Comprehensive REVIEWS in Food Science and Food Safety

Recent Applications of Spectroscopic and Hyperspectral Imaging Techniques with Chemometric Analysis for Rapid Inspection of Microbial Spoilage in Muscle Foods

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Abstract: Muscle food is one of the most perishable food products because of its vulnerability to microbial spoilage, which can result in critical food safety problems. Traditional techniques for detection and evaluation of microbial spoilage in muscle foods are tedious, laborious, destructive, and time-consuming. In recent years, spectroscopic and imaging technologies have shown great potentials for the assessment of food quality and safety due to their nondestructive, noninvasive, cost-effective, and rapid responsive nature. This review focuses on the applications of several valuable spectroscopic techniques including visible and near-infrared spectroscopy, Fourier transform infrared spectroscopy, fluorescence spectroscopy, Raman spectroscopy, and hyperspectral imaging for the rapid and nondestructive detection of microbial spoilage in common muscle foods such as meat, poultry, fish, and related products. Combined with chemometric analysis, such as spectral preprocessing and modeling methods, these potential technologies have been successfully developed for the determination of total viable count, aerobic plate count, *Enterobacteriaceae*, *Pseudomonas*, *Escherichia coli*, and lactic acid bacteria loads in muscle foods. Moreover, the advantages and disadvantages of these techniques are discussed and some perspectives about future trends are also presented.

Keywords: hyperspectral imaging, microbial spoilage, muscle food, spectral pre-processing, spectroscopy

Introduction

Muscle foods such as livestock and poultry meat and fish flesh are a rich nutrient matrix with important dietary components for people and represent an accessible source of high-quality protein for consumption (Ikutegbe and Sikoki 2014). However, these muscle foods also provide a suitable and comfortable environment for the proliferation of meat spoilage microorganisms and common food-borne pathogens. Therefore techniques such as refrigeration (Sun 1997; Sun and Eames 1996; McDonald and others 2001; Wang and Sun 2004; Kiani and Sun 2011; Zheng and Sun 2004) and drying (Cui and others 2008; Delgado and Sun, 2002) could be used to enhance product quality and safety. Spoilage is most rapid and evident in muscle or proteinaceous foods such as pork, beef, lamb, chicken, fish, and shellfish. These foods possess a neutral or slightly acid pH and high moisture content that allows the growth of a wide range of microorganisms (Huis in't Veld 1996). More specifically, meat spoilage during distribution can be considered as an ecological phenomenon because of the fact that microbial spoilage is by far the most common cause of spoilage, which may manifest itself as visible growth (slime, colonies), textural changes (degradation of polymers), or off-odors and off-flavors during the presence of a particular microbial association, known as the so-called specific spoilage organisms (SSO) (Borch and others 1996). Initially, SSO are present in low quantities and constitute only a minor part of the natural microflora. During storage, SSO generally grow faster than the remaining microflora and produce metabolites that are responsible for off-odors, off-flavors, or slime and finally cause sensory rejection (Gram and Huss 1996). In fact, meat spoilage commonly depends on an even smaller group of SSO, called ephemeral spoilage organisms (ESO). The ESO are the consequence of factors that dynamically persist or are imposed during processing, transportation, and storage in the market (Nychas and others 2008). The spoilage microorganisms are commonly divided into 6 broad categories: Gram-negative rod-shaped bacteria, Gram-positive spore-forming bacteria, lactic acid bacteria (*Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus* spp.), other Gram-positive bacteria, yeasts, and molds (Dainty 1996).

However, spoilage is often subjectively judged by the consumer, which may be influenced by cultural and economic considerations and background as well as the sensory acuity of individuals. Indeed, when spoilage progresses, most consumers would agree

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that unpleasant discoloration, strong off-odors, and the generation of slime would constitute the main qualitative criteria for meat rejection. Therefore, muscle food safety problems caused by microbial spoilage have attracted increasing attention from food manufacturers, distributors, and official regulators (Aymerich and others 2008). To reduce the number of food-borne outbreaks and control microbial spoilage, some regulatory frameworks and risk management systems, such as good hygienic practices (GHP), good manufacturing practices (GMP), good agricultural practices (GAP), and hazard analysis critical control points (HACCP), have been developed for the production of safe foods (Balzaretti and Marzano 2013). Nevertheless, the level of safety that these food safety systems are expected to deliver has seldom been well-defined in quantitative terms (Van Schothorst and others 2009). To eliminate consumer concerns over emerging microbial hazards, microbial spoilage detection technology is desperately needed to secure the safety of muscle foods. Some sensitive and possibly specific microbial methods based upon enumeration, immunological methods, and molecular techniques have already been conducted for the detection of microbial spoilage. The culture and colony counting methods such as the plate rubbing and pouring methods are basic tools widely used for detection of food-borne pathogens because of their reliability in efficiency, sensitivity to target organism, and applicability to a wide range of food matrices (Yeni and others 2014). However, these microbiological methods have major drawbacks being labor-intensive and time-consuming, and often take 2 to 3 d for initial results, and up to 7 to 10 d for confirmation. This is obviously inconvenient in modern food industrial applications (Velusamy and others 2010). In addition, the immunology-based methods, involving antigen–antibody interactions, have been successfully employed for the detection of bacterial cells, spores, viruses, and toxins alike. Methods based on antigen–antibody bindings are also commonly used for determining food-borne pathogens (Iqbal and others 2000). However, when quantities of the pathogen are too high, the immunoassay-based methods are indicative of low assay sensitivity, low affinity of the antibody to the pathogen or other analyte being measured, and potential interference from contaminants (Meng and Doyle 2002). The polymerase chain reaction (PCR) method involving DNA analysis is also a broadly used method for the detection of pathogens in the food sectors (Nugen and Baeumner 2008). In spite of its advantages, PCR is considered to be excessively expensive and complicated to be utilized in industrial settings, based on the industrial point of view, and skilled workers are needed to carry out the tests (Velusamy and others 2010).

Apparently, these aforementioned analytical methods are destructive and not suitable for online and real-time detection of microbial spoilage in a rapid and nondestructive/noninvasive manner. Furthermore, the meat industry also needs rapid analytical tools for quantification of these microbial indicators to estimate the remaining shelf-life of their products. Therefore, spectroscopic and imaging techniques have gained great significance in the measurement and evaluation of food quality and safety because they can solve some of the existing problems presented by the traditional methods and instruments. In recent years, although these spectroscopic techniques have been developed for measuring microbial spoilage in muscle foods, no review has been published to specifically address the applications of these important spectroscopic techniques, including visible (VIS) and near-infrared (NIR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, fluorescence spectroscopy, Raman spectroscopy, and hyperspectral imaging (HSI) technique for the rapid and nondestructive detection of spoilage microorganisms in common muscle foods. Therefore, the objective of this paper was to review the applications of these spectroscopic and hyperspectral imaging techniques together with chemometric analysis, for inspection and evaluation of microbial spoilage in muscle foods based on the determinations of total viable count (TVC), aerobic plate count (APC), *Enterobacteriaceae*, *Pseudomonas*, *Escherichia coli*, and lactic acid bacteria (LAB) loads.

Chemometric Analysis

Chemometric analysis as an effective support means has been widely developed for multivariate data processing and analysis in spectroscopy techniques and hyperspectral imaging with the aim of establishing the calibration and prediction models for practical applications of classification, identification, quantification, measurement, detection, and assessment of quality and safety of food. Chemometric methods can build a multivariate model that best describes the system under analysis. The spectral data can be analyzed directly after following pretreatments.

