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# Optical measurements of pH in meat

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**Abstract**

The pH changes occurring in a carcass during the first 24 h after slaughter are important for the quality of the final meat or meat products. Protein denaturation will occur if pH falls to too low a level or if a relatively low pH sets in at a time after slaughter where the carcass temperature is still high. This will result in meat with poor water holding capacity and in extreme cases in meat that is PSE. pH is measured electrochemically using either glass or solid state (IS-FET) electrodes. However, electrochemically based methods are slow to use and do not offer good precision on unhomogenized meat. In this work it has been investigated whether pH can be measured spectroscopically in reflectance using the visual and near infrared spectral regions. On a limited number of pig meat samples (46 *longissimus dorsi* and 46 *semimembranosus muscles*) correlations of 0.85 have been achieved using the fast spectroscopic techniques opposed to glass electrode measurements done in duplicate. The prediction errors for the spectroscopic techniques are found to be comparable to the precision of the reference method. 

## 1. Introduction

The quality of pork is greatly affected by the development in pH during the first 24 h after exsanguination. This is due to the anaerobic metabolic decomposition of the glycogen reserves in the muscles that result in the production of lactic acid and a subsequent decline in pH. This process can lead to denaturisation of muscle proteins if the decline in pH is too great or if the carcass temperature is too high at even moderately low pH levels which in turn may result in meat with poor water holding capacity and in extreme cases pale, soft and exudative (PSE) meat. This degrading of meat quality may be minimised by a gentle treatment of the animals prior to slaughter and by organizing the slaughter process in such a way that the carcasses are chilled quickly after slaughter (EAAP, 1985; Maribo, 1998). Therefore pH measurements are an important tool for optimizing meat quality and for giving a reasonably good indication of the final (equilibrated) meat quality.

pH is normally measured electrochemically in the carcass using either glass electrodes or solid state (IS-FET) electrodes. However, protein material tends to build up on these electrodes during use which means that they have to be cleaned carefully at regular intervals. Also, they are quite slow and rather imprecise when measuring, — especially on warm carcasses. Therefore, alternative methods for measuring pH in meat would be welcome.

Among the possible alternative methods that are available for measuring pH in meat are the optical methods. These are distinguished by being fast and relatively uncomplicated. Optical measurements on meat have been applied for a number years, —usually for simple meat colour measurements (Francis & Clydesdale, 1975). Fibre optics have also been employed as a means of performing remote measurements inside muscles on the intact carcass (MacDougall, 1980; Swatland, 1989). Optical detection of PSE meat has been attempted many times (Barton-Gade & Olsen, 1984; Bendall & Swatland, 1988; MacDougall, 1980; Swatland, 1983, 1986), but direct determination of pH has not attracted much attention. (See, however, Swatland, 1983 & Mitsumoto et al. 1991).

Whether it is possible to utilize optical methods for determining pH in meat was therefore investigated. Optical/spectroscopic methods are characterized by giving an immediate response, contrary to electrochemical methods which are based on the establishment of a chemical equilibrium at the electrode surface. A

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spectroscopic method will not measure pH directly as is the case with $H^+$ sensitive electrodes. The spectrometer working in the visual or near infrared spectral region will register differences in absorbance patterns due to changes in e.g. inter-molecular forces or structural changes in the meat at different pH levels. Since meat is a very complex medium a spectroscopic method for measuring pH will have to be calibrated on a large number of samples representing a wide variation in meat quality. A method that has been calibrated on e.g. pork *longissimus dorsi* muscles may thus not be applicable for measurements on pork *semimembranosus* muscles. Sample temperature and time that has passed from exsanguination will also be factors that have to be taken into account for the calibration.

If a spectroscopic method is to be useful for in-line measurements the instrument will have to be based on a relatively simple, robust, inexpensive pocket size equipment. However, for this study standard laboratory spectrometers have been used in order to test if optical pH measurements are possible on meat samples.

