

Honors Thesis

Presented by Maranda Sauerland

Effect of pH and Temperature on Myoglobin in fresh Meat

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Abstract

Color or appearance of a food product is one of the most important attributes that influences consumers' purchasing decisions. In living muscle tissue, the function of myoglobin is to bind and store oxygen; however, in meat products the concentration and chemical state of this water-soluble protein influences the visual color of meat. Denaturation of myoglobin can result in less desirable appearance and may be associated with other undesirable palatability characteristics in fresh pork, such as Pale Soft Exudative (PSE) pork, which is one of the largest economic burdens to the pork industry. The characteristics of PSE include undesirable color, soft texture, and excessive purge. Pork carcasses that produce PSE product often have an accelerated rate of post-mortem metabolism, which can produce a combination of high temperature and low pH in muscle tissue. The purpose of this study is to determine the effects of specific pH and temperature combinations on the denaturation of porcine myoglobin, mimicking the range of potential environments of postmortem metabolism. Bovine myoglobin was isolated and purified from a fresh beef heart. The myoglobin was reconstituted and subjected to multiple pH levels (ranging from 7.0 to 5.4 in increments of 0.4 pH units). The stability of myoglobin was evaluated at each pH level from 36° C to 44°C in 4°C increments. Samples of myoglobin were evaluated within each of the 15 pH and temperature combinations. Myoglobin fractions were evaluated using spectrophotometry to determine the extent of myoglobin denaturation within each pH and temperature combination. The major finding from this project is that myoglobin is not significantly altered or damaged from the pH and temperature combinations within the physiological range of postmortem metabolism. The combination of high temperature and low pH did not significantly affect the function of myoglobin. The changes in percent oxymyoglobin were not to the extent that would explain the pale color observed in PSE pork.

Background

The swine industry has shifted from lard type hogs to heavier muscled and leaner pigs over the last 50 years. This change was facilitated by genetic selection and improved nutrition. During the last 15 years, the swine industry dramatically increased the percent lean and weight of its carcasses as it has continued to select for heavier muscled and leaner pigs. Although the increase in muscle volume and size has brought about some inherent efficiencies in processing, these changes have also been accompanied by an increasing incidence of some undesirable traits in

fresh pork quality - namely PSE. PSE is the acronym used to describe pale, soft and exudative pork, not only does PSE have an undesirable appearance in the retail case, it often results in dryer and/or tougher products when the pork is cooked and also results in lower processing yields when the fresh pork is further processed into ham (Brewer and McKeith, 1999). PSE has been related to porcine stress syndrome (PSS), which is a genetic mutation that often referred to as the “stress gene”. Pigs that have PSS are more likely to exhibit PSE products. However, pigs that have not inherited the genetic mutation associated with PSS are also susceptible to producing PSE product when they are handled or subjected to considerable stress prior to harvest. Pale, Soft, and Exudative pork is one of the largest economic burdens to the pork industry.

Color is one of the most important attributes of fresh meat. Consumers often use color as an indication of freshness or quality when making purchasing decisions. Thus, poor color is a detriment and often results in economic losses at the retail level due to product being discounted to facilitate quick sales or even discarded. Myoglobin is a water-soluble sarcoplasmic protein that is primarily found in cardiac and skeletal muscle and stores oxygen to facilitate aerobic respiration by supplying the mitochondria with oxygen for oxidative phosphorylation.

Myoglobin is an extremely compact heme protein (MW \approx 17,800), which consist of a single polypeptide chain of about 153 amino acids. Approximately 70% of the main chain is folded into eight major, right-handed alpha helices. Each myoglobin contains one heme group that has a central iron surrounded by four nitrogen molecules.

Normally the iron molecule is found in the ferrous state (Fe^{2+}) and when oxygen is bound to the iron myoglobin is referred to as oxymyoglobin; however, when oxygen is not bound to the ferrous iron it is referred to as deoxymyoglobin. Oxidation of myoglobin results in the iron

converting to the ferric state (Fe^{3+}). Oxidation renders the molecule incapable of normal oxygen binding and the myoglobin is then referred to as metmyoglobin.

Fresh meat color is directly related to both the concentration of myoglobin in a muscle and the chemical state of the myoglobin on the exposed surface of the muscle. The concentration of myoglobin is different among different species and also among different muscles. Beef tends to have a brighter red color than pork; this is a direct result of beef containing a higher ratio of red:white muscle fibers than pork as red muscle fibers have a greater concentration of myoglobin.

