SOL-GEL IMMOBILIZED LUCIFERASE-BASED ATP BIOSENSOR FOR MEAT QUALITY DETERMINATION IN POSTMORTEM PIG MUSCLE

A.D. Weaver[1], S.S. Jedlicka[2,4], J.L. Rickus[2,3,4] B.C. Bowker[3] and D.E. Gerrard[1]*

[1] Departments of Animal Sciences and [2] Agricultural and Biological Engineering, Weldon School of Biomedical Engineering, Physiological Sensing Facility at Bindley Bioscience Center, West Lafayette, Indiana USA, [3] USDA-ARS, Food Technology and Safety Laboratory, Beltsville, MD USA. Email: dgerrard@purdue.edu

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Introduction

Pale, soft, exudative (PSE) and associated meat quality defects represent a serious problem facing the pork industry. Not only do these undesirable quality attributes negatively impact further processing potential of a valuable commodity, they also create a level of indecisiveness on the part of consumers wishing to purchase a highly satisfying and nutritious food item. In addition, production of pork with reduced quality robs the industry of potential revenues. R.G. Kauffman (Univ. of Wisconsin) has suggested in his "*Report Assessing the Definable and Potential Losses Due to Quality Problems in the US Pork Industry*" that lowered pork quality costs the industry $100 million annually. Granted, the industry has responded to an increased incidence of adverse quality development and has instigated new processing procedures to alleviate some of the negative attributes of this problem by using various injection or "pumping" strategies; however, the bottom line is that pork possessing these abhorrent traits represents an inefficiency in the food production chain, as muscle protein functionality, and therefore product quality, is less than optimal in some pork carcasses. Unfortunately, the industry has perpetuated this problem by aggressively selecting for improved meat-producing genotypes. As a result, the incidence of pork quality problems has and will continue to increase. Classically, PSE pork results from a rapid rate of muscle metabolism (energy) that is stimulated by ATP depletion during the early postmortem period (Kastenschmidt *et al.*, 1968). This accelerated metabolism results in a low muscle pH while the carcass temperature is still high and leads to severe muscle protein denaturation and thus, diminished pork quality attributes. The other major pork quality aberration, known as 'acid meat', which possesses quality characteristics similar to PSE pork (a pale color, soft texture and an exudative cut lean surface) results from an exaggerated or prolonged energy metabolism postmortem, which results in a greater than normal pH decline in muscle tissues at 24 hr after slaughter. The enzyme luciferase is commonly used to measure ATP in biological samples. This enzyme catalyzes the ATP-dependant oxidation of luciferin and produces light as a consequence:

$$ATP + luciferin + O_2 \xrightarrow[Mg^{2+}]{luciferase} oxyluciferin + AMP + PP_i + CO_2 + light(\lambda_{max}=560nm)$$

In an excess of co-substrates, the light produced correlates with the ATP concentration present. Despite the widespread use of luciferase as a lab-based bioassay for ATP, there are few practical ATP biosensors available, due to the challenge of luciferase immobilization and the instability of the luciferase enzyme in solution. Recently, a biologically friendly method of immobilizing luciferase in a silica-based sol-gel was discovered by Cruz-Aguado, *et al.* (2004). The objective of this study was to explore the use of a silica immobilized luciferase-based optical biosensor in monitoring muscle metabolism postmortem and correlating these results to pork quality.