## 2. Materials and methods

### 2.1. The meat samples

At a commercial slaughterhouse pork carcasses were measured in the *longissimus dorsi* and *semimembranosus* muscles with an Ingold (Urdorf, Switzerland) LOT type 3120 glass electrode and a Knick (Berlin, Germany) Portamess 751 pH-meter the day after slaughter. Samples of both muscles were taken from 46 carcasses representing a reasonable range in pH values. The samples were brought to the laboratory and each sample was divided in two portions,—one being thoroughly homogenized by grinding twice through a 2 mm hole plate.

In the laboratory the pH was measured once more with the standard set-up mentioned above on both the homogenized and unhomogenized meat samples. All measurements were done in duplicate and if necessary in triplicate (if deviation between two measurements was greater than 0.2 pH units). The average pH value for each sample was then used for further analysis as reference value for calibrating the spectroscopic readings. In Table 1 the relevant statistics for the 46 samples are presented. In Fig. 1 the agreement between pH reference measurements on homogenized and unhomogenized samples is given for all 2×46 samples (*longissimus dorsi* and *semimembranosus*),—the correlation being 0.989 and the slope 1.01.

The reproducibility of the pH measurements on cold pork has been investigated (E. V. Olsen, 1997) and found to be 0.064 for *semimembranosus* and 0.072 for *longissimus dorsi* muscles. This means that the largest difference between two neighbouring measurements on the same muscle with 95% confidence will be of the order $2 \times \sqrt{2} \times 0.072 = 0.20$ pH units. If measurements, as is the case here, are done in duplicate the precision will be improved by a factor of $\sqrt{2}$ resulting in an overall precision of $0.072/2 = 0.05$. This means that pH values for measurements on meat should be represented by only one digit after the decimal point.

Table 1
pH reference values: average, standard deviation, maximum and minimum values, based on the entire number of samples

| | Unhomogenized | | Homogenized | |
|---|---|---|---|---|
| | LD[a] | SM[b] | LD[a] | SM[b] |
| Average | 5,69 | 5,77 | 5,69 | 5,75 |
| Standard deviation | 0,25 | 0,27 | 0,26 | 0,28 |
| Maximum | 6,67 | 6,97 | 6,74 | 7,01 |
| Minimum | 5,37 | 5,46 | 5,38 | 5,45 |

[a] LD = *longissimus dorsi*.
[b] M = *semimembranosus*.

### 2.2. The spectroscopic measurements

With two different spectrometers the following were measured

- Near infrared (NIR) reflectance as a continuous spectrum.
- Visual reflectance as a continuous spectrum.

on all 46 *longissimus dorsi* samples and 46 *semimembranosus* samples. With the NIR equipment measurements were done on both homogenized and unhomogenized samples. With the visual spectrometer, measurements were only performed on the unhomogenized samples. Due to technical problems only 43 samples were measured in the visual spectral range.

### 2.3. Spectroscopic equipment

The near infrared measurements were carried out on a MB series 160 FT-NIR instrument with a Diffusir sample compartment both made by the company BOMEM (Quebec City, Canada). The wavenumber region was set from 3799 $cm^{-1}$ to 10,000 $cm^{-1}$ corresponding to the wavelength region 2630 nm down to 1000 nm with the wavenumber resolution set at 4 $cm^{-1}$ (1 nm steps on average over the spectral region). The spectrometer measures a surface area of approximately 13 $cm^2$.

The visual spectra were acquired with a MCS 310 diode array spectrometer and a CLX 111 Xenon lamp, both by Zeiss (Oberkochen, Germany). Wavelength range and spectral resolution was 362 nm to 777 nm and 1 nm, respectively. The spectra were acquired through a remote probe with randomized illuminating and receiving optical fibres arranged in a 0.5×10 mm slit at the tip.