Spectral preprocessing methods

After collection of a large number of spectral data in spectroscopy, preprocessing of the corresponding spectral data is an important procedure for improving the subsequent multivariate regression, classification, model development, or exploratory research, and it has become an integral part of chemometric analysis. In a spectroscopic experiment, the process of spectral data collection can be appreciably affected by nonlinearities caused by light scattering (Rinnan and others 2009). In addition, because of the comparable size of the wavelengths in NIR electromagnetic radiation and particle sizes in biological samples, NIR spectroscopy is vulnerable to undesirable scattering effects such as baseline shift and nonlinearities, which have a significant impact on the recorded sample spectra (Zeaiter and others 2005; Rinnan and others 2009). Therefore, applying suitable spectral preprocessing methods to some great extent can reduce and eliminate these negative effects. The most commonly used preprocessing techniques in spectroscopy (in both reflectance and transmittance mode) can be divided into 2 groups: scatter-correction methods and spectral derivatives. The first group consists of multiplicative scatter correction (MSC) (Maleki and others 2007), inverse MSC, extended MSC, extended inverse MSC, de-trending, standard normal variate (SNV), and normalization (Fearn and others 2009). Spectral derivatives mainly include Norris–Williams derivation and Savitzky–Golay derivation (Rinnan and others 2009). Derivative pretreatment is able to eliminate both additive and multiplicative effects in the spectral data and has been applied in analytical spectroscopy for decades. Specifically, the first derivative mainly removes the baseline and the second derivative eradicates both baseline and linear trend (Chang and others 2009; Sharma and others 2014). In fact, these spectral preprocessing methods mentioned above show their individual advantages and their combinations are very helpful and effective in subsequent modeling analysis. The implementations of these preprocessing techniques were conducted by the chemometric software (Unscrambler version 9.7, CAMO, Trondheim, Norway).

Common modeling techniques

The spectral data obtained can be used to develop the multivariate model by utilizing the appropriate chemometric algorithms such as principal component analysis (PCA), cluster analysis (CA), linear discriminant analysis (LDA), quadratic discriminant

analysis (QDA), soft independent modeling by class analogy (SIMCA), partial least square-discriminant analysis (PLS-DA), artificial neural network (ANN), support vector machine (SVM), least square-support vector machine (LS-SVM), multiple linear regression (MLR), principal component regression (PCR), and partial least square regression (PLSR) for classification and regression purposes in spectroscopic and imaging techniques (Forina and others 2008; Prats-Montalbán and others 2011; Dai and others 2014). Some commonly and importantly used approaches for supporting the applications of spectroscopy and hyperspectral imaging are highlighted and discussed later in the paper.

PCA. Multivariate analysis shows great advantages for dealing with a large number of complex colinear spectral data and allowing for the reduction and simplification of these data to a lower dimension that retains the most informative and useful information. PCA as one of the most popular methods has been widely carried out to do an initial investigation and to visualize the spectral data for examining any possible grouping of samples according to spectral features of the tested meat species (Barbin and others 2012). It means that PCA can be performed on the whole spectral data (X) to identify the deviant spectral outliers and the most important directions of variability in a multivariate data space (X). As an unsupervised pattern recognition, PCA transforms the original variables through orthogonal transformation into new uncorrelated variables, called principal components (PCs), which are linear combinations of the original spectral data and ranked in such a way that the first PC covers as much of the variation in the data as possible and the second PC covers as much of the remaining variation as possible, and so forth (Abdi and Williams 2010). This means that only the first few PCs retain most of the variation present in all of the original variables. Each PC can be interpreted independently that permits an overview of the data structure by revealing the relationship between the objects. The matrix expression of the PCA for the spectral data is described later:

$$R = SP + E \tag{1}$$

where R is the spectral reflectance matrix ($n \times k$); S is the score matrix ($n \times p$); P is the eigenvector matrix ($p \times k$); E is a residual matrix ($n \times k$); n is the number of spectra; k is the number of wavelengths, and p is the number of principal components (Park and others 2001).

MLR. MLR is a classic, simple, and efficient algorithm for linear modeling. It makes linear fitting for independent variables (X) and dependent variables (Y), and obtains an optimal result in a least-squares sense (Sousa and others 2007). However, the main disadvantage of this approach is that it requires a larger number of samples than variables and its performance can be easily affected by the colinearity between variables (Guillén-Casla and others 2011). A fixed regressor model of the following form is used,

$$y = Xb + e \tag{2}$$

where b is the unknown parameter vector; X matrix and y vector are the measured calibration data for regressor variables x and response variable y, respectively; and e is the error vector. The fitting degree of MLR model is usually evaluated by standard statistical methodology.

PLSR. Compared with MLR, PLSR is suitable to solve the multi-colinearity problem. Recently, PLSR has been a widely used bilinear-modeling and multi-analysis method for quantitative regression analysis in the food industry, which can be considered as a standard calibration technique for spectroscopic analysis. It is especially suitable for situations when the number of variables is greater than the number of samples, and when there is colinearity among variables (Mehmood and others 2012; Mahesh and others 2015). During the process of model establishment, PLSR merges the functions and advantages of linear regression analysis, canonical correlation analysis, and PCA. Therefore, the results of PLSR cannot only provide a more reasonable regression model, but can also perform some other analyses, such as PCA and canonical correlation analysis. It proves that PLSR can provide abundant and deeper information (Vongsvivut and others 2014). PLSR decomposes both independent variables X and dependent variable Y into several principal components (PCs), where the orthogonal score T of X is correlated with Y by using the following formulas:

$$Y = Xb + E = XW_a^* C + E = TC + E \tag{3}$$

$$W_a^* = W_a \left(P^{\mathrm{T}} W_q\right)^{-1} \tag{4}$$

where b is the regression coefficients; E is a residual error matrix; W_a is the PLS weights; a is the number of latent variables (LVs) adopted; P and C are loadings for X and Y, respectively. As indicated above, when regression coefficient b is finally determined for a specific problem, the Y values for new samples can be predicted with reasonable errors by multiplying the spectra (X) of these new samples with the obtained regression coefficients.

LS-SVM. LS-SVM is an evolutionary version of the standard SVM and has been widely developed to involve equality instead of inequality constraints and to work with a least squares cost function for optimal control of nonlinear systems and spectral calibration (Suykens and others 2001; Bao and others 2014). This approach utilizes nonlinear map function, projects input features to a high-dimensional space, and adopts the Lagrange multiplier to compute the partial differentiation of each feature for converting the optimization problem into resolving the linear algebraic equation (Sadik and others 2004; Thissen and others 2004). LS-SVM not only possesses the advantage of good generalization performance as SVM, but also exhibits the simple structure and shorter optimization time. Given a training set of N samples $\{y_n, z_n\}_{n=1}^N$, where z_n is the input features and y_n is the output pattern, the classifier of the form based on LS-SVM can be expressed as:

$$y(z) = \mathrm{sign}\left[\sum_{n=1}^{N} \alpha_n K(z_n, z) + \beta\right], \tag{5}$$

where $K(z_n, z)$ is the kernel function, β is a bias term, and α_n is the support weight vector. LS-SVM provides a generic mechanism that fits the hyperplane surface to the training data using a kernel function. The radial basis function (RBF) as a popular kernel function is normally used in LS-SVM and the grid-search technique is usually applied to determine the optimal parameters obtained from the RBF kernel (Suykens and others 2001). RBF kernel function (denoted as K_{RBF}) is defined as follows:

$$K_{\mathrm{RBF}}(z_n, z) = \exp\left(-\|z_n - z\|^2 / 2\sigma^2\right) \tag{6}$$

where σ is the width of Gaussian function. Before the application of LS-SVM, 2 parameters, γ (the regularization parameter) and σ^2 (the width parameter of RBF kernel) are optimized by the LS-SVMLab toolbox (Pelckmans and others 2002).

Table 1–A comparison of common regression algorithms.

