6. Early Post-Mortem Biochemical Events

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Learning objectives

This chapter provides an understanding of the biochemical events that occur in muscle early post-mortem and their significance on subsequent meat quality.

By the end of this chapter you should:

• Understand that cells continue to metabolise after an animal is dead.
• Recognise that this metabolism can affect meat quality.
• Know the sources of cellular energy following slaughter.
• Recognise that the amounts of cellular energy can affect meat quality.
• Understand why rigor mortis occurs.

Key terms and concepts

Rigor mortis, anaerobic metabolism, lactic acid, muscle extensibility, glycolysis, creatine phosphate, pH decline.

6.1 Introduction

Regardless of the species being harvested, death is accompanied by an inability to deliver oxygen within the body and subsequent anoxia. When normal life processes are halted, many of the biochemical reactions present in the living state retain some degree of activity. For the conversion of muscle to meat the non-living or post-mortem period can be divided into two stages, pre-rigor and post-rigor. This chapter of the course is concerned only with the former particularly the biochemical aspects involved in the conversion of muscle to meat.

6.2 Consequences of circulatory failure

Circulatory failure within an animal occurs due to either cessation of cardiac contraction (heart beat stops) or loss of blood pressure, with both factors occurring at slaughter due to stunning and exsanguination. Without this vital supply of blood the environment within the muscle cells change rapidly. The following sequence of events summarise some of these changes:

- At the moment the animal is stunned and circulatory failure occurs, its various tissues are continuing their particular types of metabolism under local control.
- Although muscle is not actively contracting after death, energy is being used to maintain its temperature and the organisational integrity of cells against their spontaneous tendency to break down.
- Non-contractile ATPase of myosin is actively consuming energy to maintain temperature and cell integrity.
The early post-mortem events have a large impact on the ultimate meat quality from a carcase.

- Blood borne oxygen supply is eliminated and consequently there is a fall in oxidation - reduction potential within muscle.
- Aerobic metabolism of glucose in the mitochondria ceases, along with aerobic production of ATP.
- Creatine phosphate within the cell is rapidly utilised in an attempt to maintain ATP concentrations. As this reserve is depleted, and the readily available supply of ATP is diminished, actomyosin cross-bridges accumulate and the loss of muscle extensibility begins (onset of rigor mortis).
- The continuing operation of the non-contractile myosin ATPase reduces ATP levels, increasing ADP and inorganic phosphate concentrations.
- These increased levels of inorganic phosphate stimulate the breakdown of glycogen which is metabolised anaerobically producing lactic acid (via glycolysis).
- The accumulation of lactic acid reduces the pH, diminishing the structural integrity of cellular proteins.
- This fall in pH also causes water to be lost from the carcass due to protein denaturation and proteins approaching their isoelectric point.
- Endogenous proteases within muscle further breakdown the muscle proteins.
- Fat solidifies as the temperature falls.
- Glycolysis and therefore ATP regeneration ceases at an ultimate pH of about 5.5.
6.3 Metabolism in muscle post-slaughter

As discussed earlier in the section on energy production of muscle, the two pathways available for energy production (ATP) in anaerobic conditions (such as post-slaughter or strenuous exercise) are from:

- Creatine Phosphate; and
- Glycolysis

ATP production via the TCA cycle ceases as soon as oxygen is no longer available, therefore fat cannot be utilised as a fuel under anaerobic conditions (as it can provide substrate, acetyl CoA, for the TCA cycle only). In a resting state the myofibre stores glucose as the polysaccharide called GLYCOGEN and this is mobilised when there is an ATP deficit.

**Creatine Phosphate (CP)**

During intense muscle activity, when direct ATP production by mitochondria is limiting, creatine phosphate provides a rapid, short-term supply of ATP. The reversible nature of this reaction, catalysed by creatine kinase, is important in that it allows regeneration of creatine phosphate from creatine and ATP during periods of rest. See below:

![Figure 6.2 ATP Production. Source: Gardner, (2006).](image)