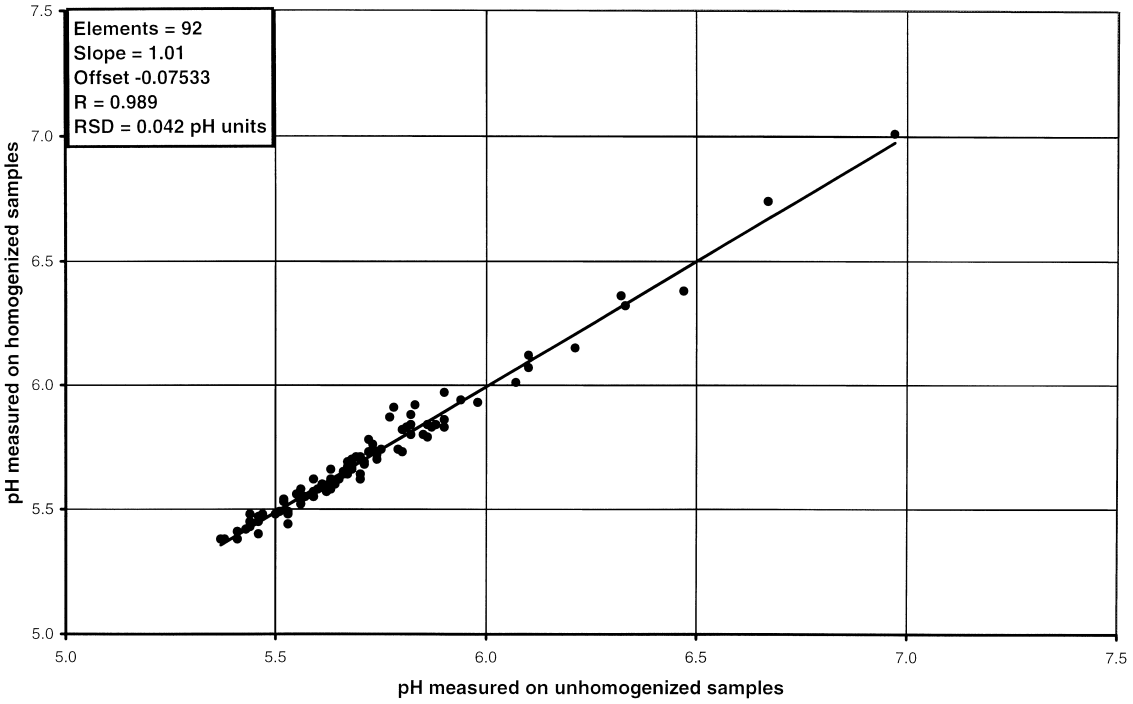


Fig. 1. pH measured on unhomogenized samples versus pH measured on homogenized samples. R = 0.989, slope = 1.01 and RSD = 0.042 pH units.

## 3. Results and discussion

The data analysis (linking of spectra with pH reference values) was carried out in two ways

- by identifying regions in the spectra which have a high correlation with pH; and
- by different kinds of multivariate data pretreatment techniques and PLS regression (see below).

### 3.1. Correlations between spectral regions and pH

In Fig. 2 is shown the cross-correlation between the pH reference value and absorbance recordings at each of the wavenumbers between 3799 $cm^{-1}$ to 10,000 $cm^{-1}$. These correlations are based on spectra from homogenized samples from both *longissimus dorsi* and *semimembranosus* muscles. By choosing only one wavelength at 5177 $cm^{-1}$ it is possible to estimate the pH value of the 92 samples with a correlation of $R = 0.5$. If this analysis is done on homogenized *longissimus dorsi* samples alone the correlation increases slightly to 0.55. A change from negative to positive correlations is seen in Fig. 2. The reason why the absorbance correlates positively with pH in the high energy NIR region and negatively in the low energy domain is intriguing, however a speculative explanation may be offered:

Meat with high pH contains less free water than meat with low pH. The low energy (long wavelength) region of the spectrum is dominated by a number of very strong water absorption bands (6900 $cm^{-1}$ and below 5400 $cm^{-1}$). Thus when pH increases the absorbance caused by free water will decrease giving a negative correlation between pH and absorbance.

In the high energy (short wavelength) region water absorption is not nearly as dominating as in the low energy region. In this region the amount of reflected light is dominated by the mean penetration depth of the light into the meat sample. For 'high energy' photons (around 10,000 $cm^{-1}$ or 1000 nm) this mean path length decreases with increasing denaturation of the muscle proteins (low pH condition). Consequently for low pH samples much more light is reflected back to the detector system. By this mechanism a low pH sample will give low absorbance recordings resulting in a positive correlation.

