The Measurement of pH in Muscle and its Importance to Meat Quality

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Introduction

The pH of muscle tissue is extremely important to meat science since the pH at specific times during the conversion of muscle to meat, as well as the ultimate pH of meat, affects many quality factors. The quality factors affected by pH include: color, grading characteristics and shrink of carcasses and wholesale cuts; texture, cooking loss and tenderness of steaks and roasts; and processing and binding characteristics of comminuted and restructured meats. This paper is not intended to be a complete review of the involvement of pH in meat quality, but will outline several of the important relationships, and will indicate many of the parameters which might affect pH measurements. Some of the points discussed may seem self-evident, and are primarily included because of their obvious influence.

Effects of pH on Meat Quality

Meat Color

One of the most widely recognized problems of meat color is that of dark cutting in beef. This phenomenon has been related to the ultimate pH of meat for over 40 years (Davey and Graafhuis, 1981) and was the subject of a recent symposium (Hood and Tarrant, 1981). The higher ultimate pH of dark cutting beef produces meat having a very dark color, which, in turn, has been attributed to reducing consumer acceptance of the product (Hood and Tarrant, 1981).

The condition of dark cutting is an extreme which can be manifest to a lesser extent in the form of heat-ring (a dark area in the rib-eye adjacent to the subcutaneous fat). Recently, heat-ring has been shown to be caused by a higher pH in the heat-ring area (as compared to the central portion of the longissimus muscle), substantiating the relationship of beef color to pH (Orcutt et al., 1983). When the rate of pH decline is increased by electrical stimulation, a significant improvement in lean color (a lighter, cherry red color) and a reduction in heat-ring occur (Savell et al., 1978; Orcutt et al., 1983).

The relationship of pH to the color of porcine muscle is well documented (Briskey, 1964; Davis et al., 1974). The research on pH and color of porcine muscle indicates that the same relationship observed in beef exists in pork; i.e., a higher pH gives a darker color.

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Meat Tenderness

Recent reviews on the relationship of postmortem pH decline and muscle tenderness have been conducted by Dutson (1983) and Marsh (1983a). Most authors agree that a high ultimate muscle pH (6.0 or greater) is associated with more tender muscle (Fredeen et al., 1974; Dransfield, 1981, Dutson et al., 1981). However, there appears to be an interaction with sex, in that dark-cutting bulls are, on average, more tender than are dark-cutting heifers (Fredeen et al., 1974).

The relationship of early postmortem pH to meat tenderness is less clear. Dutson (1983) indicated most studies show that a low pH at an early time postmortem, while muscle temperature is high, produces more tender meat. This is supported by the fact that high temperature conditioning and electrical stimulation produce a more rapid pH decline and more tender meat (Dutson and Pearson, 1983). However, Marsh et al. (1981; 1983a) have stated that high pH early postmortem, in conjunction with high muscle temperature (37°C), produces more tender meat. Further research in this area will likely resolve the differences between these studies and provide greater insight into the mechanisms of tenderness.

Another method of improving meat tenderness is that of pressure treatment (Macfarlane et al., 1982). When applied pre-rigor, this treatment also reduces muscle pH, but temperature and time of pressure application cause differences in pH decline (Macfarlane et al., 1982). Further study of pressure-induced tenderness changes may also expand our understanding of the mechanisms of tenderness.

Meat Processing

The relationship of muscle pH to water-holding capacity is well understood (Briskey, 1964; Davis et al., 1974; Honikel et al., 1981a; 1981b) and is evident in many species. The effect of water-holding capacity on the processing properties is also important in numerous meat products, including comminuted and restructured meats.

Many of the factors that increase the binding properties of comminuted and restructured meat products are directly or indirectly related to muscle pH. Water-holding capacity is highly correlated to meat particle binding (Moore et al., 1976), and is directly affected by muscle pH (Honikel et al., 1981a; Hamm, 1960). One of the effects of polyphosphate on the increased binding of meat is due to enhancement of water-holding capacity through an increase in pH (Hamm, 1960; 1970). Maximum gel strength of myosin and actomyosin occurs at a pH of 6.0 and is reduced at lower pH's (Ishioroshi et al., 1979; Yasui, 1980). Amount of protein

extraction has been related to binding strength of processed meats (Miller et al., 1980) and protein extraction is increased in pre-rigor meat which has a high pH (Solomon and Schmidt, 1980). Also, Saffle and Galbreath (1964) have shown a direct effect of muscle pH on protein extraction.

