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COMMUNICATION

Application of Diode-Laser Raman Spectroscopy for In situ Investigation of Meat Spoilage

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Abstract Raman spectroscopy is well suited for non-invasive and non-destructive analysis. The spectra provide detailed information about the composition of the matter like a fingerprint on molecular level. Here, we have applied Raman spectroscopy for the characterization of meat spoilage. For this purpose, pork chops (musculus longissimus dorsi) were ice-stored at 5 °C, and time-dependent Raman spectra were measured daily up to 3 weeks post mortem. A prototype Raman probe for meat was constructed featuring a miniaturized optical bench combined with a customized 671-nm microsystem diode laser for the integration into a handheld device. During the time-dependent investigations with this laser scanner, the Raman spectra preserve their basic spectral features, but small changes of the protein Raman signals occur during storage. The time correlation of the complex spectra were analyzed with principal components analysis leading to a distinction of spectra on the time scale between day 8 and 10 typically. This corresponds to the transition from unspoiled meat to meat at and beyond the end of shelf life identified by means of visual inspection.

Keywords Raman spectroscopy · Portable Raman sensor · In situ · Meat spoilage · Diode laser

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Introduction

Due to the value and perishable nature of meat samples requiring strong hygienic regulations and careful handling during the processing, there is a demand for methods to detect meat spoilage. Besides the time-consuming traditional microbial analyses involving complex laboratory investigations (Diaz et al. 2008; Dainty 1996), alternative methods like the application of electronic noses (Blixt and Borch 1999; Balasubramanian et al. 2009) or the analysis of volatile organic compounds as markers for meat spoilage (Lovestead and Bruno 2010) were presented. Nevertheless, these methods are not suited for a non-invasive monitoring because they require a more or less complicated sample pretreatment.

Hence, there is a need for non-destructive optical methods. In that way, diffuse reflectance spectroscopy (Lin et al. 2004) as well as Fourier transform infrared spectroscopy (Ammor et al. 2009; Ellis and Goodacre 2001; Ellis et al. 2004) were applied for the detection of microbial spoilage. Alexandrakis et al. (2010) additionally presented a combination of near-infrared reflectance spectroscopy and Fourier transform infrared spectroscopy. Another approach with a great potential is the use of Raman spectroscopy where the frequency-shifted signals correspond to molecular vibrations which are characteristic

for the material and therefore can provide a fingerprint of the sample. Here, Fourier transform Raman spectroscopy with an excitation in the near-infrared spectral range is well-established (Keller et al. 1993; Schrader et al. 2000). Additionally, in contrast to the infrared absorption method, due to the rather weak Raman signal of the water-bending mode in the fingerprint range, water is not disturbing the Raman measurement. This is an important precondition for in situ measurements of meat because it has a high water content of about 75 % (Fjelkner-Modig and Tornberg 1986). Nevertheless, in Fourier transform Raman setups, problems with sample heating or destruction of biological materials have been reported (Marigheto et al. 1996; Li-Chan 1996).

Within the framework of the BMBF-funded project "FreshScan" (contract number 16SV2332), we performed Raman spectroscopic measurements to identify parameters for a rapid in situ monitoring of meat quality and spoilage. In parallel, our aim was to develop portable equipment to apply this Raman spectroscopic approach in situ. The basic idea was the usage of Raman spectra to monitor the biochemical and physical changes of meat which take place during storage. For that reason, storage time-dependent Raman spectra were measured in the laboratory and correlated with concurrent chemical reference analyses (Schmidt et al. 2009a).

Materials and Methods

For Raman spectroscopic measurements of meat samples, it has to be considered that the laser radiation must not be absorbed by the tissue and that the generation of fluorescence should be kept as low as possible. The major absorbing components of meat in the 300–1,000-nm range are the heme pigments of myoglobin and water as the main component. We focused on excitation in the red spectral region because of the lowest absorption in this range (Takatani and Graham 1979; Segelstein 1981). An excitation at 671 nm offers moreover the advantage that the interesting spectral features can be measured in the range of the highest sensitivity of silicon-based detectors. Herewith, the disadvantage of the poor Raman signals from meat can be partially overcome. Moreover, this enables shorter measuring times and allows monitoring the full Raman range up to shifts of 4,000 cm^{-1}. For that reason, at Ferdinand-Braun-Institut, Leibniz-Institut für Höchstfrequenztechnik, a compact 671-nm diode laser system (Maiwald et al. 2008) was designed and realized.

