



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

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ABSTRACT

The performance of a multispectral imaging system has been evaluated in monitoring aerobically packaged beef file 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 log₁₀ CFU/g), Class 2 (5.5 log₁₀ CFU/g < TVC < 7.0 log₁₀ CFU/g), and Class 3 (TVC > 7.0 log₁₀ 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 filets.

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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

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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 $1280 \times 960 \times 18$. 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.

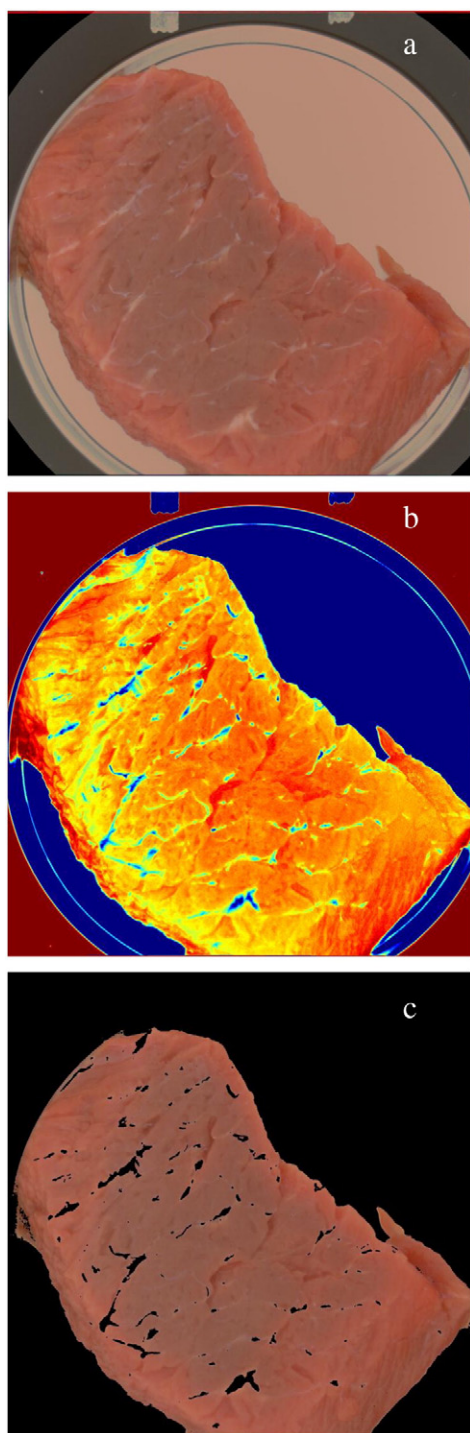


Fig. 1. Processing of an image of beef filet: (a) raw image in sRGB mode, (b) image transformed by canonical discriminant analysis (CDA) to divide foreground (meat tissue) from background information (fat and connective tissue), and (c) mask obtained by threshold operation.

2.4. Data analysis

Data analysis was performed using multivariate statistical methods including both qualitative and quantitative approaches. For qualitative analysis a three microbiological quality class evaluation scheme was employed. Specifically, according to the values of TVC, beef filets were divided into three distinct classes namely, Class 1 with TVC values up to $5.5 \log_{10}$ CFU/g, Class 2 with TVC between 5.5 and $7.0 \log_{10}$ CFU/g, and Class 3 with TVC exceeding $7.0 \log_{10}$ CFU/g. The rationale behind

this discrimination was based on legislation and published data. For example, 1st class samples were considered those that fulfill the microbiological criteria set on the EU legislation (EC 2073/2005) for beef carcasses ($5.0 \log_{10}$ CFU/cm²), while the 3rd class ($>7.0 \log_{10}$ CFU/g) on published data where it was indicated that spoilage was evident (Stanbridge and Davies, 1999). From the industrial perspective, the first class corresponds to samples that are characterized by the absence of off-flavors and are suitable for consumption (fresh); the second class is associated with the presence of slight off-flavors but is still of acceptable quality (semi-fresh); and the third class corresponds to clearly off-flavor development for which the sample is of unacceptable quality (spoiled). Overall, 86 beef filet images were captured with the VideometerLab 2 system and discriminated into the defined groups as Class 1 (26), Class 2 (12), and Class 3 (48).

Hierarchical cluster analysis (HCA) was initially performed as an unsupervised technique to explore the relationship between variables (wavelengths) and meat samples (quality classes). HCA was implemented using Pearson's correlation as a similarity measure and Ward's linkage as a clustering algorithm. The results are graphically illustrated in the form of a heatmap using the MetaboAnalyst 2.0 software (Xia et al., 2009). Furthermore, PLS discriminant analysis (PLS-DA) was used as a supervised multivariate technique to allow the discrimination of beef filets in the selected microbiological quality classes. The aim of PLS analysis is to define the relationship between a set of predictor variables X (spectral data from the VideometerLab 2 device) and a set of responses Y. The PLS method projects the initial input–output data down into a latent space, extracting a number of principal components, also known as latent variables (LVs) with an orthogonal structure, while capturing most of the variance in the original data. The first LV conveys the largest amount of information, followed by the second LV and so on. The optimal number of LVs is determined with the help of the residual variances to model useful information and avoid over-fitting of the data (Brereton, 2000). For PLS analysis, spectral data were first arranged in a 2-D matrix (X) where the rows represent the meat samples stored at different temperatures and time intervals and the columns represent the variables (18 wavelengths). One column vector (Y) containing the dependent variable (the property measured) was assigned to this matrix. For class discrimination, the Y variable was coded in a numerical format by assigning 1 to “Class 1”, 2 to “Class 2”, and 3 to “Class 3” with a cut-off value of 0.5 (Argyri et al., 2010). The classification accuracy of the PLS-DA classifier was determined by the number of correctly classified meat samples in each quality class divided by the total number of samples in the class (sensitivity, %). The overall correct classification (accuracy, %) of the model was also calculated as the number of correct classifications in all classes divided by the total number of samples analyzed (Sokolova and Lapalme, 2009).