The formation of ATP from creatine phosphate in muscle. Post-slaughter, the reaction only proceeds one way, to maintain ATP levels in the myofibre. Once all the creatine phosphate is converted to ATP, the overall level of ATP drops as glycolysis is unable to generate sufficient ATP rapidly enough to support all the energy requiring processes within the muscle. It is important to note that the onset of the loss of muscle extensibility (i.e., the onset of rigor) correlates approximately with depletion of the creatine phosphate reserve. ATP is necessary for contraction and the subsequent release of myosin from actin during a contraction cycle, so the depletion of ATP following death, results in a greater percentage of myosin heads remaining fixed to the actin filament (actomyosin cross-bridges). Thus, as ATP concentration decreases and approaches zero, the number of actomyosin cross-bridges increases and extensibility of muscle decreases (see figure below).
**Glycolysis**
At death the animal’s ability to obtain and deliver oxygen to body tissues is lost. Very small amounts of oxygen can penetrate at the surface of the carcase allowing some aerobic metabolism at the muscle surface, however almost all remaining tissues within the carcase can no longer function aerobically, relying exclusively on anaerobic energy production via glycolysis. The metabolism of glucose (derived from muscle glycogen) through the glycolytic pathway, results in the accumulation of lactic acid and the generation of 2 ATP molecules per molecule of glucose. Due to cessation of blood flow glucose can no longer be sourced from outside the muscle cell, thus glucose is sourced endogenously from the cells store of carbohydrate — Glycogen. As glycogen is catabolised as a fuel and lactic acid accumulates, the pH of the cell steadily drops until a point is reached at which glycolysis is inhibited (either by a low pH or insufficient glycogen) and metabolism ceases. This is known as ultimate pH (pHu), and when this occurs the ability to produce ATP is completely lost.

**Figure 6.4 Glycolytic Pathway.** Source: Gardner, (2006).
Simplified scheme of the glycolytic pathway in the conversion of muscle glycogen to lactate.

**Changes in muscle pH**

The conversion of glycogen to lactic acid (via glycolysis) will continue until a pH is reached at which the enzymes involved in this process are inhibited, or the glycogen supply is depleted. In a typical mammalian tissue the pH at which the enzymes are inactivated is 5.4 - 5.5. However in pigs susceptible to porcine stress syndrome, the pH drops lower than 5.4. This is possible because the enzymes are able to function at a lower pH than normal.

The final pH attained is called the ultimate pH and is normally about 5.5 which is approaching the isoelectric point of muscle proteins. At this pH the water holding capacity is lowered. An ultimate pH significantly above or below this can cause meat quality problems such as pale soft exudative meat (PSE) and dark firm dry meat (DFD).

Both the rate and extent of the post-mortem pH fall are influenced by intrinsic factors such as the species, and muscle type (see figure (a) and (b) below).

**Figure 6.5 Rate of post-mortem pH fall at 37°C. Source: Gardner, (2006).**

The effect of (a) species and (b) type of muscle on the rate of postmortem pH fall at 37°C. Zero time is one hour postmortem.

The drop in muscle pH is faster in pig LD muscle than in beef LD, which is intermediate in rate. It is slowest in horse LD muscle. The differences between pH values are most noticeable during the early post-mortem period (first three hours). By five hours post-mortem, the pH values for all three species were essentially the same. This is related to the variation of glycolytic rate between species and perhaps varying concentrations of free Ca²⁺. Different muscles within the same species have different post-mortem pH curves, due to variable glycolytic rates. There is also variability between animals resulting from important extrinsic factors such as environmental temperature, carcase fat cover, and stress (see figures below).
Temperature affects the rate of any chemical reaction, thus high post-mortem temperatures accelerate glycolytic rate increasing the rate of pH decline. Therefore the rates of glycolysis will tend to be higher in muscles which are slow to cool and vice versa. This is influenced by their proximity to the surface of the carcase and their insulation by fat or other muscles. Given their small carcase size relative to cattle, sheep are particularly prone to slow rates of pH decline, causing a meat quality problem called cold shortening.

**Figure 6.6 Variability in post-mortem pH. Source: Gardner, (2006).**

Variability in the rate of post-mortem pH fall in L.dorsi muscles at 37°C between individual pigs.

### 6.4 Rigor mortis

The most easily observed structural change during the development of rigor is the change from soft pliable tissue to rigid inextensible tissue. This rigidity is brought about by a series of biochemical events that take place within muscle following death of the animal. Ultimately these events lead to a fall in ATP concentration and the gradual accumulation of actomyosin cross-bridges resulting in the loss of muscle extensibility. The development of rigor can be measured by the degree of stretching while alternatively loading and unloading the muscle, and in most cases a carcase will have reached rigor mortis (or completely lost muscle extensibility) when the pH of the muscle has fallen to about pH 6.