Experimental Setup

The experimental setup is shown in Fig. 1. The collimated beam of the 671 nm diode laser module (1) passes inside the Raman optical bench (2) which has a length of 180 mm and a diameter of 25 mm. The laser radiation then is guided by mirrors and optical filters to a lens (with a focal length of 10 mm) which focuses the beam through a quartz glass window onto the sample (3). The filters were purchased from Semrock, USA, and the lenses, the quartz window, as well as the mirrors were purchased from Thorlabs, Germany. The backscattered radiation from the sample is collected by the same lens and filtered by a Raman edge filter which is blocking the Rayleigh scattered radiation. Finally, the Raman Stokes signal is launched into a single 100-µm optical fiber (4) by a lens with a focal length of 16 mm. A more detailed description of the Raman optical bench can be found in Schmidt et al. (2009b).

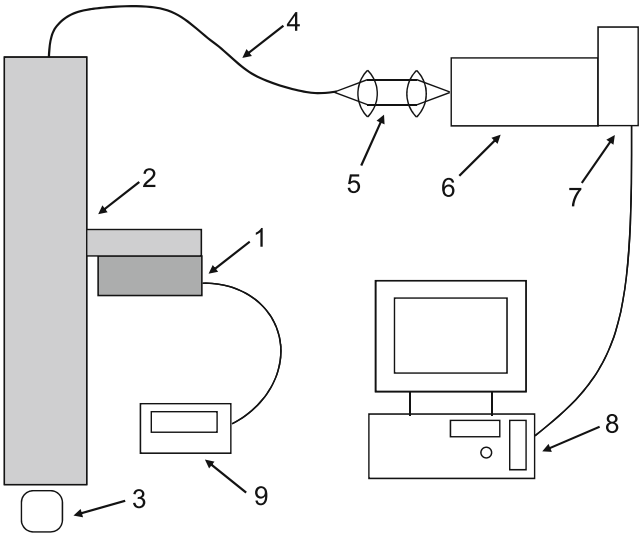

Fig. 1 Experimental set up; (*1*) laser module, (*2*) Raman optical bench, (*3*) meat sample, (*4*) optical fiber, (*5*) launch optics, (*6*) spectrometer, (*7*) CCD, (*8*) computer, (*9*) laser driver

The quartz glass fiber of the Raman optical bench was coupled with two lenses (5) from Thorlabs, Germany to a laboratory Raman spectrometer (Chromex 300IS) (6) equipped with a deep depletion CCD camera (EHRB 1340×400, Princeton Instruments) (7) cooled down to −70 °C. For the Raman measurements of meat, integration times of 5 s were applied, and the laser power was set to 35 mW at the sample. The spectra were recorded using computer (8) running Winspec software from Roper Scientific. A PilotPC laser driver (9) from Sacher Lasertechnik was used to control the injection current and the temperature of the laser module.

Testing Material and Test Procedure

For our investigations, meat samples from musculus longissimus dorsi from pork were chosen due to their relative homogeneity. After stunning of the pigs in a local slaughterhouse (Vion Lausitz GmbH in Kasel-Golzig,

Germany), the entire muscle was removed from the carcass and cut into 16 slices of 2 cm thickness, and all slices were split in halves and stored separately in polyethylene bags at 5 °C for a period of 3 weeks. One half was used for the Raman measurements at Technical University Berlin, while the corresponding other half was transported to the Analysis Division at Max Rubner-Institute in Kulmbach for chemical reference analyses. For the Raman measurements, the packaged meat slices were investigated each day through the polyethylene foil at three different positions. For each selected position, ten spectra were recorded with an integration time of 5 s. After that, the packaging foil was removed, and two subsamples were cut out of the slice using a stainless steel cylindrical cutting tool. Each of the two cylindrical samples with a diameter of 2 cm was measured at three different positions with an integration time of 5 s and ten spectra recorded in each case.