Importance of pH Measurement

From the above brief description of the involvement of pH in many of the important quality parameters of meat, it is clear that the measurement of pH is essential to the study of these parameters. One of the problems with pH measurement in muscle is its apparent simplicity: One merely inserts an electrode into a muscle, ground product, or homogenate, and reads the pH value displayed. However, the apparent simplicity of the procedure can be misleading, and can result in the acceptance of erroneous data. In view of the importance of pH in the ultimate determination of meat quality, meat scientists must understand the problems that can be encountered when measuring pH.

Measurement of pH in Pre-Rigor Muscle

Many of the problems related to pH measurement in pre-rigor muscle are associated with the rapid rate of glycolysis which occurs in pre-rigor muscle, particularly if the muscle has been subjected to a treatment which causes membrane disruption, such as cutting, grinding or powdering.

Direct Measurement of Muscle pH Using a Probe-Type Electrode

As with any type of pH measurement, care must be exercised to standardize or calibrate the electrode and meter properly. Fresh buffer solutions must be used and, if possible, should be at the same temperature as the material to be measured. Electrodes must be rinsed when removed from a buffer solution and between readings to insure that the buffering capacity of the meat sample is not overcome by any residual buffering agent. Slow electrode response can be a problem in both calibrating and measuring, and is usually due to clogging of the electrode membranes. Soaking electrodes in a standard buffer solution of pH 7.0 when not in use and between measurements minimizes clogging and sluggish response. Electrodes should be cleaned regularly to remove accumulated fat and protein from membrane pores. Either a cleaning solution (such as supplied by electrode companies), or a combination of detergent followed by alternate solutions of 0.1N NaOH and 0.1N HC1 can be used. Most companies that produce electrodes supply information on their proper care and cleaning.

Another problem associated with measuring pre-rigor pH utilizing probe-type electrodes is the variance in pH among different areas of the muscle. To obtain a representative pH reading in pre-rigor muscle, numerous readings must be taken at various positions within the muscle, and care must be taken to ensure that proper contact is made between the meat sample and the electrode membrane junctures: This tends to be more of a problem in samples which are dry, and can be alleviated by coating the electrode surface with distilled water prior to insertion. If individual readings are recorded at each position within the muscle, this becomes a very tedious and rigorous process. To expedite pH measurements (particularly on-line in a packing plant), the probe electrode is moved to different positions within the muscle, readings are observed, and the different values are mentally averaged by the operator. The person taking the measurements must be well trained and able to obtain meaningful measurements by mental averages. This can be accomplished by comparing the operator's mental averages with averages mechanically calculated from recorded readings obtained from the same series of samples.

As with any technique, the skill and experience of the technician are very important in determining the accuracy and precision of the measurements. Inaccurate pH readings can occur if the electrode is placed in connective tissue or fat, or if insertion of the electrode creates an air pocket. By understanding the anatomy of the muscle and by taking multiple readings, these erroneous readings can be recognized, discarded and/or prevented.

Utilization of a probe-type electrode to obtain pH measurements in a coldroom is complicated by the fact that many pH meters do not function properly in a cold environment. Either a method of maintaining the pH meter at room temperature must be devised, or the pH meter must be limited to periods of approximately 15 minutes in the coldroom. In order to accommodate this time limitation, measurements could be spaced to allow the pH meter to return to room temperature between measurements, or more than one portable meter can be used.

Pre-rigor muscle is extremely sensitive to stimulation by cutting or other physical trauma to the muscle cells. Thus, the pH can be altered just prior to measurement. If care is taken to cut only the epimysial cover of the muscle sufficently to allow penetration by an electrode with a sharp spear point, pH can be measured without unduly traumatizing the muscle by physical means.

