

NON-INVASIVE PH MEASUREMENT EARLY POSTMORTEM IN PORCINE *M. SEMIMEMBRANOSUS* USING RAMAN SPECTROSCOPY

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Abstract – We have examined the feasibility of using a portable Raman system as a rapid non-invasive optical device to measure the early postmortem pH of pork meat.

M. semimembranosus samples (n=10) were excised in a commercial abattoir and pH, lactate and Raman kinetics were measured 0.5 – 9 hrs p.m. to follow the metabolic rate of the samples.

The Raman spectra were correlated with pH using PLS regression analysis and multi-linear regression (MLR) with a reduced number of spectral channels. For the prediction of pH, PLSR resulted in a coefficient of determination of $R^2 = 0.87$ and a standard error of cross validation RMSECV = 0.15 pH units. With 90.5% correctly identified samples this model allows for a discrimination of the Raman spectra according to the pH<5.8 criterion and only 3.5% of the samples were misclassified as deviating. With the MLR model the accuracy was only slightly reduced to 84.2%.

These first results demonstrate the feasibility of predicting the pH from Raman spectra measured with a portable Raman device during the first hours postmortem. This could be useful for the identification of PSE deviation of ham in the meat production.

Key Words – pH₄₅, PSE detection, handheld Raman

I. INTRODUCTION

Ensuring a high quality of meat is an ongoing concern of industrial meat production. Here, the identification and reduction of deviating meat qualities such as PSE are of interest because the reduced water-holding capacity (WHC) is causing problems for subsequent meat processing and economic loss. It is accepted that an accelerated postmortem metabolism is a key factor for the incurred changes of muscle structure and reduced WHC [1, 2]. This can be identified, amongst other, by an increased muscle temperature, lactate formation and rapid pH decline [3]. The pH₄₅ is a good indicator for WHC [4], but the measurement is

invasive and susceptible to faults. Hence, a great deal of work has been performed to assess and predict PSE deviation [5] and WHC early in the production process by non-invasive methods such as color [6, 7], NIR [8] or vibrational spectra [9].

In this context, Raman spectroscopy is of interest as it is a non-invasive technique providing direct information of the molecular composition without being biased by variation in water content. The effect is based on inelastic scattering of light leading to the excitation of molecular vibrations in the sample. Therefore, Raman spectroscopy has shown considerable use in the food sector [10].

In the early work of Pedersen et al. [9] the use of a bench top instrument was a limiting factor for industrial on-line application. Since then, the development of portable Raman equipment has significantly advanced [11] and a handheld Raman probe has recently been developed for meat based on a laser diode emitting light at 671 nm [12].

In this study, we report on the first use of this portable Raman device to measure the pH of pork meat during the first 9 hrs after slaughter.

II. MATERIALS AND METHODS

The topsides (*M. semimembranosus*) from 10 pigs which were slaughtered in a commercial abattoir were removed 15-30 minutes after exsanguination and transported to the laboratory. The muscles were cut into three parts which were used in parallel for pH, Raman, and lactic acid measurements which started 25-40 minutes postmortem. The pH was recorded with a polymeric electrolyte pH electrode, InLab[®] solids Pro (Mettler Toledo, Giessen, Germany). For lactate analyses of each muscle, 8-9 samples of 5 g each were taken with increasing intervals according to the slowing down of the metabolism. The samples were processed according to the method of Boehringer [13] and the lactate concentration was determined photo-

metrically using an enzymatic test assay (Megazyme International Ireland Ltd., Wicklow, Ireland). Raman spectra were measured with the 671 nm handheld Raman probe described by Schmidt et al. [12] with a laser power of 100 mW. The probe was connected via optical fiber to a custom-made portable spectrograph (HORIBA Jobin-Yvon, Longjumeau, France) with thermoelectrically-cooled CCD camera covering the spectral range from 300 to 2100 cm^{-1} with a spectral resolution of 8 cm^{-1} . For each sample and time of the kinetics, 5 spectra with 5 s integration time were recorded at the meat surface and stored with a net book using a self-written Labview program (Labview 9.0f3, National Instruments, Austin, TX, USA). The intervals between the set of 5 spectra were increased from a few seconds during the first 2 hours to 20-30 minutes at the end of the measurement.