Type	Algorithm	Advantages	Disadvantages
Linear	PCR	Simple and intelligible	Too abstract
	MLR	Easy to fit models	Powerless for predicting complex problems
	PLSR	Easy to determine statistical properties	
Nonlinear	LS-SVM	More efficiency	High computational complexity
	ANN	Suitable for analyzing complex problems	Indigestible

ANN. ANN is a frontier field that has been rapidly developed and widely used in many fields because of its good predictability and practicality. It is helpful to solve the problem for transforming the nonlinear regression into linear regression by variable substitution (Zupan and others 1997; Wang 2003; Guo and others 2015). Then linear regression methods are applied to achieve predictive purposes. In the practical applications of using ANN for spectroscopic and hyperspectral imaging analysis in food, back propagation artificial neural network (BP-ANN) is the most commonly used algorithm. BP-ANN is a multilayer feed-forward neural network and can learn and store many input–output model mapping relationships, without revealing the mathematical equations that can be used to describe these mapping relationships (Petritis and others 2003). It is usually composed of 3 neuron layers: an input layer, one or several hidden layers, and an output layer. The spectral value at each wavelength is first imported into the input layer, and then the output layer yields the corresponding prediction values after some complicated transformation among hidden layers (Ramadan and others 2005). The functions that connect different layers are based on nonlinear mapping. Besides, this method usually has one hidden layer or more, which shows greater potential for dealing with nonlinear and complex correlation problems despite the need of more training time (Syu and Chen 1998). Table 1 illustrates the comparison of common regression algorithms. As to how to carry out these algorithms, the common used implementation softwares mainly related to the chemometric software (Unscrambler version 9.7, CAMO, Trondheim, Norway) and Matlab2010a software (The Mathworks Inc., Mass., U.S.A.).

Model evaluation. A mandatory check is required to validate the integrity and applicability of the developed calibration model in predicting unknown samples to make sure that the model could work in the future for new and similar data. Full cross-validation, also called leave-one-out cross-validation, is commonly utilized to validate the established models. Meanwhile, regardless of the purpose of qualitative description or quantitative regression, the analytical procedure of spectroscopy and hyperspectral image data is subjected to the processes of calibration, cross-validation, and prediction based upon the above-mentioned chemometric methods. More importantly, it is necessary to look for effective methods to evaluate the predictive effectiveness, robustness, reliability, and accuracy for practical applications. Generally, the evaluation indicator systems are mainly related to the correlation coefficient (R) or determination coefficient (R^2), and the corresponding root mean square errors (RMSEs) in calibration (R^2_C, RMSEC), cross-validation (R^2_{CV}, RMSECV), and prediction (R^2_P, RMSEP), as well as the integrated index of residual predictive deviation (RPD). Generally, speaking, a good model should have higher values of R, R^2_C, R^2_{CV}, R^2_P, and RPD, and lower values of RMSEC, RMSECV, and RMSEP, as well as a small difference between them. In detail, R^2 indicates the proportion of the variance in reference data that can be explained by the variance in the predicted data. In fact, the value of R^2 in the range of 0.82 to 0.90 usually indicates good performance of a model, whereas the value of R^2 lower than 0.82 reveals inaccurate and relatively poor performance, and the value of R^2 higher than 0.90 shows excellent performance (Williams 2001). The values of RMSEC, RMSECV, and RMSEP are measurements of the RMSEs in the analysis and assessment of the fitting degree of regression during calibration, cross-validation, and prediction with lower values implying better predictive capacity (Hernández-Martínez and others 2013). RPD indicates the relative prediction performance of a model more directly than situations when either R^2 or RMSECV is used separately. The values of RPD are considered satisfactory, good, or excellent in the range of 3.1 to 4.9, 5 to 6.4, or 6.5 to 8, respectively (Cozzolino and others 2004). The values of RMSEC, RMSECV, R^2, and RPD are defined and calculated below:

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n} (y_{cal} - y_{act})^2}{n}} \tag{7}$$

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n} (y_{pred} - y_{act})^2}{n}} \tag{8}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_{bcal} - y_{act})^2}{\sum_{i=1}^{n} (y_{cal} - y_{mean})^2} \tag{9}$$

$$\mathrm{RPD} = \frac{SD}{\mathrm{RMSECV}} \tag{10}$$

where n is the number of samples; y_{act} is the actual value; y_{cal} is the calibrated value; y_{pred} is the predicted value; y_{mean} is the mean of the reference measured value; and SD is the standard deviation of the reference values.

Applications of Spectroscopic Techniques

Based on the spectral preprocessing and modeling methods described above, the applications of some common spectroscopic techniques for inspection of microbial spoilage in muscle foods are discussed.

VIS and NIR spectroscopy

VIS spectroscopy is a kind of electromagnetic spectrum that can be perceived by human eyes. The wavelength of the VIS spectrum is generally in the range of 380 to 780 nm. In this wavelength region, absorption spectra are original from the transition of electrons from their ground state to higher electronic states. The maximum absorption in this spectral region for some particular compounds corresponds with the structure, geometry, and symmetry of the material (Lin and others 2004). In the NIR spectral region (780 to 2500 nm), the occurrence of the overtones and combinations of fundamental vibration responses is related to the changes of chemical bonds such as O–H, N–H, C–H, C–O, and other organic molecules. Based on the basic knowledge of

VIS and NIR spectroscopy, diffuse reflectance spectroscopy in the spectral range of 600 to 1100 nm was used to quantify the APC of chicken breast muscle samples with PCA and PLSR analysis, and it was noted that PCA analysis showed clear classification of samples held for 8 h or longer, compared with the 0 h control, and the quantitative PLSR model with 8 latent variables presented good performance for predicting microbial loads with R^2_P of 0.83 and SEP of 0.48 log CFU/g. Similarly, in another study, VIS/NIR spectroscopy in the wavelength region of 400 to 1000 nm was applied to predict the freshness of packaged sliced chicken breast based on determination of APC value, and the PLSR-based prediction model with 13 optimal wavelengths showed tolerable accuracy with R^2_{CV} of 0.82 (Grau and others 2011). On the basis of the above studies, although it has been demonstrated that this spectroscopic technique is feasible to determine the APC value, the prediction performance is still relatively poor, which could be because of the fewer tested samples or the effects of the different storage times and temperatures. In addition, the above-mentioned spectroscopy technology is also used to detect the microbial loads of vulnerable fish muscle. For example, Tito and others (2012) used NIR spectroscopy combined with PCA and PLSR to predict microbial loads on Atlantic salmon. It was demonstrated that qualitative PCA analysis showed a clear separation between the fresh salmon fillets and those stored for 9 d at 4 °C. The PLSR model was established for the prediction of APC and a competitive performance was obtained ($R^2 = 0.95$ and RMSE = 0.12 log CFU/g). Sone and others (2011) utilized a VIS/NIR spectroscopy (400 to 2500 nm) to investigate spectroscopic changes occurring during storage of Atlantic salmon fillets with and without bacterial growth. PCA was used to detect the spectroscopic changes. Results showed that VIS/NIR spectral changes occurred in the control as well as the treated group of samples within a single day after filleting. After 2 d of storage, the samples obtained were discernible from those fresh in both groups and it was only after the microbial spoilage became pronounced (8 to 9 log CFU/g) that the spectra of the spoiled control samples could be differentiated from spectra of the treated samples with no bacterial growth. It could be concluded that VIS/NIR spectroscopy could detect autolytic changes occurring in salmon muscle during the early stage of storage, which was independent of microbial growth. To explore the wide application in other fish species, Duan and others (2014) developed a portable NIR spectrometer for the nondestructive determination of total bacteria counts in flounder fillets. Results revealed that the pretreatment of NIR spectrum by the wavelet transform could significantly improve the accuracy and precision of the analysis. Combination of genetic algorithm (GA) and BP-ANN exhibited much better efficiency, with R of 0.985 and RMSE of 0.095 log CFU/g. Based on these results, it has been suggested that VIS and NIR spectroscopy in tandem with chemometric analysis is a promising technique for nondestructive and onsite monitoring of microbial spoilage in muscle foods. More importantly, it can be concluded that selection of the suitable modeling methods and spectral preprocessing approaches plays an important role in the improvement of prediction performance.