Measurement of Pre-Rigor Muscle Using Iodoacetate

Iodoacetate (an inhibitor of glycolysis) has been utilized to stop the glycolytic process before pH is measured (Bendall, 1973). This procedure allows flexibility for timing of pH measurements. However, samples must be frozen in liquid nitrogen, or some other cryogenic material, so that glycolysis can be arrested and samples can be stored. When samples are collected and frozen for subsequent pH measurements, ultra-low storage temperatures must be maintained since glycogen and ATP can be depleted at normal freezer temperatures (Winger et al., 1979). The muscle can be minced into small pieces while immersed in the iodoacetate, which allows storage of muscle for up to 24 hr before homogenization and measurement (Marsh, 1983b). Alternatively, freshly excised muscle samples can be homogenized immediately in iodoacetate solution to prevent rapid glycolysis; however, the length of time required to mince or homogenize fresh muscle prevents large numbers of measurements from being taken over a short period of time.

Samples which have been frozen for pH measurement must not be allowed to thaw before iodoacetate has inhibited the glycolytic process, since freezing disrupts the membrane systems of the muscle and thawing will cause an extremely accelerated glycolysis. For this reason, it is preferable to powder (pulverize the muscle using a Waring Blender cooled

to liquid nitrogen temperatures) samples in liquid nitrogen, maintaining these samples at close to liquid nitrogen temperatures (using liquid nitrogen cooled spatula, etc.), until they can be measured into the iodoacetate solution. Homogenization of the powdered sample should occur immediately, since small muscle pieces may thaw before the iodoacetate has penetrated the sample. If samples are homogenized directly (without powdering), homogenization should be very rapid to allow the iodoacetate to inhibit glycolysis immediately.

According to Bendall (1973), one disadvantage to homogenizing muscle in dilute solutions of iodoacetate is that the pK's of the muscle buffers are altered by lowering the ionic strength. Bendall (1973) suggests making up the iodoacetate in 150 mM potassium chloride to alleviate this problem, and has shown that the relationship of muscle pH with iodoacetate alone (pH_I is related to the muscle pH with iodoacetate and potassium chloride (pH_{ICK1}) by the following formula:

$$pH_I = 1.019\ pH_{IKC1} + 0.04$$

Bendall (1973) also states that the action of iodoacetate causes unavoidable pH shifts, resulting in an alkalinization of about 0.2 pH units at a natural pH of 7.0 and about 0.1 pH units at a natural pH of 6.0. Figure 1 illustrates values presented by Bendall (1973) and shows the pH measured with iodoacetate (·) and the calculated natural pH (▽) for measured pH's ranging from 7.3 (1) to 5.7 (9). Figure 1 shows that the measured and calculated actual pH are identical at pH 5.7, and 0.2 pH units apart at a calculated actual pH of 7.1.

Figure 1.

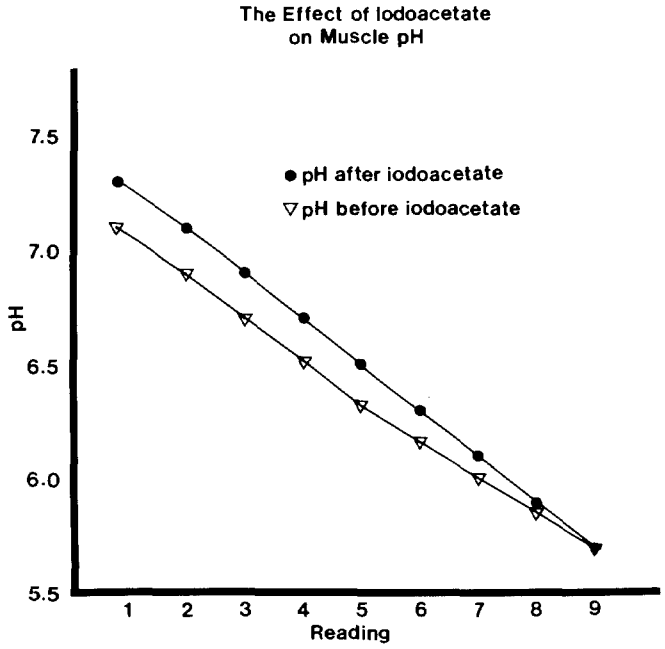

Figure 1. A plot of pH of samples which were measured with iodoacetate (●) and pH values that were calculated by considering the effect of iodoacetate on muscle components (▽). Adapted from Bendall (1973).