In addition, PLS regression (PLS-R) models were built for the quantitative analysis of the microbial population of total viable counts, *Pseudomonas* spp. and *B. thermosphacta*, using the spectral information as input variables and the counts of each individual microbial group as output variables. The underlying objective of this analysis was to investigate the potential of estimating the counts directly from the acquired images during storage of meat samples. The performance of the developed PLS-R models was evaluated by the calculation of root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV), the correlation coefficient in calibration (r_c), and the correlation coefficient in cross-validation (r_{cv}). A good model should have high values for r_c and r_{cv} and low values for RMSEC and RMSECV. The optimum number of latent variables (LVs) for the best regression models was assigned at the minimum prediction residual error sum of squares after cross-validation using a leave-one-out method during the calibration step to avoid overfitting (Barbin et al., 2013). Prior to analysis spectral data were preprocessed with standard normal variate (SNV) transformation (Feng and Sun, 2013). Multivariate data analysis was carried out with the chemometric software Unscrambler® ver. 9.7 (CAMO Software AS, Oslo, Norway).

2.5. Model validation

The developed models were further evaluated to determine whether they could predict the microbiological class quality and the respective counts of the selected microbial groups at different storage temperatures and batches of meat. For this purpose, two independent experiments were undertaken at intermediate storage temperatures of 2 °C and 10 °C using two different batches of meat, one for each storage temperature. Specifically, additional beef filet samples (60 packages) were prepared as described previously and stored aerobically at the specified temperatures. At predetermined time intervals, triplicate packages were removed from each storage temperature and subjected to microbiological analysis and image acquisition as mentioned before. The acquired spectra were used as an independent input data set in order to (i) predict the microbiological class quality, and (ii) estimate the microbial counts of TVC, *Pseudomonas* spp., and *B. thermosphacta*. For qualitative analysis, model (PLS-DA) performance was evaluated with the calculation of the sensitivity and accuracy indices. For quantitative analysis, model (PLS-R) performance was based on the calculation of the room mean square error of prediction (RMSEP) and the correlation coefficient (r_p) between the observed and estimated counts. Moreover, the bias (B_f) and accuracy (A_f) factors were also calculated (Ross, 1996). The bias factor indicates whether there is a structural deviation

in a model, i.e., if the observed values lie above or below the line of equivalence. A bias factor = 1 indicates perfect agreement between predictions and observations. The accuracy factor is a measure of the average deviation between predictions and observations, i.e., how close predictions are to observations, taking values ≥ 1 . The lower the value the more accurate is the average estimate.

3. Results and discussion

3.1. Microbial association

The main microbial groups enumerated on beef filets at the onset of storage were *Pseudomonas* spp., *B. thermosphacta*, and lactic acid bacteria, whose population was greatly affected by storage temperature (Fig. 2). Aerobic storage accelerated spoilage due to the fast growth of *Pseudomonas* spp. that became the dominant microbial group at all storage temperatures, followed by *B. thermosphacta*, whereas lactic acid bacteria remained at lower levels. Thus, for multivariate analysis the first two microbial groups were selected to be correlated with spectral data because they constitute the main spoilage microbiota during aerobic storage of meat (Doulgeraki et al., 2012; Ercolini, 2013). Further information on the development of the microbial association and population dynamics of beef filets during aerobic storage can be found in a recently published

