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Non-destructive mobile monitoring of microbial contaminations on meat surfaces using porphyrin fluorescence intensities

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ABSTRACT

A non-destructive mobile system for meat quality monitoring was developed and investigated for the possible application along the whole production chain of fresh meat. Pork and lamb meat was stored at 5 °C for up to 20 days post mortem and measured with a fluorescence spectrometer. Additionally, the bacterial influence on the fluorescence signals was evaluated by different experimental procedures. Fluorescence of NADH and different porphyrins could be correlated to the growth of diverse bacteria and hence used for contamination monitoring. The increase of porphyrin fluorescence started after 9 days p.m. for pork and after 2 days p.m. for lamb meat. Based on the results, a mobile fluorescence system was built and compared with the laboratory system. The corrected function of the meat slices showed a root mean square error of 1156.97 r.u. and a mean absolute percentage error of 12.59%; for lamb the values were 470.81 r.u. and 15.55%, respectively. A mobile and non-invasive measurement system would improve the microbial security of fresh meat.

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1. Introduction

The growing global demand for fresh meat leads to increasing industrialization of the production. Throughput rates of up to 1000 animals/hour require new approaches for quality monitoring. Increasing price pressure leads to increased production rates and the need for higher automation degrees in the processing chain. Therefore, new requirements to ensure highest hygiene and quality standards are needed. Currently, in the European Union each carcass must be visually inspected for pathological changes by a veterinarian (regulation (EC) No. 854/2004). The analysis of the microbial meat condition directly after slaughter is required only randomly or in suspected cases because of the cost intensive and time consuming realization and evaluation of microbial studies (Bunthof & Abee, 2002; Ellis & Goodacre, 2001).

The microbial contamination of fresh meat can be subdivided into primary and secondary contamination. Primary contaminants are more prevalent in weak, sick or stressed animals. However, most contaminations occur on secondary routes, i.e. during the slaughter process, meat production and meat processing (Krämer, 1997). The meat inspection is a critical point, which may lead to cross contamination after cutting of lymph nodes below the upper jaw. During this statutory procedure, bacteria present in the lymph nodes may contaminate the knife or hands of the meat inspectors and subsequently other parts of the carcass (Nesbakken, Eckner, Høidal, & Røtterud, 2003; Edwards, Johnston, & Mead, 1997). During these inspections, but also during the slaughter process and subsequent processing of meat, subcutaneous and intramuscular abscesses may be injured. Aerobic (e.g. *Arcanobacterium pyogenes*, *Streptococcus* spp., *Pasteurella multocida*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and anaerobic (e.g. *Clostridium* spp., *Bacteroides* spp., Gram-positive coccobacilli, *Fusobacterium* spp.) bacteria (Engvall & Schwan, 1983; Jones, 1980) can be released this way and spread on the meat surface. However, the presence of fecal matter and its distribution on the carcass during the slaughter process is the major source of foodborne diseases caused by e.g. *E. coli* or *Salmonella* (Ashby et al., 2003). The most frequently found spoilage organisms on meat are *Pseudomonas* spp., *Moraxella* spp., *Psychrobacter* spp. and *Acinetobacter* spp. (García-López, Prieto, & Otero, 1998). Additionally, human pathogenic bacteria such as *Salmonella* spp., *Campylobacter* spp. and *Yersinia enterocolitica* can be found and possibly transmitted from meat to humans (Borch, Nesbakken, & Christensen, 1996). Therefore, in terms of a higher control density, fast and non-destructive methods for the determination of meat quality attributes including the microbiological conditions along the entire processing chain are required.

The specific detection and quantification of viable and damaged human pathogenic bacteria and spoilage bacteria is of great importance. A particular challenge lies in the development of real-time detection with minimal sample preparation, which should ideally be automated. In this context, the application of optical methods is particularly suitable because of their short measuring times and non-invasive data

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acquisition directly on the meat surface at different stages in the production line. The direct detection of bacteria on the basis of specific autofluorescences of intracellular biomolecules with characteristic excitation and emission spectra is already possible. These biomolecules include the amino acids tryptophan and tyrosine, the coenzyme NADH, flavins, carotenoids, chlorophylls and porphyrins (Bao, Jagadeesan, Bhunia, Yao, & Lu, 2008; Bhatta, Goldys, & Learmonth, 2005; Leblanc & Dufour, 2002; Seaver, Roselle, Pinto, & Eversole, 1998; Hairston, Ho, & Quant, 1997; König & Schneckenburger, 1994).

