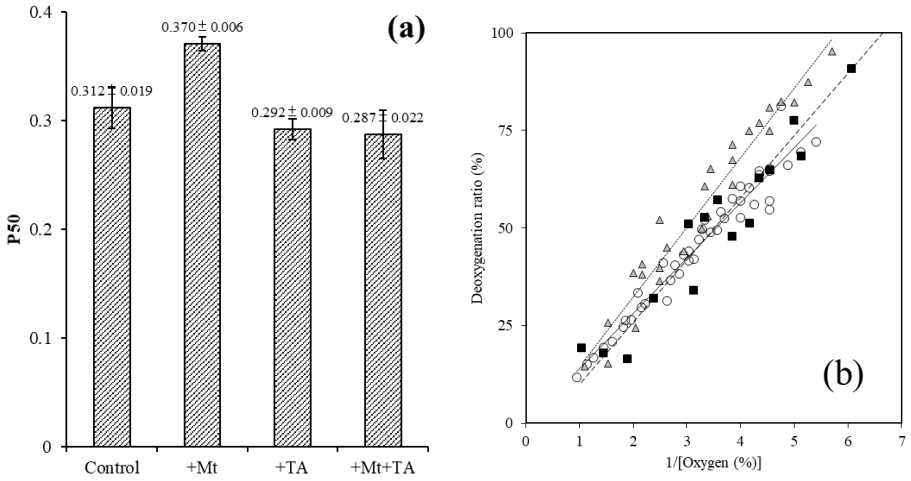


Fig. 1 The effect of tartrate on myoglobin affinity for oxygen.
(a) Mean ± standard error of P50 values are shown. +Mt: myoglobin with mitochondria; +TA: myoglobin with tartrate; +Mt+TA: myoglobin with mitochondria and tartrate. (b) The deoxygenation ratio is shown against the inverse of the oxygen concentration. White circles with the solid line: Control (myoglobin); gray triangles with the dotted line: +Mt; black square with the dashed line: +Mt+TA.

Fig. 2 demonstrates how oxygen concentration influences MetMb (%) levels in beef. It is observed that when the oxygen concentration was above 1%, the MetMb (%) content in the beef treated with tartrate was lower than the control ($P < 0.001$). However, when the oxygen concentration was below 1%, the MetMb (%) was higher than that of the control ($P < 0.001$). Tartrate produces nicotinamide adenine dinucleotide (NADH) via dehydrogenase [6], potentially stabilizing meat, similar to lactate [7]. However, this hypothesis does not explain the accelerated oxidation at low oxygen concentrations. A more plausible explanation is that the increased $MbAfO_2$ shifts the oxygen concentration range, leading to earlier oxidation at lower oxygen levels.

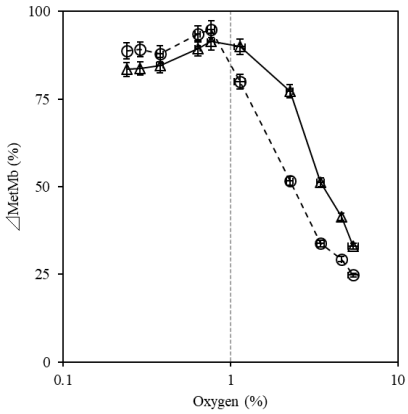


Fig. 2 The effect of tartrate on the oxidation rate of beef in low oxygen concentrations.
The increase in the MetMb ratio during storage (mean ± standard error) is shown against the oxygen concentration. Open triangles with solid lines are used for control samples; circles with dashed lines are used for samples treated with

IV. CONCLUSION

Tartrate treatment revealed that oxygen affinity regulates the oxygen concentration at which beef discoloration promotes. As beef ages and mitochondrial activity declines, its oxygen affinity rises, making oxidation occur at lower oxygen levels.

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