Meat is approximately 75% water. Of the water in a meat product 85% is found within the muscle cells. This water is often described in three categories: tightly bound (4 to 10% of the intracellular water), loosely bound or immobilized (approximately 20% of the water), and the largest proportion is referred to as free water. Free water is held in the meat via capillary activity associated with the intracellular or myofibrillar protein matrix (meat is approximately 18.5% protein). One of the main effects on water holding capacity is the pH of the fresh meat. As the pH approaches the isoelectric point of meat, which is approximately 5.1, the protein matrix denatures and/or shrinks and thus there is less space between the myofillaments and less capillary activity. This leads to water being pushed out of the fresh meat product hence the term “exudative”. This loss of water from the cells also leads to the product being less firm, which is described as a “soft” texture or surface. Myoglobin is a water-soluble protein so as some of the water is pushed from the muscle, it carries myoglobin with it and produces purge (the liquid seen retail package that resembles blood). Many consumers mistake purge for blood, but this solution is predominately a combination of water and myoglobin. Because myoglobin and hemoglobin have similar functions (both bind oxygen) they have very similar structures and therefore they

reflect light at a similar wavelength, thus they resemble one another when they are in a water-based solution. The loss of this myoglobin may be contributing to the “pale” color; however, most of the myoglobin found in purge would be from the interior of the muscle (escaping from the meat product as it was cut into chops or roasts). Thus, this phenomenon does not explain the pale color that is often seen on the surface of cut or uncut fresh pork that has been labeled as PSE.

During harvest, a pig is stunned to render it insensible prior to being exsanguinated. From this point on the muscle enters into a phase known as postmortem metabolism. Because the blood has been removed from the carcass, there is no means to supply additional oxygen to the muscle cells, nor is there a means to remove heat (a byproduct of normal cellular respiration) from the muscle tissue. As the intracellular supply of oxygen is depleted from the myoglobin, aerobic respiration ceases and only anaerobic respiration or glycolysis continues to provide ATP, however, two common by-products of glycolysis are heat and lactic acid. When pigs have the genetic mutation known as PSS or if they are subjected to stress prior to harvest, they may have already started to enter anaerobic respiration prior to the stunning and exsanguination. Thus, the muscle may already have some lactic acid accumulation and a slightly higher temperature. This temperature can result in an acceleration of postmortem metabolism resulting in an even higher temperature while simultaneously increasing the production of lactic acid, which in turn results in a further decrease in muscle/meat pH (Figure 1). Thus, the myoglobin would simultaneously be subjected to both a lower pH and a greater temperature than would be present under normal physiological conditions. As both temperature and pH are known to denature proteins, this combination may explain, in part, the denaturation of myoglobin resulting in the pale color that is observed with PSE products on both the surface as well as in the interior of the meat product.

Experimental Focus

Approximately 100 million pigs are harvested each year in the United States. The incidence of PSE has been estimated to from 10% to 16% in various reports (Kauffman et al., 1992; Cannon et al., 1995) and is certainly affected by seasonality, however, Ellis (2006) estimated that PSE cost the pork industry \$0.90 per carcass (if the cost of PSE is spread across all pigs harvested in a given year).

Pork carcasses that result in PSE product often have an accelerated rate of postmortem metabolism, which can produce combinations of high temperatures and low pH in muscle tissue. We hypothesize that the relatively immediate post mortem environment in PSE pork carcasses (combination of high temperature and low pH) leads to the denaturation of myoglobin and formation of metmyoglobin. Hence, the fresh meat product will not “bloom” because the ferric iron cannot bind oxygen; resulting in the pale color associated with PSE pork.

Objective

Determine the effect of pH and temperature combinations (mimicking the potential muscle environment during postmortem metabolism) on the denaturation of myoglobin.

Materials and Methods

Isolation and Preparation

The isolation procedure was adapted from the described method in Trout and Gutzke (1996). In preparing the myoglobin extract, a freshly harvested beef heart was ground through a plate (10 mm). Two 150 g samples of ground beef heart were homogenized for one minute in a blender with 10mM *Tris*-HCl buffer and 1mM EDTA (pH 8.0).

The two homogenized samples were pooled and centrifuged at 10000 g for 10 minutes. The resulting supernatant was filtered through several layers of cheesecloth and the pellet was discarded. The filtered supernatant was adjusted to pH 8.0 with 10% (w/w) NaOH, then adjusted to 70% saturation with ammonium sulfate, and stirred for 60 minutes.

The sample was centrifuged for 20 minutes at 18000g. The supernatant was again filtered through cheesecloth and adjusted to pH 8.0. The sample was adjusted to 100% saturation with ammonium sulfate and stirred for 60 minutes.

The sample was centrifuged for 60 minutes at 18000g. The supernatant was discarded and the resulting pellet was resuspended in 5mM *Tris*-HCl buffer (pH 8.5) with the ratio of 2 pellets:1 buffer. The sample was then applied to a gravity column (Sephadex G 100) washed with 10mM *Tris*-HCl buffer.

