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Postmortem glycolysis and pork quality

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Abstract. After an animal is harvested for meat, the skeletal muscle initiates a myriad of biochemical pathways in an attempt to maintain energy homeostasis. Anaerobic glycolysis is responsible for the generation of ATP to help meet energy demand and for the decrease in pH by generating H^+. Both the rate and the extent of the *post-mortem* pH decline are paramount in the context of the development of pork quality attributes, such as color, water holding capacity, and texture. Pale, soft and exudative meat and dark, firm, and dry meat are two of the major quality defects facing the pork meat industry. Because glycolysis has the potential to affect meat quality attributes either positively or negatively, evaluating its regulation *post-mortem* is fundamental to understanding meat quality. Therefore, the aim of this study was to evaluate factors that affect mechanism of glycolysis. Special consideration will be given to meat quality attributes and development of pork quality defects.

1. Introduction

During the *post-mortem* period, a myriad of energetic, biochemical, and physical changes takes place in the muscle that results in its conversion to meat. As the animal succumbs to exsanguination and resulting anoxia, skeletal muscle still attempts to achieve its *ante-mortem* homeostatic balance through adenosine triphosphate (ATP) synthesis and its utilization. ATP is synthesized through the catabolism of stored glycogen to ultimately produce lactate [1]. The end products of ATP hydrolysis and *post-mortem* glycolysis, hydrogen ions (H^+) and lactate, respectively, accumulate in the muscle due to the lack of an effective elimination mechanism. This accumulation of H^+ ions acidifies the muscles and consequently causes the pH to drop from 7.2 in living muscle to an ultimate pH (pHu) of approximately 5.5 [2]. Under normal circumstances, muscle pH declines gradually until the onset of *rigor mortis*. Any alterations in the rate or extent of pH decline in *post-mortem* muscle can have substantial influence on meat quality. Hastened, extended, or insufficient *post-mortem* pH decline adversely influences meat color, water-holding capacity (WHC) and texture and can lead to occurrence of two classical fresh meat quality problems in the pork meat industry: pale, soft and exudative (PSE) meat and dark, firm and dry (DFD) meat [1,3]. PSE pork is characterized by pale color, low WHC, and soft texture. Conversely, DFD meat has a dark color and a firm, dry, and sticky texture due to its enhanced water binding capability [1]. A greater understanding of *post-mortem* metabolism during the conversion of muscle to meat and the mechanisms that govern glycolysis are critical to characterizing pork quality development. Therefore, the aim of this study was to summarize current understanding and new discoveries in the *post-mortem* regulation of glycolysis and their impacts on meat quality.

2. The conversion of muscle to meat

The biochemistry behind the transformation of muscle to meat is a heavily researched topic. The basic biochemical reactions and physical changes underlying the conversion of muscle to meat are well recognized. As ATP is utilized and depleted, actomyosin cross-bridges become permanent and the muscle extensibility decreases. This loss of extensibility signifies the onset of *rigor mortis*. The magnitude, extent, and timing of *post-mortem* metabolism can dramatically affect meat quality development. The rate of pH decline during the conversion of muscle to meat reflects the intensity of *post-mortem* metabolism. Many factors influence the rate and the extent of the *post-mortem* pH decline, including intrinsic factors such as species, the type of muscle, anatomical location, carcass fatness, and variability between animals, and extrinsic factors such as animal feeding, pre-slaughter handling, the environmental temperature. Typically, pH gradually declines from around 7.2 to around 5.7 within 8 h *post-mortem*, with pHu of about 5.6 achieved at 24 h [1, 4].

3. *Post-mortem* glycolysis in pigs

One of the key metabolic pathways in the conversion of muscle to meat is glycolysis. Under anaerobic conditions, glycogen is catabolized to glucose 6-phosphate through glycogenolysis. Glucose 6-phosphate is the initial substrate in the glycolytic pathway (glycolysis), a sequence of ten reactions, all of which occur in the sarcoplasm of the muscle fiber [5]. Glycogen is the primary and possibly sole carbohydrate source utilized in the rephosphorylation of adenosine diphosphate (ADP) in *post-mortem* muscle [2]. One glycogen moiety generates three ATP molecules and two lactate molecules *post-mortem*, which is why glycogen has been studied for its role in determination of pHu and meat quality [2]. Classically, glycolysis is considered to generate H^+ and lactate at a 1:1 ratio, but this does not necessarily mean the H^+ is derived from the dissociation of lactic acid [6, 7]. Within the past decade, new theories have ignited a debate challenging the notion of 'lactic acidosis' [7]. Once pyruvate is formed by glycolysis, it is converted to lactate under anaerobic conditions. The conversion of pyruvate to lactate by lactate dehydrogenase serves as a buffer by removing a H^+ ion from the system and attaching it to the lactate molecule, thus limiting muscle acidification [7, 8]. Rather, the hydrolysis of ATP produces the H^+ ions responsible for *post-mortem* acidification during the conversion of muscle to meat [5]. Lactate was also investigated to predict pHu and meat quality [9, 10, 11, 12]. High concentrations of exsanguination lactate were associated with lower meat quality such as decreased WHC, lighter color [9], and lower pH 45 mins *post-mortem* [10].