The corresponding cross-correlations between the reference pH and the visual spectra (362 nm to 777 nm) acquired on unhomogenized *longissimus dorsi* samples are shown in Fig. 3. The maximum correlation $R = 0.8$ is achieved at wavelengths between 362 nm and 365 nm. For unhomogenized *semimembranosus* samples the best single wavelength correlation is only $R = 0.5$. Fig. 3 furthermore reveals a rather sharp and pronounced decrease in the correlation in a narrow spectral range around 420 nm, i.e. coinciding with the Soret band. Since haemoproteins such as myoglobin and (residual) haemoglobin have strong absorptions here it may well be that corrections for these would result in a smoother

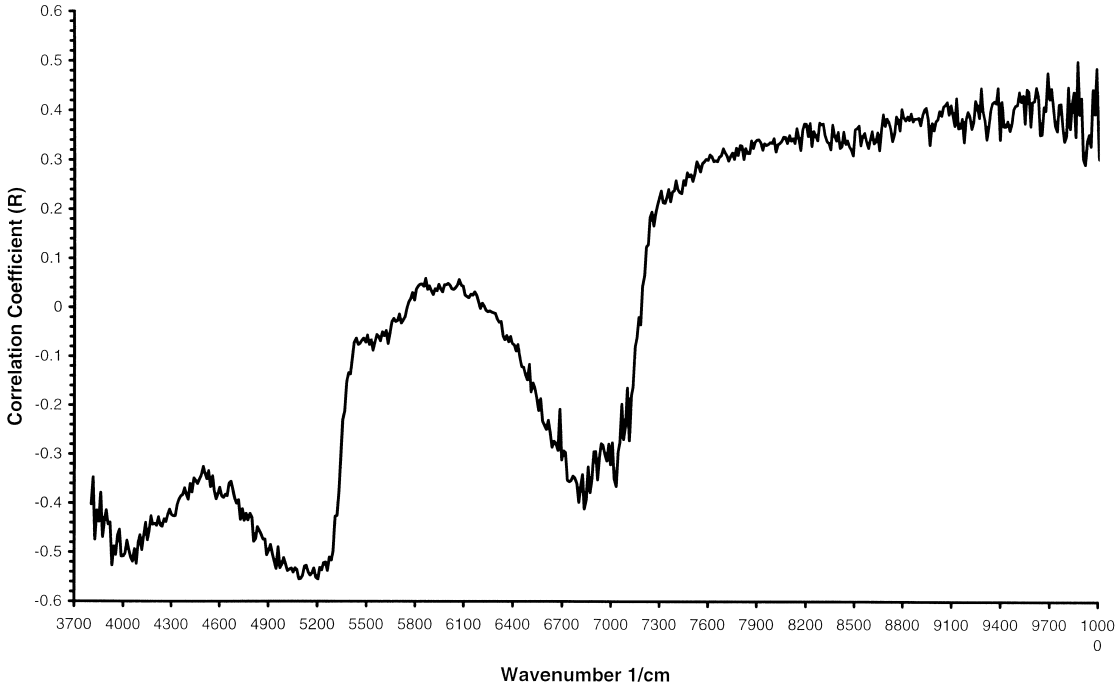


Fig. 2. Single wavelength correlations between pH and NIR spectra of homogenized samples.


Fig. 3. Single wavelength correlations between pH and VIS spectra of unhomogenized *longissimus dorsi* samples.

curve for the correlation versus wavelength. However, such corrections are difficult to make and the overall best correlation is not expected to be above 0.8 even with corrections.

