

## **Project Summary**

**Engineering Optical Biosensors to Detect Calpastatin, a Meat Tenderness Indicator**

**Principal Investigator: C.L. Lorenzen, Ph.D.,  
University of Missouri**

**Study Completed  
May 2008**



*Funded by The Beef Checkoff*

# Engineering Optical Biosensors to Detect Calpastatin, a Meat Tenderness Indicator:

## Project Summary

### Background

The development of instrument grading tools has been a priority of the beef industry for a number of years. Great strides have been made through this research and the USDA Agricultural Marketing Service has adopted standards that allow graders to use both yield and quality grade factors collected by instruments online. Previous research has focused on developing a biosensor that can accurately predict calpastatin and then be incorporated into an instrument that can be used at the time of grading to sort cattle based on their predicted tenderness value.

The objectives of this project were to:

1. Establish a relationship between an in-solution dual binding technique, calpastatin assays and Warner-Bratzler shear force;
2. Immobilize the dual binding agents onto optical fibers and test in a series of complex homogenized beef samples.

### Methodology

Standard calpastatin assays were performed according to Koohmaraie et al. (1995). *Longissimus* muscle samples (n = 18) were collected at 0, 24, 36 and 48 hours postmortem and used for calpastatin activity determination to simulate potential grading time in industry. Calpastatin activity was determined according to procedures of Koohmaraie (1990). The fractions that were eluted were screened to determine which fractions were active for calpastatin. The active and partially active fractions were pooled. Each assay was run in triplicate.

As an instrumental measure of tenderness, Warner-Bratzler shear force (WBSF) was performed on 2.54 cm *longissimus* muscle steaks 14 days postmortem. These values were correlated with calpastatin quantities and pre- and post-column biosensor technique readings. Using a procedure modified from Molecular Probes labeling kits, mouse anti-calpastatin IgG was labeled to the donor fluorophores, Alexa Fluor 546 (AF546), while secondary antibodies (mouse anti-calpastatin IgG2) was labeled to the acceptor fluorophores, Alexa Fluor 594 (AF594). The tips of silica optical fibers (600  $\mu\text{m}$  core diameter) were tapered. Tapering increases the capture of fluorescence into the fiber tip and results in higher signals.

Following the silanization procedure, spectra files from spectrofluorometer scans were exported and converted to text files and pasted into a spreadsheet. Each value from a fiber's background scan was subtracted from the donor-only and final scans at the corresponding wavelength. This removes the background absorption from the scans, leaving the emission spectra of the donor and acceptor peaks. Because baseline shifts occur between scans, the difference between the baseline value (560 nm) from the donor-only scan and the final scan was added to each value of the donor-only curve. To quantify the amount of binding that had taken place, a ratio of donor peak/isosbestic point (D/I) was found. The donor peak was calculated by averaging the intensity values from 570 - 575 nm, and the isosbestic point was calculated by averaging the intensity values near the point where the curves intersect, from 590 - 595 nm. The average donor peak intensity value was divided by the average isosbestic point intensity value to give the D/I value. Finally, the percent change in D/I was calculated and found.

Because the amount of decrease in donor results from the concentration of bound acceptor to which it transfers its energy, the percent change in D/I is a quantitative measure of the amount of acceptor bound to the system relative to the amount of donor. The result is a relative measure of calpastatin concentration in the test sample.

### **Findings**

Initial experiments were conducted to determine the most efficient blocking solution for the proposed system. The change in Acceptor/Donor (A/D) ratio after addition of acceptor-labeled antibody for the fiber exposed to dextran as a blocking solution was 8.8%, whereas the change in A/D ratio for the fiber exposed to nonfat milk as a blocking solution was 4.7%. The purpose of the blocking solution was to minimize this nonspecific binding. Therefore, the most efficient blocking solution would result in negligible differences in A/D ratio after addition of the acceptor-labeled antibody. Consequently, the nonfat milk blocker was utilized in subsequent experiments.

In order to demonstrate that the proposed biosensor was able to detect calpastatin, the system was tested using a known concentration of calpastatin and compared to the same concentration of a nonspecific analyte. The nonspecific protein used was bovine serum albumin (BSA). After addition of either specific or nonspecific antigens and the acceptor-labeled antibody, there was a greater decrease in Donor/Acceptor (D/A) value for the fiber exposed to calpastatin than the fiber exposed to BSA. The ratio was changed to D/A because the donor was scanned first on the biosensor. This indicated an increased acceptor signal and/or decreased donor signal, and that fluorescence resonance energy transfer (FRET) was taking place.

The drop in D/A value for the optical fiber exposed to calpastatin showed that higher levels of binding occurred. Because the binding was higher for calpastatin, this experiment illustrates that the biosensor detects specifically calpastatin. The low D/A value when there was only donor present on the fiber exposed to calpastatin suggested that protein A was poorly immobilized onto the fiber tip surface.

Overall, the calpastatin vs. BSA experiments illustrated that the proposed biosensor had variable results in detecting calpastatin specifically. It was therefore decided to proceed with development of the biosensor using the Fluoromax spectrofluorometer, which provides spectral data that can be analyzed qualitatively as well as quantitatively. Through the experiments, it was decided that more accurate ratiometric measurements can be obtained by determining the D/I ratio instead of the D/A ratio. The biosensor developed in this research significantly correlates the 48 hour pre-column biosensor readings as well as 48 hour post-column with the 48 hour post-mortem traditional calpastatin assay, indicating it is a good measurement of the amount of calpastatin in a beef carcass 48 hours postmortem. It was important to read the calpastatin assays and the biosensor assays around the same time frame to assure that the biosensor readings were true to the amount of calpastatin present in the sample.

The correlation of the biosensor to WBSF with post-column biosensor readings at 24 hours postmortem indicates that not only is the biosensor a representation of calpastatin activity but also of the WBSF of a steak at 14 days postmortem. The highest correlation of calpastatin activity with WBSF was 0.2504 with  $P = 0.316$  for this study. However, the WBSF range reported from the current research was 2.15 to  $5.52 \pm 0.87$ , and there were four readings that were above the consumer acceptable level of tenderness of 2.27 to 3.58 kg. With the variety of genetic variation and

small sample size seen in this project, it would not be discounted to believe that there would be low correlations of calpastatin assays and WBSF.

The recommendation from researchers following this project follows that the calpastatin activity of 48 hr postmortem is most closely correlated with pre-column biosensor readings at 48 hours, therefore these are the measurements that should be taken for detection of calpastatin in a beef carcass.

### **Implications**

The aim of this research was to determine if the previously developed biosensor was capable of detecting specific, biologically active levels of calpastatin in meat and to determine if those levels are correlated with tenderness measurements. This was accomplished with the research and the most correlated measurements were taken at 48 hours postmortem, suggesting that this is the best time for use of the biosensor. Though there is still development that must take place before the integration of this biosensor as a tool in online meat tenderness evaluation, significant progress has been made. The current biosensor would be useful in laboratory determination of differences in biologically active calpastatin concentrations.

### **References**

Koohmaraie, M., S. D. Shackelford, T. L. Wheeler, S. M. Longeran, and M. E. Doumit. 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. *J. Anim. Sci.* 73:3596-3607.

Koohmaraie, M. 1990. Quantification of Ca<sup>2+</sup>-dependent protease activity by hydrophobic and ion-exchange chromatography. *J. Anim. Sci.* 68:659-665.

---

### ***For more information contact:***

National Cattlemen's Beef Association  
9110 East Nichols Avenue  
Centennial, Colorado 80112-3450  
(303) 694-0305