The temperature effect on the ionization of the muscle buffer system is another problem associated with pH measurement in muscle (Bendall, 1973; Westcott, 1975; Willard et al., 1981). This effect is somewhat compensated for by proper standardization and temperature calibration of the pH meter as described previously; however, the change in the various buffer systems in muscle due to temperature is likely different from the NBS reference buffer systems. Bendall (1973) has calculated a ΔpH of 0.2 between 0° and 20°C (at a 20°C pH of 6.90), and the NBS phosphate buffer standard has a ΔpH of 0.103 between 0° and 20°C (at a 20°C pH of 6.881) as shown by Willard et al. (1981). Thus, to obtain an accurate pH measurement of muscle at a specific temperature, the pH of the muscle/iodoacetate solution should be measured at that temperature and the electrode/pH meter should also be calibrated at that temperature.

Comparison of Iodoacetate and Probe pH Measurements

Although Bendall (1973) states that probe pH measurements also have inherent problems, such as temperature gradients in the probe itself and membrane potentials in early postmortem muscle, in general, probe-type measurements will give a more accurate pH than those obtained using iodoacetate solutions (providing the precautions discussed previously have been considered). We have conducted some determinations of pH using the probe electrode and iodoacetate solutions on the same muscle samples, and have made some comparisons for the purposes of this discussion (Dutson and Swatland, 1983).

Figure 2 and Figure 3 present comparisons between probe measurements (P) and iodoacetate measurements (I)

Figure 2.

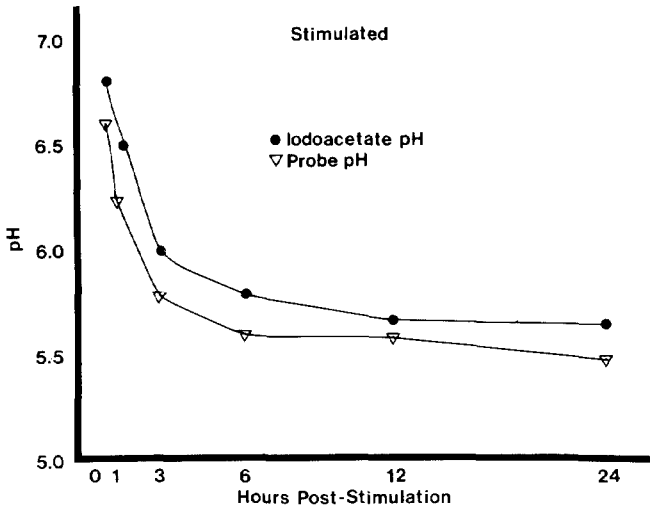

Figure 2. A graph showing the difference in pH values obtained by probe-type measurements and measurements using iodoacetate of electrically stimulated beef longissimus dorsi muscle. Each value is a mean of measurements from 30 sides (Dutson and Swatland, 1983).

Figure 3.

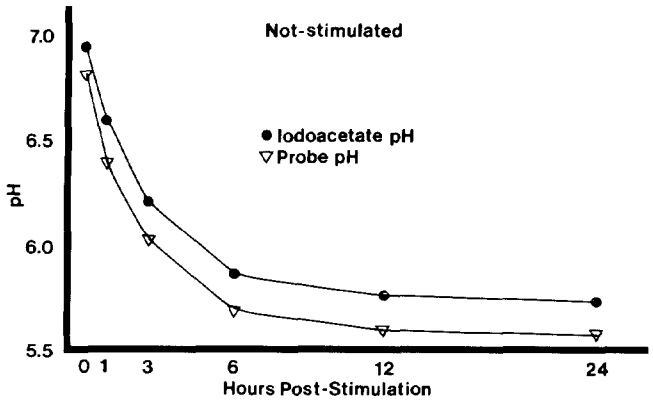

Figure 3. A graph showing the difference in pH values obtained by probe-type measurements and measurements using iodoacetate of the non-stimulated sides from the same carcasses as Figure 2 (Dutson and Swatland, 1983).

Figure 4.

