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## **A novel biosensing technique to detect calpastatin, a meat tenderness indicator**

We have been investigating a fluorescence biosensor to detect calpastatin. Calpastatin is a protein found in meat and it is a regulator of meat tenderness. The ability to accurately predict the calpastatin concentration of beef with a biological sensor at the time of grading would lead to a more accurate assessment of the overall palatability of beef. The biosensor technique utilizes FRET (fluorescence resonance energy transfer). FRET requires the use of two fluorophores, termed a donor and acceptor. The FRET dual binding sensing method requires the integration of two binding agents, calpain I and a monoclonal antibody, Mab, with the donor and acceptor fluorophores respectively. When the two labeled binding agents are separated, most of the fluorescence will be at the emission wavelength of the donor. During the binding event, the two FRET labeled “receptors” bind to calpastatin. This binding results in a change in distance between the fluorophores, which initiates a fluorescence from the acceptor fluorophore. The FRET dual binding technique was tested in heated and unheated meat extract (beef). A volume of 1 ml of the meat homogenates was added to 4-ml microcuvettes. Appropriate amounts of labeled calpain I and Mab were added. These pre-calpastatin samples (controls) were then scanned using a spectrofluorometer. Then, the meat homogenate solutions were spiked with various concentrations of calpastatin. The results demonstrated a limit of detection for calpastatin of 120 ng/ml in heated meat extract with no significant response in the unheated meat extract. The proof-of-principle of utilizing a FRET dual binding technique to detect calpastatin in heated meat extract has been established. The next step in development is to determine if the biosensor can detect specific, biologically active levels of calpastatin in meat and to determine if those levels are correlated with tenderness measurements.