Evaluation of Isolated Myoglobin

Every third sample was transferred to a 1 mL spectrophotometer cuvette and absorbance readings were recorded for the wavelengths of 280 nm, 525 nm, 572 nm and 730 nm. The values were evaluated to determine the percent metmyoglobin using the following equation from Trout and Gutzke (1996):

$$\% \text{ Metmyoglobin} = \left\{ 1.395 - \left[\frac{[A_{572} - (A_{730} \times 1.45)]}{[A_{525} - (A_{730} \times 1.73)]} \right] \right\}$$

Samples with a low percentage of metmyoglobin and a high concentration of oxymyoglobin were subjected to SDS-PAGE electrophoresis. The samples applied to the SDS-PAGE gels consisted of 25 μ L of the desired fraction and 25 μ L of urea/thiourea buffer. Samples of 20 μ L were applied to each lane and were compared to a broad standard and a sample of horse myoglobin. Samples containing myoglobin were pooled and concentrated.

Testing the effects of pH and temperature on Myoglobin

The concentrated myoglobin was added to a buffer solution at a concentration of 2 mg/g to resemble the concentration in pork muscle. The stock solution was divided into five 100 ml beakers, adjusted to pH 5.4, 5.8, 6.2, 6.6, 7.0, and placed in a water bath and percent metmyoglobin was recorded at 36, 40 and 44° C.

Statistical Analysis

The effect of temperature and pH on myoglobin (mg/ml) and percent metmyoglobin were evaluated using PROC GLM procedures of SAS. Means were separated using Fisher's protected LSD test, and differences were considered significant when $P < 0.05$.

Results

Elutions from the gravity column can be seen in Figure 2. The samples with the highest percent oxymyoglobin were taken from elutions 91 to 110. The SDS-PAGE gel is shown in Figure 3. The gel shows samples, equine myoglobin and a broad standard to display the presence of myoglobin in the samples.

Statistical analysis results for the effect of temperature are summarized in Table 1. When myoglobin was subjected to a temperature of 44° C a greater percent of metmyoglobin was formed; however, the increase was numerically small. Furthermore, during postmortem metabolism the postmortem physiological temperature would not exceed 40° C under conditions resulting in PSE pork. Statistical analysis results for the effect of pH are summarized in Table 2. There was a numerical trend for percent myoglobin to increase as pH decreased from 7.0 to 5.4. However, this trend was not significant ($P > 0.05$).

Conclusion

The data indicates that the range of temperature and pH in postmortem muscle would not denature myoglobin to the extent that would explain the pale color observed in PSE pork. Although Myoglobin did not appear to be responsible for the conditions in PSE pork, several other proteins were found in the extracted matrix that could be tested in a similar fashion.

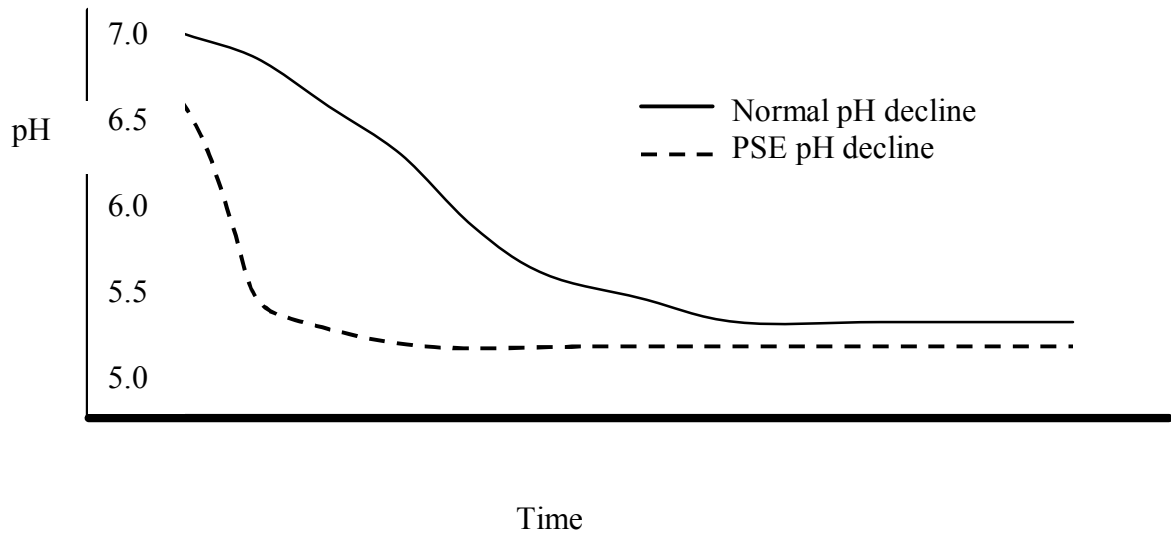


Figure 1. Rate of pH decline during postmortem metabolism of pigs that produce normal appearing fresh pork versus that of pigs producing pork with visual characteristics of PSE