For data evaluation, the measured spectra for each day (30 spectra recorded through the packaging and 60 spectra obtained for unwrapped meat) were averaged and preprocessed by mean centering followed by second derivative computation and Savitzky–Golay smoothing. To analyze the apparent changes of the Raman spectra, we applied principal components analysis (PCA) (Beebe et al. 1998; Brereton 2007) using the program MATLAB (MathWorks Inc., Natick, MA) in combination with PLS toolbox (Eigenvector Research, Inc., Wenatchee, WA).

Results and Discussion

The probe head of the Raman optical bench was optimized for measurements of fresh raw meat so that the probe simply had to be put on the sample. The laser radiation was focused about 0.5 mm inside the meat sample which also allows measurements through various packaging materials. In that case, the Raman signals arising from the packaging foil could be eliminated by subtracting the spectrum of the package.

The employed laser spot size of about 50 µm is sufficiently large to determine representative information of the status of the meat sample. The spot size was investigated theoretically using optical simulation software (WINABCD, FBH, Eppich) as well as experimentally by applying measurements of the laser power after focusing through well-defined pinholes.

Selected spectra of a storage time-dependent series for a period of 3 weeks for unwrapped meat samples are shown in Fig. 2 (left). Meat samples show a typical protein spectrum containing the dominant amide I and amide III signals of the polypeptide backbone as well as strong signals attributed to the aromatic amino acids phenylalanine (Phe) and tyrosine (Tyr) (Carew et al. 1975, 1983). Nevertheless, everyday spectra were recorded; for clarity, not all spectral data are shown. Depending on the storage time, the Raman spectra generally kept their basic structure, but gradual changes of all major Raman signals, notably the amide bands, took place (Schmidt et al. 2009a). An increase of the baseline of the Raman spectra in the time slot around 10 days after slaughter, which we attribute to a laser-induced fluorescence resulting from porphyrins (Schneider et al. 2008), is additionally observed (Fig. 2, left).

To determine more detailed information about the spectral changes, the multivariate statistical tool of PCA was applied. A plot of the scored Raman data for the first two principal components (PCs) is displayed in Fig. 2

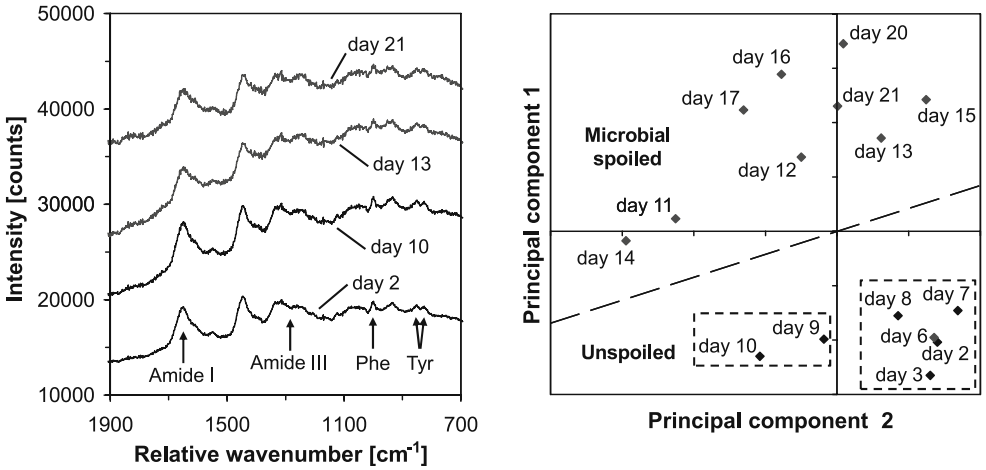

Fig. 2 Raman spectra of unwrapped pork meat depending on storage time, stored at 5 °C and packaged in polyethylene bags during storage. The signals attributed to the aromatic amino acids are marked with Phe (phenylalanine) and Tyr (tyrosine) (*left*). Scores of the Raman data plotted for PC 1 and PC 2. The *dashed line* (PC1=0.425 PC2) indicates the distinction of spectra separating spoiled from unspoiled samples. The *dashed rectangles* mark the two groups corresponding to exponential bacterial growth (day 2 to day 8) and the onset of a steady state of bacterial surface coverage (day 9 and day 10) (*right*)

(right). Here, the scored spectra of the meat samples with an age up to 8 days form a compact cluster with PC 1 smaller than zero and PC 2 larger than zero. The days 9 and 10 mark a kind of transition area where PC 1 stays below zero, but the sign of PC 2 changes to negative values with a decreasing tendency from day 9 to day 10. The scored Raman spectra of meat samples with an age above 11 days are clearly separated from those with an age of 10 days or less.