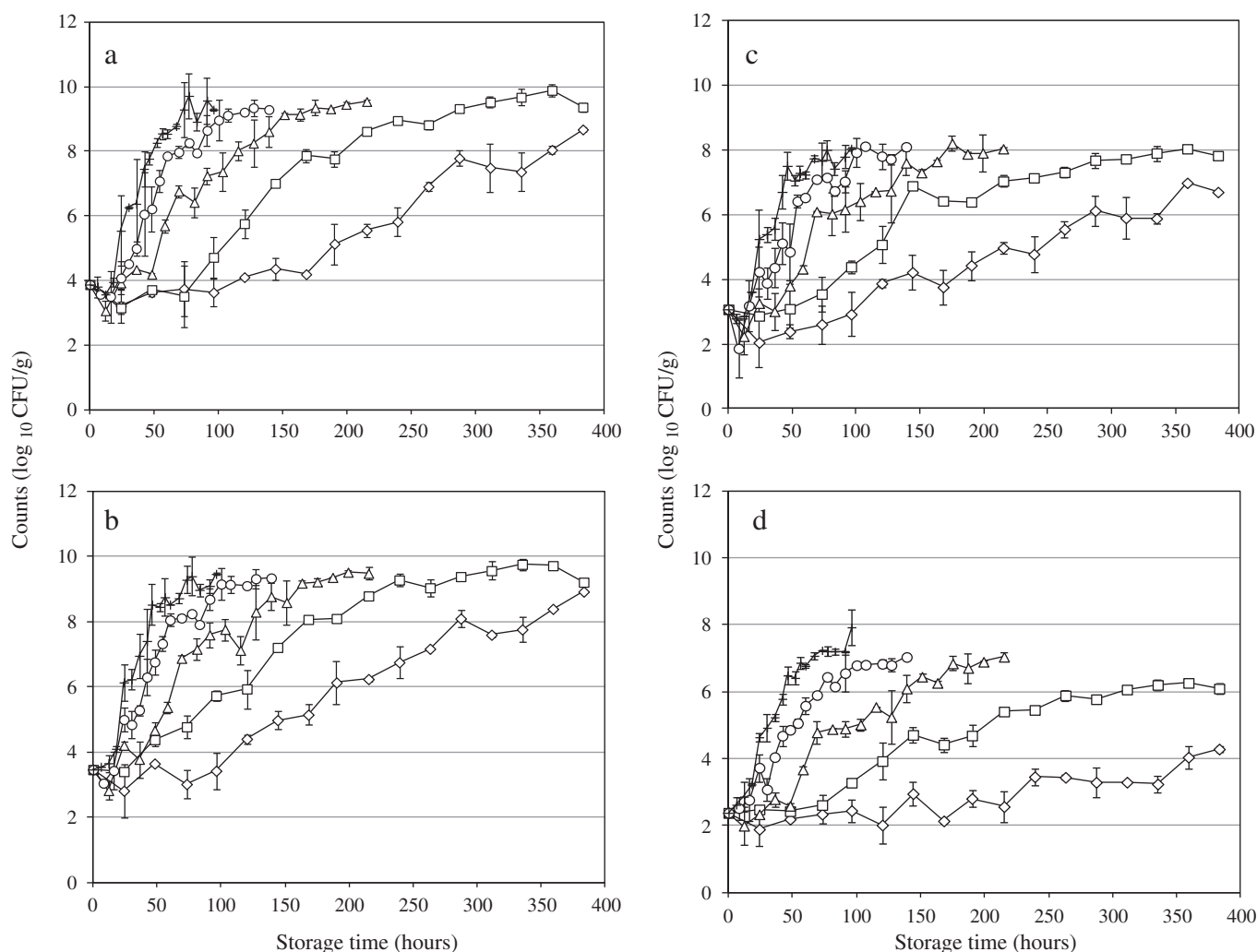


Fig. 2. Population dynamics of total viable counts (a), *Pseudomonas* spp. (b), *B. thermosphacta* (c), and lactic acid bacteria (d) on beef filet samples stored aerobically at 0 °C (●), 4 °C (□), 8 °C (Δ), 12 °C (○), and 16 °C (+). Data points are mean values of duplicate samples $\pm$ standard deviation.

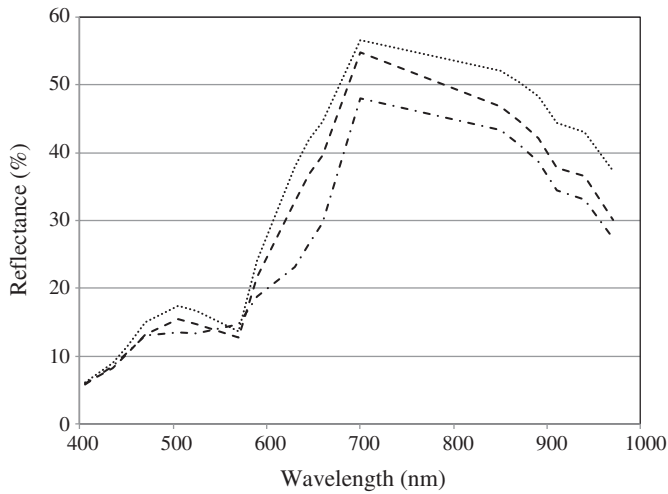


Fig. 3. Selected spectra of the examined beef filet samples corresponding to microbiological Class 1 (dotted line, 8 °C for 12 h), Class 2 (dashed line, 8 °C for 81 h), and Class 3 (dotted–dashed line, 8 °C for 103 h).

work of the same research group (Papadopoulou et al., 2013). In this work beef filets were subjected to the same packaging conditions and storage temperatures, but different non-invasive techniques were employed to monitor spoilage. The changes in beef file microbiota were typical for meat spoilage under aerobic conditions and they were in line with previous published works for both beef and pork

(Koutsoumanis et al., 2000, 2006; Skandamis and Nychas, 2001, 2002; Ercolini et al., 2006, 2009).

3.2. Spectral features

Typical spectral reflectance curves collected from beef filets stored at 8 °C for different storage times are presented in Fig. 3. Although a similar pattern was observed in the spectral information extracted from the meat samples, some difference in the magnitude of reflectance existed between the three conditions. Specifically, with increasing storage time, reflectance was gradually reduced so that samples belonging to Class 3 presented lower values compared with Class 2 and finally Class 1 meat samples. This was especially evident in the visible area between 600 and 700 nm corresponding to the reddish color of meat samples. Particularly, the spectral intensity variation of the curves at 630, 645, 660 and 700 nm could suggest a dynamic conversion and degradation for a number of myoglobin derivatives (oxymyoglobin, deoxymyoglobin, metmyoglobin) resulting in gradual browning of meat tissue (Liu et al., 2003; Cozzolino and Murray, 2004; Prieto et al., 2009). A similar pattern was observed in the near infrared region (850–970 nm) where reflectance values were also decreased with increasing storage time. This part of the spectrum could also contain interesting information about the properties of the meat matrix. For example, the absorption bands at 940 and 970 nm could be associated with fat and moisture contents, respectively (Barlocco et al., 2006; Dissing, 2011). Most of the spectral information used for meat discrimination is contained in the visible and near infrared region due to the presence of pigments on the meat tissue and matrix characteristics (e.g., fatty acids, moisture) as reported by other authors (Cozzolino and Murray, 2004).