### 3.2. Multivariate PLS regression

It is outside the scope of this paper to give a detailed description of the Partial Least Squares regression

(PLS-R) technique (see Martens & Naes, 1989 for details). PLS-R is a standard statistical method used for creating calibration models for spectroscopic data. In the following the results obtained with this regression technique in developing a model for predicting pH in meat with the two spectrometer types will be presented. All results presented below are given as RMSEP values (root mean square error of prediction) defined as:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{N}(pH_i^{ref} - pH_i^{pred})^2}{N}}$$

where $N$ is the number of samples being predicted by the model, $pH^{ref}$ is the reference pH value for sample No. 1, and $pH^{pred}$ the predicted pH value for that same sample.

This measure for the validity of a prediction model has the advantage over presenting results as $R^2$ (the coefficient of determination) in that it is independent of the range in the reference value for the samples being studied (a single sample with a very high or low pH value will give an impressive $R^2$ even though the model gives a large RMSEP). From the standard deviation (SD) of the reference values for any given population (in this study given by Table 1) and the model RMSEP one can calculate the $R^2$ (and consequently $R$) that can be achieved with that model using the relationship:

$$R^2 = 1 - \frac{\mathrm{RMSEP}^2 * N}{SD^2 * (N-1)}$$

Also in the following, the RMSEP is estimated by using the cross-validation technique. This method of validation is performed by leaving one or more samples at a time out of the calibration and then using the remaining samples to construct a PLS model. The model is subsequently tested on the samples that have been left out. This procedure is repeated until all samples in the data set have been used as test samples. For this study full cross validation is used, i.e. the samples are left out one at a time.

### 3.3. Data pretreatment

Before the calibration models were made, the reflectances were converted to absorbance $A = \log(1/\text{reflectance})$.

A common problem in reflectance spectroscopy is the shift in the spectra from different samples caused by the varying amount of scattered radiation with no chemical information.

One way of compensating for this is to use a mathematical transformation such as multiplicative scatter correction (MSC; Geladi et al., 1985). Each spectrum is transformed to minimize the difference from the mean spectrum of the calibration samples by adding one constant and multiplying by another. The two constants are found by regressing the spectrum against the mean spectrum. As can be seen from Figs. 2 and 3, there are large differences in the correlation with pH at different wavenumbers, and the calibration model is improved by using only spectral regions with high correlation with pH. The wavenumber selection procedure used here consists of finding the number of variables that gives the minimum value of RMSEP found by full cross-validation. In this study, the NIR spectra were transformed using MSC, and subsequently the optimal number of variables (wavenumbers) was found.

### 3.4. PLS regression results

In Table 2 are shown the results obtained with the NIR instrument on homogenized and unhomogenized samples and with the visual spectrometer on unhomogenized samples. Before performing the PLS regression spectral outliers were identified by graphing all the spectra and evaluating them visually and by using principal component analysis (Martens & Naes, 1989; Sharaf et al., 1986). These outliers were removed from the calibration data.

Table 2
Cross-validation results for predicting pH with NIR and visual spectrometers

| Spectral region | Sample type | Number of outliners | SD | PLS factors | RMSEP | R[e] |
|---|---|---|---|---|---|---|
| NIR (1000 nm–2630 nm) | UL[a] | 5 | 0.153 | 3 | 0.104 | 0.73 |
| | HL[b] | 6 | 0.159 | 1 | 0.100 | 0.77 |
| | US[c] | 5 | 0.128 | 1 | 0.077 | 0.79 |
| | HS[d] | 5 | 0.135 | 1 | 0.091 | 0.73 |
| VIS (362 nm–777 nm) | UL[a] | 4 | 0.189 | 3 | 0.099 | 0.85 |
| | US[c] | 4 | 0.132 | 1 | 0.121 | 0.37 |

[a] UL = unhomogenized *longissimus dorsi* muscle.
[b] HL = homogenized *longissimus dorsi*.
[c] US = unhomogenized *semimembranosus*.
[d] HS = homogenized *semimembranosus*.
[e] R is the correlation coefficient and the number of PLS factors is a measure of the complexity of the PLS regression model.