3.1. Regulation of rate and extend of glycolysis

While most authors believe glycogen depletion arrests pH decline, some suggest that glycogen content alone cannot fully explain the limitation in pH decline [3]. This raises the question, why does glycolysis stop? Although the most logical reason is depletion of the muscle's glycogen depot, metabolizable carbohydrate is rarely depleted entirely in muscles [13]. The extent of *post-mortem* metabolism is dictated by the glycogen content of the muscle as long as levels are between 0 and 53 mmol/kg muscle, and for normal *post-mortem* pH decline, the minimum content of glycogen is about 53 mmol/kg muscle [14]. Further increase in glycogen levels beyond 53 mmol/kg muscle is not associated with further decline in pH [14]. Muscles with limited glycogen reserves, which were reduced below normal levels at slaughter due to *ante-mortem* stress, usually have limited pH decline and resultant elevated pHu (>6). The resulting meat can exhibit inferior quality attributes, like a dark color and reduced overall acceptability. Thus, when reduced, muscle glycogen content can affect the extent of *post-mortem* pH decline and meat quality. The role of glycogen content in dictating normal pHu is less established when glycogen is not reduced [2]. When oxidative muscles were provided with excess glycogen in an *in vitro* glycolytic system, they still produced a high pHu compared to glycolytic muscles [3]. Therefore, according to some authors, glycogen is not an exclusive explanation for higher pHu (near 5.8-6.0), but rather, muscle glycolytic capacity, i.e. the maximal ability of a cell to convert carbohydrate (i.e. glycogen) to pyruvate or lactate, controls the extent of pH decline in pig muscles [3, 15]. Hence, evaluation of enzymes regulating glycolytic capacity is critical to further understand the meat quality attributes developed during *post-mortem* transformation of muscle to meat [2]. According to some authors, phosphofructokinase-1 (PFK-1) is the most likely candidate for regulation of *post-mortem* glycolysis [2, 13]. Glycogen can be converted to lactate and H^+, provided PFK-1 maintains its activity. This enzyme begins to lose activity around pH 5.9 and becomes completely inactive at pH 5.5, which could explain why the pHu of pork is bracketed in a fairly consistent range between pH 5.5-5.9 [2, 13]. However, PFK-1 inactivation does not explain why some muscles produce meat with a high pHu (pH > 5.9) in the presence of residual glycogen [2]. Further investigation of factors regulating PFK-1 activity *post-mortem*, such as adenonucleotide availability (ATP and AMP) and AMP deaminase abundance and activity, could clarify the regulation of glycolysis and *post-mortem* pH decline [2, 3, 16]. Recent evidence increasingly suggests that mitochondrial content and function both likely influence *post-mortem* metabolism and variability of pHu within a species [17, 18].

4. Meat quality attributes

Meat quality has always been important to consumers and is a particularly important issue for the meat industry of the 21st century. The innate characteristics of muscles and extrinsic conditions during slaughter influence the development of three key traits important to fresh meat quality: WHC, color, and texture. Fresh meat quality is a broad area of investigation encompassing many topics that include glycolysis and *post-mortem* pH. pHu is widely regarded as an indicator of fresh meat quality [19]. The normal pHu value of pork ranges between 5.5 and 5.7 [4]. Meat with pHu < 5.4 has a pale color, lower WHC, reduced protein extractability, and poor processing yield, while meat with pHu ≥ 6.0 appears darker in color and has a shorter shelf life.

