Final report

Laboratory experimental testing of potential pH devices across meat samples with differing MSA marble scores

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1 Abstract

This study evaluated pH meters on the market to see whether any show potential as an alternative to the cumbersome TPS-WP80M. One of the challenges identified when measuring meat with high levels of Intramuscular fat (IMF) is the possibility of the pH probe's aperture being blocked leading to erroneous readings. Five meters were evaluated for their accuracy and ease of use on 86 samples. The IMF and pH of the samples varied (2.5-35g/100g meat) IMF; 5.17-6.02 pH). Cleaning the aperture regularly gave more accurate readings. The Halo pH meter was found to be less cumbersome and could be a replacement for the TPS-WP80M meter. One of the positive features of the Halo2 is that it is a small, light pH meter that uses blue tooth technology to record the readings on an external device. The Halo pH meter was then compared under commercial conditions on 126 carcasses and again on 94 carcasses. The meat pH values ranged between 5.39 to 7.01. The Halo meter gave similar readings to that of the TPS-WP80M (R^2 = 0.9587) when all procedures (calibration of pH and temperature, cleaning of probes) were carried out correctly.
The Halo2 showed potential as an alternative to the TPS-WP80M.

2 Executive summary

Background

- There is a perception amongst beef producers that the pH readings of carcases that contain high levels of Intermuscular fat might be erroneous due to the pH probe's aperture being contaminated.
- This study investigated whether, when proper procedures are followed during the MSA grading and/or just measurement of pH, this perception holds true.
- Various pH meters were evaluated to make recommendations on whether there are more modern meters available on the market that are comparable to the TPS WP80M and provide alternative options for commercial use that can be endorsed by MSA.

Objectives

- Conduct a literature review that focuses on pH measurement combined with temperature technologies that may be applicable to the Australian meat industry to measure muscle pH as part of the MSA grading process.
- To test any potential devices for accuracy and repeatability ensuring that the tested devices could be used in a commercial environment – like the current industry standard handheld device (TPS-WP80MM). Within this second objective, meat samples were tested across varying intramuscular fat scores.
- The identified pH meter (Halo) was evaluated on-line in at least 2 commercial abattoirs against a diverse range of cattle types to establish the practicality of the suggested devices. The Halo pH meter (model 1 and 2) were evaluated against the TPS-WP80MM on two occasions in a commercial abattoir on chilled carcasses, as well as under other MSA measuring conditions by the MSA R&D Strategy and Integrity Systems Manager.

Methodology

Five pH meters were evaluated under laboratory conditions on their suitability to measure the pH of meat samples containing different levels of intramuscular fat.
The Halo pH meters was found to be a potential alternative for the more cumbersome TPS-WP80MM and was evaluated under commercial conditions.
Advantages and disadvantages of the Halo was compiled during more in-depth field evaluations.

Results/key findings

It was shown that if the standard operating procedures (regular cleaning of the aperture on the pH probe, thorough calibration) that the level of fat in the meat did not influence the pH readings.

The Halo pH meter was found to be less cumbersome, and it is suggested that this meter be evaluated further as a potential alternative for the TPS-WP80M currently being used in the MSA grading system.

Benefits to industry

The benefit to the industry is indirect in that the taking of the pH readings during the MSA grading will be less strenuous and cumbersome.

Future research and recommendations

It is recommended:
- that the commercial entity that owns the Halo2 pH meter continue to work with MSA to further test and evaluate the Halo2 pH meter across larger numbers of carcases across beef and also look at capability in lamb and for longer time periods to understand impacts on battery life to ensure robustness of the pH probe and meter.

- to include measuring of rate of decline in hot carcases as part of the further commercial testing.

- to engage with the manufactures of the TPS WP80M meter to test their appetite for a meter that has blue tooth capability and a more modern design.

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1. Background

Presently there is a belief amongst beef producers, particularly those who produce carcases with high levels of IMF that the pH readings taken during the MSA classification are sometimes erroneous. The MSA method used at grading is a puncture glass electrode. It is theorised that the fat and protein particles block the small portal/aperture containing the membrane in the meat pH probe thereby limiting the flow of ions which the probe measures and converts into a pH reading.

The conventional practice of measuring pH in meat is based on simple electrochemical methods. A puncture glass electrode is inserted into the muscle, whole, or homogenised meat to determine its pH. The accuracy of this method of measurement has been queried before. In 1986, the pH of 80 beef and 120 pork muscles were measured using electrochemical pH meters in two different electrode combinations: puncture electrode and surface electrode with three different meat presentations via. muscle, meat and water mixture, and muscle homogenate. A significant difference in the pH values was observed and the differences were higher with different electrode systems than with different meat presentations (Korkeala et al., 1986). Another experiment with portable pH meters and puncture electrodes revealed that a significant difference exists in these values and this difference is said to bring some practical changes to the meat as well (Mäki-Petäys et al., 1991). The fat thickness in the meat apart from directly influencing pH and water holding capacity is also said to affect pH measurements when using puncture electrodes (Dallantonia et al., 2015). The probes' membrane become contaminated with muscle proteins and fat which affects the accuracy of the readings (Dutson, 1983).

The pH of meat influences its colour, water-holding capacity, flavour, tenderness, and shelf-life. Its measurement at different times post-mortem provides information about forthcoming quality characteristics. The pH value at rigor mortis is termed the ultimate pH (pHu). To monitor ultimate pH in a meaningful way, a proper measurement is necessary. However, because of the inherent characteristics of such measurements, it has not always been easy to achieve accuracy and reliability (Honikel, 2004).

Optical or spectroscopic methods are alternatives to conventional pH probes. The spectrometric methods monitor the change in absorption spectra at different pH levels (Monin, 1998; Andersen et al., 1999). Near infrared (NIR) spectroscopy is being used in various laboratories to accurately monitor a variety of quality parameters in beef and several other meat products (Zhang, 2013). However, spectroscopic methods require extensive calibration on different types of meat samples to monitor the pH levels. None the less, an added advantage using spectroscopic methods is that the in-line methods for measuring pH are supposed to be instant, compact, and cost expensive. A compact device based on the principles of spectroscopy would be a great alternative to current pH meters.

This research project was designed to evaluate the effect that different levels of intramuscular fat might have on the accuracy of pH measurements. At the same time, alternative pH meters were to be evaluated and their suitability tested, first in a laboratory setting, whereafter, the selected pH meters were to be evaluated for their suitability under commercial conditions.

Conflict of Interest: As there was a possibility that the results of this research could have economic implications for the manufacturers of the different pH meters, none of the manufacturers were contacted regarding their products. All pH meters were purchased from the suppliers with the information supplied that the meters were to be used for research. It was only with last objective,

evaluation under commercial conditions, where the supplier was contacted and requested to supply additional technical information.

2 Objectives

This project had 3 objectives

1) The first was a literature review that focuses on pH measurement combined with temperature technologies that may be applicable to the Australian meat industry to measure muscle pH as part of the MSA grading process. Within this review a number of potential pH meters as well as the use of NIR were identified for possible evaluation.
2) From this review, in consultation with the MSA team, the second objective was to test any potential devices for accuracy and repeatability ensuring that the tested devices could be used in a commercial environment – similar to the current industry standard handheld device (TPS-WP80M). Within this second objective, meat samples were tested across varying intramuscular fat scores. Five pH meters identified were evaluated for suitability to be used in Objective 3 (in an abattoir). The pH meters were first tested against a Tabletop pH meter that was designated as the "gold standard".
3) Thereafter the identified pH meters were evaluated on-line in at least 2 commercial abattoirs against a diverse range of cattle types (MSA marble score 100-1190) to establish the practicality of the suggested devices. The final output, post approval and endorsement by the MSA Supply Chain Taskforce and Pathways committees, will be a recommendation around pH measurement devices and any potential considerations for measuring pH in high MSA marbled carcases. The Halo pH meter (model 1 and 2) were evaluated against the TPS TPS-WP80M on two occasions in a commercial abattoir on chilled carcasses, as well as under other MSA measuring conditions by the MSA R&D Strategy and Integrity Systems Manager to evaluate its suitability.

3 Methodology and Results

3.1 Literature Review

The objectives of this literature review were to compile a review that focuses on pH measurement combined with temperature technologies that may be applicable to the Australian meat industry to measure muscle pH as part of the MSA grading process. A second objective is to evaluate from the literature, how other countries measure pH and lastly, to report back on any alternative technologies that might be of interest to measure meat pH.

3.1.1 History/background of pH measurements in Australia and Meat Livestock Australia

Typically, pH is used routinely in meat processing plants. Manufacturers of sausages and cooked and uncooked hams usually test the pH on incoming raw materials. In South Africa, it is not uncommon for some retailers to measure the pH of incoming beef carcases where carcases with a pH above a certain value are returned, this cut-off pH point differs between retailers – the main reason for taking a pH measurement is to identify dark cutters who are DFD (Dark, firm and dry). However, when prepacked products such as steaks are brought into the retail display cabinets, no pH measurements are taken. Measuring pH gives important information about potential microbial spoilage/shelf-life, colour and water- holding capacity of the meat – parameters that are important during the processing of meat products. pH measurements are also an important parameter in fermentation processes, where pH is measured to establish whether the fermentation is proceeding in a normal way. This is relevant when

producing salami type products (Boggard & Andersen, 2004). Typically, a pointed electrode is inserted into the meat to measure the pH – sometimes, a sharp blade is attached around the pH electrode point to cut a slice in the muscle to minimise the possibility of the glass electrode breaking.

pH measurements play an important role in the pork industry where a pH reading taken between 30- and 45-mins post-mortem is an indication of whether a negative meat quality phenomenon called PSE (pale soft and exudative) might occur. Pigs are prone to *ante-mortem* stress and when experienced this results in a rapid fall in muscle/meat pH whilst the carcase temperature is still high resulting in denaturation of the integrity and structure of the muscle fibres causing the meat to lose its water binding capacity. This phenomenon causes huge economic losses in the pork industry. Although pork can also exhibit the DFD phenomenon, this is not so common in porcine. Similarly, although cattle and sheep can develop PSE, this is not as common as the development of DFD. There have been a few documented cases of PSE-like conditions in wild ungulates, also known as white muscle capture myopathy, typically induced during long *ante-mortem* stress conditions.

The measurement of pH plays a key role in the Meat Standards Australia (MSA) as it is a disqualifier for MSA grading. If an inaccurate pH measurement is taken, the result could have commercial consequences for the producers (McGilchrist et al., 2012; 2014; 2019). MSA requires that a carcase must have a pHu <5.71 to be eligible for grading – producers face a financial loss for carcases with a pHu >5.71. Using kill data from 2009, McGilchrist et al. (2012) calculated that for the 1,157,781 cattle slaughtered and MSA graded in Australia that year, 5.45% had a pHu >5.71 and with a penalty of $AUS0.50 per kg this equated to a loss of $7.09 per animal graded with an average weight of 260kg. In the 2012-13 financial year, 4.8% of the carcases subjected to MSA grading were non-compliant as pertaining to the pHu. In the previous financial year (2019-2020), 4.01 % were non-compliant (Table 1) whilst for 2019/20, the price differential between MSA and non-MSA grainfed cattle was $0.11/kg and price differential between MSA and non-MSA non-grainfed cattle was $0.27/kg (based on Hot Standard Carcase Weight). It is speculated that the high non-compliance during 2017/18 and 2018/2019 is linked to the wide-spread drought experienced throughout Australia. In the 2020-2021 financial year, 3.80% of the carcases graded were non-compliant as pertaining to the pHu.

Table 1: Indication of the total number of carcases graded and the number non-compliant due to pHu >5.71.

Year	Number carcases graded	Number non-compliance due to pHu	% non-compliance due to pHu
2020/21	3,300,000	125,400	3.80
2019/20	3,760,266	150,872	4.01
2018/19	3,484,058	339,830	9.75
2017/18	3,132,102	303,336	9.68
2016/17	2,781,465	130,690	4.70
2015/16	3,1034,97	163,243	5.26

It is not the objective of this literature review to discuss the MSA program nor debate the role and weighting of the various factors that make up MSA. A thorough description of MSA can be found on the Meat and Livestock Australia's (MLA) webpage, whilst several scientific papers have been delivered at international conferences and/or published after undergoing a rigorous peer review (see as example Polkinghorne et al., 2008; Polkinghorne & Thompson, 2010; Watson & Polkinghorne 2008a,b; Yuan et al., 2016). What is important to note is that MSA is seen as being a world leader in measuring and predicting consumer expectation of meat quality of various muscles prepared under different cooking methods. This literature review is focused on (1) the technology of pH measurement, particularly in meat samples that have high levels of intramuscular fat (IMF) and, (2) alternative devices commercially available for determination of meat pH – highlighting their advantages and disadvantages.

None the less, a brief overview of the post-mortem physiology and the biochemistry linked to the transformation of muscle into meat and the role that pH plays in influencing the meat quality is required.

3.1.2 Post-mortem physiology of meat and role of pH

When an animal is slaughtered, its physiological muscle's pH is around 7.0, immediately after slaughter, the pH might rise to 7.2 before decreasing to an ultimate pH (pHu) of 5.3-5.8. The rate of decrease as well as the pHu is determined by numerous *ante*- and *post-mortem* factors that include *ante-mortem* stress experienced by the animal, the slaughtering method/technique and electrical stimulation (ES), chilling rates after the animal is dead and as it enters the chiller (Balan et al., 2019), muscle type, sex of the animal (Fig. 1, North et al., 2016).

The conversion of muscle into meat *post-mortem* has been well reviewed, even though there are still some areas where our scientific knowledge is scarce (see reviews by Ferguson & Gerrard, (2014) and Ponnampalam et al. (2017) for more details on causes of DFD in beef). A muscle cell will not immediately enter an apoptosis state as the animal dies. From a muscle physiological point, the muscle cells enter an anaerobic state due to the animal dying and no longer providing oxygen to the cells. One of the products of this anaerobic glycolysis is lactic acid: 0.1 mol/l lactic acid forms from glycogen in the anaerobic glycogenolytic pathway resulting in a decrease in pH. However, due to the buffering

capacity of other constituents (side chains of amino acids, peptides such as anserine and carnosine, phosphate ions, etc.) in meat, the pH of an aqueous 0.1 mol/l lactic acid solution (pH = 2) differs from that of a similar concentration lactic acid in meat (pH = 5.3-5.8). These buffers differ in their buffering capacities – the buffers bind the H^+ ions from the lactic acid formed and the concentration of lactate anions increases. Typically, less than 1% of the lactic acid formed is in its dissociated form; more than 99% is undissociated lactic acid (Honikel, 2004).

Post-mortem energy metabolism or glycolysis in muscle is highly relevant to ultimate meat quality, particularly tenderness. Typically, estimates of glycolytic rate in post-mortem muscle are obtained from measurements of pH over time (as example see Cadavez et al. (2019) for different parameters influencing the exponential decay models for meat pH/temperature). The rate of glycolysis post-mortem can influence two central mechanisms, which ultimately govern myofibrillar tenderness; the degree of myofibrillar contraction and the rate and extent of proteolysis during ageing (Ponnampalam et al., 2017).

Anaerobic glycolysis is driven by enzyme activities which are strongly influenced by temperature. Historically, after slaughter carcases were hung in a 'chilling' corridor before being moved into the chiller. The chilling corridor allowed for the gradual chilling of the carcases. As abattoirs became more efficient and slaughtered large numbers of animals per time unit, carcases were moved into the chillers more rapidly. To counteract the rapid loss of heat, and the subsequent decrease in proteolytic enzymes' activities, carcases were electrically stimulated (ES) to activate the enzymes whilst the carcase temperatures were still high. Modern abattoirs could have numerous interventions including electrical inputs from restrainers, stimulation systems and rigidity probes which all interact to determine the rate of pH decline. Over stimulation causes a too rapid decrease in muscle pH whilst the carcases are still too warm, causing denaturation of the proteins including the enzymes resulting in a decrease in various meat quality attributes (Balan et al., 2018).

