

Non-invasive assessment of the bioburden of minced pork using a hand-held fluorescence device

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Abstract To speed-up monitoring of the hygienic status of meat, fast and non-invasive measuring techniques are required. The feasibility of a hand-held fluorescence device (freshdetect) was investigated to quantify the bioburden of minced pork meat. In total, 144 minced pork samples from 18 batches were stored at 2°C for up to 8 days. Fluorescence spectra, $L^*a^*b^*$ colour and total viable mesophilic plate counts (TVC) were measured in parallel during this period. Correlation of the fluorescence spectra with $\log(\text{TVC/g})$ using partial least squares regression yielded cross-validated predictions with $R^2_{cv} = 0.72$ and a prediction error of $\text{RMSECV} = 0.97 \log(\text{TVC/g})$. Cross-validated limits of detection and quantification were determined as 1.03 and 3.38 $\log(\text{TVC/g})$, respectively. This would allow for a quantification well below the alarm threshold of 5.7-6.7 $\log(\text{TVC/g})$. The spectra correlated also well with storage time ($R^2_{cv} = 0.81$), but less strong with $L^*a^*b^*$ colour.

Keywords: Meat inspection, fluorescence spectra, minced pork.

1 Introduction

Minced meat is a very perishable commodity requiring special attention with respect to hygiene requirements and control of bioburden. Conventional analysis of bioburden is based on destructive sampling and methods such as colony counts or flow cytometry which are labour

intensive, time consuming and expensive. With these restrictions, they are only suitable for a random monitoring, but not for on-line control. In this regard, spectroscopic methods are a promising alternative as they are fast, non-destructive and allow for on-line applications. A number of spectroscopic methods such as fluorescence, FT-IR, NIR or Raman have been shown to be suitable for an assessment of the microbial status of meat.

Fourier transform infrared (FT-IR) spectroscopy using attenuated-total reflectance (ATR) was proven to rate the microbial cell density on minced beef [1], minced pork [2] or beef fillets [3–5]. Quantification was shown using partial least squares regressions (PLSR) and artificial neural network (ANN) models. While FT-IR is fast and non-invasive, a number of drawbacks are limiting the applicability of the mid-infrared technology in practice. For example, the ATR crystal is brittle, toxic and water is strongly interfering. Complementary information to the mid-infrared is obtained with Raman spectroscopy, but avoiding interference from water and the material restrictions of IR. Microbial spoilage of pork was qualitatively detected with a handheld Raman scanner [6,7] and it was shown that PLSR models were able to predict microbial spoilage as accurately from Raman spectra as from the FT-IR spectra [8].

Similarly, NIR hyperspectral imaging was shown to allow for a prediction of bacterial surface counts [9–11]. With pork ($n = 51$) as example, NIR-hyperspectral imaging proved feasibility of predicting bioburden with $R^2_{cv} = 0.82$ and a prediction error of $0.83 \log(\text{TVC}/\text{cm}^2)$ [10].

The prediction of bacterial surface counts by fluorescence spectroscopy is based on different fluorophors depending on the excitation wavelength [12–16]. With 280 and 380 nm, fluorescence of NAD(P)H, flavins, Lipids and porphyrins are excited and used for the correlations [14]. The authors obtained prediction errors below one log-unit, but the number of samples was very low ($n = 28$). Using synchronous fluorescence spectroscopy with excitation between 250 nm and 500 nm, aromatic amino acids were excited. Given a low number of samples ($n = 21$) rather optimistic prediction errors of $0.2 \log \text{TVC}/\text{cm}^2$ were reported [15]. Oto et al. have correlated tryptophan and NADPH fluorescences of pork ($n = 23$) with ATP content and bioburden based on excitation-emission matrices (EEM) resulting in cross-validated coefficients of determination of R^2_{cv} of 0.84–0.88 [13].