FT-IR spectroscopy

FT-IR spectroscopy in the mid-infrared region (4000 to 400 cm^{-1}) is a biochemical fingerprinting technique. In combination with multivariate statistical approaches, it has been demonstrated to be a very rapid and reasonably accurate method for bacterial detection and enumeration in muscle foods. For instance, FT-IR spectroscopy combined with machine learning algorithm was used to detect microbiologically spoiled or contaminated beef at room temperature for 24 h (Ellis and others 2004). In the study, FT-IR measurements were collected every hour directly from the sample surface using attenuated total reflectance (ATR), in parallel the TVC of bacteria were obtained by classical microbiological plating methods. PLSR was used to accurately model and estimate the bacterial loads, and GA and genetic programming (GP) were used to elucidate the wavenumbers of interest related to the spoilage process. The results obtained demonstrated that FT-IR spectroscopy combined with machine learning was possible to detect bacterial spoilage in beef, and the most significant functional groups selected could be directly correlated to the spoilage process, which arose from proteolysis, resulting in changes in the levels of amides and amines. FT-IR spectroscopy in tandem with chemometric analysis was also developed to differentiate fresh and modified-atmosphere-packaged beef. The wavenumbers (1714 to 1710 cm^{-1}, 1614 to 1211 cm^{-1}, and 1031 to 1000 cm^{-1}) correlated with the spoilage process, and were identified by PCA, and a good estimate of TVC ($R^2 = 0.80$) from the spectral data was obtained by PLSR (Ammor and others 2009). To improve the prediction accuracy, Argyri and others (2010) introduced another machine-learning strategy of multilayer perceptron neural network based on back-propagation to correlate FT-IR spectral data with beef spoilage during aerobic storage. Three quality classes of fresh, semi-fresh, and spoiled samples were determined by sensory evaluation. The results demonstrated that this machine learning algorithm was capable of classifying the beef samples with high accuracy for fresh (91.7%), semi-fresh (81.2%), and spoiled samples (94.1%). The performance of the network in the prediction of TVC was also satisfactory, with good correlation of microbial loads on the beef surface ($R^2 > 0.92$). In another study, the same authors used FT-IR spectroscopy coupled with PLSR and feed-forward ANN analysis to classify and predict the microbial spoilage in beef fillets. Both approaches showed good performance in discriminating spoiled beef samples. The PLS-DA classification models showed the correct classification rates (CCR) ranging from 72.0% to 98.2% and 63.1% to 94.7% in the training and testing dataset. The ANN model performed equally well in classifying meat samples with CCR from 98.2% to 100% and 63.1% to 73.7% in the train and test sessions, respectively (Panagou and others 2011). A recent study by Argyri and others (2013) utilized PLSR, ANN, RBF-SVM, GP, and GA to compare and predict the microbial spoilage of minced beef samples stored under aerobic and modified atmosphere packaging at 5 °C using FT-IR spectroscopy. It was observed that FT-IR calibration models showed good performance for predicting TVC and *Enterobacteriaceae* loads, and the multivariate analysis methods (RBF-SVM, PLSR) showed similar performances ($R^2 > 0.80$) and offered better predictions compared to GA-GP, GA-ANN, and GP ($R^2 < 0.71$). Another kind of neuro-fuzzy network modeling using a prototype defuzzification scheme in combination with PCA was applied to explore the potential of FT-IR spectroscopy for determining beef spoilage microorganisms during aerobic storage, and results showed that the adopted methodology was effective to classify the samples with high CCR for fresh of 95.8%, semi-fresh of 87.5%, spoiled of 100%, and overall CCR of 96% (Kodogiannis and others 2014b). Similarly, the extended normalized RBF-ANN and the Bayesian Ying-Yang expectation maximization algorithm were also successfully applied in FT-IR spectroscopy (Kodogiannis and others 2014a). In another work, ATR-FT-IR spectral information of intact chicken breast muscle packaged under aerobic conditions and stored at 4 °C for 14 d was collected and investigated for detection

of TVC using PCA, PLS-DA, and outer product analysis (OPA) based on SNV transformed FT-IR spectra. Results indicated that PCA and PLS-DA methods could not discriminate completely between days 0 and 4 samples, but could classify correctly days 8 and 14 samples. The performance of OPA on FT-IR spectral datasets revealed the positive correlations between the spectra and the effect of proteolysis because of the possible fact that the increase of free amino acids and peptides could be the main factor in the discrimination of intact chicken breast muscle (Alexandrakis and others 2012). Similarly, PLSR analysis was employed to estimate TVC, LAB, *Pseudomonas* spp., *Brochothrixthermosphacta*, and *Enterobacteriaceae* counts based on FT-IR spectral data. Analysis of an external set of samples allowed an evaluation of the predictability of the method, and the R^2_P of 0.798, 0.832, 0.789, 0.810, and 0.857 and RMSEP of 0.789, 0.658, 0.715, 0.701, and 0.756 $\log_{10}$ CFU/g were obtained (Vasconcelos and others 2014). Moreover, PLSR models were also conducted to correlate spectral data from FT-IR with minced pork meat spoilage during aerobic storage and good performance in classifying meat samples with the overall CCR of 94.0% and 88.1% in calibration and validation model was obtained. Similarly, PLSR models were employed to provide quantitative estimations of microbial counts during meat storage, and the calculated values of the accuracy factor showed that the average deviation between predictions and observations was 7.5% and 7.9% for TVC and *Pseudomonas* spp., and 10.7% and 11.3% for LAB and *B. thermosphacta* (Papadopoulou and others 2011). The above studies indicated that FT-IR spectral information in combination with an efficient choice of a learning-based modeling scheme could be considered as an alternative methodology for the accurate evaluation of meat spoilage.

Fluorescence spectroscopy

Fluorescence refers to the light emission when a fluorescent molecule or substructure, called a fluorophore, is subjected to ultraviolet or visible light. Fluorescence spectroscopy is more sensitive and selective in terms of organic and inorganic compounds and enables valuable analytical information to be obtained for quantification of fluorescent components and assessment of quality changes of food during storage (Guzman and others 2015). Recently, fluorescence spectroscopy has become quite popular as a tool in biological science related to food technology. The potential of fluorescence spectroscopy was investigated to determine TVC on pork meat surface stored aerobically at 15 °C for 3 d. Excitation (Ex)-emission (Em) matrix of fluorescence intensity was acquired and fluorescence from tryptophan (Ex = 295 nm and Em = 335 nm) and triphosphopyridine nucleotide (NADPH; Ex = 335 nm and Em = 450 nm) was detected, because of the fact that tryptophan and NADPH fluorescence changed with the growth of microorganisms, and thus microbial spoilage on meat could be detected from fluorescence. For example, Oto and others (2013) used fluorescence spectroscopy to predict the TVC value of pork based on PLSR analysis, and TVC was predicted with satisfactory determination coefficients of R^2_C = 0.94 and R^2_{CV} = 0.88. In another study, to improve the measurement accuracy, Shirai and others (2014) developed a 2-dimensional Savitzky–Golay second-order differentiation method to preprocess the excitation–emission matrix. The plate count on pork meat surface was predicted with good result of R_P = 0.90 to 0.94 and RMSEP of (0.68 to 0.79) $\log_{10}$ CFU/g. In addition, better prediction accuracy was obtained when the sensitivity of the fluorescence spectrophotometer was set to focus on fluorescence from NADPH than that from both tryptophan and NADPH. Similarly, the potential of front-face fluorescence fingerprint (FF) spectroscopy coupled with PLSR analysis was also investigated to develop a nondestructive method for predicting APC on beef muscle stored aerobically at 15 °C for 0, 2, 4, 6, and 10 h. FFs were collected in both excitation and emission wavelength ranges of 200 to 900 nm. The PLSR model showed high accuracy with a prediction error of 0.75 $\log_{10}$ CFU/g. Furthermore, the regions where the regression coefficient of the PLSR model was relatively higher were consistent with those of the FF peaks of 5 intrinsic fluorophores of tryptophan, NADPH, vitamin A, porphyrins, and flavins. It was suggested that changes in the autofluorescence of these intrinsic fluorophores because of the metabolism of bacterial flora on meat were reflected in the PLSR model for predicting APC from the FF dataset (Yoshimura and others 2013). Therefore, FF spectroscopy coupled with multivariate analysis was considered to be applicable to the nondestructive determination of APC on the surface of lean beef.