To counter these potential negative interactions, an 'abattoir window' was established and incorporated into the MSA standard to take the effect of the temperature pH decline relationship into account. This window requires temperatures to be above 12°C and below 35°C at the point that the meat's pH reaches 6.0 as measured in the loin (Polkinghorne et al., 2008).

Numerous *ante*- and *post-mortem* factors as well as interventions during the slaughter process (such as electrical stimulation of the carcases and cooling rates – Warner et al., 2014) are known to influence the pH_u of meat (see reviews by Ponnampalam et al., 2017; Balan et al., 2018; Cadavez et al., 2019) – most of these have been included into the MSA grading system. Australian meat scientists have accessed the MSA data and from these have identified some of these factors and indicated strategies to minimise their impact - see for example McGilchrist et al (2012; 2014) where the effect of eye muscle area, ossification, carcase weight, marbling (IMF) and rib fat depth, season of slaughter, etc., were evaluated. Interesting in their 2012 paper, IMF had no effect on dark cutting however, marbling score is a factor taken into consideration in MSA and in their data analyses, MSAmarb scores (range of 100-1100) were no higher than a mean of 327.4 ± 38.31 (±s.d) for the 2008 data. Few of these carcases were Wagyu where higher MSAmarb scores are recorded. McGilchrist et al. (2012) did note though that for every 100 points increase in marbling score, the mean pH_u decreased by 0.0034 pH units. The challenge is to find a representative sampling site as the level of marbling is known to vary between locations in the same muscle (Elmasry et al, 2012), and with a probe, the region where the aperture lies is not visible, therefore it is not known whether this aperture is surrounded by IMF or not.

Numerous researchers have indicated that muscle pHu is determined by the energy levels in the muscles at slaughter (strongly determined by available feed/nutritional status prior to slaughter, see for example: Chingala et al., 2019; McGilchrist et al., 2014) and it is expected that the presence of IMF is an indication of sufficient *ante-mortem* nutrition resulting in sufficient energy levels in the muscle. However, it is not clear how the higher IMF typically found in Wagyu meat will influence the meat's pHu.

Time of measurement: As the anaerobic glycolysis proceeds over time, the muscle's pH will change/normally by decreasing (Fig. 1). The rate of this change is temperature dependant. It is therefore self-explanatory that the time that a specific pH measurement is taken is crucial, particularly when it is taken soon after death. The ideal time to take an ultimate pH reading is when the carcase has gone into full *rigor mortis*. It has been noted that during prolonged ageing, the pH of the meat could start increasing again. It has been suggested that this increase could be due to bacterial activity and/or the denaturation of proteins and the NH^{2+} becoming NH^{4+}, thereby removing the H^{+} and causing an increase in the muscle pH readings. An interesting phenomenon noted in the ostrich fan fillet (*M. iliofibularis*), is that in this specific muscle, the pH increases over time, irrespective of whether the ostriches had been electrically stimulated or not (Fig. 2). When ostrich muscle had been aged, the pH decreased once more (Fig. 3) – this decrease is typical of that found in most red meat (Figure 1).

Figure 1: **The decline in the pH of the *Longissimus thoracis et lumborum* (LTL) muscle in female (r^2 = 0.85) and male (r^2 = 0.76) springbok and *Biceps femoris* (BF) muscle in female (r^2 = 0.87) and male (r^2 = 0.81) springbok during the first 30 h *post-mortem* as described by non-linear regressions (North et al., 2016).**

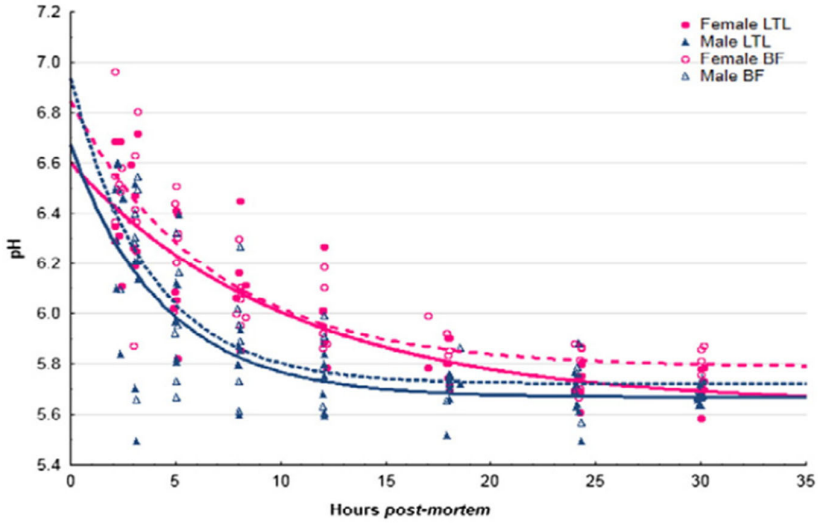


Figure 2: **Trends in the pH of the fillet muscle (*M. iliofibularis*) of ostrich carcasses subjected to electrical stimulation and untreated controls over time. Vertical lines about the means represent standard errors (Hoffman et al., 2009).**

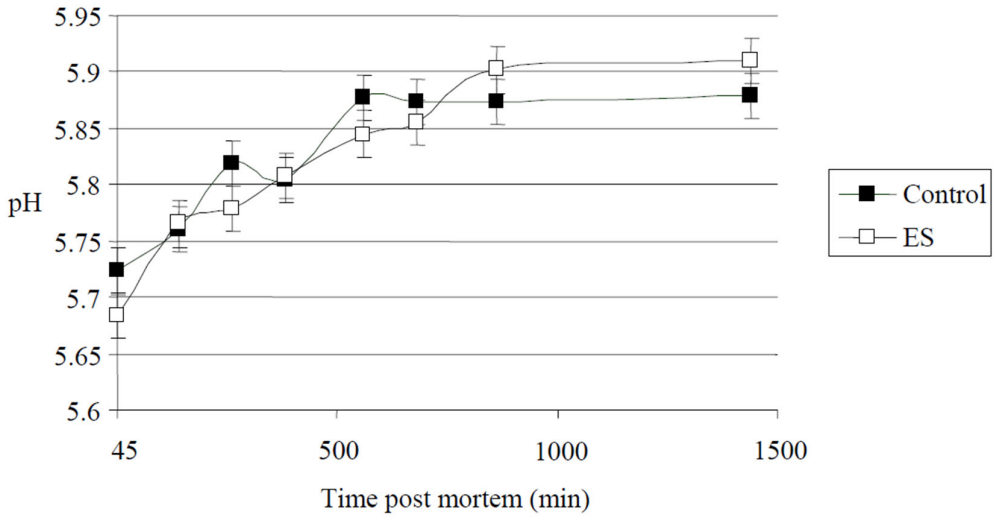
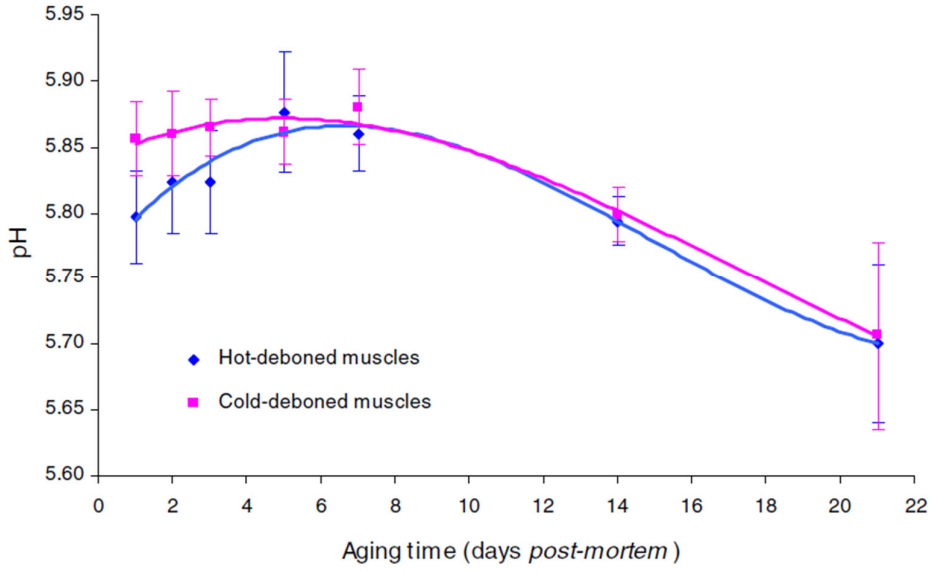


Figure 3: **Third order polynomial trend lines for muscle pH (± standard error) over aging time of 21 d *post-mortem* for respectively, the hot-deboned ($y = 7 \times 10^{-5}x^3 - 0.0033x^2 + 0.0343x + 5.7635$; $R^2 = 0.9733$) and the cold-deboned ($y = 3 \times 10\text{-}5x^3 - 0.0016x^2 + 0.0135x + 5.8401$; $R^2 = 0.9864$) *M. gastrocnemius, pars interna* of ostriches (Botha et al., 2007).**



It is worth noting that there is variation between muscles and within muscles as pertaining to the anaerobic metabolic rates. These differences can be linked to numerous factors including fibre types, enzyme activities – both these factors being influenced by numerous animal production factors such as exercise, age, sex as well as environmental/genetic factors such as *ante-mortem* stress and

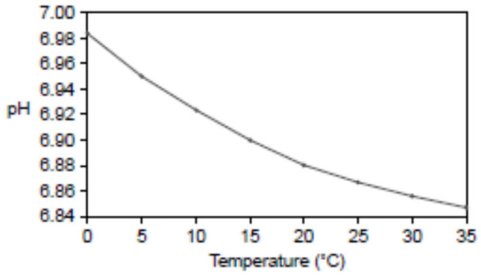
disposition towards stress. The within-muscle variation is interesting and to minimise the influence thereof, MSA require that the pH reading be taken at a specific point (normally between the 12th/13th rib along the quartering line). As mentioned, MSA takes this and numerous other factors into account when calculating the final MSA score of a carcase (Watson & Polkinghorne, 2008a,b).

3.1.3 Theory behind pH measurement

3.1.3.1 Effect of temperature?

Most chemical reactions are temperature dependent, including the dissociation of acids and bases. Internationally, pH is recommended to be measured at ambient temperature of 20–25 °C. However, in meat, temperatures between 0 and 43 °C are common, so this must be kept in mind in interpreting pH measurements in meat, as in most cases calibration is done at around 20 °C, yet the meat may be at a different temperature when the pH reading is taken. Even a temperature adjustment at the glass electrode itself corrects only the temperature dependence of the glass electrode and not the changes within the meat matrix or meat product, which may follow a different temperature dependence. Therefore, it is recommended to accept pH readings of meat to no more than one decimal place, if the calibration temperature and the temperature of the meat differ by more than 10 °C. Greater accuracy demands a calibration at the temperature of the meat. In these cases, the temperature drift of calibration buffers must also be considered (**Fig. 4**).

Figure 4: The effect of temperature on a phosphate buffer (Honikel, 2004).



Care should be taken to ensure that the measurements are standardised and where required, adjusted to a standard final temperature using Bendall's equation (Bendall, 1978).

3.1.3.2 What is pH?

The pH of a solution is defined as the negative base 10 logarithm (log10) of the concentration of hydrogen (H^+) ions or, the concentration of the reaction of H^+ with a water molecule to produce a hydroxonium ion (H_3O^+). For example, as described by Honikel (2004), a concentration of 10^{-5} mol/l H_3O^+ in an aqueous solution has a pH of 5. The pH scale in aqueous solutions ranges between 0 and 14. Pure water has a pH of 7.0. Hydrogen ions are formed when acids such as lactic acid (CH_3–CHOH–COOH) dissociate according to eqn [1].

$$CH_3\text{–}CHOH\text{–}COOH \leftrightarrow CH_3\text{–}CHOH\text{–}COO^- + H^+ \quad [1]$$

The dissociation of acids can be complete in strong acids such as hydrogen chloride, or it can be in an equilibrium in medium strong acids like lactic acid. Like pH, the degree of dissociation is defined as the p*K* of an acid (HA), where *K* is the dissociation constant of the reaction. The relationship between p*K*, concentration of hydrogen ions (C_{H+}), concentration of anion (C_{A-}) and concentration of acid (C_{HA}) is shown in eqn [2].

$$\mathrm{p}K = -\log\left(\frac{C\mathrm{H}+ \times C\mathrm{A}-}{C\mathrm{HA}}\right) \quad [2]$$

p*K* values are found in published tables. For example, for a 0.1 mol l^{-1} solution at 20–25 °C, the given p*K* value for lactic acid is 3.1. Using the p*K* value, the pH value of a solution of a weak or medium strong acid can be easily calculated. Using lactic acid as an example, the pH of a pure 0.1 mol l^{-1} lactic acid solution in water can be calculated using eqn [2], where it can be assumed that the concentration of H^+ is equal to the concentration of A^- (anion CH3–CHOH–COO^-). Thus $C_{H+} = C_{A-}$. Substituting the published p*K* value into eqn [2] results in eqn [3], which can be solved to give a pH value of 2.04.

$3.1 = -\log (C_{H+} \times C_{H+})/10^{-1}$ [3]

$8.4 \times 10^{-4} = (C_{H+})2 \times 10^{-1}$

$8.4 \times 10^{-5} = (C\mathrm{H}+)^2$

$C_{H+} = (8.4 \times 10^{-5})^{1/2}$

$C_{H+} = 9.16 \times 10^{-3}$

pH = 2.04

The pH of an aqueous solution of 0.1 mol/l lactic acid is therefore around 2.0–2.1 (Honikel, 2004).

3.1.4 Standard pH measurements and equipment used.

pH meters consist of two main parts, a pH electrode connected to a device that is programmed to display the pH (and frequently also the temperature). In fact, this device is a precision voltmeter that measures the charge/potential difference between the solution's electrolytes. Most of the electrodes used consist of a glass tube ending in a glass bubble, the latter is the active part of the electrode. For the measurement of meat pH, the round glass bubble has been replaced with a pointed glass spear. Some pH electrodes are also supplied with a blade that fits around the pointed sphere and aids in piercing through the muscle thereby helping to protect the fragile glass spear-point from fracturing (Fig. 5). Inside the bubble/spear-point is usually filled with buffered solution of chlorides in which silver wire covered with silver chloride is immersed. This solution could be either Ag/AgCl or $Hg/HgCl_2$ (ISO_2917_1999: Meat and meat products – Measurement of pH – Reference Method). The pH of the internal solution varies - for example it can be 1.0 (0.1M HCl) or 7.0. Surface of the glass is protonated by both internal and external solution till equilibrium is achieved. Most commercially available pH electrodes are combination electrodes that have a glass H^+ ion sensitive electrode and an additional reference electrode, both placed in one housing (Fig. 5). Both sides of the glass are charged by the adsorbed protons, this charge is responsible for potential difference measured by the meter after it has been enhanced. There is normally an aperture that allows for the ions outside and inside to reach equilibrium. This hole is blocked by a porous membrane, or a ceramic (asbestos in older models) wick. Internal solution flows very slowly through the junction; such electrodes are called flowing electrodes. To slow down leakage through this membrane, the internal solution is gelled.

As the electrode is placed into the meat, a potential difference builds up on the sides of thin glass in the bubble due to the difference between H^+ activities on both sides, this potential difference is measured with the help of reference electrodes - and is known to be proportional to the pH on the outside of the bubble. Although the reliability and precision of today's pH meters are good, they are still limited by the electrode construction and properties of the solution/meat itself. The potential measured by pH meter and indicated as a pH, is a sum of all potentials present in the system. There are three sources of electromotive forces that result in electoral potentials. (1) a potential that builds

up on the glass electrode, thanks to different activities of the H^+ ions on both sides of the glass. (2) is the glass electrode silver wire covered with AgCl and immersed in the solution of chlorides, and (3) is the reference electrode - silver chloride or calomel, depending on the application. Use is made of the Nernst equation (which takes these three potentials into account) to calculate a potential which is directly proportional to the pH difference between the solutions both sides of the glass electrode (http://www.ph-meter.info/pH-meter).