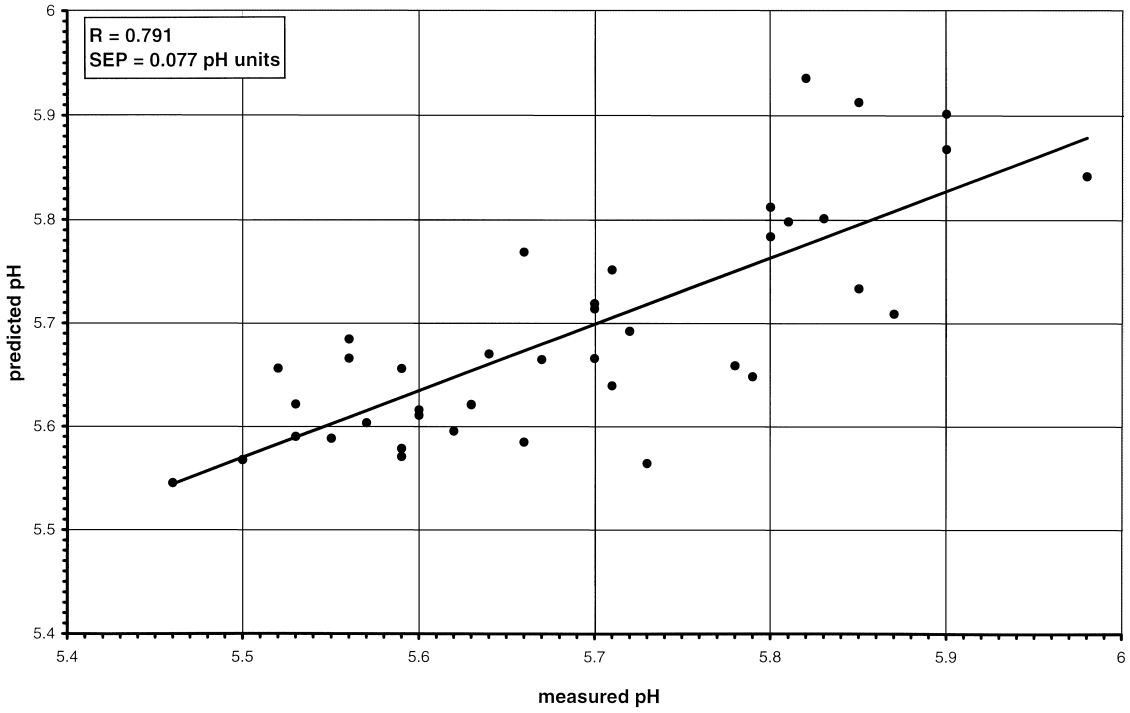


Fig. 4. Predicted pH-values (cross-validation results using the model from NIR data) versus measured pH-values for unhomogenized *semimembranosus*. RMSEP = 0.077 pH units.

To get an idea of how well these models actually work one must compare the RMSEP values in Table 2 with the population standard deviation (SD in Table 2) for the model. The measurements done in the visible spectral region do not give good results on the unhomogenized *semimembranosus* muscles. This may be due to the very small measuring window of the optical probe that was used and the high degree of inhomogeneity of these samples.

In Fig. 4 is shown an example of a graphical representation of the cross-validation results. The abscissa values are the measured pH, and the ordinate values are the predicted pH using the model from the NIR data describing unhomogenized *semimembranosus*.

The correlations listed in Table 2 do not look very impressive at a first glance. However, the ranges and standard deviations of the reference pH values for the calibration data sets are very small, and as the precision of the reference values is almost 0.1 pH units there is obviously very little room for improving these results. It should be borne in mind that the spectroscopic method can never be proven to be more accurate than the reference method which in this case is the standard pH electrode.

## 4. Conclusion

The results disclosed here indicate that it may indeed be possible to develop a spectroscopic method for measuring pH in intact muscles and in homogenized meat. It is furthermore to be expected that such a method should be a reliable and rapid alternative to pH measurements using standard electrodes. It was shown that the accuracy achieved with the NIR and visual range spectrometric methods are comparable to the precision of the standard glass electrode pH meter.

As a consequence continuation of the work is warranted with the aim of developing a well documented, robust and inexpensive optical method capable of measuring pH at a slaughter line.

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