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Potential of multispectral imaging technology for rapid and non-destructive determination of the microbiological quality of beef filets during aerobic storage

Efstathios Z. Panagou [a,*], Olga Papadopoulou [a], Jens Michael Carstensen [b], George-John E. Nychas [a]

[a] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens GR-11855, Greece
[b] DTU Compute, Technical University of Denmark, Richard Petersens Plads 321, Kgs. Lyngby DK-2800, Denmark

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ABSTRACT

The performance of a multispectral imaging system has been evaluated in monitoring aerobically packaged beef filet spoilage at different storage temperatures (0, 4, 8, 12, and 16 °C). Spectral data in the visible and short wave near infrared area (405–970 nm) were collected from the surface of meat samples and correlated with microbiological data (log counts), for total viable counts (TVCs), *Pseudomonas* spp., and *Brochothrix thermosphacta*. Qualitative analysis (PLS-DA) was employed for the discrimination of meat samples in three microbiological quality classes based on the values of total viable counts, namely Class 1 (TVC < 5.5 $\log_{10}$ CFU/g), Class 2 (5.5 $\log_{10}$ CFU/g < TVC < 7.0 $\log_{10}$ CFU/g), and Class 3 (TVC > 7.0 $\log_{10}$ CFU/g). Furthermore, PLS regression models were developed to provide quantitative estimations of microbial counts during meat storage. In both cases model validation was implemented with independent experiments at intermediate storage temperatures (2 and 10 °C) using different batches of meat. Results demonstrated good performance in classifying meat samples with overall correct classification rate for the three quality classes ranging from 91.8% to 80.0% for model calibration and validation, respectively. For quantitative estimation, the calculated regression coefficients between observed and estimated counts ranged within 0.90–0.93 and 0.78–0.86 for model development and validation, respectively, depending on the microorganism. Moreover, the calculated average deviation between observations and estimations was 11.6%, 13.6%, and 16.7% for *Pseudomonas* spp., *B. thermosphacta*, and TVC, respectively. The results indicated that multispectral vision technology has significant potential as a rapid and non-destructive technique in assessing the microbiological quality of beef fillets.

1. Introduction

The current EU beef market with ca. 8,000,000 tonnes annual consumption and similar levels of production, ranks globally 2nd in size in terms of both consumption and production, while the latest report from Eurostat states that the 44,000 enterprises across the EU-27 for which the production, processing and preservation of meat and meat products were the main area of activity in 2006, generated an estimated EUR 30.0 billion of added value in the same year (Evans, 2005). Consequently, the assessment of meat quality which covers many aspects, such as functional, technological, sensory, nutritional, toxicological, microbiological, regulatory (European Commission, 2005) and ethical, can be considered as an essential issue for this industry. Meat quality is traditionally measured by chemical, physical, and sensory methods some of which are quite time consuming, laborious, and destructive (for a review see Nychas et al., 2008). Thus, a major challenge of the meat industry in the 21st century is to obtain reliable information on meat quality and safety throughout the production, processing, and distribution chain, and finally turn this information into practical management support systems to ensure high quality final products for the consumer (Damez and Clerjon, 2008; Sofos, 2008) and satisfy the requirement for market expansion and segmentation. These systems must be readily available to the industry and easy-to-use without requiring special expertise from end-users. In addition, they must be accurate and reliable providing rapid, non-destructive, low cost analysis with minimum or no sample preparation and have the potential to analyze multiple food attributes simultaneously (Alexandrakis et al., 2008; Kamruzzaman et al., 2012).

Recently, optical sensing techniques, namely spectroscopy (Prieto et al., 2009) and computer vision (Brosnan and Sun, 2004; Du and Sun, 2004; Teena et al., 2013), have been widely explored as a potential tool for the automated quality and safety evaluation of plant and animal food commodities. However, spectroscopy does not provide spatial information of a food sample and at the same time computer vision is not able to record spectral information. By combining the advantages of computer vision and spectroscopy, hyperspectral imaging has been evolved as a promising technology that has been extensively investigated in several aspects of meat quality and safety (Cozzolino and Murray, 2004; ElMasry et al., 2012a; Feng et al., 2013; Wu and Sun, 2013a; Liu et al., 2013). Another way to combine the strengths of computer vision

* Corresponding author. Tel./fax: +30 210 5294693.
E-mail address: stathispanagou@aua.gr (E.Z. Panagou).

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technology with spectroscopy is to use multispectral imaging in the visual and short wave near infrared range of the spectrum. The main difference between the two techniques is that in hyperspectral imaging a continuous spectral range is obtained, whereas in multispectral imaging the spectral data obtained are in discrete bands (Mehl et al., 2004). This is the case with a videometer, an instrument able to record spectral reflection properties in narrow bands, thereby making it possible to assess the surface chemistry maps or hypercubes of the object of interest (Carstensen et al., 2006). The system has been developed to guarantee the reproducibility of images collected, which means that it can be used in comparative studies of time series or across a large variety of different samples including quality monitoring during continuous frying of meat (Daugaard et al., 2010), changes in meat color during storage (Christiansen et al., 2012; Trinderup et al., 2013), and identification of different *Penicillium* species (Clemmensen et al., 2007), with varying degrees of success.

The potential of multispectral imaging as a rapid and non-destructive technique for the assessment of the microbiological quality of minced pork during aerobic storage at different isothermal conditions has been reported previously by the same group (Carstensen et al., 2009; Dissing et al., 2012). The purpose of this study was to confirm this potential with beef filets stored aerobically at different isothermal conditions (0, 4, 8, 12, and 16 °C) by (a) developing PLS models, (b) validating the developed models with independent experiments undertaken at intermediate storage temperatures (2, 10 °C) using different batches of meat, and (c) estimating microbial loads of *Pseudomonas* spp. and *Brochothrix thermosphacta* which are the dominant microbiota during beef spoilage under aerobic conditions beyond the 'conventional' enumeration of total viable counts.