An important factor to remember is that the pH will differ according to the temperature of the medium. Most modern pH meters and electrodes come with automatic temperature compensation (ATC) where the signal from a separate temperature probe (sometimes built into the electrode) is fed into the pH meter, so that it can accurately determine a pH value of the sample at that temperature (Fig. 6). As this function differs between different electrodes and pH meter brands, care should be taken that the temperature of the meat is considered – this also includes the temperature of the buffers when calibrating the meter. The most exact pH value is obtained when the temperature of the calibration solutions and measured meat are identical.

As the pH probe is made of very thin glass, and easily breaks off, some pH probes are fitted with a blade that shears the meat as the pH probe is inserted (Fig. 5).

Figure 5: Automatic temperature compensation pH probe fitted with a blade.

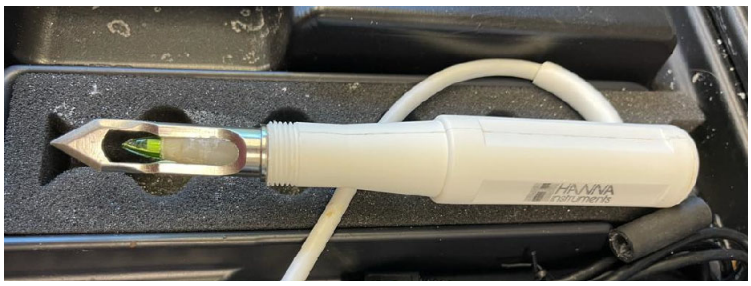
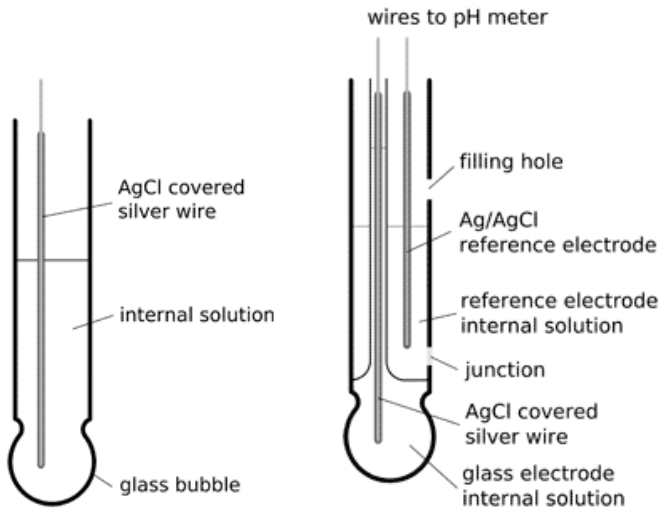


Figure 6: pH electrodes showing the basic structure, the probe on the right has a reference electrode.



A major problem that could occur with the pH measurement is that fat and protein could block the membrane placed at the open junction between the external medium (meat) and the internal solution. This is particularly of concern when there is a high IMF content (as found in Wagyu cattle) containing a high proportion of unsaturated fat. The ISO standards (ISO_2917_1999) suggest that the electrodes be cleaned by wiping them with pieces of cotton wool wetted with diethyl ether (saturated with water) followed by ethanol ((C_2H_5OH), 95 % volume fraction). Finally, the electrode is washed with water. There are alternative suitable cleaning solutions such as 0.1 mol/L HCl acid or in an ultrasonic bath or any one of the commercial cleaning solutions available on the market. It is suggested that after each reading the electrode be cleaned by rinsing with deionised water; the electrode should not be wiped clean with a tissue paper as this will scratch and damage the pH-sensitive glass membrane causing the signal being read to be unstable.

3.1.5 Carcase grading and meat quality standards/classification in other countries

Polkinghorne & Thompson (2010) define classification and grading. Broadly speaking classification describes the different features of a carcase and places it into a specific class. South Africa as example uses a classification system where carcases are defined according to various criteria including sex, age, fat cover, conformation, etc. Grading on the other hand, places a specific value on a carcase/cut – this value being defined by a set of quality ques. From within the (MSA) grading system, a consumer grading system was developed which defines consumer satisfaction of a specific cut cooked in a specific manner – this is typical of a meat quality system with a consumer's potential eating experience/satisfaction linked to it.

Most countries have a standard beef grading system whilst meat quality systems with the consumer's need/perception as basis is lacking. Typically, these grading systems are developed from measurements taken on the carcase itself and include various combinations of carcase weight, sex, age or maturity (using either teeth eruption or level of ossification), marbling, meat colour, fat colour, firmness, texture, lean maturity, and fat thickness. Some of these measurements are then included into equations to predict the saleable meat yield. There has however been movement in various countries to develop meat quality standards, frequently based on MSA. One such standard is the

development of a Meat Standards Wales (MSW) grading system (BeefQ), to be used in addition to the compulsory EUROP classification system, where they hope to improve the eating quality and increase the value of Welsh beef (http://www.beefq.wales/index.html). South Africa has also visited the MSA team in Australia early in 2020 to gain insight into the MSA system as a possible model for implementation – this need was identified by the retail industry and is being driven by the South African Red Meat Producers Association.

The main beef grading system in use in the UK and Europe is the EUROP system, introduced in 1981 under the European Commission. Grading is done at the end of the slaughter line just before chilling. The EUROP system evaluates carcase conformation and fat cover with six conformation classes (S=superior, E=excellent, U=very good, R= good, O=fair, P=poor) and 5 fat cover classes (1=low, 2=slight, 3=average, 4=high, 5=very high) respectively using either a certified human grader or a video image analysis system. The aim of the EUROP system is to describe carcases for those involved in slaughtering, cutting, distribution and retailing according to terms relevant to trading. The EUROP system is a good indicator of yield but has the limitation of not being linked at all to the consumers' eating quality experience. In fact, Bonny et al. (2016) reported that Polish, French and Irish consumers found no correlation between sensory quality scores and the EUROP system – they evaluated 18 muscles from 455 carcasses using the MSA protocols.

While EUROP is the standard system of evaluating beef for pricing, there are many different quality assurance schemes and processes in Europe that are applied in addition to this. These schemes take advantage of consumers' willingness to pay a premium for foods associated with a particular geographic or production system of origin. These schemes include European Commission controlled labels such as "Protected Designation of Origin (PDO)", "Traditional Speciality Guaranteed (TSG)", "Protected Geographical Indication (PGI)" and Organic as well as other national schemes such as "Label rouge'' from France and ''Red-Tractor'' in the UK with production system descriptors such as "free range" or "grass-fed'' also being used by retailers (Erasmus, Muller, & Hoffman, 2017). Being granted a status under these different schemes has economic importance for that specific red meat industry (producers, processor, etc), as it identifies the origin and unique production qualities of that meat (beef, lamb, etc).

Although these schemes are important from a marketing point of view, it does not provide any guarantee as to the eating quality experience for consumers. In fact, most beef grading systems around the world are yield focused and therefore tailored for meat traders, not consumers. These grading systems may either be compulsory or voluntary. The European (EUROP), Canadian, United States of America (USDA), South Korean (Korea), Japanese (JMGA – Japanese Meat Grading Association) and South African (SA) grading systems are compulsory. In Australia there is a chiller assessment system AUS-MEAT which involves the measurement of traits such as carcase conformation, marbling, ossification, and meat colour. The AUS-MEAT chiller assessment data feeds into a voluntary grading system (MSA system in Australia) that does include eating quality prediction. Similar voluntary systems are in operation in New Zealand (e.g., Silver Fern Farms quality guarantee in New Zealand). The EUROP and SA grading systems are focused on yield and fat cover and classify carcases based on carcase weight and conformation as well fat cover (sex and age are also included in the SA classification system). For Canada, USDA, Korea and JMGA, eating quality is inferred from subjective assessments of intramuscular fat content (marbling) and carcase maturity (ossification score and dentition). These inferences are made based on known scientific associations between the observed characteristics with eating quality. The assessments may be wholly visual or make use of standards, such as the colour standards used in AUS-MEAT chiller assessment which are also used in the MSA grading system. The South African (SA) and AUS-MEAT grading systems use dentition to

determine age or maturity whilst the MSA, USDA, Canadian and Korean systems use ossification scores. Additionally, other characteristics such as meat texture, meat colour and lean maturity are used in the MSA, USDA, AUS-MEAT, Canadian, Korean and JMGA systems.

Japan has a Meat quality grade (JMGA grade) where numerous parameters are assessed after quartering the carcasses between the 5th and 6th ribs (Polkinghorne & Thompson, 2010). The parameters assessed include 12 beef marbling scores that are converted into 5 beef marbling grades. There are also 5 beef colour and brightness scores, 5 firmness classifications and 5 texture classifications that are combined to provide a final firmness and texture grade. There are also 7 beef fat standards used to describe the fat colour, texture, lustre, and quality – all combined to form a beef fat colour and lustre grade. These grades are then all combined to allocate a carcase quality grade from 1 (lowest) to 5. Japan also has a beef quality assurance program called "Kobe beef" which is only applicable to animals from the Tajima-gyu strain of Wagyu cattle that have been raised in the Hyogo Prefecture. To qualify the carcase must have a weight <470kg, have an A or B yield grade, have quality scores of 4 or higher, have marbling scores of 6 or higher as well as have a fine meat texture and excellent firmness.

New Zealand has a Beef and lamb quality mark, and the animals must originate from within New Zealand to qualify. No growth promotants ae allowed, carcases must be electrical stimulated and follow an approved chilling and aging regime. This scheme also requires an ultimate pH <5.8 or else the meat must have undergone a supplementary ageing period followed by a shear force testing thereafter.

In the USA, there are several quality Assurance programs linked to specific brand names. These include Nolan Ryan tender-aged beef, Cattleman's Collection, Swift's Chain of Tenderness, and Safeway Rancher's Reserve Angus (Yuan et al., 2016). Interestingly, there are only a few common measurements/interventions between these Quality Assurance Programs in the US and include electrical stimulation and an ageing period. Some have additional colour measurements or shear force testing with maximum cut-off forces. Most of the measurement are done on the *Longissimus* muscle, which is in fact a poor predictor of quality attributes in other muscles within a carcase.

The USDA and the MSA are the only quality grading systems that predict the eating experience of the consumer; although these two use different approaches. The USDA is based only on measurements made on the carcase and raw meat, particularly the rib eye muscle, whilst the MSA grading system uses mathematical models developed after extensive consumer testing to assign quality grades. Furthermore, while the USDA quality grading system is focused on the point of harvest, the MSA employs a holistic farm to fork approach commonly referred to as a Total Quality Management System. The USDA grading system consists of two separate grade designations, the yield grade, and the quality grade. Carcases are assigned either a quality or yield grade or both at the same time. The USDA quality grading system assigns a quality grade according to the degree of marbling and physiological maturity. Physiological maturity is used to account for the effects of maturity/age on beef tenderness with five maturity classifications designated A to E and the approximate ages for each group are:

A — 9 to 30 months

B — 30 to 42 months

C — 42 to 72 months

D — 72 to 96 months

E — more than 96 months

Within each physiological maturity class (determined by ossification) the principal determinant of beef quality grade is the degree of marbling using the rib eye (*longissimus dorsi, complexus* and *spinalis* muscles) at the 12^{th}–13^{th} rib interface as an indicator muscle. There are four main grades: prime, choice, select, and standard and a further four lower grades: commercial, utility, cutter, and canner. Each of the four main grades is further divided into three which are in turn discriminated by dividing each marbling degree into 100 sub-units, although marbling scores are generally discussed in tenths within each degree of marbling. The different beef grades are used to reflect eating quality with prime expected to have the highest eating quality while canner is expected to have the lowest eating quality. The top four grades best predict tenderness of the middle meats (rib and loin) which are usually cooked with dry heat (e.g. broiling, grilling, roasting). They are however less useful predictors of tenderness of meat when cooked with moist heat.

Both the MSA and USDA quality grading systems are determined by visual evaluation of the size, shape and ossification of the bones and cartilages in the carcase with further evaluation of the colour and texture of the ribeye muscle. Another important feature of the USDA system is the reliance of the grading on the gender or type of animal. There are gender and type limitations with bulls not being quality graded, cows never being graded prime and bulls being graded separately from steers, cows, and heifers.

An interesting phenomenon worth mentioning is the pH threshold for dark cutting (DFD) beef as evaluated by Asian consumers; this may have implications for Australia's export of beef into Asia. Colour development in meat is strongly influenced by meat pH, mainly by the influence the latter has on the oxygen consumption and reducing activity of myoglobin (Neethling et al, 2017; Ramananthan & Mancini, 2018). As discussed, MSA have a pHu below 5.71 as a requirement for grading and meat with a higher pH_u is typically classified as DFD, in Brazil and Canada the pH value for DFD classification is 5.8 (Holdstock et al, 2014; Rosa et al., 2016), in the USA it is 5.9 and in China it is as high as 6.1 (Du et al., 2009 as quoted by Zhang et al 2021). However, other researchers have used different classes linked to the pHu, as example Silva et al. (1999) had the following classification: Normal (pH 5.5 to 5.8) moderate DFD (mod DFD) (5.8<pH<6.2) and DFD (pH 6.2 to 6.7) and found that the higher pHu meat to be more tender, even after an ageing period of 13 days. This phenomenon of high pHu meat (DFD) being tender is attributed to the curvilinear relationship between pH and WBSF tenderness, although it should be remembered that there are other quality attributes associated with DFD meat that result in negative consumer perceptions (colour and juiciness) as well as shelf-life and potential negative attributes that could develop due to microbial growth (Shange et al., 2019). None the less, in an Asian study amongst 4322 respondents, it was noted that the respondents preferred darker meat – typically that with a pH around 6.20 when the a* colour ordinate was taken to be the effective variable (Zhang et al., 2021). As correctly noted by the authors, meat with a pH this high could have tenderness issues, although this would not be a factor when the meat is consumed as hot pot or sauced products (Mao et al, 2016). Zhang *et al* (2021) further notes that this acceptability of DFD meat could be correlated with a high incidence of DFD meat in abattoirs in China resulting in a familiarity within the consumer cohort.

3.1.6 Other measurements/Latest technologies

The meat industry is dynamic and complex, needing low cost and environmentally friendly, fast techniques to determine the quality of the meat and meat products. Although most of the traditional techniques can determine pH with reasonably high reliability, they are time consuming and open to

erroneous readings. Alternatively, non-destructive techniques focused on vibrational spectroscopy are being researched and developed that can provide rapid, consistent, and objective assessment of the samples under investigation. In conjunction with appropriate multivariate data or image analysis techniques, these could be effective tools for the determination of multiple meat quality attributes. These methods also have the potential for automation, thus eliminating tedious and time-consuming traditional methods.

However, some major limitations preventing the commercial implementation of these non-destructive techniques include high-cost equipment although, in the last decade, a range of more affordable miniaturized NIR sensors and robust instruments coupled to long fibre-optic probes have appeared in the market, which make it possible to implement NIRS sensors in a processing plant for real-time *in situ* measurements and decision making. All these sensors generate large data sets that require advanced modelling to ensure accurate interpretation of the data. Scientific knowledge is required when selecting an efficient and practical classification algorithm, as there is no universally ideal technique; this could be a challenging task.

Once data has been collected and a calibration model developed, a validation process is required. Usually an internal or cross-validation, where the same data set used for calibration, is used in the validation. However, external validations using a separate and independent sample set (test set) provide more reliable and relevant estimates of the future prediction ability of the model. It is important to select a representative set of samples providing the largest information for the calibration data set, since it is of critical importance that this data set represents as much variation as possible that will be encountered in future samples.

An additional challenge faced is the accuracy of determination of the base-line measurement using traditional techniques, e.g., pH measured with a probe. As mentioned, there is frequently more variation between the readings provided by different types of probes than in the readings obtained between muscles.

Downey & Hildrum (2004) give an early comprehensive review on the use of NIR in meat and meat products. In this review, they classify the applications as being either quantitative or qualitative. The Monogram from which this review is given gives a good background to the use of spectroscopy in whole and ground meat samples.