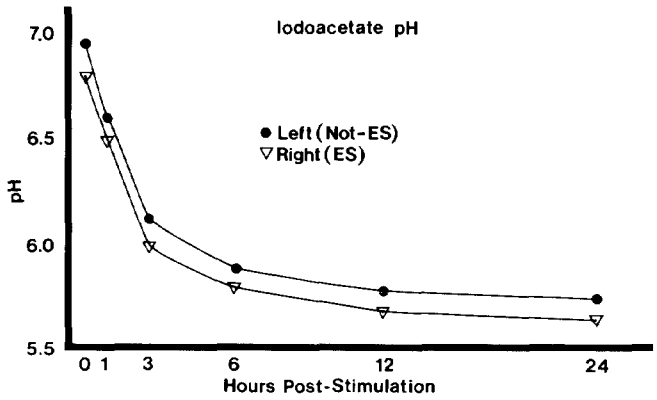

Figure 4. A graph showing differences in rate of pH decline of longissimus dorsi muscle between electrically stimulated and non-stimulated sides of the same animals as shown in Figure 2. Measurement of pH was conducted in homogenates of muscle in 0.005M iodoacetate (Dutson and Swatland, 1983).

for electrically stimulated (ES) and non-stimulated (NS) sides, respectively, from 30 beef carcasses (60 sides). As demonstrated in these figures, the I measurements are higher than the P measurements for both ES and NS sides at all postmortem times, which is in agreement with the calculations of Bendall (1973). However, the differences between I and P are greater at early postmortem times (high pH) when compared to later postmortem times in the ES muscles (Figure 2). The opposite is true (less difference between I and P at early postmortem times) for NS sides (Figure 3). The differences between the values presented in Figures 2 and 3 and those calculated by Bendall (1973) may be due to the effect of ES on nucleotide concentrations (Calkins et al., 1983), as these concentrations were used by Bendall (1973) in a portion of his calculations.

When comparing the pH decline between ES and NS sides within each pH measurement method (I and P; Figures 4 and 5), it is evident that both measurement methods show a faster rate of pH decline for the ES sides. However, the relative differences between the pH of ES and NS are greater at early postmortem times for the P measurements, and greater at later postmortem times for the I measurements. Therefore, different interpretations may be made from pH data, depending on the method of pH measurement, particularly if the treatments under investigation do not produce large differences in pH.

In order to determine if pH measurement by one method is closely related to that of another method, correlations between I and P pH values were computed at each postmortem time period and for each stimulation treatment. These data are presented in Table 1 (Dutson and Swatland, 1983). The correlation coefficients were generally small (0.10 to 0.60), which indicates a rather low relationship between pH measurement methods when specific treatments are being compared.

One conclusion to be drawn from the above information is

Figure 5.

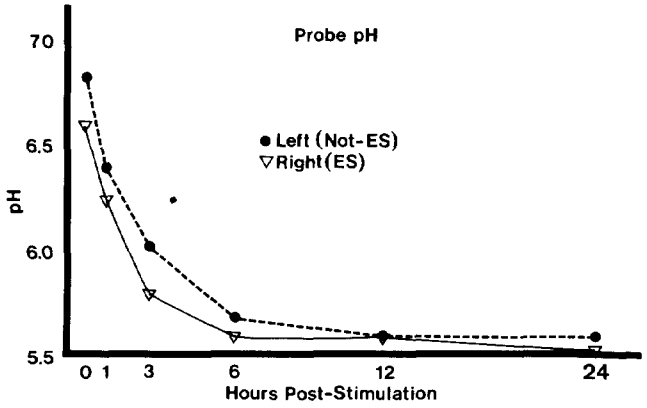

Figure 5. A graph showing the differences in rate of pH decline of longissimus dorsi muscle between electrically stimulated and non-stimulated sides of the same animals as shown in Figure 2. Measurement of pH was conducted by insertion of a probe electrode into the muscle (Dutson and Swatland, 1983).

that direct comparisons cannot necessarily be made between pH data that have been collected by different measurement techniques. Corrections could be made for differences in measurement technique, but this effect cannot be accurately determined due to the interaction between treatment effect and measurement method.