4.1. Color

Color is considered the most important sensory attribute of fresh meat, and is what consumers primarily use as an indicator of quality and freshness of the products [20]. The distribution and amount of myoglobin species, deoxymyoglobin, oxymyoglobin and metmyoglobin, together with internal reflectance influence the color of the pork [21]. Fresh meat color is also significantly influenced by *post-mortem* metabolism. Abnormally low pHu causes denaturation of muscle proteins, including myoglobin, and reduces their ability to bind water. As a result, large amounts of water migrate from inside the muscle fibers to the extracellular space, which increases light reflectance and results in a paler meat color [22]. On the contrary, high pHu causes meat color to appear darker due to greater WHC and increased light absorbance, lower reflectance, and lower protein denaturation. High pHu also promotes greater activity of oxygen-scavenging enzymes, resulting in reduced available oxygen to bind myoglobin, and more deoxymyoglobin formation [23].

4.2. Water-holding capacity

The ability of pork to retain its inherent moisture has a dramatic impact on consumer acceptance of fresh pork. WHC is an important quality attribute as it influences the yield and the quality of fresh and processed meat products [24]. Many factors, such as pH and *post-mortem* proteolysis, influence WHC by altering the amount and location of moisture in muscle [24]. Rapid pH decline coupled with high muscle temperature in early *post-mortem* causes the denaturation of approximately 20% of muscle proteins, leading to their loss of functionality and their ability to hold water [24]. WHC is closely related to the color of meat and influences other physical properties including texture and firmness of raw meat and eating properties of cooked meat [25].

4.3. Texture

Texture is a complex concept that involves several attributes including, tenderness, juiciness, firmness, and cohesiveness. *Post-mortem* factors that contribute to the texture of the meat include *post-mortem* pH, carcass temperature, contractile state, proteolysis, and their interactions [5]. Meat tenderness decreases as pHu increases from 5.4 to 6.0, and then improves as pHu increases from 6.0 to 7.0 [26, 27]. Meat texture is also affected by the rate of pH decline. Rapid pH decline increases protein denaturation and decreases WHC, resulting in an undesirable soft texture in pork.

5. Abnormal *post-mortem* metabolism and meat quality defects

The meat industry has focused selection pressure on producing animals that are efficient feed converters, fast growing and have high lean meat content. These characteristics have been achieved through genetic manipulation and careful selection of breeds [22]. However, this focus has also resulted in the production of animals that are much more susceptible to stress, and consequently, to the development of meat quality defects, ranging from PSE to DFD pork [12]. Due to the considerable economic losses, PSE and DFD pork have attracted significant research attention.

5.1. Pale, soft, and exudative meat

PSE meat is characterized by an abnormally light color, soft texture, and impaired ability to hold water. Hastened glycolysis coupled with a corresponding increase in H^+ accumulation and heat production causes rapid pH decline when carcass temperature is still high [22]. The resulting meat is pale in color due to the loss of meat pigments or the oxidation of heme pigments, and due to the increase in light reflectance at meat surface [22]. This meat is exudative as a result of impaired WHC, and is soft in texture because of protein denaturation [22]. The development of PSE is mainly associated with genetic factors and pre-slaughter stressors including improper handling, and mixing with unfamiliar animals [12]. Acute stress immediately prior to slaughter can also accelerate glycogenolysis and glycolysis resulting in PSE meat [28]. Under stressful situations, epinephrine is secreted from the adrenal medulla and functions primarily to raise blood glucose levels through the stimulation of glycogen degradation [29].

5.2. Dark, firm and dry meat

DFD meat is characterized by its dark color, firm texture, and dry sticky surface. When animals are exposed to chronic or long-term stress before slaughter, DFD meat can result. Chronic stress prior to slaughter leads to the depletion of stored glycogen, so less glycogen is available *post-mortem*, affecting the normal process of acidification and leaving the meat pH high [22]. The high pHu (> 6.0) results in relatively little denaturation of proteins, water is tightly bound, and little or no exudate is formed [30]. High pHu minimizes meat pigment losses and denaturation, thereby increasing light absorbance, which gives the meat its darker appearance [22]. Meat with elevated pHu also exhibits higher WHC as the pH is further from the isoelectric point than normal [22].

6. Conclusions

The rate and extent of *post-mortem* glycolysis has a profound effect on pork quality. Therefore, understanding mechanisms controlling *post-mortem* glycolysis and pH decline should improve the probability of producing high-quality pork meat. Recent research in this area further elucidated the biochemical mechanisms regulating glycolysis and improved the understanding of *post-mortem* muscle glycolysis and meat quality development. Despite this, further efforts are necessary to explain the rate and extent of *post-mortem* pH decline in order to ensure consistent production of the highest quality meat possible. Also, those involved in the meat industry must understand these biological processes and implement management practices that optimize them.

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