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Principal Component Analysis in the Development of Optical and Imaging Spectroscopic Inspections for Agricultural / Food Safety and Quality

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1. Introduction

The American Society for Testing and Materials has defined the principal component analysis (PCA) as "a mathematical procedure for resolving sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy. As successive components are calculated, each component accounts for the maximum possible amount of residual variance in the set of data. In spectroscopy, the data are usually spectra, and the number of components is smaller than or equal to the number of variables or the number of spectra, which is less."(ASTM, 1990) Many books are available that explain the theory and mathematical basis of PCA implementation in vibrational spectroscopy covering the visible, near-infrared (NIR), mid-infrared (IR), and Raman regions (Burns & Ciurczak, 2001; Mark & Workman, 2007; Ozaki et al., 2007; Williams & Norris, 2001). This Chapter only outlines the usefulness and effectiveness of PCA in extracting valuable information from optical and imaging spectroscopy of complex agricultural /food matrixes and subsequently the development of optical and imaging spectroscopic tools for their safety and quality assessment within the recent ten years.

Interpretation of PCA pattern correctly is very important and many types of plots are available. The most frequent and essential elements are score-score and loading-loading plots. The correlations among samples are indicated by their scores (or projections) on new principal components (PCs) or the latent variables. Similar samples tend to group together in the score-score plot, and in turn, atypical samples (i.e., outliers) could be easily detected by the simple visualization.

The relationships between the new PCs and the original variables are revealed in the loading plots, in which the degree of importance decreases with the increasing PCs. A loading-loading plot displays the contributions of variables to a model directly, that is, proportional to the square of the distance from the origin. Variables far away from the origin mean their importance in discriminating the samples, whereas those close to the origin exhibit little effects on sample separation. Variables located in the same or opposite domain reflect similar or completely different information, and those with mutual locations share the common attributes.

For the comparison, standardized scores and loadings might be used, and also scores and loadings could be simultaneously displayed in biplots. In some cases, visual observation is extremely useful for elucidating the multivariate data, especially when the numbers of samples and variables are small.

2. PCA and spectroscopic sensing

Concurrent improvements in analytical techniques and data processing enable the spectroscopic devices based sensors to be more sensitive and selective, smaller, cheaper, and more robust than their laboratory version. Both optical and imaging spectroscopy is making critical judgment in assessing the safety, security, and quality aspects of agricultural and food products.

Hyper- and multi- imaging spectroscopy, which combines the features of imaging technique and vibrational spectroscopy, has been developed as an inspection means for quality and safety assessment of a number of agricultural and food products, mostly thanks to its non-invasive nature and capacity for large spatial sampling areas. However, spectral imaging technology currently has met some degree of hindrance as automated on-line systems, because current image acquisition and data analysis speeds are too slow for on-line operations.

To design rapid optical and imaging sensing systems, several essential spectral bands (usually two or three) are first sought through a variety of strategies, such as through the analysis of spectral differences in conventional visible/NIR spectra (Liu et al., 2003a), the use of PC loadings from PCA on conventional visible/NIR spectra (Windham et al., 2003), and the use of PCA on hyperspectral imaging data (Kim et al., 2002a). The selected wavebands should not only reflect the chemical /physical information in samples, but also maintain successive discrimination and classification efficiency.

Multivariate quantitative models from principal component regression (PCR) and partial least squares (PLS) require a large number of training samples to build accurate and reliable calibrations. It takes a number of initial work collecting the samples and measuring the references of targeted constitutes by the established or standard methods. In general, quantitative models will always predict reasonable values for the calibrated constitutes, given the spectra of the unknown samples are fairly similar to the training set. In other words, the reported concentrations alone will not indicate if the samples are contaminated or defected. In some scenarios, the constituents' information is not easy to determine or the samples are hard to collect. Nevertheless, the spectrum of a sample is unique to its compositions, and samples of similar compositions should have spectra that are very similar as well. Therefore, it might be possible to highlight a difference between a "good" sample and a "bad" one by only comparing the spectra with such simple methods as visual inspection or spectral subtraction. Unfortunately and apparently, these subjective methods cannot be applied for large and complicated spectral sets. Consequently, PCA based discriminant analysis, a process of classifying the samples on the basis of their spectral characteristics and also their logical assignment, is preferred and utilized considerably.

It is conceivable that the "traditional" procedure of developing the calibration models for quantitative analysis could be replaced in many applications by the operations based on discriminant analysis that identifies samples to be kept and those to be discarded or degraded. As an example, this could be applicable to automatic grading of grains, where such a factor as damage incurred by weather or storage conditions could result in lowering of the commercial value. Also, it could be used in screening for quality indices in plant breeding research, such as kernel texture and water absorption, by developing classes with high, medium, and low levels of the respective parameters.

