QUANTIFYING BACTERIA ON PORK USING A PORTABLE RAMAN SCANNER

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Abstract – In this work, the feasibility to quantify the microbial surface concentration of pork with a portable Raman device was investigated. Thirty eight pork samples from three LT muscles were stored at 4°C for 1 to 14 days allowing for bacterial growth. Raman spectra and total viable mesophilic plate counts (TVC) were daily measured during this period. The Raman spectra were correlated with the microbial surface concentration in the range from 10^2 to 10^{14} TVC/cm² using partial least squares regression. The PLSR model was able to predict the logarithm of total viable counts precisely ($R^2cv = 0.83$ and RMSECV = 1.35 log TVC/cm²). Thus, the portable Raman device shows potential for a rapid and non-invasive evaluation of the microbial status of meat at the critical threshold of 10^5 TVC/cm² and below.

Key Words – meat spoilage, PLSR, Raman scanner.

I. INTRODUCTION

Microbial spoilage of meat is a serious risk causing considerable economic and environmental damage from farm to fork. Therefore, national and international legislation as well as recommend-dations from associations have defined microbial hygiene criteria. Corresponding to the European regulation on microbiological criteria for foodstuffs (EC Nr. 1441/2007) the alarm value and the critical limit for total aerobic colony counts are 4.0 and 5.0 log TVC/cm², respectively. This is also about the threshold, where sensible humans smell volatile compounds generated by typical spoilage organisms.

Spectroscopic methods, especially Fourier transform infrared (FT-IR) spectroscopy using a ZnSe attenuated-total reflectance (ATR) crystal to collect spectra, proved to rate the microbial cell density on minced beef [1], minced pork [2] or beef fillets [3-6]. Corresponding to the partial least squares regressions (PLSR) and artificial neural network (ANN) models of Argyri [6, 7], Papadopoulou [2] and Panagou [5], quantification of microbial spoilage on meat is feasible with FT-IR spectroscopy. While this measurement is fast and non-invasive, a number of drawbacks are limiting the applicability of the mid-infrared technology in practice. For example the window materials are either moisture sensitive, brittle or toxic and water is strongly interfering. Furthermore, when using the ATR geometry, the penetration depth of the evanescent field is very small so that a measurement through a plastic packaging is not possible.

Comparing FT-IR and Raman spectra of minced beef, Argyri et al. [7] concluded that different types of models were able to predict microbial spoilage as accurately from Raman spectra as the FT-IR spectra. The Raman effect is revealing similar (i.e. complementary) vibrational-spectroscopic infor-mation as the IR spectrum, but the Raman spectrum can be generated in virtually any spectral range. Hence, working with Raman spectra in the visible or NIR range avoids the above material issues and allows in principle for the measurement of packaged produces. Thus, it has been shown that microbial spoilage can be qualitatively detected with a handheld Raman scanner [8] and that this can be done even through a packaging foil [9]

Therefore, the aim of this experiment was to evaluate whether the Raman scanner is able to quantify the microbial surface concentration and whether the detection below a critical threshold of 5.0 log TVC/cm² is feasible with the portable Raman scanner.

II. MATERIALS AND METHODS

For quantification of total viable counts with Raman spectroscopy, pork was chosen because pork production is a major part of total meat production worldwide.

On the day of slaughter, three porcine *longissimus thoracis* (LT) muscles were removed from the carcasses and the samples were prepared as follows.

The LT's were cut into thirteen 1 cm thick slices. The slices were bisected and stored in petri dishes with a moistened cellulose filter to protect the meat samples from dehydration. The slices were stored at 4°C up to 14 days. One slice per animal was investigated on each measuring day, in total three slices per day. One half of a slice was analyzed by Raman spectroscopy and the other half was used to determine the microbial colony counts.

For the microbial analysis, a cylindrical sample of 5 cm² surface was cut from the slice with a cylindrical knife and homogenized in a Stomacher bag with 20 ml 0.9% sodium chloride solution. The homogenized samples were serially diluted and 100 µl aliquots were spread on tryptic-soy-agar (TSA) plates (Oxoid). For each sample three dilution levels were plated in triplicate and incubated at 25 °C for 24 h to determine the total viable mesophilic plate counts (TVC).

The Raman measurements were performed with a portable Raman scanner described previously [8, 9]. The spectra were recorded at seven randomly selected spots of each slice with a Laser power of 80 mW and an integration time of 50 s per spectrum. The seven spectra per sample were averaged for data analysis.

For the correlation of the Raman spectra with the total viable counts, partial least squares regressions (PLSR) using the MATLAB 7.9.0 R2009b software (The Mathworks Inc., Natick, MA, USA) and PLS Toolbox 7.5 (Eigenvector Research Inc., Wenatchee, WA, USA) was applied. The averaged Raman spectra were preprocessed with Savizky-Golay second derivative, standard normal variate normalization (SNV) and mean-centering. The "random subset" method with 8 data splits and 20 iterations was employed to cross-validate the models.

III. RESULTS AND DISCUSSION

After a lag phase of 2-3 days bacterial growth was observed throughout the 14 days of storage (Fig. 1). Starting with an initial bacterial concentration of about 2 log TVC/cm², microbial spoilage increased to a maximum of about 14 log TVC/cm². The critical threshold of 5.0 log TVC/cm² was reached between day 5 and 7 of storage. It is noted that while the growth rate of approximately one logarithmic unit per day of storage is in accordance with previous studies, the bacteria grew in one case to much higher surface concentration when stored in petri dishes as compared to storage in plastic bags [9].

