PS3.05 Hand-held Raman-Sensor for In-situ Characterization of Meat Aging 272.00

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Abstract — **The applicability of Raman spectroscopy is shown for the in-situ characterization of the aging of meat. Due to its fingerprinting nature, Raman spectra provide information about the complex matrix meat, i.e. the molecular structure and the composition of the samples. Miniaturized diode lasers are utilized as light sources with excitation wavelengths of 671 nm and 785 nm with a view to the development of a portable field device for meat. As test sample, *musculus longissimus dorsi* from pork was taken. The chops were stored refrigerated at 5 °C and Raman spectra were measured daily from day 2 up to three weeks. They exhibit gradual changes of the Raman signals and they show a modification of the background signal depending on the storage time which arises from a laser-induced fluorescence (LIF). To analyze the time-correlation of the complex spectra, multivariate statistical methods are employed. By means of principal component analysis (PCA) a distinction of spectra is found on the time scale between day 8 and 10. This corresponds to the transition from ripened meat to meat at and beyond the limit of inedibility. After ca. 10 days of storage at 5 °C the microbial load is overwhelming and LIF increases. The results of the storage time-dependent Raman data are discussed in the context of reference analyses which have been performed in parallel. Finally a hand-held Raman sensor head for field measurements is presented.**

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***Index Terms* — hand-held Raman sensor head, *in situ*, meat aging, principal component analysis.**

I. INTRODUCTION

As is generally known meat is a very perishable food and is subject to powerful hygienic regulations and control measures during production. An approach to further increase the currently high quality standards could be to determine and document the meat quality along the production chain. Deviations in quality need to be recognized early in the production process to be avoided or eliminated. Here, optical detection techniques are of special interest because they can provide information of the status of the product with fast, non contact measurements which can be performed even through a packaging.

In the frame of the project "FreshScan" the applicability of the optical methodology of Raman spectroscopy is investigated with respect to a potential quality monitoring along the production chain. The objective of this work is to identify parameters in the Raman spectra which could be used for a rapid *in-situ* monitoring of meat quality, ripening and finally spoilage.

The final objective of the project is to demonstrate the feasibility of spectroscopic approaches *in situ* with appropriate portable equipment which is developed in parallel [1].

Due to their fingerprint nature Raman spectra can be utilized for sample identification: protein, fat and bones can be readily distinguished.

II. MATERIALS AND METHODS

For the investigations pork chops (*m. longissimus dorsi*) were chosen due to their relative homogeneity. The meat was purchased from a local abattoir (Vion Lausitz GmbH in Kasel-Golzig, Germany). Ten entire muscles were removed one day post mortem from the right half of the carcasses. The muscles were cut into 15-17 slices of 2 cm thickness and all slices were split in halves and packed in polyethylene bags. Two muscles were packaged under an atmosphere of 70 % oxygen and 30 % carbon dioxide. One half was used for the Raman measurements at the Technical University of Berlin and the other halves were transported to the Analysis Division at the Max Rubner-Institut for reference analyses. The meat samples were stored in their bags at 5 °C for a period of three weeks. At each measuring day one slice was used.

A. Experimental set-up

Two in-house constructed Raman set-ups were used in parallel, one for near infrared excitation with 785 nm and the second with visible red excitation at 671 nm. Frequency-stabilized diode laser light sources from Ferdinand Braun Institute Berlin were chosen with a view to the development of portable equipment: one

was a distributed-feedback laser [2] emitting at 785 nm with 70 mW at the sample and the second a microsystem reflection Bragg grating laser [3] emitting at 671 nm with 35 mW at the sample.

The latter was integrated into a miniaturized in-house constructed optical bench forming the central part of the hand-held Raman sensor head. All measurements at 671 nm were performed with this optical bench connected to a compact laboratory spectrometer. For further details see: [1]. The integration times of a single measurement were in between 1 and 10 s, so that a statistically representative number of ten spectra could be recorded within 1 or 2 mins – for practical use measuring times of well below one minute are envisaged.

B. Reference analyses

As physical reference analyses the colour (L*a*b*), pH-value and conductivity were recorded concurrently to the Raman experiments. The content of soluble proteins was measured photometrically after extraction according to the method of Whitaker and Granum. In addition, the microbial load on the surface was determined to follow spoilage by bacteria. Prior to Raman measurements drip loss was quantified as weight difference of the meet slices at the day of the measurement and at the day one post mortem.