Similarly, Yoshimura et al. employed EEM data exploiting signals of a variety of fluorophores to quantify bioburden of beef samples ($n = 60$) [12]. This work showed that five endogeneous fluorophors (tryptophan, NAD(P)H, retinol, flavins und porphyrins) were used in the predictive models. The authors achieved in a calibration range of 1.8 to 7.8 $\log(\text{TVC}/\text{cm}^2)$ errors of prediction of 0.8 $\log(\text{TVC}/\text{cm}^2)$. However, acquisition of excitation-emission matrices is time-consuming and thus difficult to transfer into a rapid application. An approach which is concentrating specifically on the fluorescence of porphyrins [16] has the advantage that the detection system can be less complicated and speed up the measurement. Schneider et al. have shown that protoporphyrin XI as well as the zinc and magnesium complexes thereof are suitable for a detection of meat spoilage [16,17].

To transfer these techniques from the laboratory to real-world processes, portable devices were developed. In a generic approach, the development of mobile instrumentation for fluorescence, reflectance and Raman spectroscopy was pursued [18]. Two prototype instruments were developed from this project: a handheld Raman probe head [6,19] and a prototype mobile fluorescence device [17]. The latter used a blue ray laser diode with emission at 405 nm for excitation and an 18 around 1 fibre-optic probe head for delivery and collection of the signals. The fluorescence signals were bandpass-filtered to 570–650 nm to detect specifically the fluorescence of porphyrins, namely protoporphyrin IX and Zn-protoporphyrin IX [17]. The freshdetect device is a further development of this fluorescence device. It is robust and handheld, uses 405 nm excitation, fibre-optic signal collection, and the fluorescence signals are recorded by a miniaturised spectrometer in the range 460 to 900 nm. Ait-Kaddour et al. developed a portable spectrofluorimeter based on three UV excitation wavelengths (280, 320 and 380 nm) delivered by a fibre-optic probe which was connected to a miniature spectrograph [14]. The authors investigated the quantification of spoilage of minced beef vacuum-packaged and stored at 5°C and 15°C over a period of eleven days on the basis of one mixed sample ($n = 1$) and 28 measuring days. Based on this small sample, quantification was shown feasible with cross-validated coefficients of determination ranging from 0.5 to 0.99.

The transfer to robust and portable devices, however, is still an ongoing challenge since they require in general a calibration for their specific

applications. Previous work has mainly focused on demonstrating the feasibility and evaluating performance. In general, however, data sets were too small to allow for robust predictions. Therefore, further work is required to enlarge the field of applications and to build up larger data sets for robust calibration of the devices.

The aim of this work was to show the feasibility of quantifying the bioburden of minced pork with a new prototype of the hand-held freshdetect device. Minced pork was chosen for the experiment because of its relevance as a very perishable commodity. Particularly with regard to the coarse mixture of lean meat and fat, the textural inhomogeneity of minced meat is a challenge for the detection method. As this inhomogeneity can also be monitored by the colour, the interdependence between fluorescence spectra, colour and bioburden was also investigated.

2 Materials and methods

To standardise origin and age of the meat, cuts from 18 female pigs, all crossbred of German landrace and Piétrain were obtained directly from an abattoir at day 1 after slaughtering. Cuts from the leg were chosen which are commercially used for minced meat. In total 18 batches (1 per animal) of minced pork were prepared from these cuts on day 1 after slaughtering using a Carneoline FW N/22/82 (Bizerba, Germany) mincing machine with 3 mm sieves. From these batches, 144 samples (8 per animal) were prepared in 6 measuring series and they were stored in petri dishes at 2°C from 1 to 8 days allowing for bacterial growth.

Fluorescence spectra, colour and total viable mesophilic plate counts (TVC) were daily measured during this period. Eight fluorescence spectra and 5 white light spectra were recorded per sample with the freshdetect device (Freshdetect GmbH, Germany) with an excitation wavelength of 405 nm or a white light LED and spectra were recorded from 460 nm to 900 nm. As a reference, colour readings with three repetitions each were taken at 5 different positions with a Minolta Chromameter CR-400 using the following settings: D65, 2° observation angle and normalization to a white standard ($Y = 88.3$, $x = 0.3191$, $y = 0.3367$). On each measuring day, microbial reference analyses were performed in duplicate with 5 cm^2 sub-samples which were taken from

Table 4.1: Summary results of the bioburden $\log(\text{TVC/g})$ of 144 samples.