However, most of the preliminary research concerning fluorescence of meat focused on connective tissue, amino acids and fat oxidation (Veberg et al., 2006; Allais, Viaud, Pierre, & Dufour, 2004; Wold, Lundby, & Egelandsdal, 1999). Due to numerous fluorescing meat ingredients, the detection of the bacterial fluorescence on the meat surface is challenging, because it is much weaker. Good correlations between fluorescence of adenosine triphosphate (ATP) and total viable count were achieved by using excitation–emission-matrices (Oto et al., 2013; Shirai, Oshita, & Makino, 2016). However, these studies were only conducted over 72 h and at high storage temperatures of 15 °C. In another study, pork was inoculated with *Pseudomonas fluorescens* and measured with a fluorescence imaging system (Bouchard et al., 2006). The difference of these images to the ones of the meat before the inoculation was detectable, but the bacterial concentration was relatively high (10^8 cfu/mL) and no storage of the meat was performed. However, ATP and NADH are also naturally occurring in fresh meat and may therefore cause misinterpretation. Another possibility to detect bacterial spoilage on meat may be found in the fluorescent porphyrins protoporphyrin IX (PP) and zinc protoporphyrin (ZnPP). PP is formed as a precursor of hemoglobin and ZnPP by enzymatic insertion of zinc into PP by ferrochelatase (Grinstein & Watson, 1943) or by direct conversion of heme to ZnPP in the presence of zinc ions and ferrochelatase (Taketani et al., 2007; Wakamatsu, Okui, Hayashi, Nishimura, & Hattori, 2007). Additionally, porphyrins can be formed by different microorganisms (Morita, Niu, Sakata, & Nagata, 1996; Doss & Philipp-Dormston, 1971; Kämmerer, 1924). In fresh pork, PP and ZnPP fluorescence intensities increased with storage time and temperature (Schneider et al., 2008), also when stored under different atmospheric conditions or after a freezing process (Durek, Bolling, Knorr, Schwägele, & Schlüter, 2012).

This study determined the potential use of porphyrin fluorescence intensities for a non-destructive monitoring of changes in the microbial quality during meat storage. To investigate the bacterial influence on the fluorescence signals pork was decontaminated and fluorescence signatures were recorded. Furthermore, the autofluorescence of bacterial suspensions was measured with a flow cytometer and spectrometer. Based on the results, a mobile fluorescence system was built and investigated for the possible application along the whole production chain of fresh meat.

2. Materials and methods

2.1. Meat

2.1.1. Pork

Longissimus thoracis et lumborum (LTL) was taken from 24 female pigs (mostly crossbreeds between German Landrace, German Edelschwein and/or Piétrain) at a local slaughterhouse (Vion Food Lausitz GmbH, Kasel-Golzig, Germany). They had an age of ca. six months and a weight of 90 to 110 kg or a carcass weight of 85–95 kg, respectively. After stunning with CO_2, slaughtering and flaming of bristles, pigs were eviscerated and cooled as half carcasses over night at 4 °C until cutting. LTL was removed from the 6th (7th) to 14th rib at 24 h post mortem (p.m.), cut into slices each 2 cm thick and packed individually in polyethylene (PE) bags until their measurement. PE bags were selected because they allowed a certain gas exchange (oxygen permeability was 40 $cm^3/m^{2*}d$) to achieve ambient atmospheric conditions but prevented the meat from drying over the storage period. Storage of meat with mean $pH_{24\ h}$ of 5.53 (± 0.10) was at 5 °C over 16 or 20 days p.m., respectively. In the European Union, meat of ungulates must remain under 7 °C during cutting and storage (Regulation (EC) No 853/2004). In retail, the storage temperature is usually lower (2 °C ± 2 °C, Gunvig & Bøgh-Sørensen, 1990) to prolong the shelf life, therefore 5 °C was chosen as average temperature.