Raman spectroscopy

Raman spectroscopy focuses on the polarizability response of molecular vibrations (Scotter 1997) and is also one of the vibrational spectroscopies based upon the interaction of laser radiation with molecular vibrations in order to obtain relative information about the material (Celedon and Aguilera 2002; Sowoidnich and others 2010). One major advantage of this technique is its ability to provide information about concentration, structure, and interaction of biochemical molecules within intact cells and tissues (Marquardt and Wold 2004). Raman spectroscopy is a fast and noninvasive method that has been proven for its usefulness as a tool for investigating biological matter such as the detection of bacterial contamination in cell or tissue cultures or of foodborne microorganisms on food surfaces (Scheier and others 2014). Raman microspectroscopy with Raman excitation wavelengths in the visible wavelength region is a very promising method to detect microorganisms on a single-cell level with minimal sample preparation. Thus, by applying single-cell Raman microspectroscopy, the time-consuming precultivation step can be avoided and, therefore, the detection process of microorganisms can be significantly accelerated. Therefore, Raman microspectroscopy was applied to rapidly detect the pathogens in meat and poultry. Meisel and others (2014) built a 3-level classification SVM model based on the entire amount of Raman data, and these different levels of the classification model achieved accuracies in the range of 90.6% to 99.5%, leading to the test samples being correctly assigned to their genus, and for the most part, down to the species level. This study illustrated that Raman microspectroscopy in combination with chemometrics could be a promising supplement to currently established methods.

Applications of Hyperspectral Imaging

Hyperspectral imaging technique, as an innovative and emerging tool, combines traditional spectroscopy and digital imaging or computer vision (Sun 2004; Wang and Sun 2002; Sun and Brosna 2003; Jackman and others 2008; Costa and others 2011) to acquire both spectral and spatial information from the object (Mathiassen and others 2011; Elmasry and others 2012; Sone and others 2012; Huang and others 2014). Figure 1 shows the common steps of hyperspectral imaging for the detection of food quality. In recent years, hyperspectral imaging has been widely studied for quality and safety evaluation of muscle food products (ElMasry and others 2011, 2012; Barbin and others 2012; Wu and others 2012; Wu and Sun 2013). For the rapid detection of microbial spoilage,

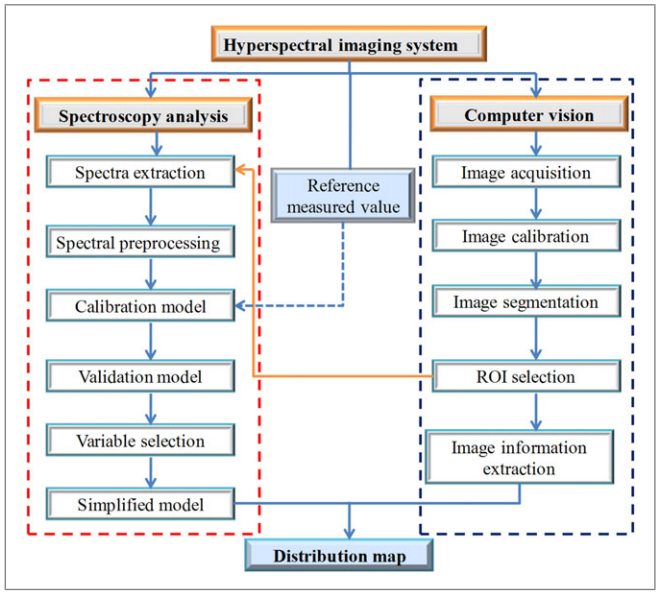

Figure 1–Main steps of application of hyperspectral imaging techniques for the detection of microbial spoilage in muscle foods.

the applications of hyperspectral imaging are mainly related to the measurement of microbial plate count, *Enterobacteriaceae*, *Pseudomonas*, *E. coli* loads, and lactic acid bacteria.

Microbial plate count measurement

TVC of bacteria is one of the most important indexes in the evaluation of quality and safety of muscle foods. Therefore, NIR hyperspectral imaging and spectroscopic transforms were used to determine TVC in chicken breast fillets (Feng and Sun 2013a). Full wavelength PLSR models were established to correlate the 3 spectral profiles with measured bacterial counts. Based on the absorbance spectral data, the corresponding PLSR model showed the best prediction performance, with R_C of 0.97, R_{CV} of 0.93, and RMSEC of 0.37 $\log_{10}$ CFU/g and RMSECV of 0.57 $\log_{10}$ CFU/g, respectively. The simplified model using 7 wavelengths (1145, 1458, 1522, 1659, 1666, 1669, and 1672 nm) selected by stepwise regression based on Kubelka–Munck spectra also showed excellent accuracy and robustness with an indicative high ratio of performance to RPD value of 3.02, R_C of 0.96, R_{CV} of 0.94, and RMSEC of 0.40 $\log_{10}$ CFU/g and RMSECV of 0.50 $\log_{10}$ CFU/g, respectively. In another work, a pushbroom hyperspectral imaging system in the NIR range of 900 to 1700 nm was exploited for determining the TVC and psychrotrophic plate count (PPC) in pork meat during chilled storage at 0 and 4 °C for 21 d. The PLSR algorithm was applied to fit the spectral data extracted from the samples to the measured TVC and PPC values. The best regressions were obtained with R^2_P of 0.93 and 0.93 for log (TVC) and log (PPC), respectively. Similarly, 11 key wavelengths (932, 947, 970, 1034, 1094, 1134, 1151, 1211, 1344, 1621, and 1641 nm) and 10 optimal wavelengths (947, 1118, 1128, 1151, 1211, 1241, 1388, 1621, 1641, and 1655 nm) were identified as good indicators by the weighted regression coefficients from PLSR analysis for optimal regression models of TVC and PPC. The optimized PLSR models also presented good accuracy ($R^2_P = 0.81$ and 0.81). The obtained results were encouraging and showed a promising potential of hyperspectral technology for detecting bacterial spoilage in pork and tracking the increase of microbial growth of chilled pork during storage at different temperatures (Barbin and others 2013). However, VIS/NIR hyperspectral imaging was also developed to detect the microbial plate count for the evaluation of muscle foods spoilage. Peng and others (2011) used spatially resolved hyperspectral scattering imaging in the spectral region of 400 to 1100 nm for predicting the microbial spoilage of beef refrigerated at 8 °C. Results revealed that the spectral scattering profiles at individual wavelengths were accurately fitted by a 2-parameter Lorentzian distribution function. The MLR model for TVC prediction was established using 6 optimal wavelengths (596, 822, 838, 841, 889, and 900 nm) and showed better prediction capability with R^2_P of 0.95 and SEP of 0.30 for $\log_{10}$ (TVC), compared with the PLSR model ($R^2_P = 0.92$ and SEP = 0.63). It was also demonstrated that MLR prediction models using only a few wavelengths were superior to the PLSR model for predicting bacterial spoilage in beef. In another study, potential of time series-hyperspectral imaging in visible and near-infrared regions (400–1700 nm) for the rapid and noninvasive determination of TVC of salmon flesh during spoilage was investigated. On the basis of full wavelengths, the LS-SVM model exhibited better predictive robustness with increase by 0.078 and 2.11 in R^2_P and RPD than the PLSR model with relatively poor performance ($R^2_P = 0.887$ and RPD = 2.978). Competitive adaptive reweighted sampling (CARS) was conducted to identify the most important wavelengths that had the greatest influence on the TVC prediction throughout the entire wavelength range. The CARS-PLSR model established using the selected 8 wavelengths (495, 535, 550, 585, 625, 660, 785, and 915 nm) was considered to be optimal for the TVC measurement of salmon flesh with R^2_P of 0.985, and RPD of 5.127 (Wu and Sun 2013b). Similarly, the feasibility of visible and near-infrared hyperspectral imaging in the range of 400 to 1000 nm for determining TVC to evaluate microbial spoilage of fish fillets was investigated. PLSR and LS-SVM models established based on full wavelengths showed excellent performances and the LS-SVM model was better, with higher RPD of 3.89, R^2_P of 0.93, and RMSEP of 0.49 $\log_{10}$ CFU/g. Seven optimal wavelengths were selected by successive projections algorithm (SPA) and the simplified SPA-PLSR was better than SPA-LS-SVM models with RPD of 3.13, R^2_P of 0.90, and RMSEP of 0.57 $\log_{10}$ CFU/g (Cheng and Sun 2015a). Another study reported by Huang and others (2013) developed a hyperspectral imaging system in the wavelength range of 430 to 960 nm for the rapid detection of TVC in pork meat. Unlike other studies, the authors combined the spectral and image information to create data fusion for modeling using back-propagation artificial neural network (BP-ANN). Results showed that the model based on data fusion (the spectra were extracted and selected by synergy interval PLS analysis and the image variables were extracted using gray level co-occurrence matrix (GLCM) algorithm) was superior to models generated by individual spectral or image information, which achieved R^2_P of 0.83 and RMSEP of 0.243 $\log_{10}$ CFU/g. Therefore, data fusion in the processing of hyperspectral image is a new thought for improving the accuracy and robustness of prediction and facilitating the development and application of hyperspectral imaging technique in the food industry.