Measurement of pH in Post-Rigor Muscle

Many of the problems encountered with measuring pH of pre-rigor muscle are alleviated when post-rigor muscle sam-

Table 1. Beef Longissimus pH Values as Measured by Iodoacetate and Probe Electrode Techniques[a]

Time Post-Stimulation	LEFT SIDE (NOT-ES)			RIGHT SIDE (ES)		
	Iodoacetate	Probe	r	Iodoacetate	Probe	r
0 hours	6.937 (.151)	6.832 (.283)	.626	6.812 (.122)	6.611 (.195)	.587
1 hour	6.608 (.197)	6.400 (.257)	.640	6.502 (.178)	6.245 (.229)	.506
3 hours	6.224 (.275)	6.036 (.299)	.472	5.999 (.261)	5.778 (.208)	.624
6 hours	5.878 (2.68)	5.675 (.210)	.407	5.794 (.243)	5.608 (.202)	.480
12 hours	5.777 (.199)	5.605 (.106)	.522	5.673 (.125)	5.601 (.087)	.427
24 hours	5.746 (.096)	5.586 (.067)	.101	5.653 (.136)	5.548 (.079)	.525

[a]Each value is a mean of 30 animals. Value in parenthesis is the standard deviation (Dutson and Swatland, 1983).

ples are utilized. However, pH of post-rigor muscle precludes the determination of rates of postmortem glycolysis, and only ultimate pH can be used for analysis. Post-rigor muscle pH can be determined using the probe electrode, or by homogenizing. Homogenization of muscle samples gives a better average of muscle pH than probe-type measurements, providing that a large quantity of muscle is homogenized. Post-rigor muscle samples may be homogenized in either iodoacetate or in distilled water, but the dilution effect must be considered (Bendall, 1973). We have compared differences in pH utilizing both iodoacetate and distilled water and found no difference in the ultimate pH measurement. Post-rigor muscle samples can also be frozen and stored at normal freezer temperatures, since almost all of the metabolic changes have been completed by this time. However, some of the problems encountered measuring pH on pre-rigor muscle also need to be monitored when post-rigor muscle pH is to be determined. These include: clogging of the electrode membranes with fat and/or protein; improper contact between the meat sample and the electrode; accuracy of the pH meter standardization; the temperature of measurement; and the ionic strength (dilution of the homogenate).

Discussion

Question: Is there one particular type of electrode that works best for specific types of pH measurements?

Answer: The spear type of combination electrode works best for measurements on intact muscle but a regular combination electrode can be used for ground muscle and muscle homogenates. There are several brands that we have tried and all of them seem to work quite well. There are some differences in dimensions of electrodes and some are more fragile than others, so personal preference in addition to application and price are usually considered when purchasing electrodes. However, the regular combination electrodes with the plastic guards over the tip are less useful for measuring pH in muscle homogenates and ground muscle than those without protectors since the guards prevent proper rinsing and allow for more rapid membrane clogging.

Question: How accurate are the types of pH electrodes that measure the pH on the surface of a sample; not the probe type of electrode, but the type that have a flat membrane to measure the pH of a flat surface?

Answer: If the conditions are right, these electrodes can produce quite good results; however, one must have a wet surface to make sure a good contact is obtained between the muscle and electrode. Also, our experience has been that the surface electrodes themselves dry out fairly rapidly, which leads to clogged electrode membranes. If pH is being measured relative to microbial growth on the surface of a meat cut, then the surface electrode is the one that should be used. However, surface measurements may not be related that closely to the internal pH of the muscle, depending on what has happened at the muscle surface to change the pH; i.e. microbial growth.

Question: When measuring the pH of an iodoacetate/muscle homogenate, would it not be advantageous to measure all pH's at a standard temperature, such as 20°C?

Answer: This depends on whether or not you are interested in the exact pH of the sample at the time (and temperature) at which the sample was frozen/homogenized. If you are comparing actual pH's, it is best to measure the pH

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at a temperature which is equal to the muscle temperature when the sample was taken. Remember, according to the calculations of Bendall (1973), the pH at 0°C is about 0.2 pH units higher than it is at 20°C for the same sample of muscle.

Question: You mentioned that numerous pH readings need to be taken on pre-rigor muscle and mentally averaged; the mental averaging being done to save time of writing down many individual values. Could not a computer system be attached to the pH meter to average many readings and give one value to the operator?

Answer: This could be done; however, the operator would still need to have control of the readings that entered the computer so that erroneous readings of fat and air pockets could be discarded. This may be accomplished by having a button on the pH meter that would be pressed each time a value was to be entered.

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