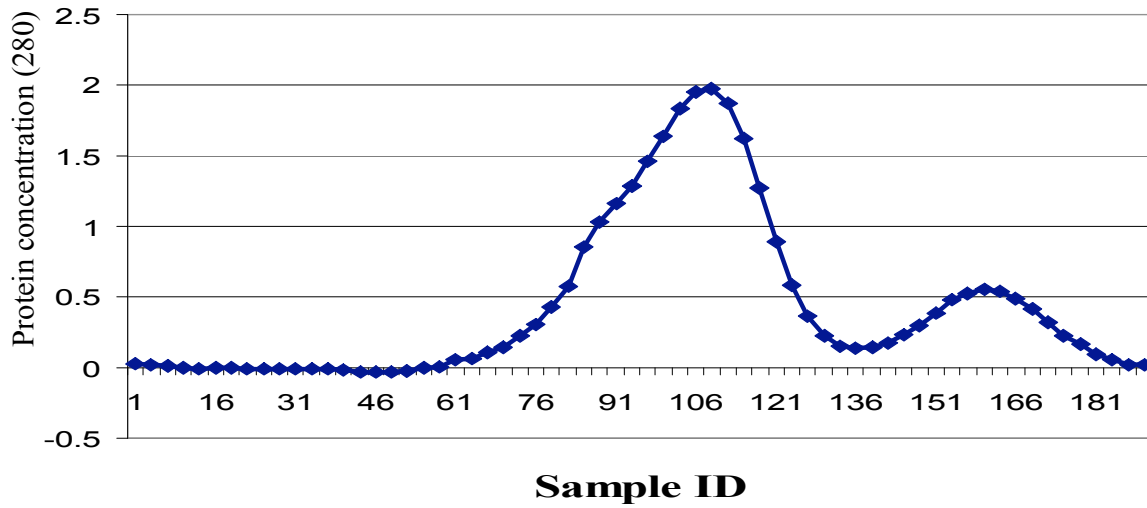


Figure 2. Elution profile of protein extracts developed on Sephadex G 100 eluted with 10mM Tris-HCl 8.0 buffer.

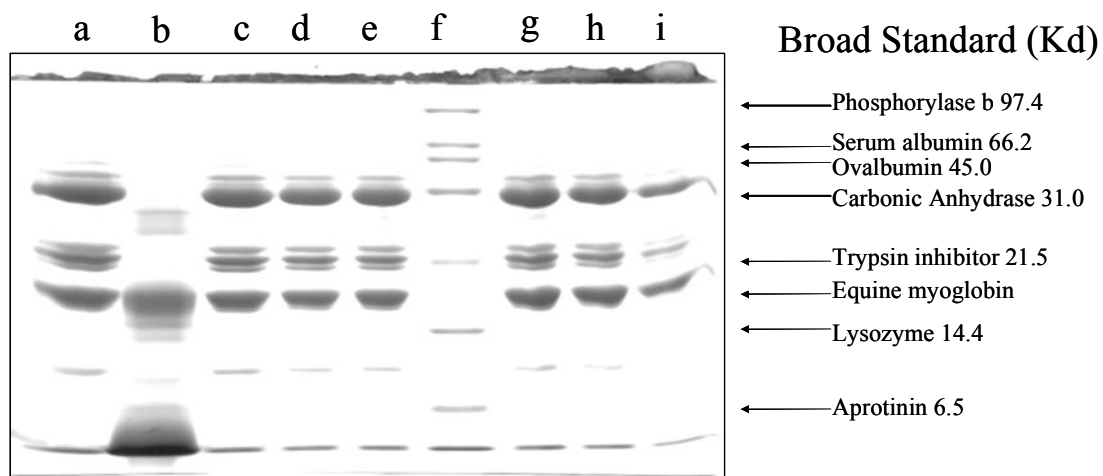


Figure 3 SDS-PAGE electrophoresis of fractions obtained from the column elution. Lanes correspond to fraction samples from Figure 2 (a= 100, b = equine myoglobin, c = 103, d = 106, f = broad standard, g = 112, h = 115, i = 118).

Table 1. The effect of temperature on the formation of metmyoglobin

	Degrees Celsius			S.E.	P-value
	36	40	44		
Myoglobin, mg/ml	0.52	0.53	0.54		
Metmyoglobin, %	0.00 ^b	0.46 ^{ab}	1.49 ^a	0.36	0.05

^{a,b} means bearing different superscripts differ (P < 0.05).

Table 2. The effect of pH on the formation of metmyoglobin

	pH					S.E.	P-value
	7.0	6.6	6.2	5.8	5.4		
Myoglobin, mg/ml	0.56	0.53	0.54	0.53	0.52		
Metmyoglobin, %	0.00	0.02	0.40	1.40	1.46	0.47	0.13

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