To realize real on-line detection, multispectral imaging as an alternative and efficient tool has been increasingly developed for the assessment of microbial spoilage. Dissing and others (2012) used a multispectral imaging system in 18 different wavelengths ranging from 405 to 970 nm for spoilage degree detection of pork meat processed by aerobic and modified atmosphere packaging as well as under different temperatures. In addition, a sensory evaluation panel was recommended to judge the spoilage degree of all meat samples into 1 of 3 classes (fresh, semi-fresh,

and spoiled). Results indicated that the multispectral imaging device to some extent was capable of differentiating the meat samples with a correct classification rate of 76.13% according to the defined sensory scale. The TVC value was also successfully predicted by this technology with SEP of 7.47%. Similarly, another multispectral imaging system in 19 different wavelengths ranging from 400 to 1000 nm was exploited to determine APC in cooked pork sausages stored at 4 °C for 28 d. PLSR algorithm was applied to establish the prediction model, and satisfactory performance was presented with R^2_P of 0.89 (Ma and others 2014). Based on these investigations, it is interesting to find that, compared with the full wavelength models, the simplified models turned out to be more robust, as indicated by a smaller difference in both correlation coefficients and root mean squared errors for both calibration and cross validation. The improvement in model capability can probably be attributed to the elimination of the uninformative or even misleading wavelengths for explaining the spoilage process of muscle foods. In addition, the reduction of spectral multicollinearity could also be part of the reasons for model enhancement. It has also been suggested that multispectral imaging could be a promising tool in developing rapid and nondestructive measurement of microbial spoilage for the meat industry.

Measurement of *Enterobacteriaceae* loads

Enterobacteriaceae are a large group of bacteria that are rod-shaped, Gram-negative, and facultatively anaerobic as well as nonsporeforming. This group is directly associated with the intestines and feces of mammals and birds and includes enteric pathogens such as *E. coli, Shigella, Salmonella,* and *Yersinia*. Therefore, the amount of *Enterobacteriaceae* is commonly used as a good indicator for food sanitation by accounting for potential fecal contamination and existence of pathogenic bacteria. Hyperspectral imaging technique in the spectral range of 930 to 1450 nm was conducted for the quantitative and direct determination of *Enterobacteriaceae* loads on chicken fillets. PLSR model established using full wavelengths performed well with R^2_P of 0.82 and RMSEP of 0.47 $\log_{10}$ CFU/g. For the further development of simplified models, 3 characteristic wavelengths (930, 1121, and 1345 nm) were selected by weighted PLS regression coefficient methods, and the new developed model was competent, and more preferred, for predicting *Enterobacteriaceae* loads with R^2_P of 0.87 and RMSEP of 0.44 $\log_{10}$ CFU/g (Feng and others 2013). The results indicated that the selected 3 optimal wavelengths were efficient and informative to specify the variations of *Enterobacteriaceae* loads in chicken meat. To determine and monitor the harmful microbial contamination occurring in edible salmon flesh, an NIR hyperspectral imaging system (900 to 1700 nm) was applied to detect the loads of *Enterobacteriaceae*. PLSR models were created based on 3 spectral transforms of reflectance, absorbance (A), and Kubelka–Munck (KM). Using full wavelengths and absorbance data, the predictive PLSR model displayed the best predictive ability with correlation coefficients of prediction (R_P) of 0.954, RPD of 3.313, and RMSEP of 0.481 $\log_{10}$ CFU/g. The simplified PLSR model based on the 9 influential wavelengths (931, 1138, 1175, 1242, 1359, 1628, 1641, 1652, and 1655 nm) selected by CARS algorithm and absorbance data provided the best prediction accuracy ($R_P = 0.964$, RPD = 3.715 and RMSEP = 0.429 $\log_{10}$ CFU/g; He and Sun 2015). These studies demonstrated that hyperspectral imaging combined with chemometric analysis is a potential tool for determining meat sanitation and detecting bacterial pathogens on a food matrix without using complicated laboratory procedures.

Measurement of *Pseudomonas* loads

Pseudomonas is one of the bacterial genera most often isolated in high numbers on spoiled meat. *Pseudomonas* is a genus of rod-shaped and Gram-negative bacteria that require only simple nutrition for growth and such easy-to-survive characteristic have contributed to its wide distribution in the environment, and it is closely associated with food waste due to spoilage species causing food spoilage (Feng and Sun 2013b). Like with other bacteria, hyperspectral imaging can be used to determine its loads. For example, a line-scan NIR hyperspectral imaging system (900 to 1700 nm) in tandem with PLSR and GA was exploited for its potential in direct and fast determination of *Pseudomonas* loads in raw chicken breast fillets. The best full-wavelength PLSR model attained based on spectral images preprocessed with SNV presented the R_P of 0.81 and RMSEP of 0.80 $\log_{10}$ CFU/g. The simplified models based on 14 wavebands selected by using a proposed 2-step method and GA produced better results than the original models with R_P of 0.88 and RMSEP of 0.64 $\log_{10}$ CFU/g (Feng and Sun 2013b).