3.1.6.1 Vibrational spectroscopy

Vibrational spectroscopy is the detection of molecular vibrations and rotations that occur when solid, liquid or gaseous samples absorb infrared (IR) light (Danezis et al., 2016). The IR region of the electromagnetic spectrum consists of three sub-regions; near-infrared (NIR, 780 – 2500 nm), mid-infrared (MIR, 2500 – 25000 nm) and far-infrared (FIR, 25000 – 200000 nm) (Esteki et al., 2018). However, the commonly used spectroscopic techniques in food and meat mainly focus on the NIR and MIR regions (Karoui et al., 2010).

The MIR region consists of absorption bands corresponding to fundamental stretching, bending and rotating vibrations of the molecules related to chemical functional groups (O-H, C-H, C=O and N-H) (Esteki et al., 2018), whilst absorption bands in the NIR region result from complex overtones and combinations of fundamental vibrations (Barton, 2002). These molecular bonds experience vibrational energy changes when irradiated with MIR and NIR frequencies (Barton, 2002; Prieto et al., 2017). Absorption of energy occurs when the light energy is equal to the frequency of the molecular bond.

A typical IR instrument mainly consists of a light source, a wavelength selection system, a detector, and a signal processor, all coupled to a computer (Prieto et al., 2017). Due to the robustness and simplicity of the instrumentation, MIR- and NIR spectroscopy have been used in food research for decades, including in meat (Andersen et al., 1999). Although both methods are widely used, they have their own strengths and weaknesses, and these attributes make them better for certain applications. The main advantages of these techniques are that they require no sample preparation and are non-destructive. Another advantage of NIR spectroscopy is the ability to make reflection measurements in addition to the absorption and transmission measurements typically used for MIR spectroscopy. In terms of sampling, some NIR instruments allow for the rotation of a sample while scanning. This allows for a larger volume to be scanned and therefore more representative sampling when compared to MIR. NIR spectroscopy is therefore a viable option for the measurement of inhomogeneous samples.

As mentioned, MIR spectroscopy measures fundamental vibrational bands related to the functional groups in the sample (Esteki et al., 2018). These fundamental bands result in spectra which are ideal for sample composition determination as well as identifying samples based on their unique MIR fingerprint. In contrast, NIR spectroscopy results in more complex spectra. Spectral bands in the NIR region tend to be broad, extensive, and generally overlap, making it difficult to determine discrete chemical species and the analytical information obtained is influenced by a number of chemical, physical, and structural variables. Compounding this problem is the fact that many compounds absorb energy throughout the entire NIR region and slight spectral variances might be caused by differences between samples, making it difficult and impractical to make a distinction with the naked eye. Therefore, various multivariate (statistical) analysis techniques can be implemented to extract the analytical data, confined in the NIR spectra. Hence, the use of chemometrics is necessary for the decomposition and interpretation of NIR spectroscopic data.

The latest development of hand-held NIR apparatuses (Fig. 7) has paved the way for rapid measurements of numerous meat quality indicators in a non-invasive manner (Savoia et al., 2020). These hand-held meters are battery charged and link via blue-tooth/wireless to the computer base where the data is captured for processing (Fig. 8). Some of the latest Micro-meters are smartphone based. A shortcoming of these handheld meters is that these smartphone-based spectrometers usually operate in one-point-detection mode, which means that only one point is detected at a time. It is inevitable to get some abnormal points and difficult to obtain the whole spectra of the sample. To perform comprehensive food quality assessment e.g., pH monitoring, hyperspectral imaging could be a better solution.

Figure 7: A typical small hand-held Micro-NIR meter suitable for on-line carcase scans.

Figure 8: Practical measurement set-up for a lamb meat steak quality measurement using a Micro-NIR (45 mm in diameter and 42 mm in height, weighing 60 g).

3.1.6.2 Near-infrared spectroscopy of post-mortem muscle.

Prieto and co-workers (2014; 2015) investigated the potential use of visible and near-infrared spectroscopy (Vis–NIRS) to separate normal and DFD beef. The authors reported 95% correct classification of both meat groups. Similarly, Reis & Rosenvold (2014) used Vis-NIRS at 20 to 40 min post-mortem and reported >90% successful segregation between carcasses with ultimate pH of >5.8 and <5.8. Crichton and co-workers (2017) showed that for beef with different freshness levels (fresh, fresh frozen-thawed, matured, and matured frozen-thawed) and using support vector machine (SVM) analysis, beef samples with a pH above 5.9, and below 5.6, were classified with an accuracy of 91% and 99%, respectively.

El Masry et al. (2012) used a hyperspectral imaging system operating in the NIR region (900-1700 nm) to measure non-contact meat surface colour, pH, and tenderness of beef. Modelling the traditional measurements' values with the spectral data using partial least square regression (PLSR) they calculated a calibration correlation of (R^2c =0.83) although in cross -validation the coefficient of determination value was less (R^2cv =0.73) with a root mean square error of estimate by cross validation of 0.06. These predictions were lower than expected due to the large number of latent factors (20 in their study) used in the study as well as the narrow pH range tested (5.37-6.13), although these are typical meat pH ranges encountered in normal abattoir situations. Interestingly, Dixit et al. (2020) developed global calibration models for predicting intramuscular fat (IMF) and pH across various red meat species (beef, lamb, and venison) and muscle types. Eight hyperspectral imaging (HSI) datasets were used from different experiments, across various muscle types, slaughter seasons and measurement conditions. Prediction models were developed using Partial Least Squares Regression (PLSR) and Deep Convolutional Neural Networks (DCNN) using a total of 1080 and 1116 samples for IMF and pH, respectively. Models for pH and IMF via both techniques yielded high R^2c (0.86–0.93) and low SEC values. Accurate prediction performance was observed with high R^2p (0.86–0.89) and low SEP values.

What should be remembered is that hyper imaging systems do not measure the H^+ concentration as is done by conventional pH probes, but measures differences in absorbance patterns due to changes in inter-molecular forces brought about by changes in the pH. Also, traditional hyperspectral imaging systems are bulky, require high precision scanning optomechanical elements and computer control and are inconvenient to carry, which hinders its wide use by consumers in an on-line system in an abattoir. This has resulted in research evaluating smaller, hand-held scanners.

Yao et al. (2019) used a low-cost hand-held portable spectrometer and collected reflectance spectra of meat samples using a push-broom scanning technique. Using a support vector regression (SVR) to predict the pH, an accuracy of 90% and a coefficient of determination of 0.93 was obtained.

Therefore, this technology (hand-held scanners) appears to be promising and has the potential to be integrated into an on-line system for prediction of meat pH early in the post-mortem period.

3.2 Laboratory comparison of selected pH meters

3.2.1 Meat Samples

A total of 86 vacuum sealed Wagyu meat samples (each weighing approximately 200g) were obtained from a local beef meat producer and stored in the freezer at -20°C until analyses at Health and Food Science Precinct, Coopers Plains, The University of Queensland. Prior to chemical and proximate analysis, each sample was thawed overnight in refrigeration conditions (2-4°C).

3.2.2 pH meters

Four (Figs. 9-12) different hand-held pH meters were used and compared to a Mettler Toledo Easy desk-top pH meter. The desk top pH meter (Fig. 13) was designated as the "Golden standard" against which the other meters' readings were compared. One of the meters was the TPS-WP80M pH meter (Fig. 9) used to measure the pH in MSA grading. The images below depict the pH meters.

To ensure total impartiality in the evaluation of the meters, new meters were purchased without the vendors knowing what the meters were to be used for, except that they were required to measure the pH in meat samples.

Figure 9: TPS-WP80M Waterproof pH-mV-Temp Meter (model used by MLA)

Figure 10: Hanna Professional Portable pH HI98163 meter

Figure 11: Hand-held, HALO PVDF FC2022 meter with a body food-care pH electrode with Bluetooth (onto cell phone).

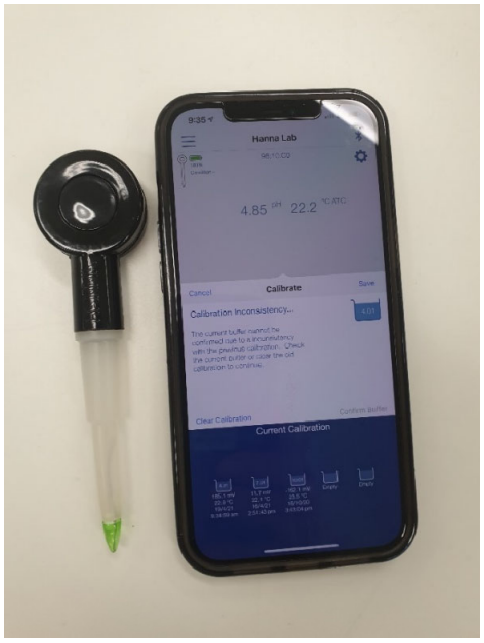
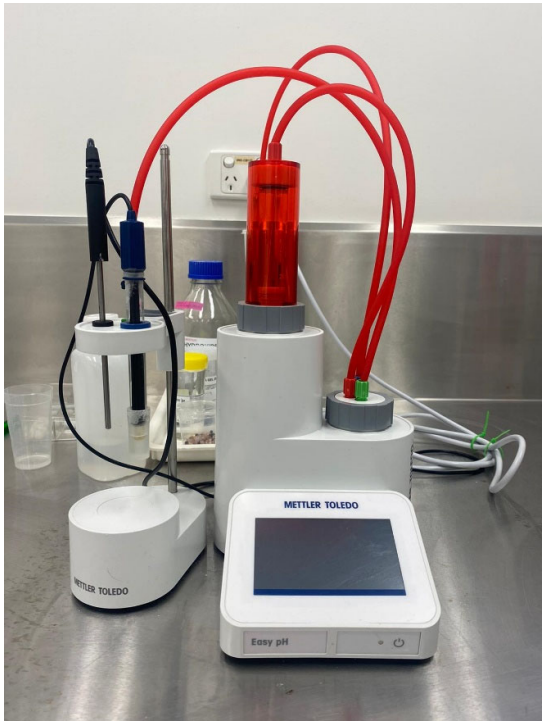


Figure 12: Hand-held Testo-205 pH meter for solids

Figure 13: The Mettler Toledo Easy bench-top pH meter used as the Golden standard.



3.2.3 pH measurements

Samples were removed from the vacuum sealed packaging and left to bloom while the four handheld pH meters (TPS-WP80M, Testo 205, Hanna HI98163 and Halo FC2022) and the desk-top meter were

calibrated using pH 4.01 and pH 7.0 buffers. The same buffer solutions were used to calibrate all the meters. The calibration of the meters was according to each meter's handbook.

pH measurements were collected following two methods. First, pH measurements were collected by inserting the pH meter probe at three different points on each sample before moving on to the next sample without cleaning the probe. A single temperature reading was also recorded for each sample. For the second set of measurements, the pH measurements were taken at three different points on each sample with the probe cleaned with distilled water and wiped dry with a Kimwipe between readings. A single temperature reading was again recorded for each sample. pH meters were recalibrated every 10 samples or every 30 readings.

The sample was then homogenised using a blender and divided into two – one half of each sample was vacuumed sealed and stored in a -20°C freezer for later chemical (proximate) analysis. Approximately five grams of the remaining meat sample was diluted 1:10 with distilled water and mixed (Guerrero, O'Sullivan, Kerry, & de la Caba, 2015; ISO 2917). A desktop pH meter (Mettler Toledo Easy pH meter) was then used to record a single pH and temperature reading – This pH readings was seen as being the Golden Standard against which the other pH readings were compared.

3.2.4 Chemical analyses

3.2.4.1 Moisture

A dry, numbered, porcelain crucible was selected for each sample, and weighed to four decimal places. This weight was tarred, and 2.5 grams of homogenised sample was placed into the crucible and weighed to four decimal places before being placed into a drying oven set at 100°C for 24 hours. Upon removal from the oven, crucibles were placed into a desiccator for 30 minutes to cool and were then weighed to four decimal places. The moisture contents of the samples were then calculated

3.2.4.2 Ash

Using the moisture-free samples and crucibles, crucibles were placed into a furnace at 500°C for four hours. Samples were then removed from the furnace and placed directly into a desiccator and left to cool for approximately 45 minutes. Cooled samples were removed and weighed to four decimal places. The ash content of the samples was then calculated.

3.2.4.3 Fat

Fat analysis was carried out as described by Lee *et al.* (1996) with modifications. A funnel lined with Whatman #4 filter paper and stoppers were placed on top of a separation funnel. Five grams ($\pm$0.1g) of each homogenised sample was weighed and placed into a 250mL fat beaker. The sample was then saturated in 40mL of a 2:1 Chloroform/Methanol solution (ratio used for high fat samples) and blended using a handheld blender for one minute. The mixture was poured into the filter paper and funnel. The fat beaker was then rinsed with an additional 10mL of the 2:1 Chloroform/Methanol solution, mixed around the fat beaker to remove sample residue, and added to the funnel containing the meat sample on the separation funnel. Once all samples had been blended and poured into the respective filter papers, samples were allowed to finish filtering (no more moisture dripping out of the funnel) before the remaining moisture was squeezed from the filter paper. Each sample's defatted meat remaining in the filter paper was dried and stored into air-tight, labelled Falcon tubes for Nitrogen determination.

Twenty mL of 5% NaCl was added to each separation funnel, and stoppered. In sequence of the NaCl being added, the separation funnels were inverted four times. The stopper was then removed to allow the release of built-up gas, before the separation funnels were allowed to stand for 40 minutes, with

stoppers in place. A 30mL fat beaker was prepared for each sample by being weighed (g) to two decimal places. An additional fat beaker per sample was prepared to collect a minimum of 5mL of the sample mixture from the bottom layer of the solution in the separation funnel. A pipette was used to measure exactly 5mL of each sample that was placed into the weighted fat beakers.

Weighted fat beakers were then placed onto an 80°C hotplate in a fume cupboard for 45 minutes to evaporate all the 2:1 Chloroform/Methanol solution from the fat beakers. Fat beakers were removed from the hotplate and allowed to cool for 10 minutes before being weighed to two decimal places. The fat contents of the samples were then calculated.

Falcon tubes storing the dried, defatted samples were placed into a 100°C oven overnight to dry out. Samples were then stored into the -20°C freezer until protein analysis could occur.

3.2.4.4 Protein

Protein was determined using a duplicated defatted and dried 0.3g meat sample and the Dumas combustion method described within AOAC International (2002) using a 928 series LECO. The % N was then converted to % protein by multiplying with a factor of 6.25.

3.2.5 Statistical analyses

Mean values were compared using paired Student's t test for the equality of means (assuming equal variances) using XLSTAT. A p value of ≤0.05 was used to determine significant differences between treatments (e.g. clean vs non-clean; pH probe A vs probe B, etc). [For ease of interpretation, Figure titles where significant differences were noted are depicted in red ink].

3.2.6 Results

3.2.6.1 Comparison of pH readings

Table 2 indicates the summary descriptive statistics of the different readings taken on the 86 meat samples with various levels of fat. A wide range of pH measurements were acquired varying from 6.01 down to 5.34 (as per the Bench top instrument; see Fig. 14). The Hanna and Bench meters showed the highest variability compared with the other instruments.

Table 2: Mean, SD, CV, median, range (max and min), quartiles Q1 and Q2 of the pH measurements taken on meat samples (n=86) containing various levels of fat.

	TPS-WP80M NC*	HANNA NC*	HALO NC*	TESTO NC*	TPS-WP80M C#	HANNA C#	HALO C#	TESTO C#	BENCH
Max	5.89	5.92	5.86	5.81	6.0	6.02	5.90	5.82	6.01
Min	5.43	5.17	5.36	5.34	5.31	5.37	5.38	5.35	5.34
Median	5.59	5.62	5.57	5.58	5.65	5.63	5.61	5.59	5.61
Q1	5.54	5.55	5.53	5.54	5.57	5.56	5.55	5.56	5.55
Q2	5.66	5.70	5.63	5.64	5.73	5.71	5.67	5.65	5.73
Mean	5.61	5.62	5.59	5.60	5.65	5.64	5.61	5.61	5.64
SD	0.08	0.12	0.09	0.08	0.12	0.12	0.09	0.08	0.13
CV%	1.4	2.1	1.61	1.4	2.1	2.1	1.6	1.4	2.3

* NC – not cleaned; C# – Cleaned

Figure 14: The desk-top's pH readings indicating the pH range of the meat samples compared.