2. Materials and methods

2.1. Sample preparation

Fresh beef filets (M. longissimus dorsi, pH = 5.6) were obtained from the central meat market in Athens and transported under refrigeration to the laboratory with minimal delay. The meat was not subjected to any pre-treatment prior to packaging such as washing, removal of fat or connective tissue. The meat was divided into portions of 50 g in a laminar flow cabinet and packed aerobically in styrofoam trays that were subsequently wrapped manually with air-permeable polyethylene plastic film ensuring that there was no direct contact of the plastic film with the meat sample. The underlying objective of the treatment was to simulate the pre-packaged meat available in retail outlets. Samples were stored under controlled isothermal conditions at 0, 4, 8, 12, and 16 °C in high precision (±0.5 °C) incubators (MIR-153, Sanyo Electric Co., Osaka, Japan) for up to 430 h, depending on storage temperature, until spoilage was pronounced (discoloration and presence of off-odors). Samples stored at 0 and 4 °C were analyzed approximately every 24 h, whereas samples stored at 8 and 12 °C were analyzed every 12 and 8 h, respectively. Finally, samples stored at 16 °C were analyzed at 4–6 h intervals. A total of 258 packages were prepared for the duration of the experiment. On each sampling occasion, randomly selected triplicate packages were withdrawn from the respective storage temperatures from which the first two were subjected to microbiological analysis and the third to image acquisition. The obtained microbiological counts from duplicate packages were averaged and associated with the acquired images. It was assumed that the microbial population in the first portion of the meat would be representative of the microbiological population in the second portion of the meat subjected to image analysis.

2.2. Microbiological analysis

Beef filet samples (25 g) were weighed aseptically, added to sterile quarter strength Ringer's solution and homogenized in a stomacher apparatus (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. Serial dilutions were prepared with the same Ringer's solution and duplicate 0.1 or 1 mL samples of the appropriate dilutions were spread or mixed on the following media: plate count agar (PCA, Biolife 4021452, Milano, Italy) for total viable counts (TVCs), incubated at 30 °C for 48–72 h; *Pseudomonas* agar base (PAB, Biolife 401961, Milano, Italy) for *Pseudomonas* spp., incubated at 25 °C for 48–72 h; streptomycin thallous acetate-actidione agar (STAA, Biolife 402079, Milano, Italy) for *B. thermosphacta*, incubated at 25 °C for 72 h; and de Man–Rogosa–Sharpe medium (MRS, Biolife, 4017282, Milano, Italy) with pH adjusted to 5.7 with 10 N HCl, for lactic acid bacteria overlaid with the same medium and incubated at 30 °C for 48–72 h. All plates were examined visually for typical colony types and morphological characteristics that were associated with each growth medium. Moreover, the selectivity of each medium was routinely checked by Gram staining and microscopic examination of smears prepared from randomly selected colonies obtained from the media.

2.3. Image acquisition and pre-processing

Images were captured using a VideometerLab vision system which acquires multi-spectral images in 18 different wavelengths ranging from UV (405 nm) to short wave NIR (970 nm). The system has been developed by the Technical University of Denmark and commercialized by Videometer A/S (www.videometer.com) (Carstensen and Hansen, 2003). The acquisition system records surface reflections with a standard monochrome charge coupled device chip. A sample of meat (50 g) was placed inside an Ulbricht sphere (a sphere painted white on the inside giving diffuse and spatially homogenous illumination) in which the camera is top-mounted. The coating together with the curvature of the sphere ensures a uniform reflection of the cast light, and thereby a uniform light in the entire sphere. At the rim of the sphere, light emitting diodes (LEDs) with narrow-band spectral radiation distribution are positioned side by side. The LEDs are placed in a pattern which distributes them uniformly around the entire rim. When an image is obtained, the LEDs are turned on successively and the reflection from that specific wavelength is recorded by the top-mounted camera. The result is a monochrome image with 32-bit floating point precision for each LED type, giving in the end, a hyperspectral cube of dimensionality 1280 × 960 × 18. The system is first calibrated radiometrically and geometrically using well-defined standard targets, followed by a light setup based on the type of object to be recorded (Folm-Hansen, 1999). The homogeneous diffuse light, together with the calibration steps, ensures an optimal dynamic range and minimizes shadows and shading effects as well as specular reflection and gloss-related effects.

The image includes information not relevant to the analysis such as the Petri dish and its surroundings as well as the fat and connective tissue of the meat (Fig. 1a). To ensure that this irrelevant information will not interfere with the analysis, a pre-processing step is needed to make a mask allowing the isolation of the segment of the image that contains only the information of the meat tissue. The pre-processing was implemented by maximizing the contrast between the sample material (meat tissue) and the other non-relevant objects, enabling thus a threshold operation (Daugaard et al., 2010). Canonical discriminant analysis (CDA) was employed as a supervised transformation building method to divide the images into regions of interest (Fig. 1b). Following transformation using CDA the separation was distinct and a simple thresholding was enough to separate meat from non-meat. This step produced a segmented image for the meat sample with the isolated part of the meat tissue as the main region of interest (ROI) to be used for the extraction of spectral data (Fig. 1c) that were further employed in statistical analysis. For each image, the mean reflectance spectrum was calculated by averaging the intensity of pixels within the ROI at each wavelength. The transformation and segmentation procedures were implemented using the respective routines of the VideometerLab software (version 2.12.39) that controls the operation of the instrument.