E. *coli* measurement

E. coli is a common bacterium with the characteristics of being rod-shaped, Gram-negative, facultatively anaerobic, and nonsporeforming (Gram and Huss 1996). *E. coli* O157:H7 is an enteric bacterium that has been implicated in food- and water-borne human illnesses worldwide, including bloody diarrhea, hemolytic uremic syndrome, and hemorrhagic colitis (Gram and Huss 1996). A lab line-scanning hyperspectral scattering technique in the spectral range of 400 to 1100 nm was used to detect *E. coli* contamination in pork meat. The scattering profiles were then fitted by Lorentzian distribution function to provide 3 parameters of α (asymptotic value), β (peak value), and γ (full width at $\beta/2$). The best predictive MLR model was attained based on parameter α with the highest R^2_{CV} of 0.77 and the lowest of RMSECV of 0.84 $\log_{10}$ CFU/g (Tao and others 2012). In a recent study, to improve the prediction accuracy, based on the previous investigation, the authors developed a novel method by modified Gompertz function to extract the scattering characteristics of pork meat from the spatially resolved hyperspectral images. MLR models were established using both individual Gompertz parameter (α, β, ε, and δ) and integrated parameters, and the results showed that Gompertz parameter δ was superior to other individual parameters. The MLR model using the integrated parameter showed the best prediction capability ($R^2_{CV} = 0.88$ and RMSECV = 0.64 $\log_{10}$ CFU/g; Tao and Peng 2014). It can be obvious to discover that different specific functions presented their individual advantages for enhancement of applications of hyperspectral imaging. Similarly, hyperspectral imaging in the spectral range of 400 to 1000 nm was developed to measure *E. coli* loads in grass carp fish for the evaluation of microbial spoilage. The full-wavelength PLSR model showed good performance with RPD of 5.47, $R^2_P = 0.88$ and RMSEP of 0.26 $\log_{10}$ CFU/g. Six characteristic wavelengths were selected by the weighted regression coefficients from PLSR analysis and used to simplify the models. The simplified MLR model exhibited more competent prediction capability than PLSR analysis (RPD = 5.22, $R^2_P = 0.87$ and RMSEP = 0.27 $\log_{10}$ CFU/g) (Cheng and Sun 2015b). The results mentioned above demonstrated that hyperspectral imaging technique combined with some effective functions and multivariate analysis was promising for the rapid and nondestructive determination and quantification of *E. coli* contamination on pork and fish muscle.

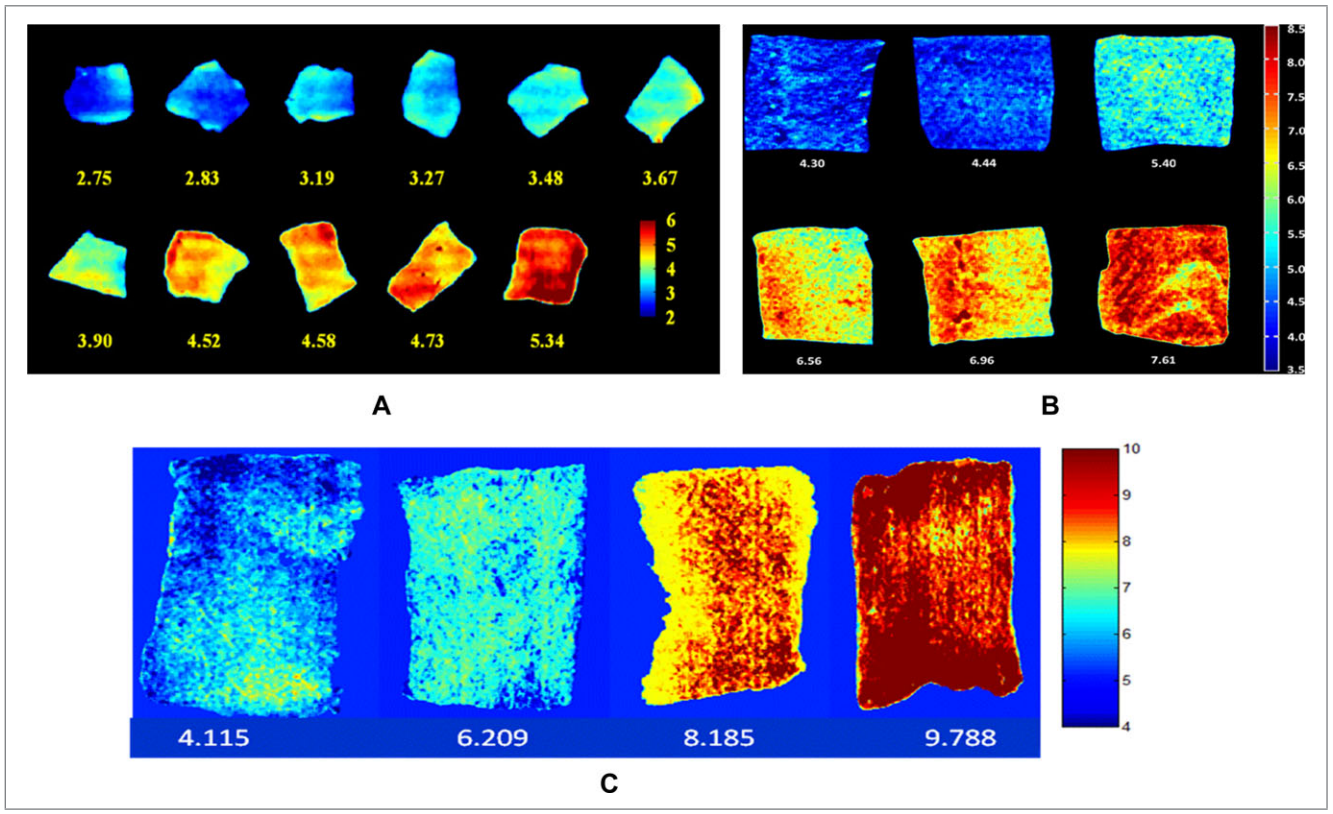

Figure 2–Visualization of microbial spoilage distribution of muscle foods. (A) *Enterobacteriaceae* distribution in chicken fillets; (B) TVC distribution in salmon fillets; and (C) *E. Coli* distribution in grass carp fish fillets.

LAB measurement

LAB are the major bacterial group associated with the spoilage of refrigerated vacuum- or modified atmosphere-packaged cooked, cured meat products. The genus or species of LAB responsible for spoilage depends on product composition (product-related flora) as well as the manufacturing site (Ringø and Gatesoupe 1998). Generally, LAB are present in the initial microflora in low numbers and are therefore rarely responsible for the spoilage of fresh proteinaceous foods. However, LAB have been identified as the major spoiling microorganisms of vacuum-packed meat and poultry and are also suggested as possible spoilers of lightly preserved fish products (Ringø and Gatesoupe 1998). Typical lactic acid bacteria are *Lactobacillus*, *Streptococcus*, *Leuconostoc,* and *Pediococcus* spp. (Gram and Huss 1996). LAB spoil foods by the fermentation of sugars and commonly cause undesirable defects, such as sour off-flavors, discolouration, gas production, slime production, and decrease in pH (Smulders and Greer 1998). Therefore, rapid and real-time monitoring the spoilage by LAB in muscle foods is very important. The emerging hyperspectral imaging technology (900 to 1700 nm) with chemometric analysis was applied to determine LAB in farmed salmon flesh during cold storage. LS-SVM algorithm using the full wavelength was used to calibrate NIR-range spectral data, generating an R_P of 0.93 with RMSEP of 0.52 $\log_{10}$ CFU/g. CARS algorithm was employed to reduce the spectral redundancy and identify the most informative wavelengths across the entire wavelength range. The optimized model (CARS-LS-SVM) built by 8 key wavelengths (1155, 1255, 1373, 1376, 1436, 1641, 1665, and 1689 nm) also generated good results with R_P of 0.93 and RMSEP of 0.53 $\log_{10}$ CFU/g (He and others 2014). The results obtained indicated that NIR hyperspectral imaging could be considered as a rapid, nondestructive, and efficient tool for the evaluation of LAB spoilage in salmon flesh.