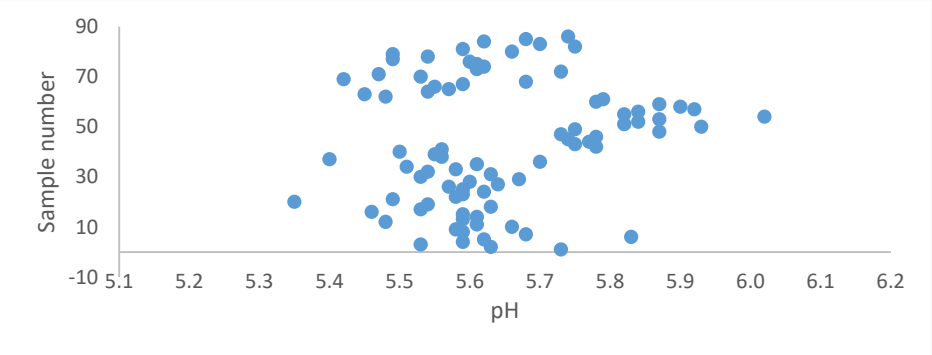


Table 3 indicates the proximate composition of the meat samples. The percentage intermuscular fat range widely with maximum values above 30% and minimum values around 2.5%.

Table 3: Mean, SD, range (max and min), of the proximate composition of the meat samples (n=86) containing various levels of fat.

	% Moisture	% Protein	% Fat	% Ash
Mean	63.3	16.0	19.2	1.1
SD	4.6	4.0	7.2	0.3
Min	52.9	10.0	2.5	0.6
Max	72.6	24.1	32.0	2.1

3.2.6.2 Comparison clean vs no clean.

Statistically significant differences between TPS-WP80M no clean (NC) vs clean (C) were observed ($p<0.05$; Fig. 15). The cleaned pH probe gave slightly higher mean pH readings, than when the pH probe had not been cleaned; Fig. 16 indicates the pH unit differences between the Clean and non-cleaned (NC) TPS-WP80M probes. No statistically significant differences with the other instruments were noted when comparing non clean vs clean (Figs. 17-19).

Figure 15: TPS-WP80M No clean vs clean – Significant differences

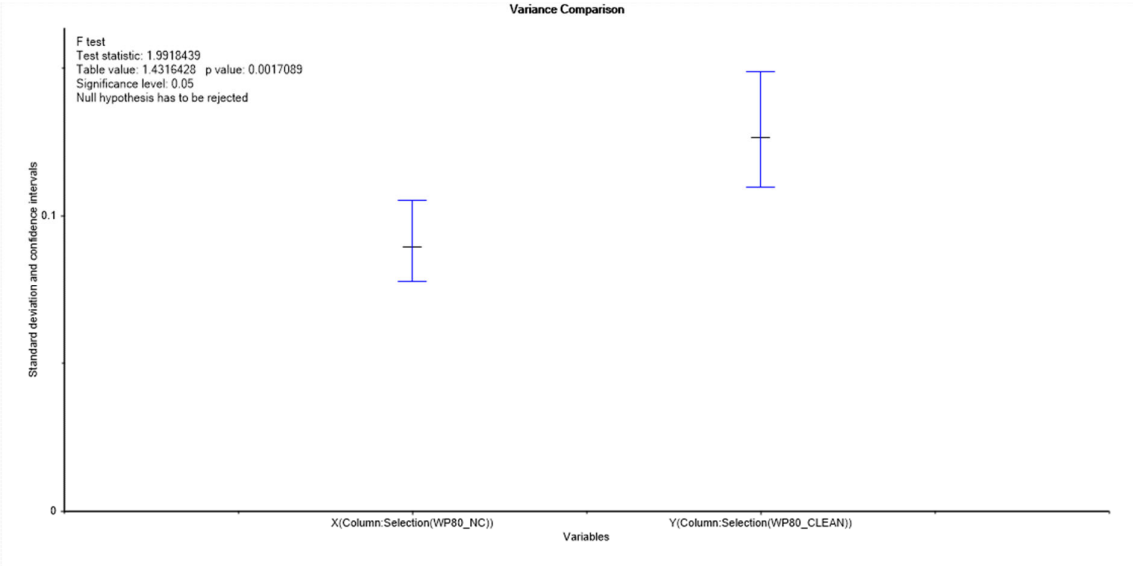


Figure 16: The difference between the pH reading taken with a cleaned (between readings) pH probe relative to the same sample's pH taken with the non-cleaned probe.

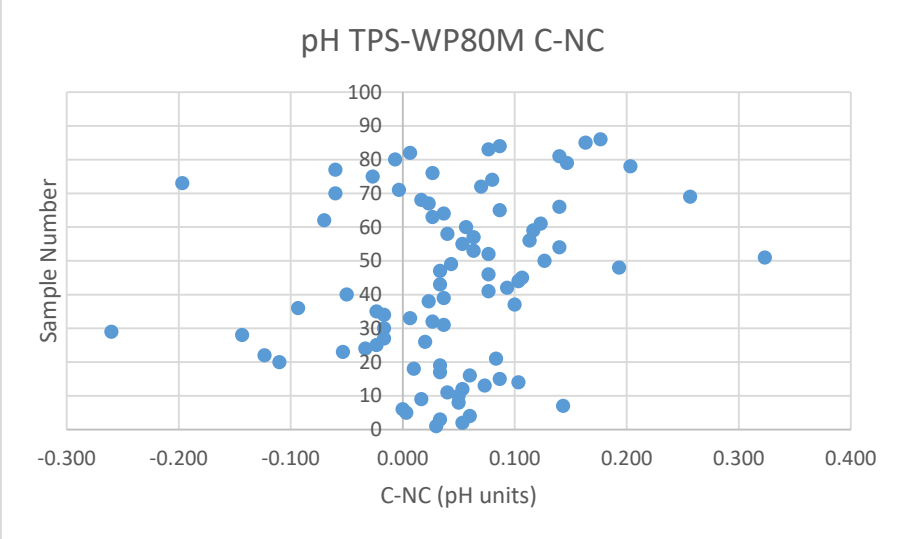


Figure 17: Hanna No clean vs clean

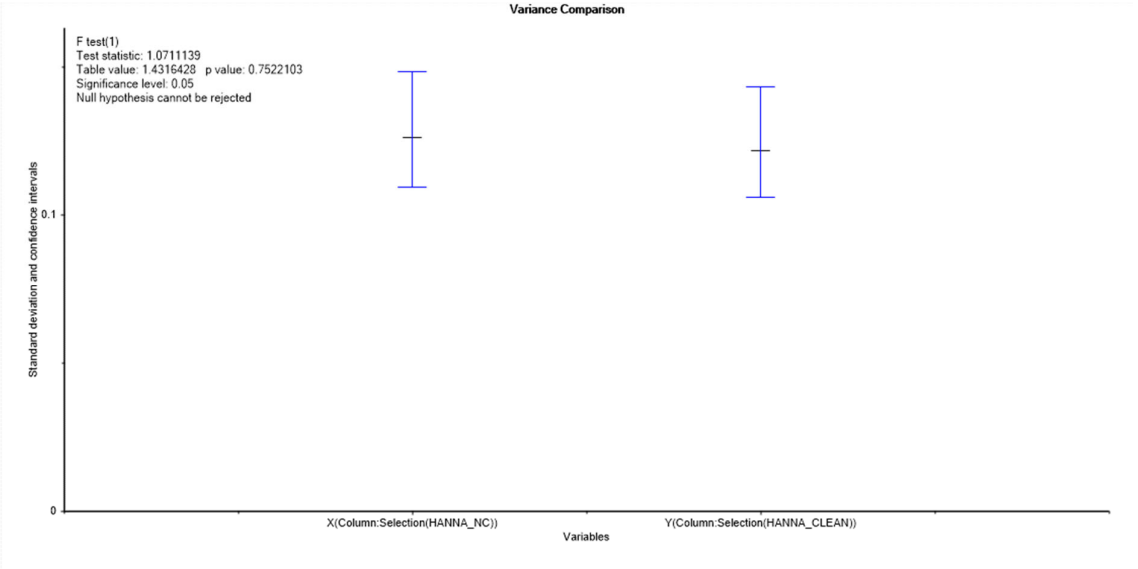


Figure 18: Halo No clean vs clean

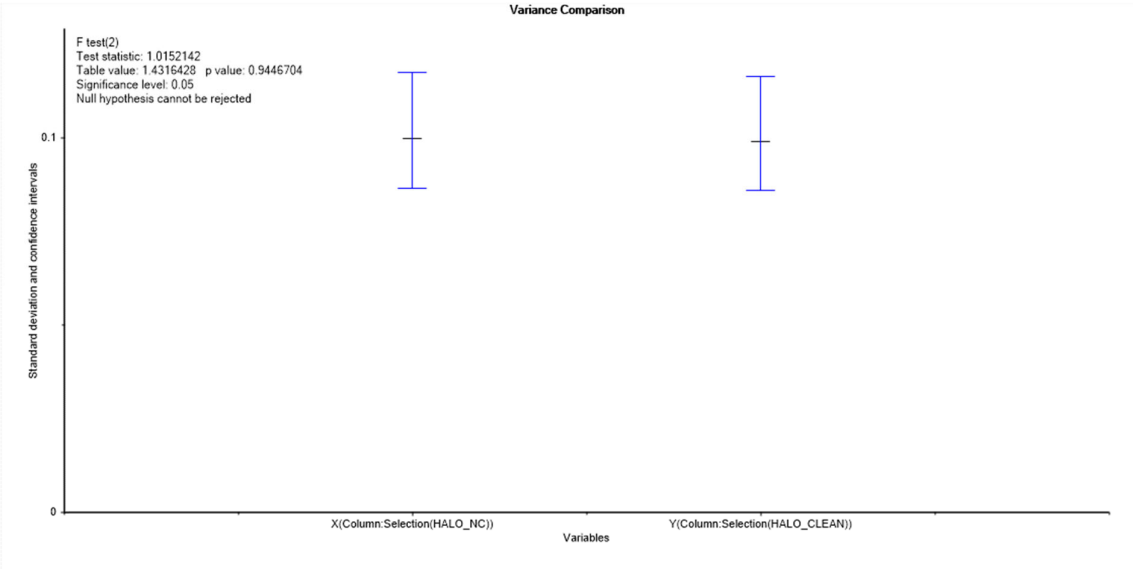
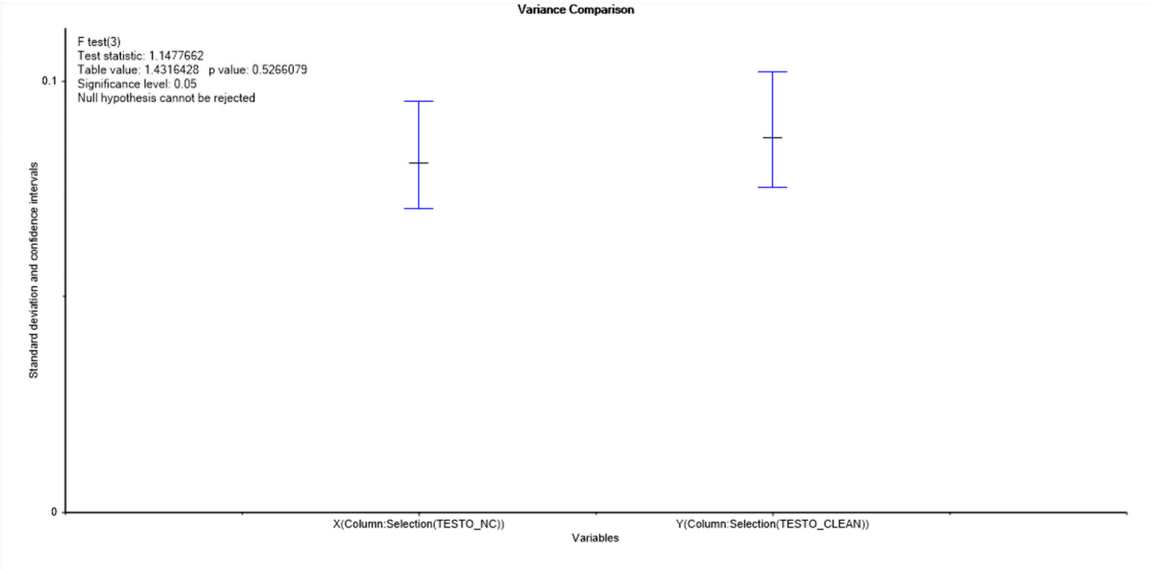


Figure 19: **Testo No clean vs clean**



3.2.6.3 Comparison clean vs bench standard (Figs. 20-25)

Statistically significant differences between HALO clean and TESTO clean vs Bench instrument were observed ($p<0.05$). Both the Halo clean (Fig. 22) and Testo clean (Fig. 23) gave lower mean readings. No statistically significant differences were noted with the other instruments when comparing clean vs bench.