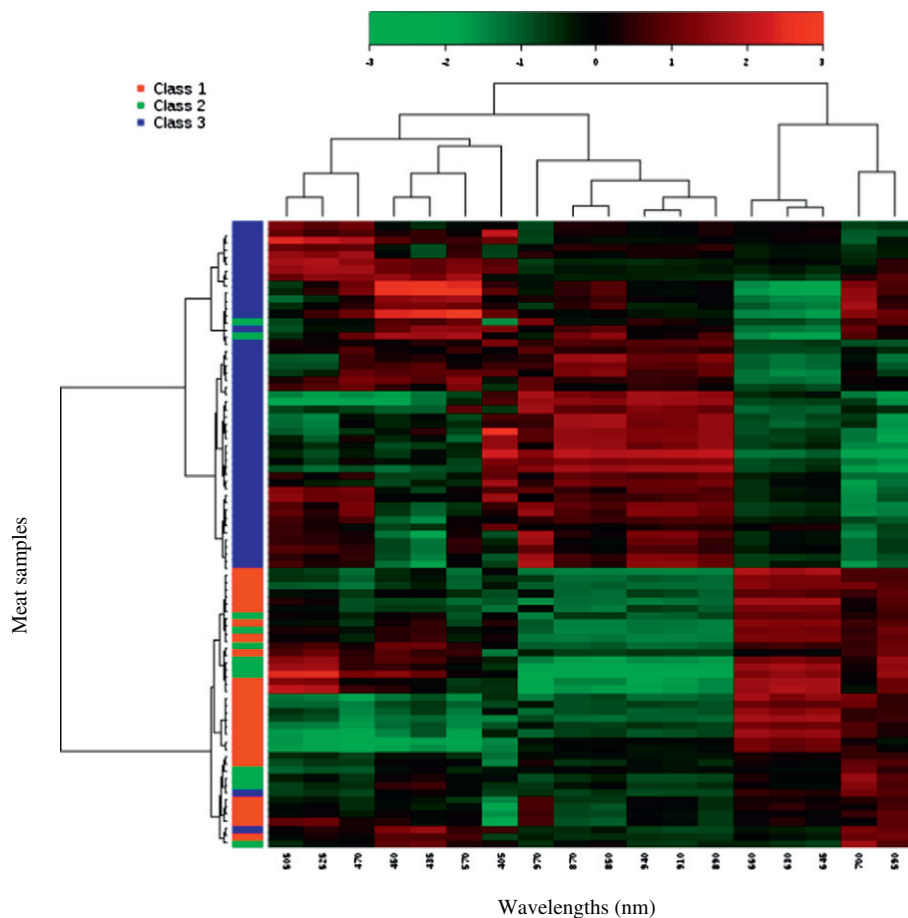


Fig. 4. Hierarchical cluster analysis (HCA) of variables (wavelengths) and objects (meat samples) shown in the form of a heatmap.

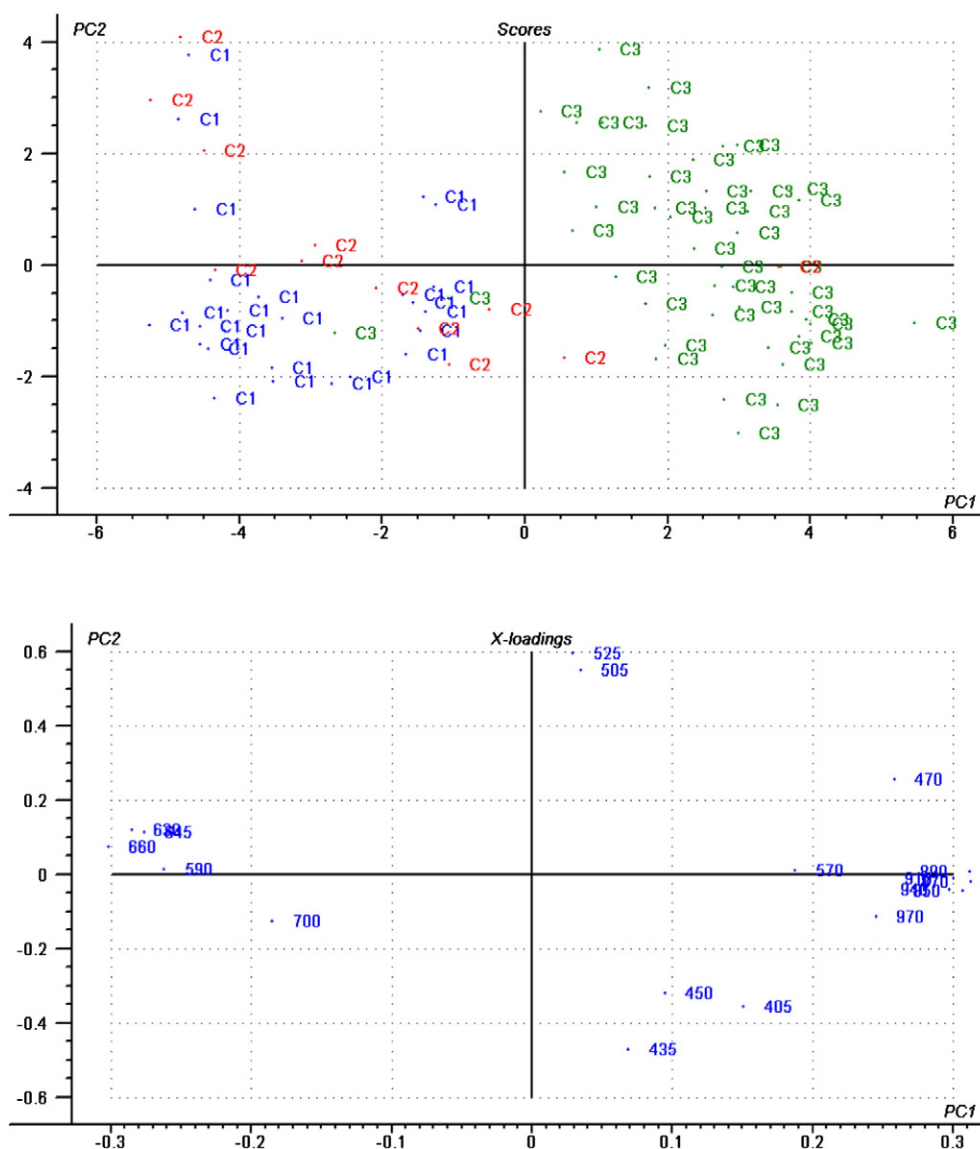


Fig. 5. PLS-DA score plot (a) illustrating the separation of the three microbiological classes (C1: Class 1; C2: Class 2; C3: Class 3), and loading plot (b) depicting the contribution of the wavelengths to the separation.

3.3. Quality class evaluation

Cluster analysis was initially employed as an unsupervised multivariate method to explore the possibility of sample discrimination in the respective microbiological quality classes. Two major groups could be visualized with Class 3 presenting clear differentiation from classes 1 and 2 together, for which overlapping was evident (Fig. 4). Concerning the grouping of variables (wavelengths), a cluster comprising of 590, 630, 645, 660 and 700 nm, corresponding to the reddish color of meat, was clearly separated from the remaining wavelengths. This cluster was highly associated (red color in the heatmap) with the samples in classes 1 and 2, whereas low association (green color in the heatmap) was evident for meat samples in Class 3, indicating that these wavelengths could describe better the samples in the first two classes. The opposite trend was observed for the wavelengths at 850, 870, 890, 910, 940, and 970 nm, all corresponding in the near infrared region, that were highly associated with meat samples in Class 3, whereas the remaining wavelengths did not show a clear pattern among the three quality classes.