C. Data analysis

The Raman spectra were analysed with multivariate statistical methods using the programme MatLab (MathWorks Inc., Natick, MA) with PLS-Toolbox (Eigenvector Research Inc., Wenatchee, WA). The spectra were preprocessed using Savitzky-Golay smoothing, second derivative and mean centering. Principal component analysis (PCA) was utilized for the evaluation of time-dependent trends and partial least squares regression analysis (PLS) to correlate the Raman spectra with the reference data.

III. RESULTS AND DISCUSSION

A. Raman spectra of meat and drip loss

Meat is loosing liquid during storage. This drip loss is to some extent an indicator of meat quality and it causes economical loss. Amongst the examined meat samples a slight increase of the weight loss was measured from 2 % (min. 1 %, max. 4 %) on the second day to 4,5 % (min. 1 %, max. 10 %) on the 17th day on average. With the drip loss the meat is losing mainly water and proteins. However, the composition of the soluble proteins washed out by the drip loss and the meat proteins are different as can be seen in figure 1. The spectra of meat [4] and of the drip loss exhibit the typical Raman bands of polypeptides and thus they are very similar. However, meat contains much more proteins in □-helical conformation than the drip loss. This is mainly due to the contractile proteins, especially myosin [5]. Hence, the largest difference in the spectra is the signal at 935 cm^{-1} which is attributed to skeletal modes of the □-helix. Similar, but smaller differences can also be detected in the amide I and amide III bands at 1650 cm^{-1} and 1250-1330 cm^{-1}.

Due to these differences the Raman spectra can be correlated with the determined drip loss and the content of soluble proteins. The PLS regression of the Raman data with the drip loss results for a measuring series in a correlation coefficient of 0.88 and an error of 9 %. This is quite good if we take into consideration that the drip loss is subject to large variations (error > 50 %) depending on animal, storage and transport conditions. As the Raman spectra are sensitive to differences in the protein composition, the correlation of Raman data with the photometrically determined content of soluble proteins is clearly better. The PLS regression yields a correlation coefficient of 0.998 and an error of 0.6 %. In this case, the content of soluble proteins of the meat is decreasing from 9.5 % on the 2nd day post mortem to 6.5 % on the 17th day.

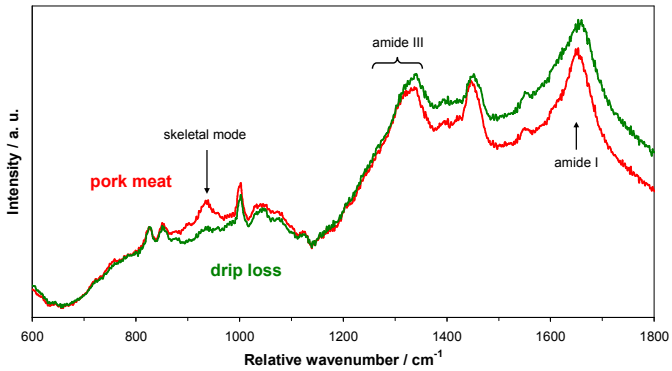

Fig. 1. Standardized Raman spectra of pork meat and drip loss measured with 785 nm excitation.

B. Raman measurements of aging

Long-time Raman measurements were performed with excitation at 785 nm and 671 nm over a period of three weeks in order to search for indicators for ripening and spoilage in the Raman spectra. The meat samples were stored at 5 °C in unsealed polyethylene bags and sealed under oxygen/carbon dioxide modified atmosphere packaging, which is commonly used for meat.

Raman spectra with excitation at 785 nm and 671 nm are yielding essentially the same spectral features and changes during storage time. Therefore, in the sequel only data for 671 nm spectra are shown. During storage for three weeks the spectra preserve their basic structure, but they exhibit gradual changes of all major

Raman signals, notably the amide bands. A striking feature is the modulation of the baseline of the Raman spectra with storage time. The background is low during the first week allowing for measurement of Raman spectra with very good signal to background ratio. After day 8 to 10 under these conditions a broadband background intensity is rapidly increasing which were attributed to a laser induced fluorescence (LIF) originating from porphyrins [6]. As expected, the intensity of LIF is much stronger at visible red excitation compared to near infrared excitation. When using the baseline of the spectra for normalization this is resulting in an overall decrease of the Raman signals upon aging.

The complex changes of the Raman spectra are analyzed by means of PCA. This method identifies spectral patterns on a statistical basis and attributes them to principal components (PCs). The largest variance is attributed to PC 1, further patterns with decreasing variance to PC 2 and so on. Having found the patterns, the spectra are scored for each PC so that the spectra are reduced to a single value for each PC. Figure 2 shows the plot of the scored Raman data for two PCs. In this case a distinction of spectra can be made between day 10 and 11. When stored at 5 °C all meat samples showed this distinction between day 8 and 10.

