

# Effects of Temperature on the NADH-dependent Reductase Activity



S. C. Spring, A. K. Abraham, B. A. Djima, R. Ramanathan  
Department of Animal Science, Oklahoma State University, Stillwater, USA.

## Introduction

- The USDA recommends to cook beef to an internal temperature of 71 °C. This ensures that the pathogens like E. coli are killed.
- Consumers do not always use meat thermometers and rely heavily on the color of the cooked meat to tell when it is fully cooked.
- Premature browning is a condition in cooked beef wherein myoglobin denaturation and subsequent browning occurs at a lower temperature that is needed to kill foodborne pathogens.
- Cooked color is due to myoglobin denaturation and it depends on the form of myoglobin present within the interior of steaks or patties. Oxymyoglobin and metmyoglobin forms are very susceptible to heat.
- NADH-dependent reductase enzyme has the ability to convert metmyoglobin to deoxy/oxymyoglobin. Hence, it can play a significant role in cooked color.
- There have not been many studies over specific enzymes and their stability during the process of being cooked and how this can better help us understand premature browning.
- The enzyme, reductase, has the capacity to converting metmyoglobin back to oxymyoglobin.
- Understanding what happens to reductase when it is heated ie cooking the meat can help us to know more about the effects of temperature on enzyme activity.

## Materials and Methods

### Enzyme Preparation:

- Enzyme reductase was isolated from beef cardiac muscle according to Hagler & Faustman method. The cardiac muscle was homogenized and the proteins were precipitated by ammonium sulfate.
- The pellets were dialyzed to remove ammonium sulfate, and the enzyme was further purified by cation and anion exchange chromatography.
- The purified enzyme was tested for the activity and the samples with NADH-dependent reductase activity were pooled.

### Determination of Enzyme Activity:

- The assay mixture contained 0.15 mM metmyoglobin in either 50 mM phosphate buffer at pH 5.6 or 6.4, 0.1 mL of 5 mM EDTA, 0.1 mL of 3.0 mM potassium ferrocyanide, and 0.1 mL of purified NADH dependent reductase.
- Using a spectrophotometer, the activity was recorded in kinetic mode with an absorbance set at 582 nm.
- Metmyoglobin reduction was expressed as nanomoles of metmyoglobin reduced per minute per milligram of reductase

### Heating of Enzyme:

- Enzyme reductase was heated to different temperatures (45, 50, 55, and 60 °C) using a hot water bath.

### Heating of myoglobin:

- In addition to determining the effect of enzyme, in an another experiment, myoglobin was heated to different temperatures to study its reducing activity of enzyme (without heating).