Furthermore, PLS discriminant analysis (PLS-DA) was employed as a supervised multivariate statistical technique to discriminate meat

Table 1

Classification matrix of the DFA model regarding microbiological class discrimination of beef filet samples stored under aerobic conditions at different temperatures based on spectral data acquired by a VideometerLab image analysis system.

From/to	Class 1	Class 2	Class 3	Row total	Sensitivity (%)
Calibration (n = 85)					
Class 1	23	3	0	26	88.5
Class 2	1	10	1	12	83.3
Class 3	0	2	45	47	95.7
					Correct classification: 91.8%
Cross-validation (LOOCV)					
Class 1	19	7	0	26	73.1
Class 2	2	9	1	12	75.0
Class 3	0	2	45	47	95.7
					Correct classification: 85.9%
Prediction (n = 20)					
Class 1	4	1	0	5	80.0
Class 2	0	3	0	3	100.0
Class 3	0	3	9	12	75.0
					Correct classification: 80.0%

samples among the three microbiological quality classes. The method of full cross-validation, following the leave-one-out procedure, was used to determine the optimum number of latent variables (LVs) to ensure the predictive ability and avoid over-fitting of the data. From a preliminary plotting of the raw data, one beef sample from the training data set, corresponding to storage at 0 °C for 434 h, was considered as outlier and removed from further analysis, resulting in a final data set of 85 meat samples. The score plot of the first 2 LVs confirmed the results obtained by cluster analysis, illustrating good discrimination between beef filets in Class 3 against classes 1 and 2 for which some overlapping was observed (Fig. 5a). The position of the samples on the graph illustrates a gradual transition from classes 1 and 2, on the left side of the graph, to Class 3, on the right side, indicating that LV1 could be associated with storage time at the different temperatures. In addition, the plot of loadings (Fig. 5b) provides interesting information about the association of the wavelengths with meat samples. Thus, the wavelengths at 590, 630, 645, 660 and 700 nm are correlated with the samples in classes 1 and 2, which is in good agreement with the results obtained by cluster analysis, whereas the remaining wavelengths are correlated with the samples in Class 3. The classification accuracy of the PLS-DA model was provided as confusion matrix together with the calculated sensitivity and overall classification rates (Table 1). Good classification was obtained in all quality classes demonstrating the efficacy of multispectral image analysis as a rapid and non-destructive screening technique to monitor a beef filet microbiological class under aerobic storage. Specifically, for model calibration the overall classification rate was 91.8%, for which the highest sensitivity was observed in Class 3 (95.7%) followed by Class 1 (88.5%) and Class 2 (83.3%), representing 3 misclassifications out of 26 samples in Class 1, and 2 misclassifications in classes 2 and 3 out of 12 and 47 meat samples, respectively. Lower classification rates were obtained during the process of model optimization (cross-validation). In this case, the correct classification rate amounted to 85.9% with respective sensitivities of 73.1%, 75.0% and 95.7% for classes 1, 2, and 3, respectively. For model prediction, the calculated percentages were lower which is somehow expected as these data were not initially included in model calibration, but used as unknown cases for prediction. Overall, 20 beef filets were analyzed from 2 to 10 °C and various sampling times. Results showed a correct classification rate of 80.0% with respective sensitivities of 80.0%, 100.0%, and 75.0% for classes 1, 2, and 3, respectively. The percentages of erroneous predictions in the safe side (i.e., meat samples in Class 1 characterized as classes 2 and 3, as well as samples in Class 2 characterized as Class 3) were 4.7%, 9.4%, and 5.0% for model calibration, cross-validation and prediction, respectively. Moreover, the misclassified cases in the dangerous side (i.e., meat samples in classes 2 and 3 characterized as Class 1, and samples in Class 3 characterized as Class 2) were 4.7% for model calibration and cross-validation and 15.0% for model prediction. It is characteristic that no meat sample of Class 1 was classified in Class 3 and vice versa, indicating a high certainty of discrimination between these two classes (fresh vs. spoiled). It must be also emphasized that the number of samples analyzed in each class was not equal due to the variable spoilage rate of beef filets at different storage temperatures, resulting in a different number of samples in each class. Thus the number of samples in Class

3 was ca. 2 and 4 times greater than the samples in classes 1 and 2, respectively. This may have affected the calibration process of the model, which is mainly a data driven approach, and could thus account for the lower sensitivities observed in certain classes.

The discrimination of meat in quality classes based on the values of total viable counts is considered as an objective approach although there is no agreement in the actual microbial size that spoilage can be easily identified (El Barbri et al., 2008; Barbin et al., 2013; Wu and Sun, 2013b). In most of these works, a cut-off value of 6.0 log₁₀ CFU/g was taken as the acceptable threshold allowing classification of samples as 'fresh' (up to 6.0 log₁₀ CFU/g) and 'spoiled' (over 6.0 log₁₀ CFU/g) because this threshold value is considered as the general microbiological safety guideline applied in food quality (Gram et al., 2002). Other authors (Ellis and Goodacre, 2001; Peng et al., 2011) have suggested that the levels at which bacterial spoilage occurs in meat are related to the muscle type and pH, and used as the indicator level in beef spoilage under aerobic conditions is the value of 10⁷ CFU/cm² or g. Stanbridge and Davies (1999) described them as fresh, meat samples with a 'meaty' smell (<10⁷ CFU/g), which gradually goes to an inoffensive but definitely non-fresh one, having a dairy/buttery/fatty/cheesy smell where bacteria reach 10⁸ CFU/g and eventually to putrid odor (10⁹ CFU/g). However, from the industrial perspective, meat does not instantaneously become 'spoiled' from 'fresh' but there is a transition period between the two quality classes. Thus, it would be interesting to define an intermediate quality class indicating the early (or intermediate) stage of spoilage where the meat has developed slight off-odors but it is still acceptable for consumption. This could assist the decision support system of the industry by selecting a shorter shelf-life for this product or a closer market for distribution.