Parameter	$\log(\text{TVC/g})$
Minimum	2.67
Mean	5.13
Median	4.57
Maximum	10.43
Standard Deviation	1.80

the minced meat in the petri dishes. The subsamples were weighed, homogenized in peptone water and analysed with the plate count method according to §35 LMBG, DIN 10161 part 1.

Dark spectra were subtracted before fluorescence spectra were averaged per sample and normalized in intensity using the fluorescence at 612 nm of a lumilass-R7 filter as standard. The fluorescence spectra were pre-processed by Savitzky-Golay smoothing (order 0, filter width 15), standard normal variate (SNV) and mean-centering and then they were correlated with the logarithm of the bioburden using partial least squares regression (PLSR) using MATLAB 7.9.0 R2009b software (The Mathworks Inc., Natick, MA, USA) and PLS Toolbox 7.5 (Eigenvector Research Inc., Wenatchee, WA, USA). For cross-validation of the models, the random subset method with 10 data splits and 20 iterations was employed.

3 Results and discussion

The kinetics of bacterial growth during storage and the distribution of the bioburden per day are shown in Fig. 4.1. Data are not normal distributed (see Tab. 4.1). Therefore, the median is used to describe the trends. The initial bioburden of the samples started at 4.34 $\log(\text{TVC/g})$ at day 1 and bacteria grew up to 7.40 $\log(\text{TVC/g})$ on day 8 (Fig. 4.1). The bacterial growth was lagging during the first 3 days with 0.05 log units per day (dotted line). Exponential growth started at day 4 at a rate of 0.96 log units per day (dashed line, Fig. 4.1). On average, the critical threshold of 5.7 $\log(\text{TVC/g})$ was reached at day 6.

The fluorescence spectra correlated well with bioburden, $R^2_{\text{cal}} = 0.80$ (see Tab. 4.2 and Fig. 4.2). The cross-validated PLSR model ($R^2_{\text{cv}} = 0.72$) was able to predict the logarithm of total viable counts with an

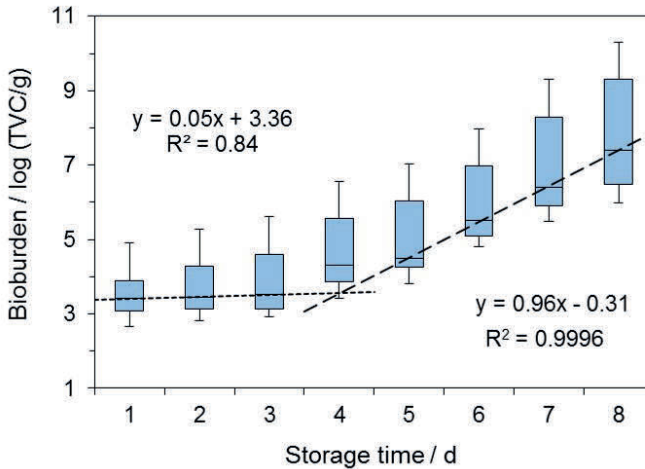


Figure 4.1: Bioburden of minced pork versus storage time at 2 °C. Lag and exponential phases are indicated by dotted and dashed lines.

RMSECV = 0.97 log (TVC/g) which is close to the error of the reference method. The congruence between calibration (filled circles) and cross-validation (open circles) was in general good. However, larger discrepancies were observed at bioburdens larger than 8 log(TVC/g). This could be due to an underrepresentation of such high bioburdens in the data set. On the other hand, bioburdens at this level are readily perceived organoleptically. In any case, the lower range, especially around and below the alarm thresholds, is most relevant for the application.

To estimate the suitability of this PLSR calibration, the limits of detection (LOD) and of quantification (LOQ) were calculated according to DIN 32465 at a 95% confidence level as $LOD = 1.03 \log(TVC/g)$ and $LOQ = 3.38 \log(TVC/g)$. This would allow for a quantification well below the alarm threshold.