A comparison of these results with the bacterial surface coverage (Schmidt et al. 2009a) shows that there is an exponential growth of bacteria up to day 8 corresponding to the mentioned cluster in the PCA scores plot. Around day 9 and 10, the colony number at the meat surface is no longer increasing exponentially and starts entering a steady state. That was also recognized by the PCA where these 2 days showed negative values for PC 2, separating them from the days 2 to 8. From day 11 up to day 21, the bacterial surface coverage remains roughly constant at a value of 10^9 cfu/cm^2 (cfu, colony-forming units). This time slot could also be identified by the PCA where the scored Raman data showed a positive value for PC 1.

Hereby, the discrimination is not only due to the above-mentioned increased fluorescence background. In fact, conformational changes in the protein matrix and the drip loss also contribute to the PCA classification. This is in accordance to experiments performed in parallel with an excitation at 785 nm (Schmidt et al. 2009a). Therefore, the specific information on molecular level provided by Raman spectroscopy is necessary to allow for a discrimination of unspoiled and microbial spoiled meat samples. This is also underlined by sensory observations during the sample preparation. Around day 10, the surface of the meat slices was covered with a slimy film and had a sourly smell indicating microbial spoilage.

The second set of Raman measurements of the same meat samples performed through the package yielded equivalent result, namely a separation of the spectra in the PCA at day 10 (data not shown). In that case, the PCA identified the Raman signals of the packaging material as additional PC. Therefore, the two data sets of meat with and without package were processed separately.

Conclusions

A Raman optical bench with integrated 671-nm micro-system laser demonstrated its suitability for in situ measurements with raw and packaged pork meat samples from musculus longissimus dorsi stored in polyethylene bags at 5 °C. Applying a laser power of 35 mW, Raman spectra could be recorded with short measuring times of several seconds which are essential for field applications. For data evaluation, principal components analysis was applied leading to a distinction of the meat samples on the time scale around day 10 post mortem, i.e., an identification of meat samples at the end of shelf life is possible. The spectral changes which lead to this separation are not yet fully understood, and more work is required to comprehend the complex processes, but in parallel studies (data not shown), we observed structural changes of the protein matrix as well as a microbial spoilage of the meat surface. Measurements of meat through the packaging resulted in the same observation.

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References

Alexandrakis, D., Downey, G., & Scannell, A. G. M. (2010). Rapid non-destructive detection of spoilage of intact chicken breast muscle using near-infrared and Fourier transform mid-infrared spectroscopy and multivariate statistics. *Food and Bioprocess Technology.* doi:10.1007/s11947-009-0298-4.

Ammor, M. S., Argyri, A., & Nychas, G. J. E. (2009). Rapid monitoring of the spoilage of minced beef stored under conventionally and active packaging conditions using Fourier transform infrared spectroscopy in tandem with chemometrics. *Meat Science, 81*(3), 507–514.

Balasubramanian, S., Panigrahi, S., Logue, C. M., Gu, H., & Marchello, M. (2009). Neural networks-integrated metal oxide-based artificial olfactory system for meat spoilage identification. *Journal of Food Engineering, 91*(1), 91–98.

Beebe, K. R., Pell, R. J., & Seasholtz, M. B. (1998). *Chemometrics: a practical guide* (1st ed.). New York: Wiley.

Blixt, Y., & Borch, E. (1999). Using an electronic nose for determining the spoilage of vacuum-packaged beef. *International Journal of Food Microbiology, 46*(2), 123–134.

Brereton, R. G. (2007). *Applied chemometrics for scientists* (1st ed.). Hoboken: Wiley.

Carew, E. B., Stanley, H. E., Seidel, J. C., & Gergeley, J. (1983). Studies of myosin and its proteolytic fragments by laser Raman spectroscopy. *Biophysical Journal, 44*(2), 219–224.