The Raman measurements of meat samples packaged under oxygen/carbon dioxide atmosphere yielded similar results, but no LIF was observed due to the high oxygen content of the atmosphere (70 %) which is efficiently quenching the fluorescence. Nevertheless the PCA distinction is made in the same time slot for both packaging types, hence LIF is not responsible for this distinction. A comparison of the spectral patterns for PC 1 and PC 2 shows that changes in the protein matrix are responsible for the distinction made in the Raman spectra. Upon aging a decrease of □-helical domains and an increase of □-pleated sheet and random coil conformations is observed. As shown earlier, the drip loss is also contributing to changes of the Raman spectra.

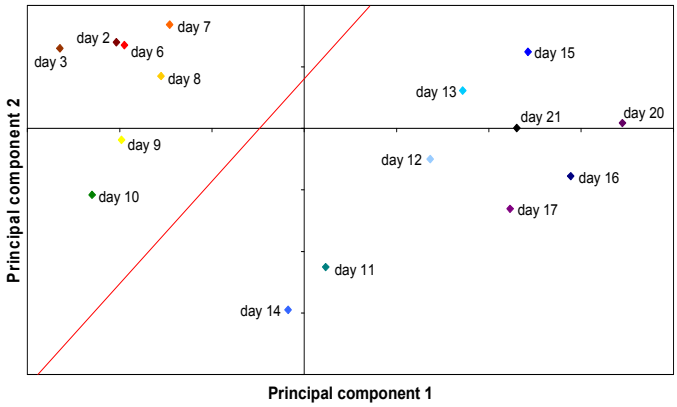

Fig. 2. Scores of the Raman data plotted for PC 1 and PC 2, meat samples stored at 5 °C in unsealed PE bags, the red line shows the clear distinction of the spectra.

C. *Microbiological analyses*

Due to its composition and the high water content meat is very susceptible to spoilage by bacteria. Depending on the hygienic status, storage conditions and packaging type bacteria grow on the surface. To follow this important parameter the number of colony-forming units on the surface was determined.

With an initial lag phase, the bacteria grow exponentially between day 3 and 9 post mortem. The surface load of 10^6 cfu/cm^2 is reached between day 5 and 6. After 8 to 10 days the bacterial growth is entering the steady state and reaching a surface load of 10^9 cfu/cm^2. When comparing with the above shown time-dependent Raman data, the quality of Raman spectra is good as long as the bacteria population has not developed too strong. Below the 10^6 cfu/cm^2 limit the spectra usually have the best signal to background ratio. The distinction in the PCA is made roughly when the bacteria are entering the steady state. And this is detected independent of the LIF, which is quenched under the oxygen atmosphere. Yet it is not fully clear what are the exact reasons for this distinction made by the PCA. Clearly, changes of the protein matrix which are the result of enzymatic digestion and denaturation in the protein matrix contribute to the observed changes of the Raman spectra. On the other hand, the coincidence with the microbial growth kinetics implies that the Raman spectra might be also altered due to destructive processes caused or induced by the bacteria living on the meat surface and exploiting the meat as source of nutrition.

D. *Physical reference analyses*

The analysis of the Raman data using PLS regression yielded correlations with reference analyses for the L*a*b* colour, and the pH-value measured on the surface. Excitation at 671 nm und 785 nm gave

equivalent results. No correlation was found for the pH-value measured inside the samples. The pH-value inside the meat is reacting with a due time delay to the metabolic products formed by the bacteria. As the physical parameters did not show unambiguous time-dependent trends we refrain from showing these correlations, but it has to be noted that the Raman spectra respond to the lightness (L*), and with 671 nm excitation to the redness value (a*) and with 785 nm excitation to the yellowness value (b*).

E. Measuring through the packaging

As the *in-situ* Raman measurements shall be applicable for packaged meat, experiments were conducted also through the package foils. The spectra contain Raman signals of the meat and of the packaging material. The latter can be eliminated mathematically so that the Raman spectrum of meat is obtained.

F. Hand-held Raman sensor head

With regard to field tests for the investigation of meat quality we have designed a hand-held Raman sensor head which is shown in figure 3. This contains the miniaturized optical bench with integrated 671 nm microsystem diode laser and a controller for the laser current and temperature. An exchangeable and rechargeable battery pack realizes the power supply and allows operating times up to 8 hours. For the portable Raman system the hand-held sensor head will be coupled to a compact spectrometer. With that system field experiments for the *in-situ* and at-line control of meat quality are planned, e.g. in the slaughterhouse or with packaged meat.