Figure 20: **TPS-WP80M clean vs Bench Gold Standard**

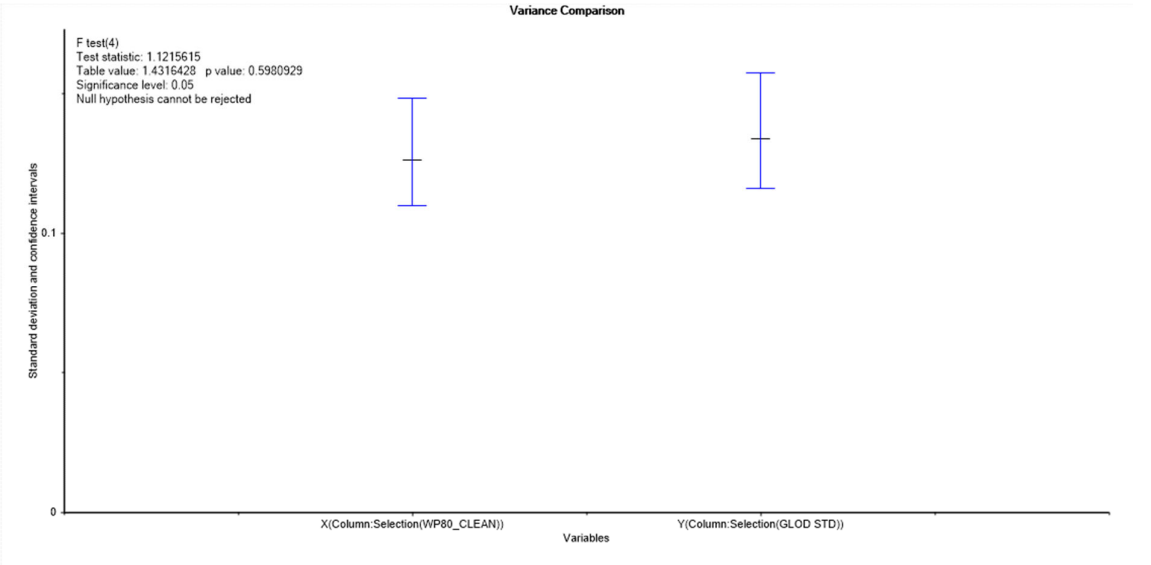


Figure 21: HANNA clean vs Bench Gold Standard

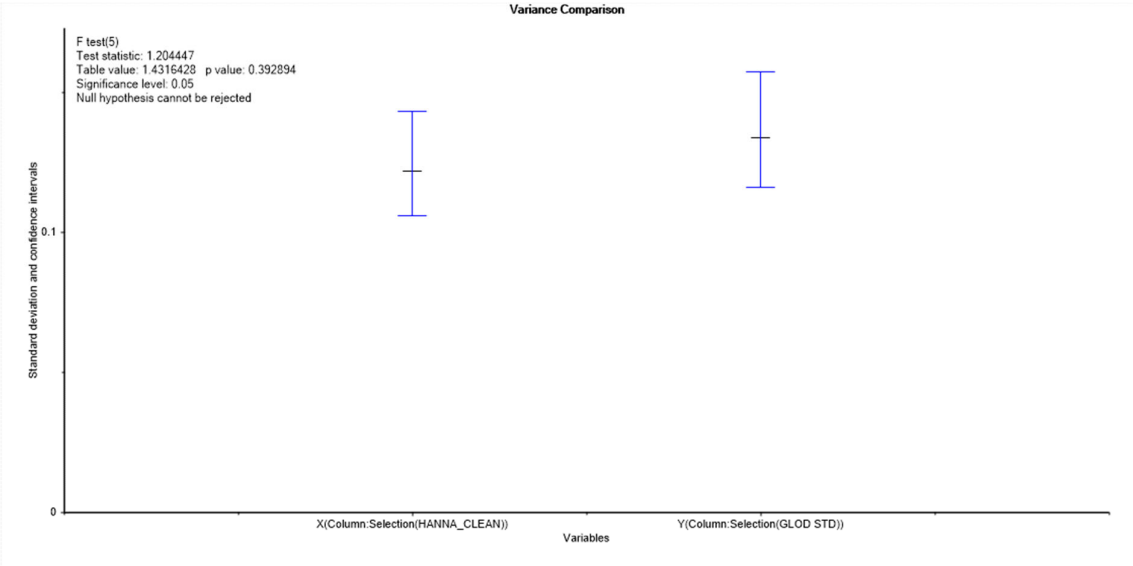


Figure 22: Halo clean vs Bench Gold Standard – Significant differences

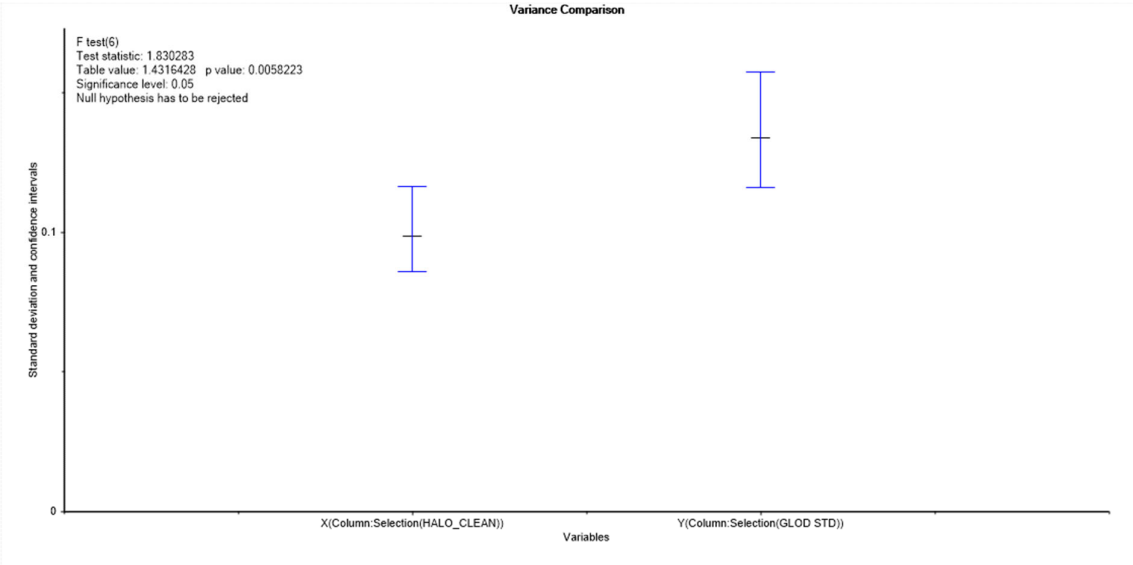


Figure 23: TESTO clean vs Bench Gold Standard – Significant differences

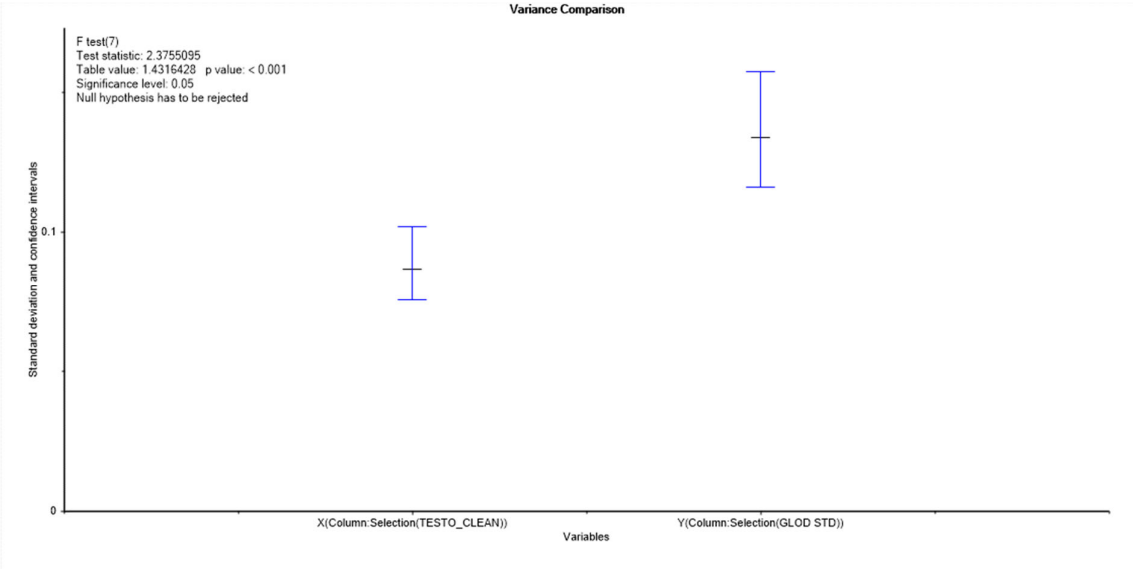


Figure 24: TPS-WP80M NC vs Halo NC – No differences

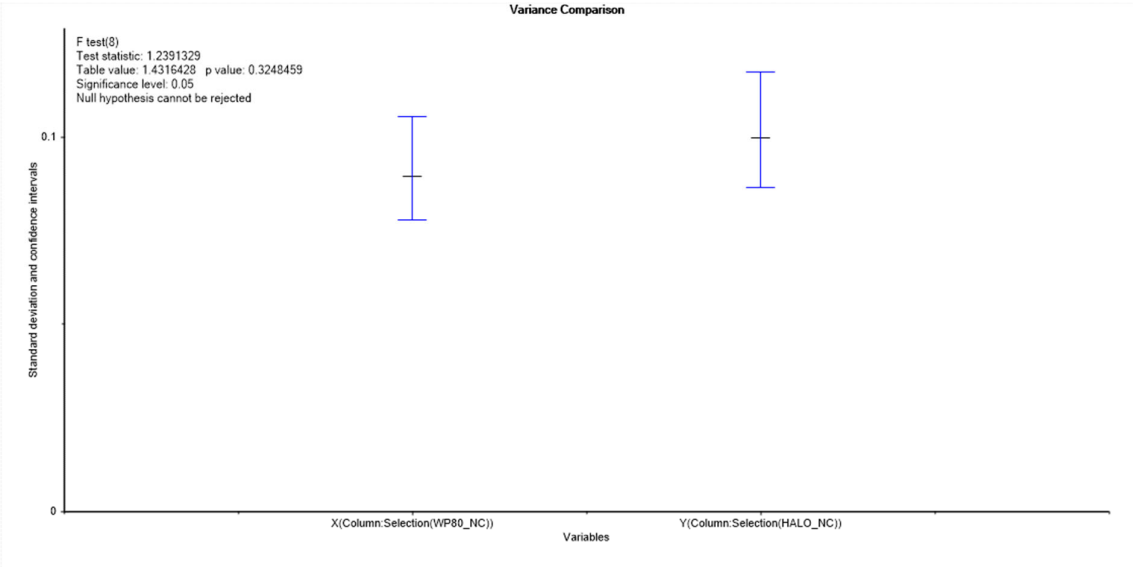
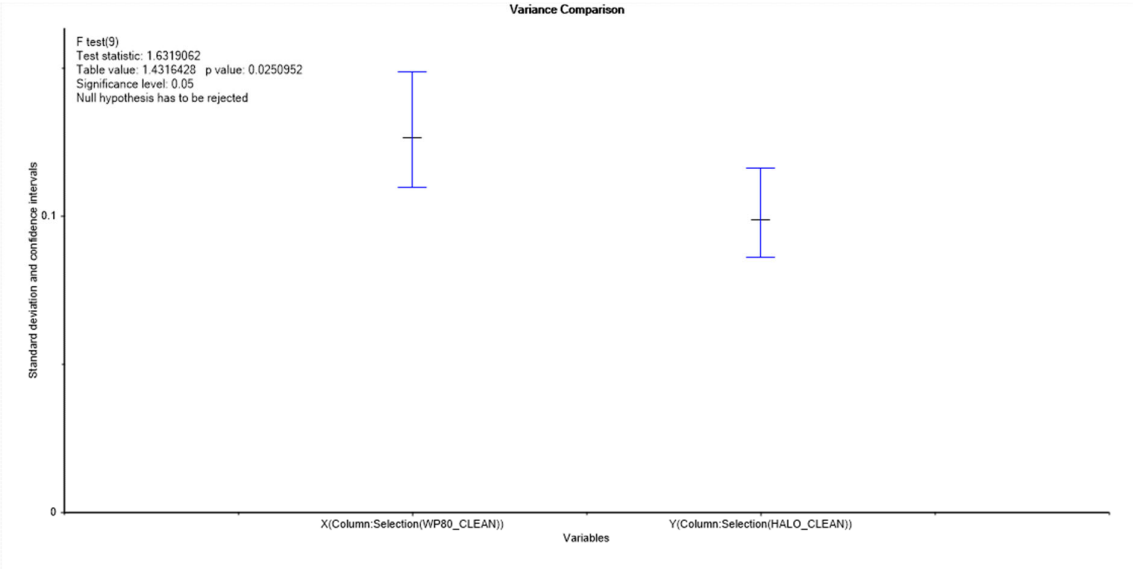
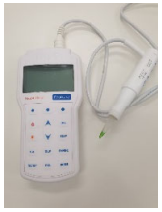


Figure 25: TPS-WP80M C vs Halo C – Significant differences



3.2.7 Advantages and Disadvantages of different pH meters

Table 4: The advantages and disadvantages of each pH meter evaluated.

	Advantages	Disadvantages	www Link
TPS-WP80M 	• Records date and time of last calibration • Data logging ability, data can be subsequently exported into Excel • Rechargeable battery • Automatically goes to sleep after a short period of time – helpful to minimise needless loss of battery charge	• Difficult to calibrate – compared to other pH meters such as the Halo, this has a fixed sequence for calibrating, e.g. it does not recognise buffers, and is therefore difficult for first time users to calibrate. You also must work through the menu for every new buffer • Probe has frame supporting it – meat, oils and solids coat this frame between each sample and is difficult to clean • Does not tell you when the reading has stabilised • Maintenance is difficult – require regular application of storage solution and additional gel on the inner probe • Two probes, one for pH, one for temperature • Difficult to insert probe into the meat • Large and bulky and has long cords, difficult to use in an abattoir environment.	TPS-WP80M Waterproof pH-mV-Temp Meter with 1m pH & Temp sensors (instrumentchoice.com.au)
Hanna 	• Designed specifically for measurement of meat • Battery run and therefore can be used in the field • pH probe has a built-in temperature sensor	• Long time to stabilise • Readings inconsistent with other pH meters – regularly records pH readings ±0.2 away from readings by other meters • Requires maintenance through regular application of storage solution • Still large and bulky and has long cords, difficult to use in an abattoir environment	Professional Portable Meat pH Meter - HI98163 (hannainst.com.au)

	• Has stainless steel piercing blade to cut into the meat to protect the probe and improve accessibility • Recognises buffers automatically when calibrating • Display prompts when the probe needs to be cleaned • Data logging ability, data can be subsequently exported into Excel • Easy to clean between samples	• Stainless piercing blade seen by the industry as being negative due to damage caused to the meat.	
Halo 	• Cordless • Readings captured with one press on probe • Calibration very easy • Device is small and compact, no cords • Records date and time of last calibration • Displays all required information on the home screen e.g., pH, temperature, reading stability, time and date of last calibration, calibration slope • Data logging ability, data can be subsequently exported into Excel • Includes notes on calibration and pH reading and accuracy • Tends to have high accuracy • Quick to stabilise • Recognises buffers automatically when calibrating • Battery run • Easy to clean between samples	• Need to have blue-tooth connection • Requires phone to attain reading • Requires maintenance through regular application of storage solution	https://hannainst.com.au/fc2022-halo-foodcare-ph-electrode-with-bluetooth-smart-technology.html

Testo 	• Cordless & light/compact body • Screen readings freeze to recognise when the reading is stable • pH and temperature probes attached • Sharp probes make it easy to insert into meat • Recognises buffers when calibrating • Battery run • Storage container keeps probe moist for ease of storage – no need to regularly apply more gel • Easy to clean between samples	• Temperature below 0°C cannot be read • Slow to stabilise • Automatic sleep after repeated measurements • No recording of previous calibration time/date, or calibration accuracy • Not data logging system • Gel becomes perforated and needs to be replaced when used often (not sure how will perform if used continuously in an abattoir environment)	testo 205 pH/temperature measuring instrument | pH | Parameters | Testo Australia
Mettler Toledo Easy EPM 	• Readings are reliable • Intuitive interface • pH measurements are reproducible	• Desktop pH meter – cannot take it onto the factory floor • Not rechargeable – must be connected to power • Probe is delicate • Separate pH and temperature probes	EasyPlus Automated Titrators (mt.com)

AOAC International. (2002b). Dumas combustion method. AOAC Official Method 992.15. In *Official Method of Analysis* (17 ed.).

3.2.8 General Discussion

From the results it would seem as if the cleaning of the probes between sampling did not always contribute to more accurate readings. A limitation to the specific protocol could be the fact that only 10 readings were taken per day (without cleaning the probe) whereafter the probe was cleaned before storage to be used the next day. In an abattoir situation, more pH readings are most probably taken before the probe is cleaned. This is due to time constraints as it takes time to clean the probe.

Both small pH meters (Testo and Halo) have the distinct advantage of being more user friendly in a practical situation such as experienced in an abattoir due to their lack of cables as well as size. Overall, the Halo was deemed the better option (See Table 4 for more details). The Halo is small, compact, user friendly and stabilises quickly to give pH readings, a quick push of the button on the 'head' of the meter transfers the readings to the cell phone. It is also easy to transfer the readings from the phone's app to a spreadsheet on a computer.

The next Phase of the project was to evaluate and compare the Halo with the TPS-WP80M and with the handheld NIR in an abattoir setting.

3.3 Evaluation of selected pH meters in abattoirs

This specific objective was to test the accuracy of the selected pH meter (HALO) on chilled carcases in an abattoir environment. Hanna's (HALO & HALO2) pH meters' performance were compared to the (TPS-WP80M) meter presently used during the grading of carcases for the MSA program. This objective consisted of three experiments. In the first experiment, the HALO, and two TPS-WP80M meters were compared and correlated with the spectra derived from a hand-held NIR. In the second experiment, the upgraded HALO (HALO2 – Fig. 26) pH meter was compared with two TPS-WP80M meters. Additionally, the HALO2 was evaluated for use in a commercial environment by the MSA R&D Strategy and Integrity Systems Manager. Other factors considered was the relative ease of using the HALO2 pH meter including calibration within the commercial environment.

3.3.1 Methodology

3.3.1.1 pH meters

In the first experiment, two different hand-held pH meters (TPS-WP80M – Fig.9, HALO PVDF FC2022 – Fig. 11) were used and compared to each other. The TPS-WP80M pH meter is used to measure the ultimate pH as required in MSA grading as well as to measure the rate of pH and temperature decline in hot carcases. A portable NIR instrument (MicroNIR) producing spectra in the wavelength range between 600 to 1600 nm was also used to evaluate the ability of the NIR to predict the pH of the meat samples. In the second experiment, the HALO2 meter (Fig. 26) used is an upgraded version from the HALO used and evaluated in the first experiment; the major difference was that a "Dual-level LCD screen" had been included into the meter to show what the actual pH and temperature readings are (Fig. 11 vs Fig. 26) without the user having to look at their receiver (cell phone) to see the pH and temperature readings.