Carew, E. B., Asher, I. M., & Stanley, H. E. (1975). Laser Raman spectroscopy—new probe of myosin substructure. *Science, 188* (4191), 933–936.

Dainty, R. H. (1996). Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology, 33*(1), 19–33.

Díaz, P., Nieto, G., Garrido, M. D., & Bañón, S. (2008). Microbial, physical-chemical and sensory spoilage during the refrigerated storage of cooked pork loin processed by the sous vide method. *Meat Science, 80*(2), 287–292.

Ellis, D. I., & Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. *Trends in Food Science & Technology, 12*(11), 414–424.

Ellis, D. I., Broadhurst, D., & Goodacre, R. (2004). Rapid and quantitative detection of the microbial spoilage of beef by Fourier

transform infrared spectroscopy and machine learning. *Analytica Chimica Acta, 514*(2), 193–201.

Fjelkner-Modig, S., & Tornberg, E. (1986). Water distribution in Porcine M. longissimus dorsi in relation to sensory properties. *Meat Science, 17*(3), 213–231.

Keller, S., Löchte, T., Dippel, B., & Schrader, B. (1993). Quality control of food with near-infrared-excited Raman spectroscopy. *Fresenius' Journal of Analytical Chemistry, 346*(6–9), 863–867.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. *Trends in Food Science & Technology, 7*(11), 361–370.

Lin, M., Al-Holy, M., Mousavi-Hesary, M., Al-Qadiri, H., Cavinato, A. G., & Rasco, B. A. (2004). Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm). *Letters in Applied Microbiology, 39*(2), 148–155.

Lovestead, T. M., & Bruno, T. J. (2010). Detection of poultry spoilage markers from headspace analysis with cryoadsorption on a short alumina PLOT column. *Food Chemistry, 121*(4), 1274–1282.

Maiwald, M., Ginolas, A., Müller, A., Sahm, A., Sumpf, B., Erbert, G., et al. (2008). Wavelength-stabilized compact diode laser system on a microoptical bench with 1.5-W optical output power at 671 nm. *IEEE Photonics Technology Letters, 20*(19), 1627–1629.

Marigheto, N. A., Kemsley, E. K., Potter, J., Belton, P. S., & Wilson, R. H. (1996). Effects of sample heating in FT-Raman spectra of biological materials. *Spectrochimica Acta Part A, 52*(12), 1571–1579.

Schmidt, H., Blum, J., Sowoidnich, K., Sumpf, B., Schwägele, F., & Kronfeldt, H.-D. (2009a). In-situ characterization of meat aging with diode laser Raman spectroscopy. In: Kim, Tu, & Chao (Eds.) *Proceedings of SPIE "Sensing for agriculture and food quality and safety", Vol 7315* (pp. 731509–731509-8), 13–17 April 2009, Orlando, Florida, USA.

Schmidt, H., Sowoidnich, K., Maiwald, M., Sumpf, B., & Kronfeldt, H.-D. (2009b). Hand-held Raman sensor head for in-situ characterization of meat quality applying a microsystem 671 nm diode laser. In: Kim, Tu, & Chao (Eds.) *Proceedings of SPIE "Advanced environmental, chemical, and biological sensing technologies", Vol 7312* (pp. 73120H–73120H-8), 13–17 April 2009, Orlando, Florida, USA.

Schneider, J., Wulf, J., Surowsky, B., Schmidt, H., Schwägele, F., & Schlüter, O. (2008). Fluorimetric detection of protoporphyrins as an indicator for quality monitoring of fresh intact pork meat. *Meat Science, 80*(4), 1320–1325.

Schrader, B., Schulz, H., Andreev, G. N., Klump, H. H., & Sawatzki, J. (2000). Non-destructive NIR-FT-Raman spectroscopy of plant and animal tissues, of food and works of art. *Talanta, 53*(1), 35–45.

Segelstein, D. J. (1981). The complex refractive index of water. M.S. Thesis. Department of Physics, University of Missouri-Kansas City, Kansas City, Missouri, USA.

Takatani, S., & Graham, M. D. (1979). Theoretical analysis of diffuse reflectance from a two-layer tissue model. *IEEE Transactions on Biomedical Engineering, BME, 26*(12), 656–664.