EVALUATION OF THE TENDERNESS OF AGED BOVINE *GLUTEUS MEDIUS* MUSCLES USING RAMAN SPECTROSCOPY AND SHEAR FORCE MEASUREMENTS

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Abstract – We have tested a portable 671 nm Raman system as a rapid non-invasive device to estimate the tenderness of aged beef meat.
M. gluteus medius **samples from 53 animals were aged for fourteen days at -1°C and 7°C. Raman and shear force measurements were performed on 104 aged samples and the Raman spectra were correlated with the shear force data using partial least squares regression analysis.**
On average, the samples aged at -1°C showed only slightly higher shear force, SF = (37.7 ± 7.3) N than the samples aged at 7°C, SF = (35.6 ± 6.5) N.
The Raman spectra correlated very well with the shear force data and good prediction models were obtained for both ageing methods. The joint model with all samples yielded for shear force a coefficient of determination of R^2 = 0.87, an error of the calibration of RMSEC = 3.0 N and an error of the cross validation of RMSECV = 4.5 N.
These first results demonstrate that Raman spectra measured with the portable 671 nm device on raw, aged beef meat can predict the shear force. Thus, the device shows potential for the assessment of tenderness of meat.

Key Words – Beef meat quality, Handheld Raman system, Non-invasive

I. INTRODUCTION

Besides taste and juiciness, tenderness is a main criterion for the consumer's acceptance of meat. Consequently, the management of tenderness is an issue for all parties involved along the chain of meat production. This is especially true for red meat which has to be aged. However, tenderness cannot be measured directly and shear force measurements which are used as an objective measure are time-consuming and laborious. Therefore, efforts have been made to estimate tenderness or shear force using non-invasive optical methods such as NIR and Raman spectroscopy [1-5]. In a recent study, a portable Raman scanner with potential for an application closer to the production environment was shown to predict shear force and cooking loss of frozen/thawed sheep meat samples [6].
Raman spectra provide information about the molecular composition of a sample with the specifity of vibrational spectroscopy. Thus, the scattered light is like a fingerprint, which can detect biochemical, structural and microbiological changes. In principal, Raman measurements can be performed through a plastic packaging [7]. This is useful as meat is often vacuum-packed during ageing.
The aim of this project was to compare two different ageing methods of beef (*M. gluteus medius)* and to evaluate whether the Raman spectra measured on the aged raw samples can predict the conventionally measured shear force values.

II. MATERIALS AND METHODS

A total of 104 beef samples were examined over a period of 5 months. To this end, the *gluteus medius* muscles of 53 animals were dissected 3-4 days p.m., separated along the tendon, and packed separately in vacuum bags. Of these samples, 53 were stored at -1°C and 51 samples at 6 - 7°C for a period of 14 days. After the aging period, Raman spectra were taken on a fresh cut along the fiber direction. The spectra were recorded with the 671 nm hand-held Raman probe described earlier [6, 7]. Each sample was scanned at 15 different positions with 5 repetitions and a 3 s integration time. The portable Raman system used for the experiments is depicted in Fig. 1.

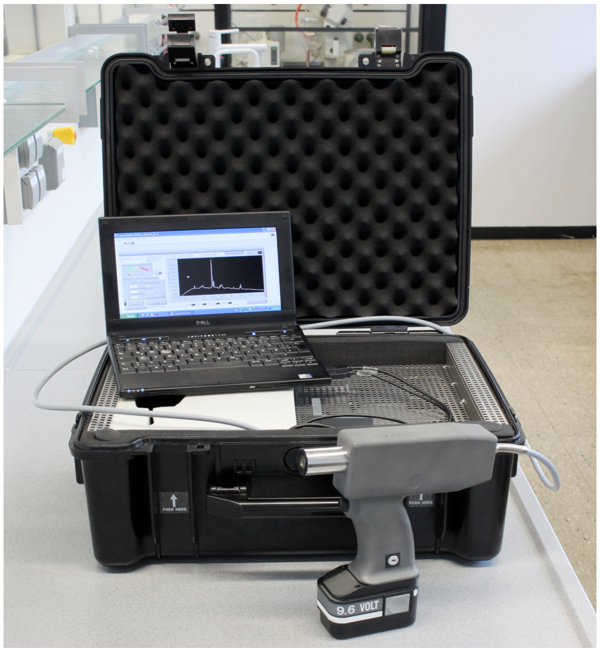

Figure 1. Portable Raman system for measurements of meat consisting of a 671 nm hand-held Raman probe (in front), netbook (left) and miniaturized spectrograph in a carrying case.