IV. CONCLUSION

The aim of this study was to find indicator parameters in the Raman spectra which could be used to describe meat quality during aging (ripening or spoilage) with a rapid *in-situ* measurement.

As a result, we could show that Raman spectra excited at 785 nm and 671 nm allow for a distinction of pork meat samples from *musculus longissimus dorsi* stored in unsealed polyethylene bags at 5 °C on the time scale between day 8 and 10 post mortem. This distinction is made by means of PCA and can be used to identify meat which is at or beyond the border of inedibility. At that time the odour and the flavour of the meat are unpleasant and the surface is microbial spoiled with germ numbers reaching 10^9 cfu/cm^2. Roughly at day 12 the laser induced fluorescence (LIF) has grown strong partially obscuring the Raman spectra.

Fig. 3. Hand-held Raman sensor head for in-situ characterization of meat quality.

Before that date the Raman spectra recognize ripened meat by a good signal to background ratio passing an optimum before the critical limit of 10^6 cfu/cm^2 which is reached under the above mentioned storage conditions between day 5 and 6. The changes in the spectra which are responsible for this distinction are not yet fully understood. Structural changes of the protein matrix are identified by an increase of β-pleated sheet and random structures whereas the content of α-helical proteins is decreasing during aging. This may have several sources: the natural enzymatic digestion of meat proteins, denaturation processes and most likely also changes in the protein structure on the meat surface due to microbial activity.

Meat samples which were stored under oxygen modified atmosphere, showed no LIF but the same distinction of spectra by PCA, sometimes with one day delay. As the bacteria showed retarded growth under the oxygen atmosphere the steady state was reached later than with samples in unsealed polyethylene bags. From that we may conclude that the major factors responsible for the distinction in the Raman spectra are structural changes in the meat matrix caused by enzymatic processes and due to the loss of liquid (drip loss), and that the microbial activity contributes to this process at the surface to a lesser extent – and that this contribution is also detected in the Raman spectra

With respect to the reference analyses, the Raman spectra can be correlated with the drip loss, the content of soluble proteins which both show slight but opposite trends with storage time. The physical parameters L*a*b* correlated too, but as these values showed high variations and no unambiguous storage time-dependent trends they cannot be regarded as suitable parameters for ripening or spoilage.

Measurements of meat through packaging were demonstrated with 671 nm and 785 nm excitation.

A hand-held Raman sensor head with 671 nm

microsystem diode laser was presented. Integrated into this sensor head is a miniaturized Raman optical bench which was used for the Raman measurements presented here connected to a laboratory spectrometer. For the portable Raman system the sensor head will be coupled to a portable spectrometer, so that this system can be used for *in-situ* control of meat quality e.g. in the slaughterhouse.

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REFERENCES

[1] Schmidt, H., Sowoidnich, K., Maiwald, M., Sumpf, B., Kronfeldt, H.-D., "Hand-held Raman Sensor Head for In-situ Characterization of Meat Quality Applying a Microsystem 671 mn Diode Laser", Proc. SPIE 7312, "Advanced environmental, chemical, and biological sensing technologies VI" (2009), in press.

[2] Maiwald, M., Erbert, G., Klehr, A., Sumpf, B., Wenzel, H., Laurent, T., Wiedmann, J., Kronfeldt, H.-D., Schmidt, H., "Reliable operation of 785 nm DFB diode lasers for rapid Raman spectroscopy", Proc. SPIE 6456, 64560W-1-64560W-6 (2007).

[3] Maiwald, M., Ginolas, A., Müller, A., Sahm, A., Sumpf, B., Erbert, G., and Tränkle, G., "Wavelength-stabilized compact diode laser system on a microoptical bench with 1.5-W optical output power at 671 nm", IEEE Photon. Techn. Lett. 20, 1627-1629 (2008).

[4] Pezolet, M., Pigeon-Gosselin, M., and Caille, J.-P., "Laser Raman investigation of intact single muscle fibers protein conformations", Biochimica et Biophysica Acta 533, 263-269 (1978).

[5] Carew, E. B., Asher, I. M., and Stanley, H. E., "Laser Raman spectroscopy – new probe of myosin substructure", Science 188, 933-936 (1975).

[6] Schneider, J., Wulf, J., Surowsky, B., Schmidt, H., Schwägele, F., Schlüter O., "Fluorimetric detection of protoporphyrins as an indicator for quality monitoring of fresh intact pork meat", Meat Science 80, 1320-1325 (2008).