3.4. Correlation of microbiological data with spectral information

Another important perspective from the microbiological point of view would be the correlation of the information residing in the spectral data with the bacteriological counts of selected microbial groups. To this end, three different PLS-R models using the entire spectral range (18 bands) were built and validated for TVC, *Pseudomonas* spp., and *B. thermosphacta*. The performance indices of the developed models for calibration, cross-validation and prediction are presented in Table 2. Results revealed good correlations between spectral data and the counts of the microbiological analysis. Correlation coefficients above 0.90 and RMSE values below 0.95 between observed and estimated counts were found for all PLS-R models for both calibration and cross-validation data sets. Based on the prediction data set obtained by the two independent experiments in two different storage temperatures and different batches of meat, the highest correlation coefficients and the lowest RMSEP values were observed for *B. thermosphacta* (0.859 and 0.996), followed by *Pseudomonas* spp. (0.837 and 1.116) and TVC (0.783 and 1.291). The performance of the external validation was also assessed by the calculation of the bias (B_f) and accuracy factors (A_f). The values of B_f were close to 1 for *Pseudomonas* spp. indicating no structural deviation of the model (i.e., systematic over or under prediction of the counts). However, for TVC and *B. thermosphacta* the values

Table 2

Performance indices of the PLS regression models correlating microbial counts in beef filet samples on the basis of spectral imaging data.

Microorganism	Range ^a (log CFU/g)	Range ^b (log CFU/g)	LV	Calibration		Cross-validation		Prediction			
				RMSEC	r_c	RMSECV	r_{cv}	RMSEP	r_p	B_f	A_f
Total viable counts	3.1–9.9 (2.2)	4.0–10.0 (1.6)	5	0.814	0.928	0.867	0.918	1.291	0.783	0.95	1.16
<i>Pseudomonas</i> spp.	2.8–9.8 (2.2)	3.5–10.0 (2.1)	5	0.890	0.911	0.949	0.898	1.116	0.837	1.01	1.11
<i>B. thermosphacta</i>	1.9–8.2 (1.9)	3.9–8.4 (1.4)	5	0.761	0.915	0.814	0.903	0.996	0.859	0.91	1.13

LV: number of latent variables of the PLS-R model; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation; RMSEP: root mean square error of prediction; r : correlation coefficient between observed and estimated counts in the calibration (r_c), cross-validation (r_{cv}) and prediction (r_p) data sets, respectively; B_f : bias factor; A_f : accuracy factor. Values in parentheses indicate standard deviation.

^a Calibration data set.

^b Prediction data set.

of B_f were <1 indicating that the PLS-R models tend to underestimate slightly the respective microbial counts. In addition, the average deviation between predictions and observations was indicated by the accuracy factor (A_f). The lowest value for A_f was calculated for *Pseudomonas* spp. (11.6%), followed by *B. thermosphacta* (13.6%) and TVC (16.7%). The performance of the PLS-R models was also graphically verified by the comparison of observed vs. predicted count plots for the enumerated microbial groups (Fig. 6). The data were uniformly distributed along the line of equity ($y = x$) and linear trends were clearly visible for both calibration and prediction data. In PLS-R models it is often desired to investigate how individual wavelengths are related to the attributes to be predicted. The important wavelengths reflecting the characteristics of spectral data for the estimation of the selected microbial groups were obtained based on the weighted regression coefficients (B_w). Wavelengths with high positive or negative values of regression coefficients play an important role in the model and carry useful information, whereas wavelengths with small regression coefficients have little contribution on the performance of the model (ElMasry et al., 2012b). The selection of the important wavelengths was based on a modified Jackknife estimation procedure (Martens and Martens, 2000) which is a standard option of the Unscrambler software. Thus, for TVC ten wavelengths at 405, 450, 505, 570, 590, 660, 850, 870, 890, and 970 nm were identified as significant for estimating the counts of this microbial group (Fig. 7). Concerning the regression coefficient importance for the *Pseudomonas* spp. model, nine wavelengths were identified as significant at 405, 450, 505, 570, 590, 660, 850, 940, and 970 nm. Finally, for *B. thermosphacta* the significant wavelengths were eight at 405, 450, 505, 570, 590, 660, 850, and 970 nm. The different wavelengths identified after the analysis of b coefficients are in accordance with previously published works. Thus, the wavelengths at 505, 570, 590 and 660 nm are associated with different forms of myoglobin in meat samples, whereas 940 nm and 970 nm are related to fat and water content, respectively (ElMasry et al., 2013; Feng et al., 2013). Generally, the trend of regression coefficients was similar for all microbial groups with the same wavelengths located in the positive and negative sides of the graph. According to the b-regression coefficients of the PLS-R models, the quantitative equations for the estimation of the enumerated microbial groups could be

$$Y_{TVC} = 7.98 + 0.290 X_{405 \text{ nm}} - 0.206 X_{450 \text{ nm}} + 0.167 X_{505 \text{ nm}} \\ + 0.370 X_{570 \text{ nm}} - 0.200 X_{590 \text{ nm}} - 0.108 X_{660 \text{ nm}} + 0.177 X_{850 \text{ nm}} \\ + 0.121 X_{870 \text{ nm}} + 0.082 X_{890 \text{ nm}} - 0.183 X_{970 \text{ nm}}$$

$$Y_{Pseudomonas} = 12.48 + 0.421 X_{405 \text{ nm}} - 0.255 X_{450 \text{ nm}} + 0.168 X_{505 \text{ nm}} \\ + 0.502 X_{570 \text{ nm}} - 0.235 X_{590 \text{ nm}} - 0.107 X_{660 \text{ nm}} \\ + 0.151 X_{850 \text{ nm}} + 0.070 X_{940 \text{ nm}} + 0.210 X_{970 \text{ nm}}$$

$$Y_{B. thermosphacta} = 10.65 + 0.345 X_{405} - 0.221 X_{450} + 0.171 X_{505} \\ + 0.367 X_{570} - 0.213 X_{590} - 0.102 X_{660} \\ + 0.167 X_{850} - 0.202 X_{970}$$