The shear force measurements were performed according to the method of Warner Bratzler using the same samples which were taken for the Raman scans. The meat samples were cut into 6 x 6 cm^2 blocks, vacuum packed and then cooked for two hours at 85°C in a Fessmann-Turbomat 1900 Ratio system (Fessmann, Winnenden, Germany). The blocks were showered for half an hour to cool to 15-20°C. Then, the blocks were stored at 2 °C for two weeks before they were cut with a slicing machine (Bizerba Typ VS8A, Meßkirch, Germany) into 1 x 1 x 6 cm^3 strips. With these stripes, at least 20 shear force measurements were carried out with an Instron 5942 (Pfungstadt, Germany) using a v-shaped blade.

For data processing the Raman spectra were averaged. PLS models were calculated using MATLAB 7.9.0 R2009b software (The Mathworks Inc., Natick, MA, USA) with the PLS toolbox 6.2 (Eigenvector Research Inc., Wenatchee, WA; USA). For cross-validation the random blocks method with 10 data splits and 20 iterations was applied.

III. RESULTS AND DISCUSSION

The shear force data of the aged samples are summarized in Table 1. On average, the samples aged at – 1°C exhibited a slightly higher shear force (SF = 37.7 N) than the samples aged at 17 °C (SF = 35.6 N). The difference was smaller than expected, but it was larger than the error of the reference measurement which was estimated from the standard deviation (SD) divided by the root of the number of samples. The number of 20 shear force measurements per sample was chosen to reduce the reference error because the correlation will finally be limited by the error of the reference method. The reference error is low compared to the biological variance of the samples.

Table 1. Mean, standard deviation (SD), minimum, maximum and reference error of shear force (N) of the beef samples aged at -1°C and 7°C.

	Shear force / N	
Ageing Temperature	-1°C	7°C
Mean	37.7	35.6
SD	7.3	6.5
Min	23.8	19.8
Max	59.7	63.4
ref. error	1.6	1.5

As the Raman spectra contain a variety of signals, which are not all equally relevant for the prediction of shear force, the number of spectral channels was reduced to exclude regions with little or no information for the prediction. This was done iteratively using variance importance in projection (VIP) plots of the calculated PLSR model [8]. Thus, spectral channels yielding VIP scores below 1 were discarded to improve the model's quality and robustness and a new model was calculated. The root mean square error of cross-validation (RMSECV) served as criterion of the robustness. Initially this error was decreasing with the number of discarded spectral channels until a global minimum was reached (Fig. 2.). This was obtained after two reduction steps (solid line) with 10 latent variables (LVs) and a coefficient of determination of R^2 = 0.87. Thus the RMSECV was reduced from 6.4 N to 4.5 N. Further reduction lead to increasing errors indicating the rejection of relevant channels (dot-dashed line).

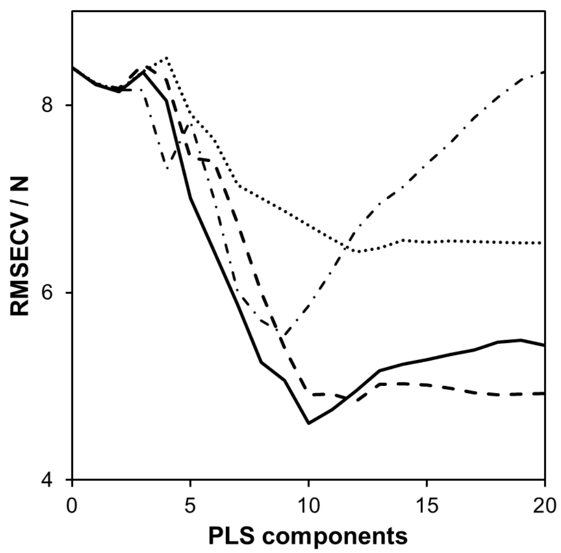

Figure 2. RMSECV of combined PLSR model versus number of PLS components without (dotted line), one (dashed), two (solid) and three (dot-dashed) reductions of the Raman data set using VIP plots.