Not unexpectedly, the individual kinetics showed a considerable variance between the samples of different animals (see Fig. 1).

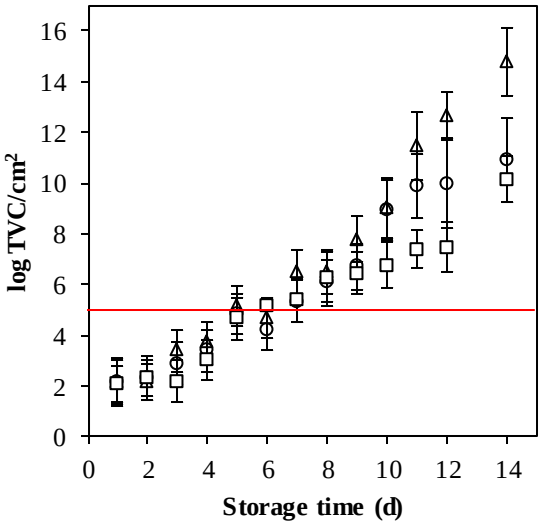

Figure 1. Bacterial growth on pork (LT) at 4 °C. Squares, circles and triangle show the kinetics for three different animals. The red line indicates the threshold of 5 log TVC/cm². Error bars correspond to one standard deviation of the TVC measurement.

The microbial data were correlated with the Raman spectra. The summary of these PLSR results is presented in Table 1. They show a cross-validated prediction of the microbial status with R^2_{cv} = 0.83 and RMSECV = 1.35 log TVC/cm².

Table 1: Summary results of the PLSR correlation of the Raman spectra with the total viable counts (TVC).

Parameter	PLSR result
R²cal	0.98
RMSEC	0.42
R²cv	0.83
RMSECV	1.35
Latent variables	4
Samples	38

A comparison of cross validation and calibration results in Fig. 2 reveals that the predictions below 7 log TVC/cm² are more accurate than compared to predictions at higher concentrations which are

encountered at longer storage periods. The leverage of these samples is high in this correlation, but their relevance in practice rather low. In practice, a quantification of the bacterial status is of higher interest at lower concentrations, for example to evaluate the shelf life of the fresh products.
Thus, the range well below 10^5 TVC/cm² will have to be evaluated in more detail for this purpose.

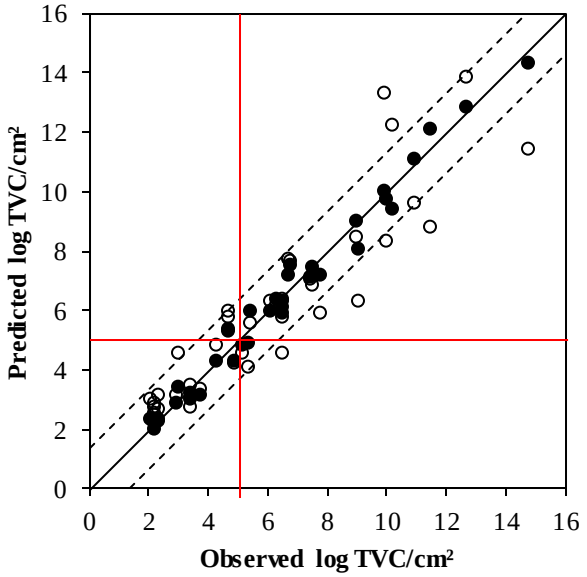

Figure 2. PLSR correlation of total viable counts (TVC/cm²) with Raman spectra. Black circles: calibration; open circles: cross-validation; red lines indicate the threshold of 5 log TVC/cm²; dotted lines indicate standard error.

On the other hand, if the detection of spoilage as an alarm is the objective an exact quantification is not required, but discrimination according to a given threshold. The ability of the PLSR model to discriminate according to 5 log TVC/cm² as threshold is estimated in Table 2 showing the confusion matrix wherein the actual group is defined by the measured TVC values and wherein the predicted group is the outcome of the cross-validation.
With the PLSR model, 87.5% of the acceptable meat samples could be correctly classified and 86.4% of the spoiled meat samples. Thus, precision and specifity are comparable. Similarly, the accuracy is high with 86.8% (Accuracy = (true positive + true negative) / (positive + negative)).

Table 2: Classification of cross-validated predictions into acceptable (< 10^5 TVC/cm²) and spoiled (>10^5 TVC/cm²) meat samples.

Predicted group	Actual group		Correctly classified
	Acceptable (n=16)	Spoiled (n=22)	
Acceptable	14	2	87.5 %
Spoiled	3	19	86.4 %

These preliminary results are showing that based on mobile Raman spectroscopy PLSR models of equal precision can be obtained as have been found for the correlations of microbial data with FT-IR spectra [2, 5-7].
The portable Raman scanner was able to quantify the concentration of bacteria on the meat surface between 10^2 and 10^{14} TVC/cm² using pork as test specimen and PLSR for data analysis with a cross-validated coefficient of determination of R^2cv = 0.83. Furthermore a discrimination of pork samples below or above a threshold of 10^5 TVC/cm² was shown.

IV. CONCLUSION

Based on these results, the Raman scanner shows potential for (i) the detection of spoilage according to a given threshold and (ii) for a quantification of the bacterial surface concentration even below the threshold of 10^5 TVC/cm². Both findings are potentially useful for hygiene monitoring during meat production.
However, while these experiments show the feasibility in principle, the number of samples is too low to evaluate the general applicability. Especially, more work is required to evaluate the performance of Raman spectroscopy for the quantification of the bacteria on meat at concentration levels well below 10^5 TVC/cm² with a larger number of cuts and animals.

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