described as follows: where X_{nm} is the reflectance at the respective wavelength and Y is the predicted value for TVC, *Pseudomonas* spp., and *B. thermosphacta*, respectively. It needs to be noted that the highest value of b-coefficients was observed at 570 nm indicating that oxyhemoglobin had a significant role in spoilage, since this wavelength is associated with the absorption band for oxyhemoglobin in beef (Peng et al., 2011). The results obtained in this study are comparable with a previous work (Dissing et al., 2012) that employed multispectral images from the VideometerLab vision system to estimate the microbial spoilage of minced pork (expressed as total viable counts) packed under aerobic and modified atmosphere packaging conditions at chill and abuse temperatures. The authors reported a satisfactory prediction of total viable counts for both packaging systems with a standard error of

prediction of 7%, whereas 83% of the predicted total viable counts were included within the $\pm 10\%$ zone of relative error. Moreover, the average deviation between predictions and observations, as calculated by the accuracy factor (A_f) was 15% which is in good agreement with the value of 16.7% obtained in this work.

Machine vision has been explored in recent years as a rapid and non-destructive technology to detect microbiological contamination in plant

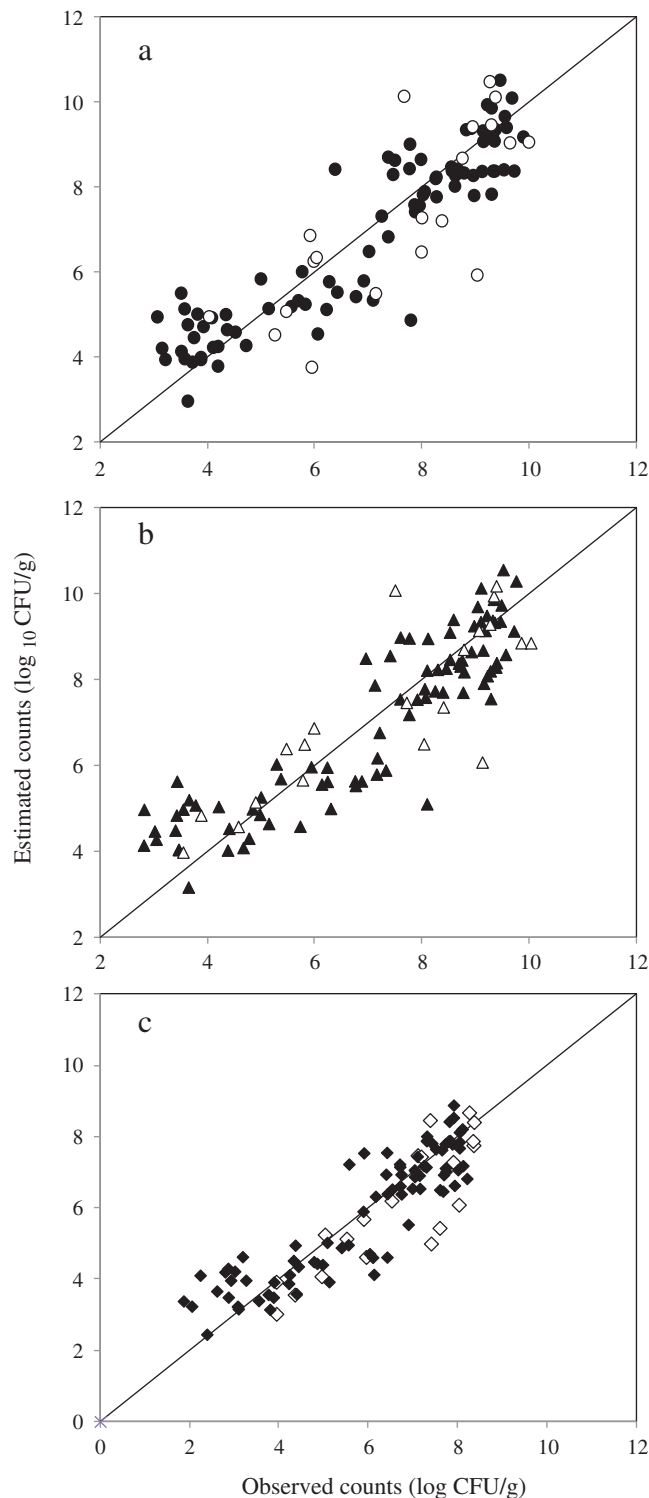


Fig. 6. Relationships between observed and estimated values of total viable counts (a), *Pseudomonas* spp. (b) and *B. thermosphacta* (c) for the calibration data set of 85 samples (solid symbols) and the prediction data set of 20 samples (open symbols) obtained from independent experiments.