The number LVs in our models is larger than those found in the earlier studies [2, 3].

At first, PLS correlations were calculated separately for both ageing temperatures -1°C and 7°C. As the results were comparable, a joint model was computed which included all samples. Table 2 gives an overview of the figures of merit of the correlation models which were optimized with VIP reduction of the number of spectral channels.

Table 2. Figures of merit of the PLSR models based on beef samples stored at -1 °C and 7 °C and combined model based on all samples.

Ageing	-1 °C	7 °C	-1 °C/ 7°C
R^2_{cal}	0.90	0.87	0.87
RMSEC	2.6	3.0	3.0
R^2_{cv}	0.69	0.61	0.71
RMSECV	4.7	5.4	4.5
LV	9	10	10
Channels	145	94	124

All three models yielded good and comparable results. The explained variance is high (87% - 90%) and the coefficients of determination were only moderately reduced by the cross validation. The errors of the cross validation were slightly increased compared to the calibration and about three times the error of the reference measurement. This suggests that the storage temperature had no impact on the Raman spectra and no impact on the correlation. This was not expected because we had anticipated a stronger growth of microbial flora on the meat surface upon storage at 7°C compared with -1°C. This would lead to a larger fluorescence background in the spectra which could impair the correlation. The slightly impaired figures of merit of the regression model for the beef aged at 7°C compared to -1°C could point at this, but the reduced number of spectral channels may also slightly reduce the performance. Apparently, the microbial flora had no big influence as we measured on a fresh cut.

Figure 3 shows the calibration and cross-validation for the combined model using the spectra of samples aged at -1°C and 7°C. The combined model yielded a coefficient of determination of R^2 = 0.87 and a root mean error of cross-validation of RMSECV = 4.5 N.

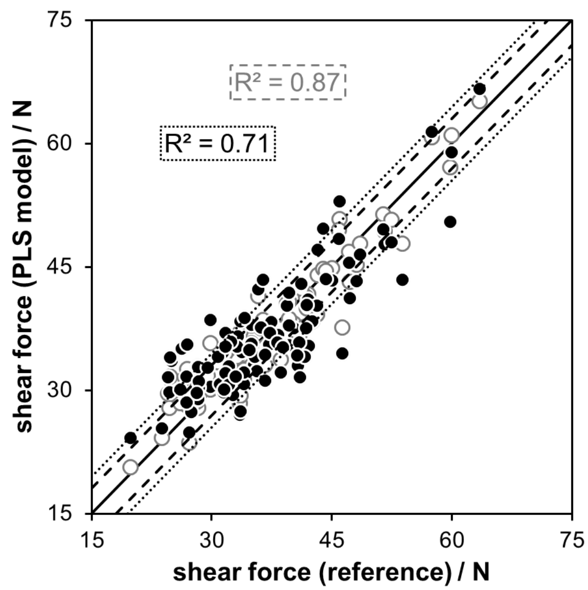

Figure 3. Shear force predicted from Raman spectra with a PLS model vs. measured shear force for beef aged at -1°C and 7°C; calibration (open symbols) and cross-validation (solid symbols).

This model utilized 124 of initially 1024 spectral channels which is a reduction of data by factor eight and the minimum RMSECV was reached after the second reduction with 10 latent variables.

IV. CONCLUSION

To our knowledge this is the first report on a correlation of Raman spectra measured with a portable Raman system and shear force data of aged beef using the *gluteus medius* muscle.

The ageing procedure at -1° C resulted on average in slightly higher shear force values (Mean SF = (37.7 ± 7.3) N) than ageing at 7°C (Mean SF = (35.6 ± 6.5) N). This difference was smaller than expected. With a reduced number of spectral channels, the PLSR correlation yielded robust and comparable models for both ageing procedures separately as well as for the combined data set of all samples.

The Raman spectra could explain 87% to 90% of the variance of the shear force data. The errors of the cross validation RMSECV's were promising and amounted to 4.5 - 5.4 N.

Thus, the ageing progress of beef meat can be non-invasively measured with a portable 671 nm Raman system. The application of this device shows great promise for a non invasive evaluation of the tenderness of aged beef.

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