For further analysis, each set of 5 spectra was averaged, smoothed with a Savitzky-Golay 2nd order filter [14] and a baseline correction was performed to remove the broadband spectral background. Preprocessing was completed by normalizing the spectra to the net intensity of the Raman peak at 1000 cm^{-1} which is assigned to the amino acid phenylalanine [15] and which serves as an internal standard to compensate for variations of the intensity.

III. RESULTS AND DISCUSSION

The pH_{45} of the samples varied from a 6.54 to 5.83 (Table 1). The pH_{24} scattered around a mean of 5.51 ± 0.13 . The lactic acid content increased between start and end of the measurements by 50 to 90 mmol/kg, except for sample 10 with virtually no increase of the lactate concentration. Therefore, and due to the low final lactate content we assign a DFD tendency to sample 10.

The normal meat samples produced 7 to 9 mmol/kg lactate per hour, whereas samples 8 and 9 showed increased lactate formation rates of 12 to 14.9 mmol/kg per hour. Considering also the low pH_{45} , Sample 8 and 9 can be regarded as PSE tendency. Schaefer et al. found in *longissimus dorsi* muscles an average lactate formation of ca. 35 mmol/kg·h [3]. The difference to our rates can be explained by the faster cooling to room temperature of our excised muscles.

Table 1 Overview of pH_{45} , pH_{24} , final lactate concentration [mmol/kg], lactate formation rate [mmol/kg·h] and estimated type of meat

#	pH_{45}	pH_{24}	Lact ₂₄	Rate	Type
1	6.54	5.80	90	7.9	normal
2	6.38	5.40	114	7.0	normal
3	6.34	5.51	99	7.1	normal
4	6.31	5.50	100	7.0	normal
5	6.25	5.53	119	6.9	normal
6	6.01	5.44	86	6.3	normal
7	5.90	5.47	89	9.4	normal
8	5.91	5.35	113	12.0	PSE tendency
9	5.83	5.38	118	14.9	PSE tendency
10	5.87	5.70	70	<1.0	DFD tendency
Mean	6.13	5.51	100	8.7	
SD	0.24	0.13	15	2.7	

The kinetics of pH and content of lactate exhibit the expected developments. While the correlation between pH and content of lactate showed a tight curvilinear relationship for each of the individual muscles, the correlation for all samples is rather low ($R^2 = 0.49$) compared to values published by Bendall et al. [16].

The Raman spectra of the time series of samples 1-9 show intensity changes of a number of Raman signals in the 500 to 1800 cm^{-1} wave number range, whereas sample 10 reveals almost no spectral changes which is in keeping with the observed lack of acidification. As expected some of the spectral changes can be assigned to the formation of lactate, notably at 853 cm^{-1} and 1414 cm^{-1} which are assigned to the C - CO_2^- stretching vibration and to the CO_2^- stretching vibration, respectively [17]. Due to the pK_A of lactic acid, only a small portion of the acid is formed in the considered pH range of 7 to 5.3 (0.07% to 3.5%) so that the signal of lactic acid at 827 cm^{-1} which is assigned to the C- CO_2H stretching vibration [17] plays a minor role. However, the co-location of the two most intense lactate and lactic acid peaks with the tyrosine doublet makes a quantitative analysis based on only these peaks impossible. On the other hand, while the content of lactate increases with progressing glycolysis, the muscles acidify to pH-values of 5.3 to 5.6 and other Raman signals indicating pH dependent protonation such as an increase of the intensity of the C=O stretching vibration of the carbonyl group of organic acids at 1720 cm^{-1} can be detected. In this region of the Raman spectrum only few other

Raman signals appear and this signal is considered as sum band of all carboxylic acid molecules. Similarly to the formation of carboxylic acid, with decreasing pH we observe the protonation of phosphate groups and inorganic phosphate in the Raman spectra. The signal at 980 cm^{-1} is assigned to the $-\text{PO}_3^{2-}$ symmetric stretching vibration of terminal phosphate groups of phosphorylated compounds and the signal at 1080 cm^{-1} to the symmetric $-\text{PO}_2^-$ stretching vibration of the $-\text{PO}_3\text{H}^-$ group [18]. Both peak intensities show diametrical behavior: while the former decreases upon acidification, the latter increases. The same holds for inorganic phosphate ions (HPO_4^{2-} and H_2PO_4^-).