The fluorescence spectra even correlated better with the storage time ($R^2_{cv} = 0.81$), while the correlation with $L^*a^*b^*$ colour was less strong, see Tab. 4.2. A cross-correlation of the five parameters showed medium to strong interdependences between the parameters, see Tab. 4.3. Storage time showed strong positive correlation with bioburden and neg-

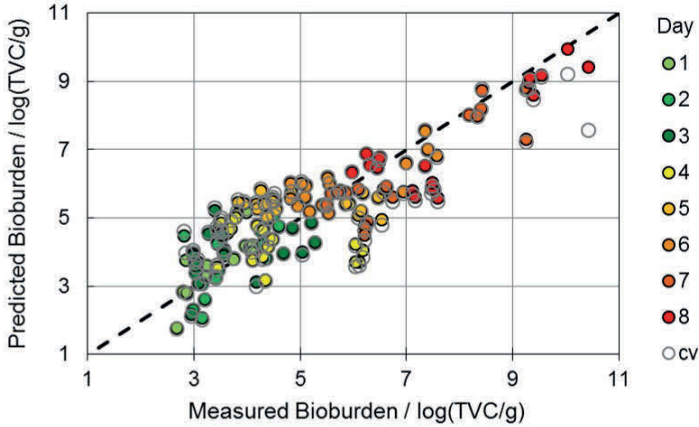


Figure 4.2: PLSR prediction of the bioburden using fluorescence spectra of the freshdetect device versus measured bioburden, filled circles: calibration, open circles: cross-validation, colours are encoding the storage time.

Table 4.2: Results of PLSR correlations of fluorescence spectra with bioburden ($\log(\text{TVC/g})$) and storage time (d) and of white light spectra with $L^*a^*b^*$ colour.

Parameter	Bioburden	Storage Time	L^*	a^*	b^*
R^2_{cal}	0.80	0.85	0.59	0.58	0.23
R^2_{cv}	0.72	0.81	0.58	0.57	0.22
RMSEC	0.80	0.9	3.78	2.22	1.47
RMSECV	0.97	1.0	3.84	2.25	1.49
Latent Variables	8	6	5	5	4
Samples	144	144	144	144	144

ative correlation with the a^* value. Bioburden was also moderately negative correlated with the a^* value. The b^* value was moderately correlated with storage time and a^* , but faintly with bioburden. It is very likely that much of this interdependence is due to the measuring protocol which links ageing of the samples to the development of bioburden and changes of the colour parameters. On the other hand, the L^* showed almost no correlation with bioburden or storage time. This could be interpreted as if there was no correlation or as the variance in the L^* incurred by the coarse mixture of meat and fat was so high that a possible correlation with storage time or bioburden was lost.

Table 4.3: Correlation coefficients of the cross-correlations of bioburden, storage time and L*a*b* colour.

	Bioburden	Storage Time	L*	a*	b*
Bioburden	1				
Storage Time	0.77	1			
L*	0.07	-0.06	1		
a*	-0.46	-0.69	-0.51	1	
b*	-0.23	-0.52	0.1	0.57	1

The measuring and sampling protocol has to be changed to overcome some of these interdependences.

4 Summary

In this feasibility study which was based on 144 samples generated from 18 independent batches, the freshdetect device proved suitable for a non-invasive evaluation of the microbial status of minced pork. The LOD was determined as 1.03 log(TVC/g) and the LOQ as 3.38 log(TVC/g), respectively. Thus, the quantification of the bioburden was shown to be feasible with an error of 1.0 log(TVC/g) at a 95% probability level well below the alarm threshold of 5.7 log(TVC/g).

The results of this study are preliminary as only one crossbred (the most common one) was used as source of meat. The number of animals and independent samples, however, is larger than in most of the previously published studies. Furthermore, the measuring protocol has to be altered to reduce the interdependence of storage time with bacterial growth and changes in the colour. Future work will have to confirm the validity of the PLSR correlation with independent samples and to improve the robustness of the model by complementing the data set with samples covering more biological variance (different animals and origins).

Acknowledgement

This work was supported by Bundesministerium für Ernährung und Landwirtschaft under the project number 2816IP003 (DIP Agrar).

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