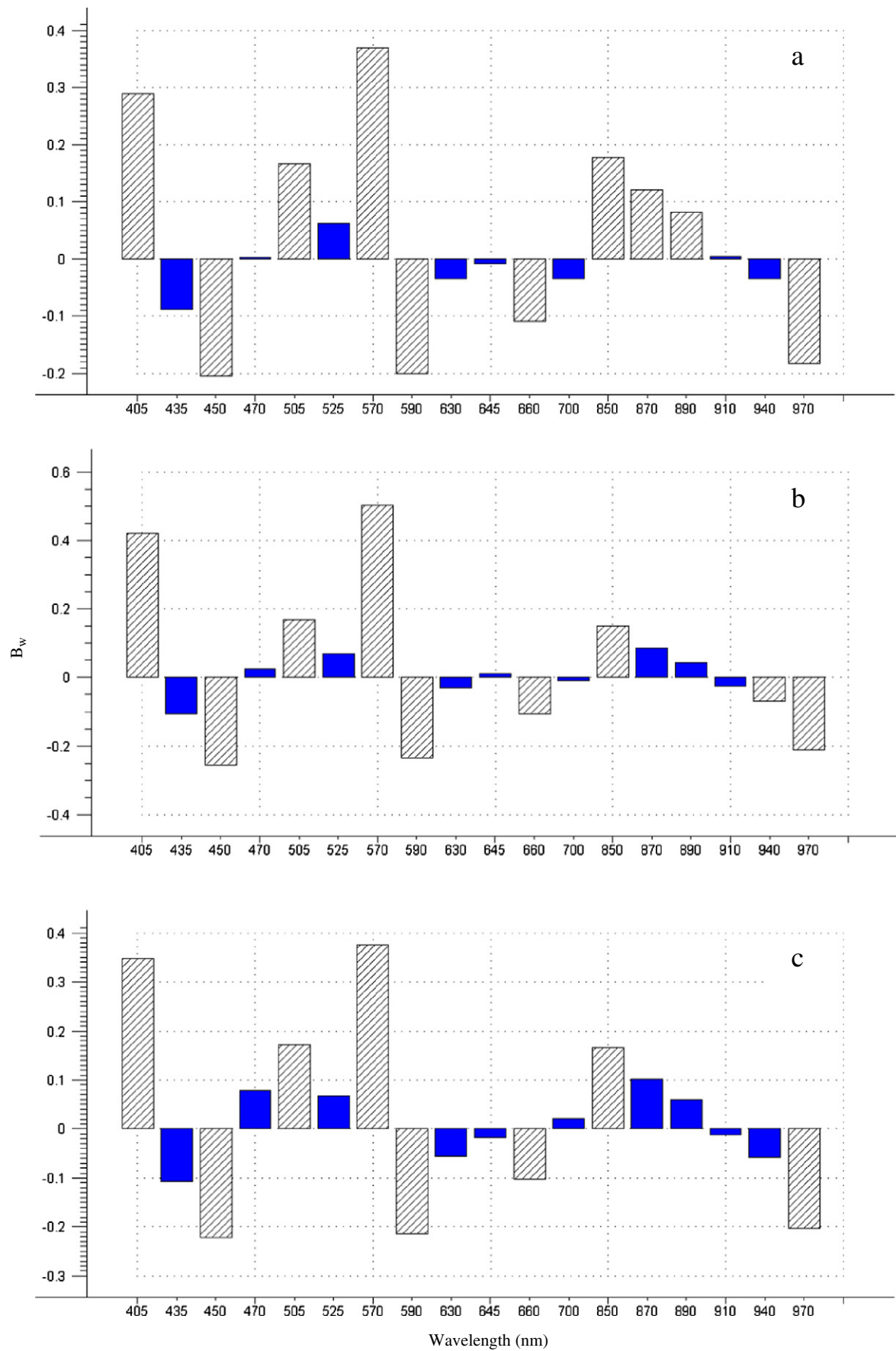


Fig. 7. Weighted beta coefficient (B_w) values of PLS-R models for total viable counts (a), *Pseudomonas* spp. (b) and *B. thermosphacta* (c). Shaded bars indicate important wavelengths (variables).

and animal food products (Teena et al., 2013). In the case of meat, the majority of works have been focused on the development of models that associate spectral data in the visible (400–800 nm) and near-infrared area (900–1700 nm) with TVC and predict spoilage during storage at refrigerated temperatures (4 and/or 8 °C) (Lin et al., 2004; Peng et al., 2011; Wang et al., 2011; Alexandrakis et al., 2012; Tito et al., 2012; Wu and Sun, 2013b). However, the enumeration of TVC does not fully reflect the spoilage dynamics in meat since a number of studies have established that spoilage is caused by a fraction of the initial microbial association that dominates, denoted as specific spoilage organisms (SSOs) (Koutsoumanis and Sofos, 2004; Nychas et al., 2007; Ercolini et al., 2011). For this purpose attention has been given on model development for the determination of specific microbial groups such as *Pseudomonas* spp. and Enterobacteriaceae with promising results (Feng et al., 2013). Moreover, as temperature abuse is not uncommon in the chill chain, model development should be undertaken at both refrigerated and abuse temperatures to provide estimations of the microbial load. Both issues have been tackled in this study by determining not only the TVC of meat samples but also the population dynamics of *Pseudomonas* spp. and *B. thermosphacta* which are the main spoilage microbiota during meat storage under aerobic conditions, whereas meat spoilage was monitored at both chill (0, 4 and 8 °C) and abuse temperatures (12 and 16 °C). It needs to be noted however that further research must be undertaken to explore the limitations of multispectral imaging for the determination of the microbiological quality of meat. Specifically, the differences in reflectance at the different wavelengths during time could be determined to a great extent by color changes associated with different states of myoglobin on the surface layer of the analyzed meat, which in turn is affected by meat factors (e.g., animal breed, muscle differences, muscle pH, postmortem age of the meat) and oxygen availability on meat surface. The latter depends on the packaging system employed (e.g., aerobic or modified atmosphere packaging, permeability of plastic film, contact of the film with the surface of meat) and the use of oxygen by microorganisms and hence by the microbial counts. It is thus necessary to undertake additional and more extensive validation with beef coming from a variety of sources taking into account also commercial samples from different processing installations and retail outlets to prove the robustness of multispectral image analysis in assessing the microbiological quality of meat.

4. Conclusion

The results obtained in this study demonstrated that the multispectral images acquired by the videometer vision system in the visible and short-wave near infrared area (405–970 nm) could be considered as fingerprints of an active biological system containing information for the discrimination of beef filets in microbiological class qualities during aerobic storage at refrigerated and abuse temperatures. Correct classification ranged from 91.8% for model calibration to 80.0% for model validation with data from an independent experiment with different batches of meat. In addition, multispectral data were employed in PLS regression models to estimate the population of TVC as well as the microbial counts of *Pseudomonas* spp. and *B. thermosphacta* on the meat surface. The developed PLS-R models showed regression coefficients between 0.90–0.93 between observed and estimated values of microbial counts during model development and 0.78–0.86 for the prediction data set depending on the microorganism. It needs to be noted that multispectral image technology provides rapid and non-invasive analysis with no sample preparation that could be an interesting alternative to laborious and time consuming microbiological analysis. However, as meat color stability plays a key role in the evolution of multispectral signals during time, a more intensive validation step must be undertaken in the future to elucidate the limitations of this technique in the determination of the microbiological quality of beef.

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