NON-INVASIVE ASSESSMENT OF THE BIOBURDEN OF MINCED PORK USING A HAND-HELD FLUORESCENCE DEVICE

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Abstract – A hand-held fluorescence device was tested to quantify total viable aerobic plate counts (TVC) of minced pork. In total, 23 samples from eight different manufacturers were stored at 2°C for up to 8 days. Fluorescence spectra and TVC were measured daily. Partial least squares regression yielded cross-validated correlations of the fluorescence spectra with log(TVC/g) and with storage time ($R^2cv = 0.83$ and $R^2cv = 0.86$). Both correlations weighted fluorescence signals of flavins and porphyrins differently. This results in a preliminary limit of quantification of bioburden of LOQ = 3.19 log(TVC/g) at 95% confidence level. Freshly prepared minced meat (t < 1 d) was discriminated with 98.8% accuracy from stored meat (t ≥ 1 d) using partial least squares discriminant analysis.

Key Words – limit of quantification, storage time, total viable plate counts (TVC).

I. INTRODUCTION

Conventional analysis of bioburden is mostly based on colony counts (TVC) which is labor intensive, time consuming and expensive. Due to the destructive sampling, the method is only suitable for a random monitoring, but not for on-line control. Spectroscopic methods such as fluorescence, FT-IR, NIR or Raman have been researched for this purpose and all have shown potential for a fast detection of spoilage. There are, however, challenges when transferring these techniques from the laboratory to production processes: (i) robust, reliable and easy-to-use devices are required and (ii) these devices have to be calibrated for the specific application with accepted reference methods.
The correlations reported so far in literature addressed mainly the detection of spoilage and were conducted with meat products which were stored to increase TVC beyond spoilage [1, 2]. Therefore, TVC was strongly linked to storage time of the samples. This link, however, prevents a clear statement whether the correlations are based on TVC or on storage time or on both. The aim of this work was to assess the quantification of the bioburden of minced pork with a hand-held fluorescence device developed by FreshDetect GmbH at the limit of detection and to reduce the correlation between bioburden and storage time in the data set.

II. MATERIALS AND METHODS

Minced pork (n=23) was procured from eight different regional manufacturers. Nine samples were prepared from cuts obtained directly from the slaughter house to ensure low initial levels of bioburden. Additional 14 samples were obtained from 4 butcher shops (n = 4) and 3 supermarkets (n = 10) to increase diversity in microbiota and variance in contamination. In 6 measuring series, out of these 23 samples, 165 subsamples were prepared in petri dishes which were stored aerobically at 2°C up to 7 days. On each measuring day, fluorescence spectra were collected using two prototypes of the hand-held freshdetect device. Seven fluorescence spectra from 458 nm to 900 nm were recorded per sample with excitation at 405 nm. The spectra were normalized in intensity with a lumilass filter. TVC was analyzed with the plate count method according to §35 LMBG, DIN 10161 part 1with 5 cm² subsamples of minced meat taken after the fluorescence measurements. The averaged and preprocessed fluorescence spectra were correlated with log(TVC/g) and storage time with partial least squares regression (PLSR) and discriminant analysis (PLSDA) using MATLAB software and PLS Toolbox. For cross-validation, random subset with 10 data splits and 20 iterations was employed.

III. RESULTS AND DISCUSSION

TVC ranged in total from 3.2 - 9.3 log(TVC/g), see Fig. 1A. Samples from the slaughterhouse were measured on each day 0 - 7, samples from food retailers on days 0 - 2, 5 and 6. The samples obtained from the slaughterhouse had on average lower levels of bioburden (see *) than samples from food retailers. This sampling reduced the correlation between bioburden and storage time from $R^2 = 0.997$ (without days 3, 4 and 7) to $R^2 = 0.66$ including all 8 days. The

fluorescence spectra correlated well with the bioburden ($R^2cv = 0.83$) with an RMSECV which was close the error of the reference, see Figure 1B. Thus, limits of detection and quantification were calculated according to DIN 32465 at a 95% confidence level as LOD = 0.94 log(TVC/g) and LOQ = 3.19 log(TVC/g). The correlation with storage time was even stronger, see Table 1. The variable importance in projection plots (not shown) showed both correlations being based on fluorescence signals of flavins and porphyrins and storage time being stronger related to protoporphyrin IX.