All experiments were conducted in the chiller rooms of commercial abattoirs on different days. In Experiment 1, two TPS-WP80M pH meters were evaluated and compared to the HALO; NIR spectra were also collected. In Experiment 2, two TPS-WP80M meters were compared to the HALO2.

In experiment 3, the HALO2 was evaluated for its performance (and comparison to the TPS-WP80M) under different commercial conditions by the MSA R&D Strategy and Integrity Systems Manager.

Figure 26: HALO2 HI9810362 pH Meter for Meat with Bluetooth connectivity.

3.3.1.2 pH measurements

Where applicable, all handheld pH meters (TPS-WP80M, HALO, HALO2) were calibrated using the same pH 4.01 and pH 7.0 buffers. The calibration of the meters was according to each meter's handbook. All the meat samples were at a similar temperature. Each pH meter's readings were taken immediately after each other on the same carcass near the position of the earlier reading(s). Where applicable the pH meters were set to adjust their readings to a fixed temperature (7°C) by correcting for the sample's temperature.

3.3.1.3 Meat Samples/experiments

In experiment 1, 126 carcasses were evaluated using two TPS-WP80M pH meters, the HALO FC2022 meter and the hand-held NIR. The first TPS-WP80M pH meter (MLA) was calibrated independently and used by the MSA R&D Strategy and Integrity Systems Manager. The second TPS-WP80M pH meter (plant grader) was the meter used within the abattoir and was calibrated by the abattoir lead grader. These readings were collected at the JBS abattoir in Dinmore. All pH values were corrected to display a pH at 7°C.

In experiment 2, 94 carcasses were evaluated using two TPS-WP80M pH meters (as for Experiment 1) and the HALO2 HI9810362 meter. These readings were also collected at the JBS abattoir in Dinmore, but a month later. Both the TPS-WP80M meters were calibrated together, and care was taken to ensure that they read similar readings. All TPS-WP80M pH and HALO2 values were corrected to display a pH at 7°C.

The HALO2 meter (experiment 2) differed from the HALO pH meter (Experiment 1) in that this upgraded meter had a "Dual-level LCD screen" to show what the actual pH and temperature readings are.

In experiment 4, 24 carcasses were evaluated (as part of MSA grading) using a TPS-WP80M pH meter and a HALO2 pH meter. All pH values were corrected to display a pH at 7°C.

3.3.1.4 Statistical analyses

Mean values were compared using paired Student's t test for the equality of means (assuming equal variances). A p value of ≤0.05 was used to determine significant differences between treatments (e.g., for different pH meters, etc).

3.3.2 Results

3.3.2.1 Experiment 1

Measurements were taken from 126 carcasses using two TPS-WP80M pH meters (JBS & MLA) and the HALO2 FC2022 (Hanna) meter (Table 4). The pH readings varied between 5.39 and 7.01 (TPS-WP80M MLA). There was a statistical difference (p=0.025) between the pH readings from the JBS versus the MLA (same type of meters – Fig. 27), although the MSA TPS-WP80M did not differ (p=0.55) from the

HALO (Fig. 28). The JBS TPS-WP80M pH meter's reading differed (p=0.007) from that of the HALO (Fig. 29).

Table 4: Basic statistics of the pH readings collected on 126 carcasses using two TPS-WP80M (JBS & MLA) meters and the HALO (Hanna) meter (Experiment 1)

	MLA	JBS	HALO
Mean	5.62	5.72	5.50
SD	0.34	0.42	0.32
Max	7.01	6.98	6.84
Min	5.39	5.46	5.27
CV%	0.11	0.18	0.10

Figure 27: Comparison of the mean pH readings between the two TPS-WP80M meters (p=0.025).

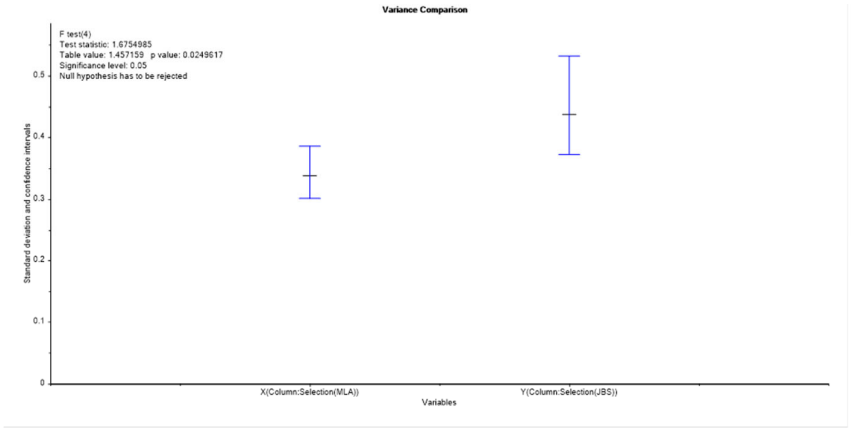


Figure 28: **Comparison of the mean pH readings between the TPS-WP80M (MLA) meter and the HALO (Hanna) meter. (p=0.55).**

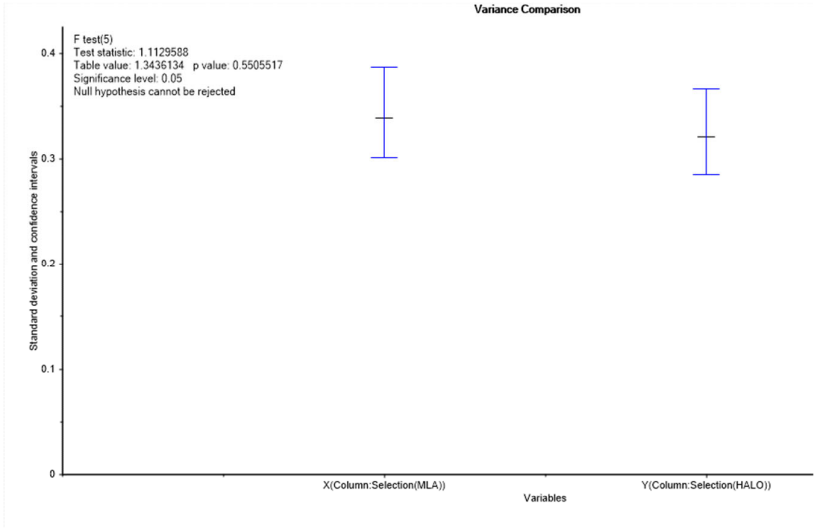


Figure 29: Comparison of the mean pH readings between the TPS-WP80M meter (JBS) and the HALO (Hanna) meter (p=0.007).

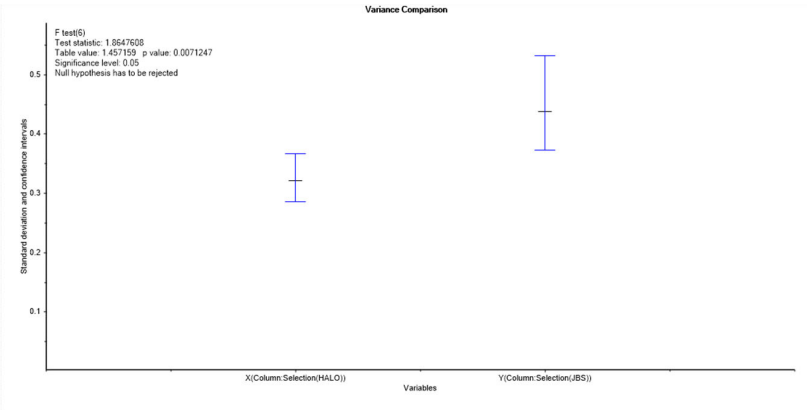


Figure 30 depicts the correlation (R^2=0.9587) between the HALO and MLA TPS-WP80M meter. To see how the two pH meters compare at different meat pH values, the differences between the two meters relative to the MLA TPS-WP80M's pH readings are depicted in Fig. 31; the indications are that the HALO readings all tended to be higher than the MLA_TPS-WP80M readings.

Figure 30: The correlation between the Halo and MLA TPS-WP80M taken on 126 carcasses in the Dinmore abattoir's chiller.

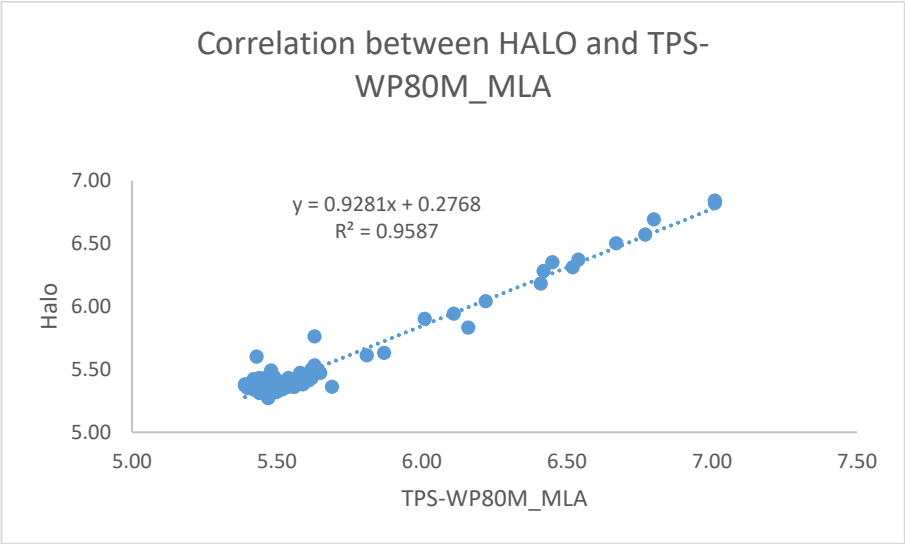
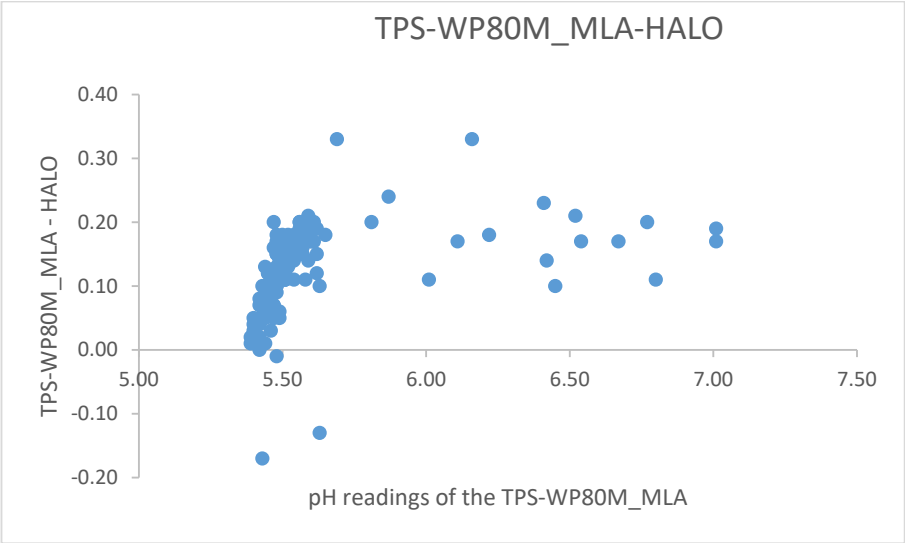
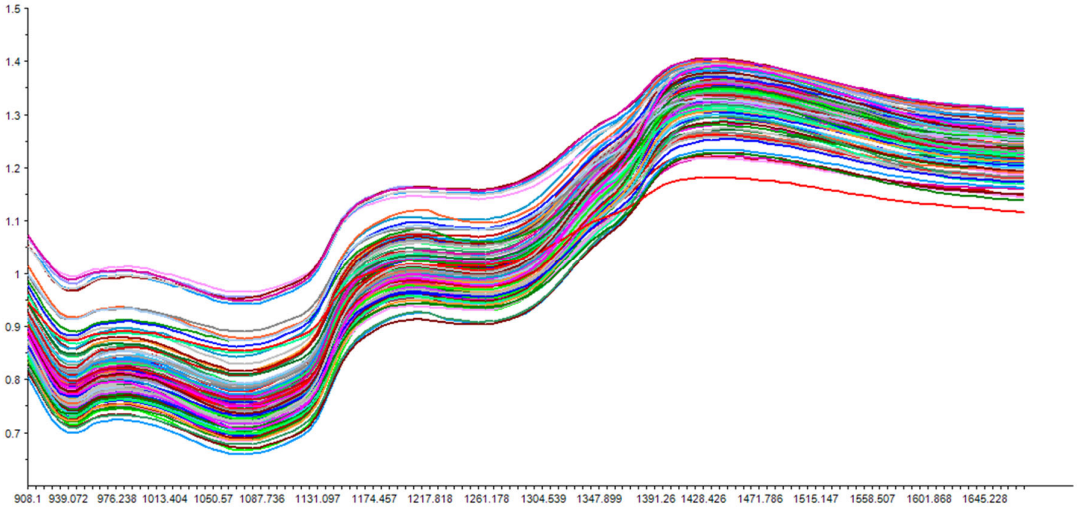


Figure 31: The difference between the pH readings of the MLA TPS-WP80M_and the HALO with increasing pH.



As pertaining to the calibrations models to predict pH in Meat using NIR spectroscopy, the spectra of the meat samples collected using NIR spectroscopy is shown in Fig. 32. The NIR spectra shown in Fig. 32 is the typical NIR spectra of meat samples. The spectra show the two main prominent bands associated with water (around 930 nm and 1400 nm).

Figure 32: NIR spectra of the meat samples analysed.



The correlations between pH and NIR data ranged from 0.16 to 0.83 (see Table 5). The optimal PLS calibrations were obtained using the optimal range in the NIR region between 930 to 1500 nm. This region has been reported in the scientific literature to be the most appropriate to measure pH in different meat sources, muscles and species. The main information from the NIR is originated from the absorption of the O-H bonds.

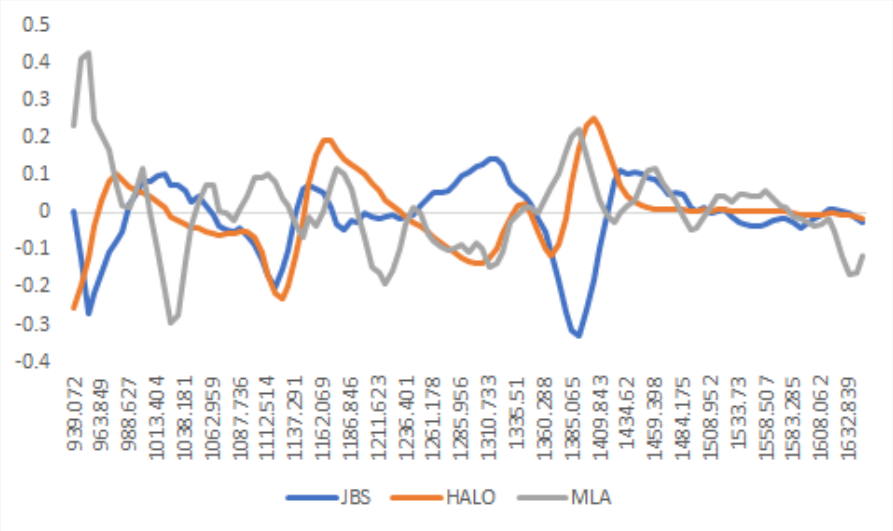
Table 5: Calibration statistics for the prediction of pH in meat samples using NIR spectroscopy.