## Results

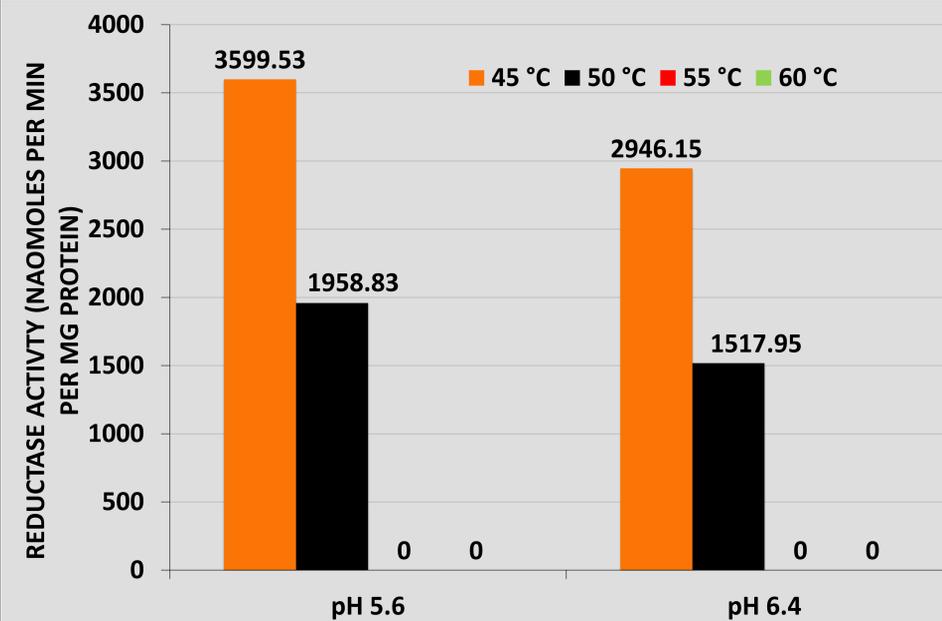


Figure 1: Effects of temperature on reductase activity at pH 5.6 and 6.4 in vitro

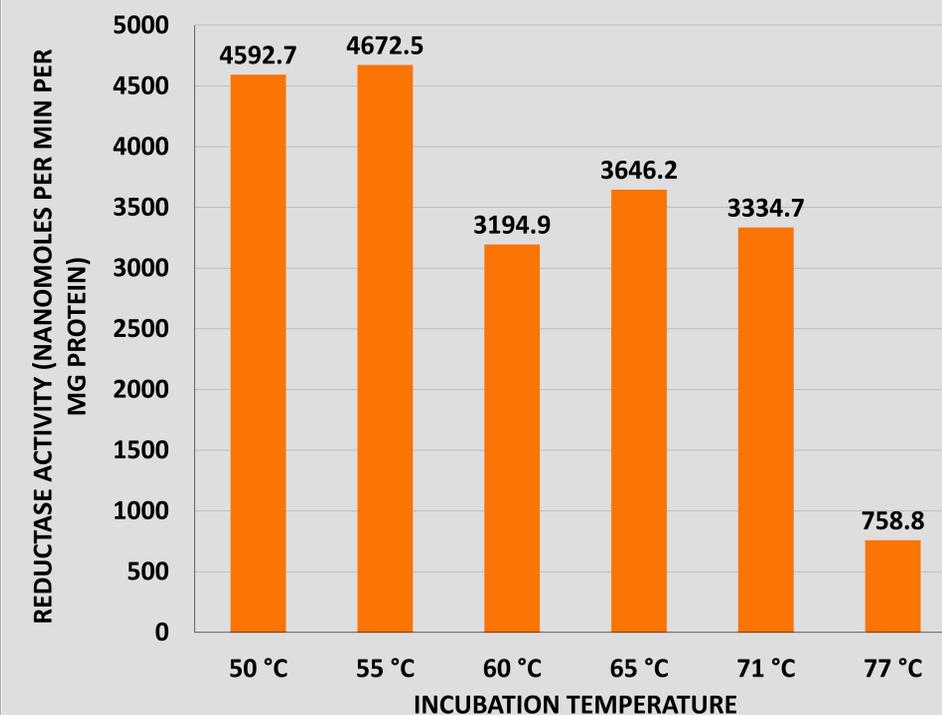


Figure 2: Effects of myoglobin denaturation on reductase activity at pH 5.6 in vitro

## Results

- The experimental design was a completely randomized design with repeated measures, and each experiment was replicated five times (n = 5).
- Data were analyzed using the MIXED Procedure of SAS.
- The diff option (LSD at P < 0.05 for significance) was used to separate least squares means from protected F-tests (P < 0.05).
- When the enzyme reductase was heated past the temperature of 50 °C there was no enzyme activity at pH of 5.6 and 6.4 (P < 0.05; Figure 1).
- This means that when the beef is cooked past the temperature of 50 °C, reductase enzyme is not doing the reverse reaction, thus being one of the causes of premature browning.
- It was also shown that for the pH of 5.6 the enzyme activity was greater compared with pH of 6.4.
- Interestingly, enzyme can reduce myoglobin heated up to 71 °C. However, myoglobin when heated to 77 °C significantly decreased the activity (P < 0.05; Figure 2).



Figure 3: Enzyme activity at different temperatures

## Conclusion

- NADH-dependent reductase enzyme is very sensitive to temperature
- NADH-dependent reductase enzyme has the ability to reduce metmyoglobin when heated to 71 °C

## Acknowledgements

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