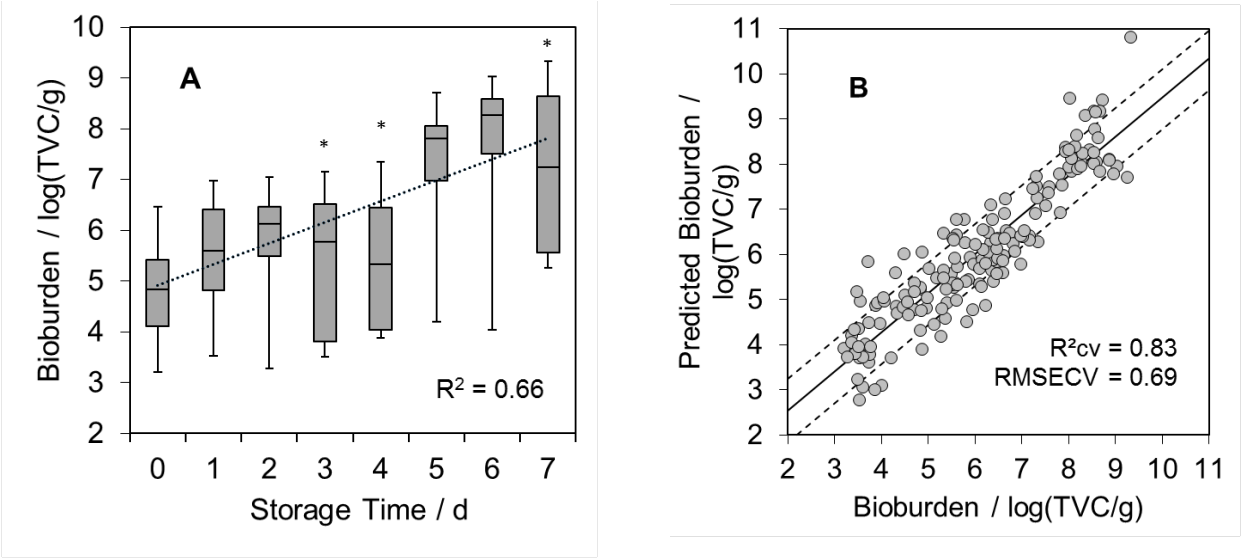

Figure 1A. Bioburden of minced pork stored at 2°C *vs.* storage time (*samples only from the slaughter house);
Figure 1B. Cross-validated PLSR correlation of bioburden with fluorescence spectra. Dashed lines indicate one RMSECV.

Table 1 PLSR results of the correlation of fluorescence spectra with bioburden and storage time of mined meat (165 measurements with two different prototypes, number of latent variables 10).

	Bioburden	**Storage Time**
R^2cal	0.89	0.91
R^2cv	0.83	0.86
RMSECV	0.69	0.87

Partial least squares discriminate analysis (PLSDA) revealed a discrimination of freshly prepared minced meat (day 0) from stored minced meat (≥ 1 day) on the basis of the fluorescence spectra with 98.8% accuracy: 100% of the freshly prepared samples and 98.6 % of the stored meat samples were correctly classified.

IV. CONCLUSION

These preliminary results show that the fluorescence spectra were well correlated with bioburden and storage time which would allow quantification beyond 3.19 log(TVC/g) and a rapid identification of minced meat which is stored for longer than one day. Both findings are useful for hygiene monitoring during meat production and retail. The results also indicate storage time as bias for correlations with bioburden and that sampling for calibration has to take this into account.

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