Visualization of microbial spoilage distribution

The great advantage of hyperspectral imaging is its ability for visualization of the detailed quality distribution in spite of spatially heterogeneous properties of the tested samples. Generally, the method used for visualizing the microbial spoilage distribution is to calculate the microbial index (such as TVC, APC, and the detailed microbial loads) of each pixel by applying chemometric analysis with the spectrum of corresponding pixels, which can be regarded as a linear or nonlinear mathematical combination of images at the optimal wavelengths selected by variable selection algorithms (Cheng and others 2014). The finally obtained chemical images or visualized distribution maps are usually shown in a linear color bar with different colors (blue color showing the pixels with low values and red color indicating the pixels with high values). Different colors in the final distribution maps represent different values of microbial index in the image in proportion to the spectral differences of the corresponding pixels, which is helpful to understand and interpret the microbial spoilage by inspecting the different color distribution. In addition, according to the distribution map, it is very useful for the muscle food processing industry to automatically select the desirable parts/sections for making the products. It is also effective to avoid and control the occurrence of food safety problems. Figure 2 shows some examples of visualization of microbial spoilage distribution in muscle foods.

Table 2–Applications of spectroscopic techniques for the detection of microbial spoilage in muscle foods.

Muscle food	Technique	Detection of	Method	R^2/CCR	Reference
Chicken	VIS/NIR	APC	PCA, PLSR	0.83	Lin and others (2004)
Chicken	VIS/NIR	APC	PLSR	0.82	Grau and others (2011)
Atlantic salmon	NIR	APC	PCA, PLSR	0.95	Tito and others (2012)
Flounder fillet	NIR	TVC	GA, BP-ANN	0.97	Duan and others (2014)
Beef	FI-IR	TVC	PCA, PLSR	0.80	Ammor and others (2009)
Beef	FI-IR	TVC	ANN	> 0.92	Argyri and others (2010)
Beef	FI-IR	TVC	ANN, PLSR	0.95	Panagou and others (2011)
Beef	FI-IR	TVC	PLSR, ANN, RBF-SVM	> 0.80	Argyri and others (2013)
Pork	Fluorescence spectroscopy	TVC	PLSR	0.94	Oto and others (2013)
Pork	Fluorescence spectroscopy	TVC	PLSR	0.85	Shirai and others (2014)
Pork	Raman spectroscopy	TVC	SVM	94.3%	Meisel and others (2014)

Table 3–Applications of HSI technique for the detection of microbial spoilage in muscle foods.

Muscle food	Wavelength range (nm)	Detection of	Method	R^2	Reference
Chicken	900–1700	TVC	PLSR	0.86	Feng and others (2013)
Pork	900–1700	TVC	PLSR	0.93	Barbin and others (2013)
Pork	900–1700	PPC	PLSR	0.93	Barbin and others (2013)
Beef	400–1100	TVC	MLR	0.95	Peng and others (2011)
Salmon	400–1700	TVC	LS-SVM	0.96	Wu and others (2013b)
Salmon	400–1700	TVC	PLSR	0.89	Wu and others (2013b)
Pork	430–960	TVC	BP-ANN	0.83	Huang and others (2013)
Chicken	930–1450	*Enterobacteriaceae*	PLSR	0.82	Feng and others (2013)
Chicken	900–1700	*Enterobacteriaceae*	PLSR	0.91	He and others (2015)
Chicken	900–1700	*Pseudomonas*	PLSR	0.66	Feng and others (2013)
Pork	400–1100	*E. coli*	MLR	0.77	Tao and others (2012)
Grass carp fish	400–1000	*E. coli*	PLSR	0.88	Cheng and others (2015)
Salmon fillet	900–1700	LAB	LS-SVM	0.87	He and others (2014)

Advantages and Disadvantages

It is well-known that traditional microbiological methods are generally laborious, time-consuming, and require a complex process for sample preparation. The obtained microbiological data cannot provide relevant information about eating quality and freshness. Compared with traditional microbial analytical methods, spectroscopic and hyperspectral imaging techniques have been proved to be rapid, nondestructive, noncontact, objective, and cost-effective, which can be used as routine procedures implemented in the muscle food industry for automated grading and detection of the muscle products and other purposes since these advanced techniques can be applied to realize online and real-time inspection, to develop labor-saving devices, to create higher economic and social benefits, and further to offer guaranteed quality of food products for human consumption and international trade. Particularly, hyperspectral imaging can provide the distribution map of microbial spoilage in muscle foods. Table 2 and Table 3 list the successful applications of spectroscopic and hyperspectral imaging techniques for detecting microbial spoilage in muscle foods. However, some disadvantages about the applications of these techniques are still needed to be noted. For quantitative analysis, VIS and NIR spectroscopy are not independent of the disadvantages arising from the reference method used for calibration, which requires a certain number of samples with known analyte concentrations. Therefore, to some extent, the predictive accuracy of NIR spectroscopy depends on the reliability and accuracy of the reference method. Moreover, due to the limited spatial field of view, NIR spectroscopy cannot provide spatial information of the samples which is essential for visualizing the distribution of the microbial spoilage in muscle foods (Cozzolino 2012). Fluorescence spectroscopy is easily affected by the interference of the mutual elements and overlapping peaks which can result in the disappearance of fluorescence (Strasburg and Ludescher 1995; Karoui and Blecker 2011). Raman spectroscopy also shows some restrictions such as inherently weaker effect of Raman-scattering, stronger interference of biological fluorescence, and higher instrumental costs; and some heat generated by the laser may affect the measurement effectiveness (Afseth and others 2006). In addition, for Fourier transform spectrum analysis, it usually causes the nonlinear problems of the curve, and the intensity of Raman scattering is easily influenced by implicit factors such as the optical system parameters. As to hyperspectral imaging, hyperspectral images usually contain much unnecessary and redundant information than a single color image, which shows considerable challenges and requires more time and superior skills to mine the hidden data by multivariable analysis and to obtain valuable information from the hyperspectral images (Cheng and Sun 2013). The speed of hardware in a hyperspectral imaging system needs to be improved to satisfy the rapid acquisition and analysis of the huge hyperspectral data cube (Wu and Sun 2013a). Considering the long time needed for data acquisition and analysis, hyperspectral imaging is not suggested for direct implementation in online applications. In addition, hyperspectral imaging needs accurate reference calibration and robust model transfer algorithms, and must not have wider detection limits compared to chemical-based analytical methods. Moreover, multivariate analysis and variable selection are usually used to reduce the effect of the problem of multicollinearity in hyperspectral imaging. Besides, it is more difficult to explore developed or novel algorithms for eliminating data redundancy and accelerating online inspection speed."

Conclusion and Future Trends

The applications of some nondestructive and noninvasive spectroscopic techniques, including VIS and NIR spectroscopy, FT-IR spectroscopy, Raman spectroscopy, fluorescence spectroscopy, and hyperspectral imaging in tandem with chemometrics such as PCA, PLSR, MLR, LS-SVM, and ANN, have been widely reported and described for their great potentials and analysis of microbial spoilage in muscle foods. These spectroscopic techniques have demonstrated great promise in the rapid and nondestructive

detection of TVC, APC, *Enterobacteriaceae*, *Pseudomonas*, *Escherichia coli*, and LAB loads for evaluating the quality and safety of muscle foods, and they have the potential to replace industrial traditional methods and enhance consumer confidence and acceptability of muscle foods. However, they also have their own defects regarding model accuracy and robustness and practical online detection speed. Therefore, further methodology development including spectral extraction and image analysis algorithms and software to enhance the sensitivity and accuracy of the technique should be strengthened. In addition, multispectral imaging should be developed for true online detection. Multispectral algorithms must be fairly simple to enhance performance and also to save time. It is also interesting to investigate the changes of spectral features and image information of the presence of microorganisms and their growth in muscle foods, and must figure out the changing information of spectra and images, which can clearly reveal the spoilage condition and growth stage of microorganisms.

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