	MLA	MLA optimal range	Halo	Halo optimal range	JBS
N	126	70	126	70	50
R^2	0.48	**0.64**	0.50	**0.71**	**0.83**
SECV	0.24	0.24	0.21	0.21	0.17
Bias	-0.0003	-0.0008	-0.003	-0.002	0.002
Slope	0.53	0.69	0.58	0.69	0.88

R^2: coefficient of determination, SECV: standard error in cross validation, N: number of samples

It was observed that the data obtained using different pH meters contributes to the observed differences in the statistics of the different calibration models. This is of importance, as this is the first time that this effect was showed on the prediction of pH using NIR spectroscopy. The pattern in the coefficients of regression (Fig. 33) is very similar comparing the different models, however, with minor differences depending on the type of pH meter used.

Figure 33. Coefficients of regression used by the different PLS models.



In conclusion, the correlations between pH and NIR data ranged from 0.16 to 0.83. Data from different pH meters contributes to the observed differences in the calibration statistics.

3.3.2.2 Experiment 2

In experiment 2, 94 carcasses were evaluated using two TPS-WP80M pH meters (as for Experiment 1) and the upgrade HALO2 HI9810362 meter. These readings were also collected at the JBS abattoir in Dinmore, but a month later. Both the TPS-WP80M meters were calibrated together, and care was taken to ensure that they read similar readings. All pH values were corrected to display a pH at 7°C.

Table 6 provides the basic information about the readings. None of the mean pH readings differed p>0.305) between the different meters (Figs. 34-36).

Table 6: Basic statistics of the pH readings collected on 94 carcasses using two TPS-WP80M (JBS & MLA) meters and the HALO2 (Hanna) meter (Experiment 2)

	JBS	HALO2	MSA
Average	5.74	5.62	5.73
SD	0.24	0.25	0.24
Max	6.55	6.49	6.53
Min	5.64	5.52	5.57
CV	4.18	4.44	4.18

Figure 34: Comparison of the mean pH readings between the TPS-WP80M (JBS) meter and the HALO2 (Hanna) meter (p=0.80).

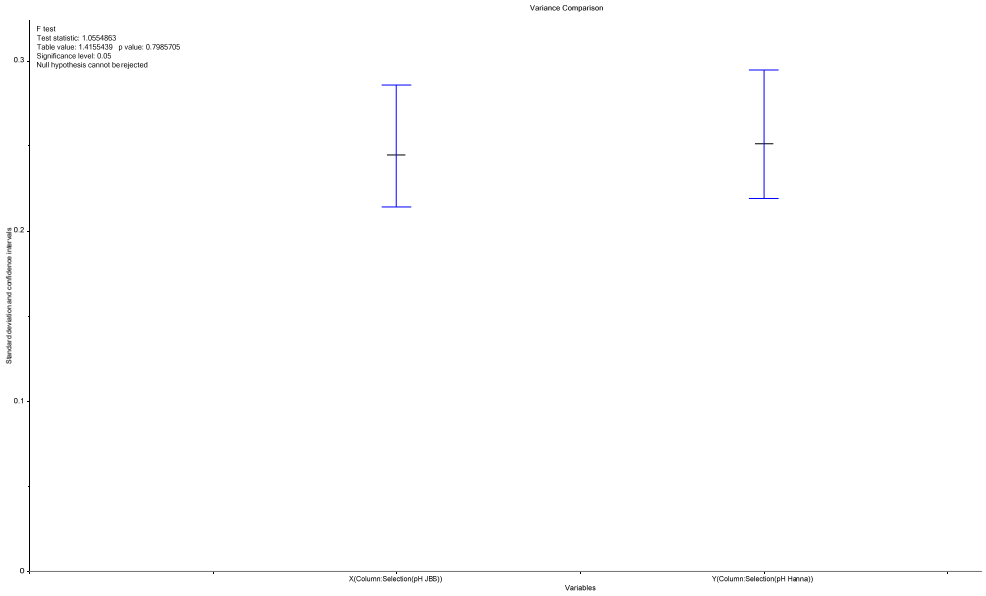


Figure 35: Comparison of the mean pH readings between the TPS-WP80M pH meter (MSA) and the HALO2 (Hanna) pH meter (p=0.72).

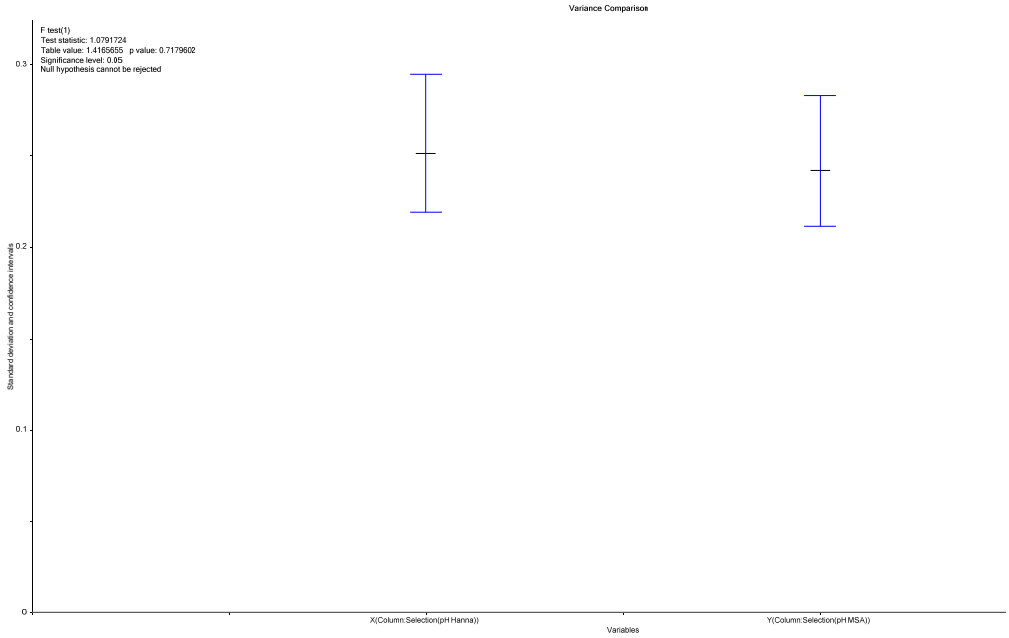
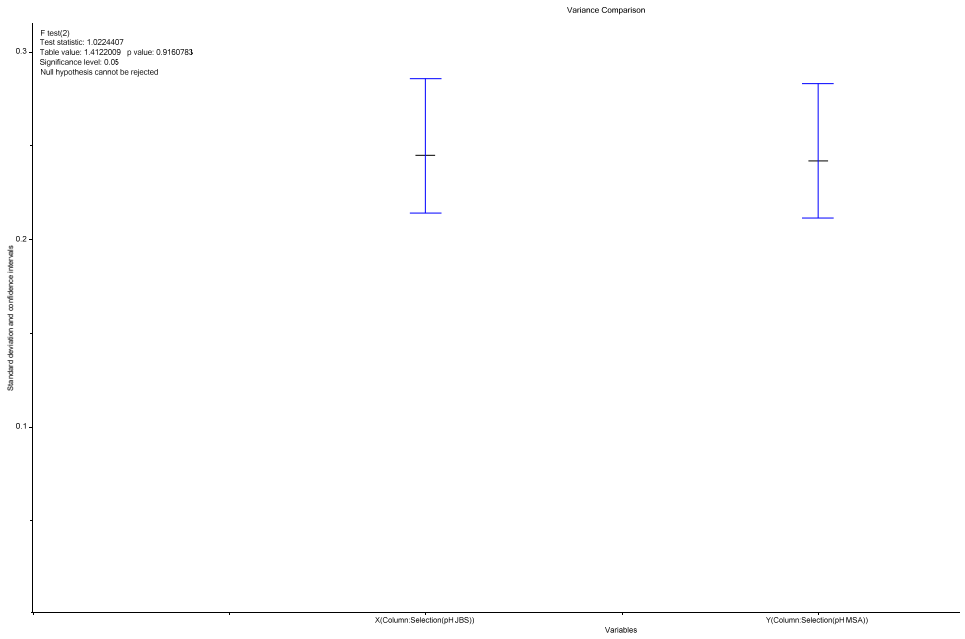


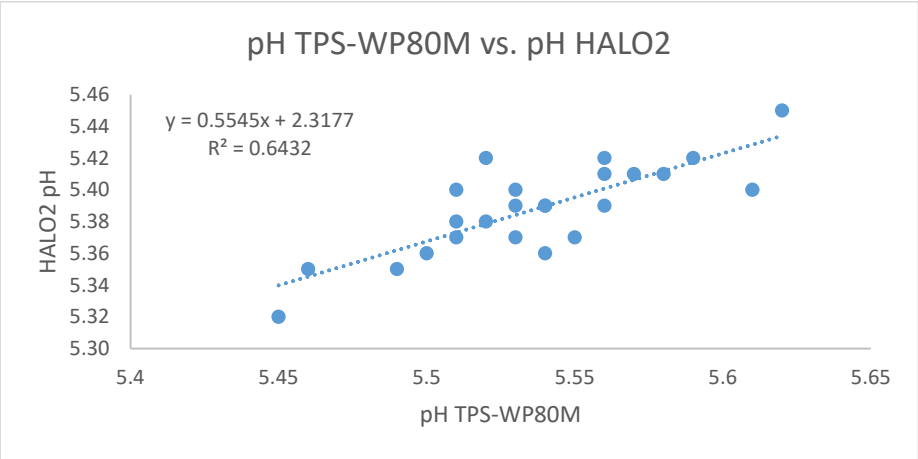
Figure 36. Comparison of the mean pH readings between the TPS-WP80M pH meter (MSA) and the TPS-WP80M (JBS) pH meter (p=0.92).



3.3.2.3 Experiment 3

Data was collected on carcasses during MSA grading by the MSA R&D Strategy and Integrity Systems Manager. For these 24 carcasses the mean (±SE) TPS-WP80M pH readings (5.54±0.007) differed ($p<0.05$) from that of the HALO2 (5.39±0.007). The correlation between the readings is depicted in Fig. 37.

Figure 37: The correlation between the pH measurements of HALO2 and TPS-WP80M taken on 24 chilled carcasses



3.3.3 Discussion

As pertaining to Experiment 1:

The difference in pH readings between the two TPS-WP80M meters is due to inaccurate calibration of the two meters – this emphasises the importance of ensuring correct calibrations are performed as was done in Experiment 3, where no differences between the two TPS-WP80M pH meters were observed.

The HALO's pH readings differed due to the readings not having been transformed from the default temperature of 25°C to the MSA pH of 7°C. When the data was transformed using the Nernst equation, the differences were even larger. It is known that as the temperature drops, the pH readings will also decrease and thus the over statement of the pH readings.

The data from different pH meters contributed to the observed differences in the calibration statistics for the NIR resulting in the correlations between pH and NIR data ranging from 0.16 to 0.83. This indicates that for the NIR to function, reference methods will need to be standardised, to either compare results or develop universal calibrations. In addition, at high pH it seems that the NIR calibrations lack linearity. One possible explanation is the effects of the concentration of the OH- on the spectra. Another explanation could be too few samples as well as a lack of variation between the pH readings.

The following are highlighted:

- Importance of ensuring that calibration is done according to guidelines and specifications.
- Importance of ensuring that temperature in which the readings are given is standard, and where applicable, adjusted for the wished fixed temperature (7°C for MSA).

- The NIR calibrations were strongly influenced by the pH meter used, this is again linked to the variation between pH meters (see earlier discussion points).

As pertaining to Experiment 2:

Care was taken to ensure that the three pH meters were calibrated according to the guidelines. A technical representative from Hanna also attended and ensure that the HALO2 was calibrated according to specifications. All three pH meters were also set to adjust their temperatures to 7°C. As a result, there was no significant difference between the readings recorded by the three pH meters.

The new Dual-level LCD screen on the HALO2 was an advantage over that HALO evaluated in Experiment 1 as it gives a fast overview of whether the pH and temperature of the sample lay within the expected parameters. No problems were encountered with the recording of the data onto the mobile phone app.

As pertaining to Experiment 3:

The MSA R&D Strategy and Integrity Systems Manager took the HALO2 with her during routine grading to test the meter under different practical conditions. It is unfortunate that the HALO2 returned to its default setting of displaying readings at 25°C which led to an overestimation of the pH readings. If this was to happen under carcass grading conditions, it could have dire financial consequences.

One of the objectives of this trial was for the Manager to see what issues could arise and to see whether these could be surmountable. The following comments were received:

I. After 20 readings, fat/meat juices were noticed on the inside of the removable sleeve on the probe. This is not ideal. It is suggested that a discussion is held with the manufacturers to look at tightening the sleeve around the probe.
II. The fat/meat juices etc were noted to be impacting the pH readings. Figs. 38 and 39 are pre- and post-clean of the probe in a buffer test. Note that before the clean, the probe was 0.05 pH units out when measuring the buffer's pH, whilst after cleaning, it read the correct pH. This emphasises the importance of ensuring that the aperture in the pH probe is clean.
III. It was reported that when working on warm carcasses where there was a lot of moisture, the head of the HALO2 probe could become slippery. It is suggested that a key chain type loop be added to the edge, or some finger grips be added to aid in holding the probe.
IV. The HALO2 probe without cables was found to be less cumbersome than the TPS-WP80M.
V. The battery life of the Halo2 would seem to be shorter than indicated – most probably due to a higher battery usage when the measurements are taken at low temperatures typically encountered in chillers.

Figure 38. The HALO2 probe after 20 readings showing fat/meat juices under the sleeve.

Figure 39. The HALO2 probe indicating the impact of the aperture being 'dirty' (left image) versus 'clean' (right image) when placed into the pH 7.01 buffer solution.

4 Conclusion

Since pH plays a critical role in the MSA grading system, it is crucial that an accurate and true pH value is measured. This is challenging as there is variation of the pH within a muscle which needs to be minimised, by typically taking the measurement at a fixed anatomical position. The experiments from this study have highlighted that there are other factors that also influence the accuracy of the pH reading which include variation between probes/pH devices as well as due to the operator(s)' measuring technique. These variations can be minimised by standardising the techniques – with an emphasis on the correct and regular calibration (and conditioning when required) of the electrodes for both pH and the temperature. Regularly cleaning of the pH probes will minimise the impact of fat and plasma on the pH readings.

The Halo2 pH meter seems to be suitable for use as an alternative to the TPS-WP80M as it gives as accurate pH readings and is less cumbersome to use. It also allows for pH and temperature readings to be displayed 'as is' – this is important for measuring the decrease in pH/Temperature over time and it also allows for the transformation of the pH readings to a fixed pre-determined temperature (e.g., 7°C). One of the challenges though is its battery life.

It is recommended:
- that the commercial entity that owns the Halo2 pH meter continue to work with MSA to further test and evaluate the Halo2 pH meter across larger numbers of carcases across beef and also look at capability in lamb and for longer time periods to understand impacts on battery life to ensure robustness of the pH probe and meter as well as any design enhancements.

- to include measuring of rate of decline in hot carcases as part of the further commercial testing.

- To engage with the manufactures of the TPS WP80M meter to test their appetite for a meter that has blue tooth capability and a more modern design.
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4.1 Key findings

- All indications are that the HALO2 could be a suitable candidate as an alternative to the cumbersome TPS-WP80M for use during MSA grading.
- Care should be taken that the pH probes (irrespective of which meter is being used) are cleaned regularly – this will also minimise the issue with fat and meat plasma blocking the pH probe's aperture.
- The pH meter (irrespective of which meter is used) needs to be calibrated for both pH and temperature on a regular basis.

4.2 Benefits to industry

The benefits to the industry are minimal in that both the TPS-WP80M and the Halo2 will give accurate pH readings if the correct cleaning and calibration procedures are followed. The benefit of having an alternative solution in the Halo2 lies in that it is less cumbersome to use.

5 Future research and recommendations

It is recommended before final decisions and Industry endorsements are made:
- that the commercial entity that owns the Halo2 pH meter continue to work with MSA to further test and evaluate the Halo2 pH meter across larger numbers of carcases across beef and also look at capability in lamb and for longer time periods to understand impacts on battery life to ensure robustness of the pH probe and meter as well as any design enhancements.

- to include measuring of rate of decline in hot carcases as part of the further commercial testing.

- To engage with the manufactures of the TPS WP80M meter to test their appetite for a meter that has blue tooth capability and a more modern design.

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