Based on the most significant spectral changes with pH, a multiple linear regression model (MLR) was calculated using eleven net intensities and one offset value to predict the pH from the Raman spectra. The model essentially relies on signals of lactate, lactic acid, phosphate and phosphorylated molecules. Only spectra to a pH above 5.6 were included in the calculation because at this pH the concentration of the phosphate bases is approaching zero so that the working range of the indicator is left. The model yields predictions with $R^2 = 0.73$ and $\text{RMSEC} = 0.12$ (Fig. 1). The model shows an overall good performance although it neglects the non-linear correlation between net peak intensity and pH-value.

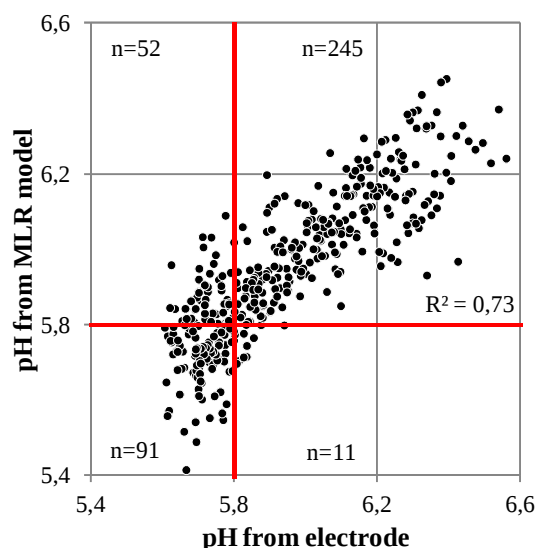


Figure 1. Predicted pH from Raman data with MLR model vs. pH measured with pH electrode

Using partial least square regression (PLSR) a better performing model for predicting the pH could be achieved (Fig. 2).

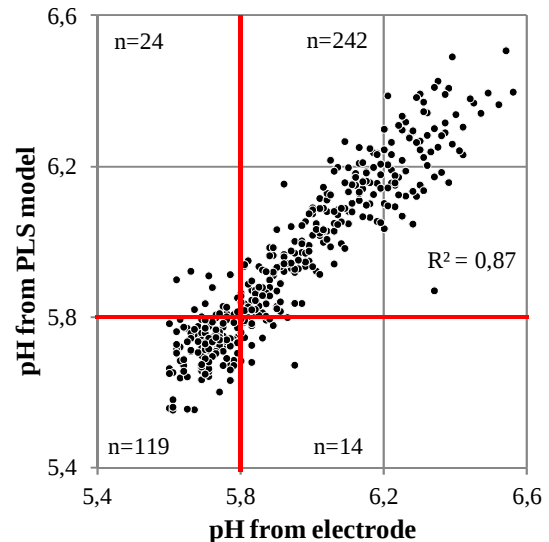


Figure 2. Predicted pH from Raman data with PLSR model vs. pH measured with pH electrode

For this model the full wavenumber range from 500 to 1800 cm^{-1} was used. With six latent variables the model yields predictions of the pH with $R^2 = 0.87$, $\text{RMSEC} = 0.08$ and $\text{RMSECV} = 0.15$. For cross validation the method “contiguous blocks” with 10 data splits was applied.

With both models the (early) post mortem pH of ham (m. semimembranosus) can be predicted from the measured Raman spectra so that the meat can be distinguished into two groups with $\text{pH} < 5.8$ and $\text{pH} > 5.8$ with reasonable precision. This criterion was chosen with a view to $\text{pH}_{45} < 5.8$ for the identification of PSE deviation. The PLSR model utilizes the full spectral information, and hence, slightly performs better with an accuracy of 90.5%. The MLR model classifies 84.2% correctly which is only slightly less good considering that only 33 spectral channels are used.

IV. CONCLUSION

That the performance of the MLR model is only slightly reduced upon reduction of the spectral information to five percent compared to the PLSR model indicates that the useful information is mainly contained in the 11 peaks of the MLR

model. Thus, the Raman prediction of the pH of ham is largely based on the increase of lactate concentration and the associated acidification monitored by phosphate signals.

Now, the models have to be tested and improved with a larger number of samples at a slaughter line, but it is obvious that Raman spectroscopy has the potential to “measure” the pH and hence to indicate the metabolic rate of a muscle early post mortem. This could be useful for the identification of PSE deviations in the slaughterhouse and prediction of a reduced WHC.

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