Materials and Methods

To test the hypothesis that muscle ATP could be used for predicting fresh pork quality, ten crossbred pigs averaging 120 kg were delivered to the Purdue Meat Science Research Laboratory and slaughtered according to normal processing practices, except some carcasses were randomly subjected to varying amounts of electrical stimulation at 10 min to simulate adverse pork quality development (Bowker *et al.*, 1999). At various times postmortem (15, 30, 60, 90 and 120 min), muscle exudates were sampled using cotton swabs. These swabs were immediately subjected to a firefly luciferase assay and luminescence was recorded. After a 24 hr chill, muscle exudates were analyzed using the same protocol and traditional pork quality characteristics were measured. An optical luciferase based biosensor was developed using the immobilization technique of Cruz-Aguado, *et al.* (2004). This method, using a biocompatible sol-gel precursor and a covalently linked stabilizing sugar moiety allows for luciferase activity retention very close to the activity of the enzyme in solution form. Diglycerylsilane (DGS) was synthesized by heating neat tetramethyl orthosilicate (TMOS) and anhydrous glycerol in a 1:2 molar ratio at 110 °C for 15 hours, followed by complete removal of the evolved methanol by distillation at 140 °C (Brook, *et al.*, 2004). DGS was hydrolyzed and mixed at an 80% molar ratio with N-(3-triethoxysilylpropyl) gluconamide (Gelest, Inc.). Luciferase (Sigma, 1:30 volumetric ratio to the sol), D-luciferin (Sigma, 2 mM final concentration), and magnesium chloride (10 mM) were added to the hydrolyzed sol in phosphate buffer, pH 8.0. Bovine serum albumin (0.1 mg/mL), EDTA (0.5 mM) and DTT (0.5 mM) were added as stabilizing agents. The resulting sol mixture was pipetted into capillary tubes containing 1000 µm optical fibres (PolyMicro Technologies). The fibre/silica/enzyme probe was allowed to sit at 4 °C overnight prior to use. To test the efficacy of a luciferase probe, the probes were connected to a jacketed fibre via a bare fibre adaptor. Jacketed fibres were connected to a hand-held USB 2000 spectrometer containing a linear CCD detector (Ocean Optics, Inc.). The

spectrometer was operated from a laptop PC using standard operating software (Ocean Optics, Inc.). Silica-based luciferase probes were tested as 'real-time' biosensors using five crossbred pigs. Pigs were slaughtered under normal processing conditions except one side of each carcass was electrically stimulated (400V, 20 pulses) at approximately 30 min postmortem. Immediately prior to and after stimulation and hourly up to 4 hr postmortem, luciferase containing probes were placed in the longissimus muscle at random locations. Luminescence was recorded using a 5 second integration time for several minutes post-insertion to ensure probe stability, and the luminescence output averaged across the given time period. A new probe was used for each time point and each side of the carcass. Probe reusability will be the focus of future work.

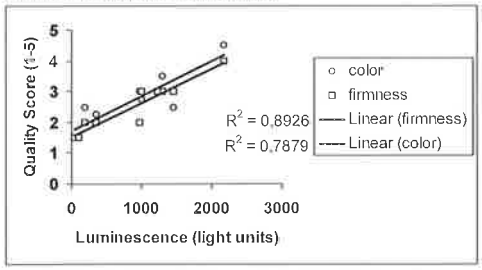

Figure 1: Relationship between luciferase-based luminescence and fresh pork colour and firmness scores.
(1=pale or soft; 5=dark or firm).

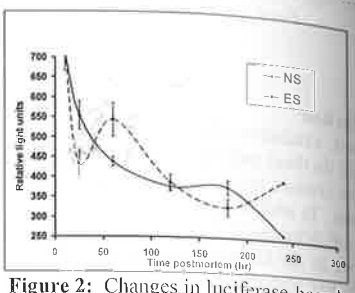

Figure 2: Changes in luciferase-based luminescence (relative light units) early postmortem in electrically stimulated (ES) and control (NS).

Results and Discussion

Figure 1 shows that luminescence in the postmortem exudates correlated with subjective fresh pork colour and firmness scores (0.79 and 0.89, respectively). These data suggest that higher levels of ATP postmortem are useful predictors of pork quality attributes. Curiously, muscle pH around this time postmortem normally correlates well with these traits. Changes in muscle luminescence postmortem parallel pH values using this model. Electric stimulation rapidly increases the consumption of ATP by the muscle (Figure 2) and as a result, anaerobic glycolysis is increased and lactate accumulation is exaggerated compared to non-stimulated carcasses. Although events leading to adverse pork quality development in this manner may not exactly simulate classic PSE development, it is a good model for rapid postmortem muscle metabolism. Additionally, the ability of the luminescence based optical biosensor for ATP to detect the changes in the postmortem muscle tissue indicates that the sensor is a potential option for rapid meat quality testing. Although more work to optimize the probes must still be done, it is a demonstration that a traditional lab-based assay can be translated into an inexpensive, hand-held device for industrial use.

Conclusions

These data suggest that the relative amount of ATP in muscle cells early postmortem is related to pork quality. Based on the results shown, the development of a hand-held real-time biosensor using a luminescence based platform is a viable possibility for the rapid detection of ATP. Additional experiments will be necessary to further support these claims.

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