**Figure 6.7 Temperature effects on post-mortem pH. Source: Marsh (1954).**
The effect of environmental temperature on the rate of post-mortem pH fall in beef LD (Marsh 1954)

**Figure 6.8** Changes in muscle extensibility of an excised psoas muscle from an anesthetised rabbit and held at 17°C. Source: Gardner, (2006).

Changes in muscle extensively over the pre-rigor time for post-mortem psoas muscle from a rabbit. In this instance the muscle was extracted from an anesthetised rabbit and was held at 17oC. As can be seen when the muscle becomes rigid rigor set in after about 10 hours post-mortem.

**Figure 6.9** Changes in muscle extensibility of an excised psoas muscle from an anesthetised rabbit and held at 38oC. Source: Gardner, (2006).

At the higher temperature the muscle loses it extensibility in half the time, ie rigor onset is hastened.

**Definitions**
Given the relationship between muscle extensibility, pH, and ATP concentrations, rigor mortis can be given the following definitions:

- **Biochemical** — The concentration of ATP falls resulting in an increasing proportion of actomyosin cross-bridges remaining in their bound state.
- **Chemical** — Post-mortem muscle pH is lower than pH 6.0.
- **Industry/practical** — The muscle has “set” and is stiff or firm to the touch. Moving the forelimb on a carcase can assess this. If it is flexible the carcase has still not gone into rigor, if it is stiff the carcase has gone into rigor.

**Biochemical processes involved in rigor mortis**
The following is a summary of the sequence of events resulting in rigor mortis. The rate at which these processes occur varies between carcases and between muscles in the same carcase due to both extrinsic (ie temperature, stress, etc) and intrinsic (ie muscle type and location, and carcase fat cover) factors, thus estimates of time and pH are just a guide.

- At slaughter the supply of oxygen to muscles is cut. Muscles continue metabolising anaerobically and the contractile units remain functional.
- ATP reserves are replenished from creatine phosphate (CP) and the breakdown of glycogen to lactic acid which leads to a drop in pH.
- The non-contractile ATPase of myosin is actively consuming energy in an attempt to maintain body heat and the structural integrity of the muscle cell.
- Creatine phosphate is depleted within two hours of slaughter and the ATP reserves gradually diminish as glycolysis can not supply ATP at a rate sufficient to meet the energy demands of the cell. The pH has dropped by some 0.5 units due to lactic acid build up.
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• As the acidity increases Ca\(^{++}\) begins to leak through the membrane of the sarcoplasmic reticulum accumulating in the cytosol around the myofibres. This is offset by ATPase calcium pumps which are continually removing Ca\(^{++}\) from around the myofibres returning it to the sarcoplasmic reticulum. However, this flux of Ca\(^{++}\) allows myosin and actin to interact triggering limited muscle contraction and further consumption of ATP.
• This process is continually repeated while glycogen breakdown continues to lower the pH, maintaining a limited supply of ATP which delays its total depletion.
• ATP levels continue to drop and the percentage of actomyosin cross-bridges increases.
• Eventually the ATPase calcium pumps fail due to the accumulating acidity resulting in major contraction and the consumption of ATP.
• The pH approaches 5.8–6 after 6 hours and only 20% of the initial muscle extensibility remains due to the actomyosin cross bridges. There is insufficient ATP to prevent the interaction of myosin and actin and thus rigor has occurred.
• Glycolysis continues until conditions are too acidic for the glycolytic enzymes to function, at which point ultimate pH is reached — usually about pH 5.5. There is no further ATP production.

Readings
There are no readings for this topic.

Summary
Summary Slides are available on web learning management systems
After death the animal goes through a series of metabolic changes:

- Blood circulation stops
- Tissues move from aerobic to anaerobic metabolism
- Available energy reserves are depleted
- ATP level drops
- Calcium concentration rises because of failure of the calcium pumps
- Glycogen is broken down to lactic acid
- pH drops due to the build up of lactic acid
- Actomyosin cross-bridges are formed and not broken due to reducing ATP concentration
- Proteases start to break down muscle proteins

Rigor mortis occurs when ATP levels are reduced and the actomyosin cross-bridges are left in a "bound" state. The onset of rigor mortis commences after creatine phosphate levels are significantly depleted, and in most cases rigor has fully developed by about pH 6.

References
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MLA Fact Sheet: The effect of pH on beef eating quality