2.1.2. Lamb meat

Lambs (n = 8) were at most one year old at slaughter and weighed 35–50 kg. They were stunned by cattle gun and subsequently slaughtered (Bayern-Lamm GmbH, Nuremberg, Germany). After skinning and eviscerating, carcass halves were cooled to 10 °C in 4–6 h and stored hanging at 4 °C. At 24 h p.m., the cutlet was removed, cut with bones in slices of 2 cm and packed in PE bags. Meat had an average $pH_{48\ h}$ of 5.71 (± 0.08) and was stored over 20 days p.m. at 5 °C.

2.2. Evaluation of bacterial autofluorescence in solution

Autofluorescence signatures of bacteria were recorded using fluorescence spectroscopic and flow cytometric techniques. *P. fluorescens* (DSM 50090) and *E. coli* (DSM 1116) were chosen as test organisms. Both bacteria were grown in a mineral solution to avoid the measurement of non-bacterial autofluorescence. The mineral solution modified after Jain, Jayaraman, Kökpinar, Rinas, and Hitzmann (2011) was prepared of 4.87 mM $MgSO_4\ 7^*H_2O$, 0.011 mM $CoCl_2\ 6^*H_2O$, 0.076 mM $MnCl_2\ 4^*H_2O$, 0.009 mM $CuSO_4$, 0.049 mM H_3BO_3, 0.009 mM $Na_2MoO_4\ 2^*H_2O$, 0.1 mM $ZnSO_4$, 0.412 mM $FeSO_4$, 97.73 mM KH_2PO_4, 18.904 mM $(NH_4)_2SO_4$, 8.85 mM citric acid and 27.8 mM glucose. The pH was adjusted to 6.9 using NaOH.

Bacteria were stored as cryo culture at −80 °C. For activation of bacteria one glass bead was transferred into 5 mL mineral solution and incubated for 24 h at 30 °C (*P. fluorescens*) or 37 °C (*E. coli*). After incubation the optical density was determined at 620 nm using a spectral photometer Unicam UV-1-100 (Nicolet Instruments GmbH, Offenbach, Germany) and 25 mL mineral solution was incubated with the bacteria suspension at a calculated OD_{620} of 0.07/mL. Bacteria were incubated at 30 °C (*P. fluorescens*) and 37 °C (*E. coli*) with shaking at 125 rpm. After 15, 18, 21, and 24 h bacteria were harvested to measure the autofluorescence at different growth stages. The measurement of bacterial autofluorescence by flow cytometric techniques was conducted using a CyFlowML (Partec GmbH, Münster, Germany) platform. The fluorescence of the samples was excited with a laser at a wavelength of 405 nm and the emission was measured using a photomultiplier and a band pass filter of 590 ± 25 nm. A total of 100,000 events were collected in all runs.

Additionally after 15, 18, 21, and 24 h, 3 mL of the bacterial suspensions were transferred in disposable acryl (PMMA) cuvettes (10 × 10 × 45 mm, Sarstedt AG & Co., Nümbrecht, Germany), which are UV-tolerant up to 300 nm. Cuvettes were measured in the fluorescence spectrometer LS55 (see 2.5.1) in front-face mode.

2.3. Decontamination treatments

2.3.1. Antibiotics

Kanamycin is an aminoglycoside antibiotic produced by *Streptomyces kanamyceticus*. It penetrates the bacterial cell membrane, binds to the ribosome and inhibits bacterial protein synthesis. Novobiocin is produced by *Streptomyces spheroides* and is one of the aminocoumarin antibiotics. In bacteria, it inhibits the initiation of DNA synthesis by inhibiting the energy-transferring component of DNA gyrase.

100 mL of antibiotic solution (1 mg/mL) was added to one pork slice in a bag which was subsequently sealed (V100 Lava, Lava vacuum packaging, Bad Saulgau, Germany). Afterwards, bags were placed on a moving platform. Every 15 min, they were turned around to treat both sides

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