2.1 Meat safety and quality

2.1.1 Diseased poultry carcasses inspection

Since the 1957 passing of the Poultry Products Inspection Act, the U.S. Department of Agriculture (USDA) inspectors have been inspecting all chickens processed at U.S. poultry plants for the indications of diseases or defects, by visually examining the exterior, the inner surface of the body cavity, and the internal organs of every chicken carcass. Especially in 1996, the USDA Food Safety and Inspection Service (FSIS) has implemented the Hazard Analysis and Critical Control Point (HACCP) program throughout the country to ensure food safety and prevent food safety hazards in the inspection of process for poultry, egg, and meat products (USDA, 1996). One requirement includes a zero tolerance standard for chickens with the signs of infections from septicemia (caused by the presence of pathogenic microorganisms or their toxins in the bloodstream) and toxemia (the result of toxins produced from cells at a localized infection or from the growth of microorganisms), and such poultry carcasses must be removed from the processing lines.

As the productivity and foreign trading arise, along with the desire from health-conscious consumers for more poultry products, workload at processing lines grows accordingly. Compared to the human inspection speed of 30-35 chickens per minute, many processing lines can run at 140 chickens a minute. To aid poultry plants in satisfying the government food safety regulations while maintaining their competitiveness and meeting consume demand, many researchers at the USDA Agricultural Research Service (ARS) during the last decade have focused on the development of new inspection technologies such as automated computer vision inspection systems.

The first-generation transportable system, developed by Chen et al. (1996), was a visible/NIR spectrophotometer based for the use in on-line and real-time classification of poultry carcasses into normal and abnormal classes. The system measured the spectral reflectance of poultry carcasses in the visible/NIR region of 471 to 963.7 nm and an optimal neural network classifier was used for the separation of poultry carcasses into two categories at the average accuracy of 97.4%. In order to facilitate the selection of optimal spectral regions and imaging camera and also further to enhance the effective discrimination, a number of strategies were taken, including two-dimensional (2D) correlation analysis on small samples and PCA approach on large sample sets. For example, Liu et al. (2000) first applied 2D correlation analysis to characterize the spectral variations between wholesome and unwholesome (diseased) chicken meats. In this work, they proposed the spectral band assignments for deoxymyoglobin (445 nm), oxymyogobin (560 nm), and metmyoglobin (485 nm) species that are mainly responsible for the meat color. Also, they concluded that the three pigments co-exist in all fresh-cut wholesome and diseased meats, but with a clear indication that wholesome meats have more variation in deoxymyoglobin and oxymyogobin and less metmyoglobin than do diseased meats.

With the consideration of significant absorptions in the 400-500 nm range, the follow-up experiments had been conducted by the use of optical spectrometers capable of scanning the spectra in much shorter wavelength up to 400 nm (Liu & Chen, 2003). They compared the PCA/SIMCA (soft independent modeling of class analogies) 2-class (wholesome vs. unwholesome) classification results between the visible spectral region (400-700 nm) and the entire 400-2500 nm spectral region. It was observed that the differentiation in the 400-700 nm range is almost as effective as that from the entire spectral region with a correct separation of 93.8%. Their findings echoed the observation that visible spectroscopy is useful in studying the variation of meat color.

Chao et al. (2003) took a different visible/NIR spectroscopic system and discrimination strategy intended to further understand how the wholesome and unwholesome carcasses could be separated. Using PCA and a linear discriminant function, they reported the best visible/NIR classification model with correctly classified 100%, 90.0%, and 92.5% rate of the whole (skin and meat) samples for wholesome, septicemia, and cadaver categories, respectively. Examination of the PCA loadings for the whole samples suggested that the better discrimination of whole samples was dependent on spectral variation related to different forms of myoglobin present in the chicken meat, i.e. deoxymyoglobin, metmyoglobin, and oxymyoglobin. In particular, key wavelengths were identified at 540 and 585 nm, which have been identified as oxymyoglobin bands, for PCs 1 and 2; 485 nm, metmyoglobin, for PC 3; and 440 nm, deoxymyoglobin, for PC 8.

The second-generation system involved a development of hyper- and multi- spectral imaging systems. At earlier stage, Park et al. (1996) first described the spectral image characterization of poultry carcasses at a variety of conditions based on the gray-scale intensity, Fourier power spectrum, and fractal analysis, and then reported a neural network classifier performance of 91.4% accuracy for the separation of tumorous carcasses from normals based on the images scanned at both 542 and 700 nm wavelengths.

Through the accumulated knowledge from extensive studies over the years, the line-scan image camera capable of scanning the visible region of 389 to 753 nm was adopted for the use in the most recent investigations, as reported by Chao' team (Chao et al., 2007; Yang et al., 2010). They documented the achievement of 90.6% classification accuracy for wholesome birds and 93.8% accuracy for diseased birds in the calibration data set as well as 97.6% accuracy for wholesome birds and 96.0% accuracy for diseased birds in the testing data set during the in-plant trial. Finally, they concluded that the hyper- and multi- spectral line-scan imaging systems can be used for automated on-line inspection of chicken carcasses for the detection of systemically diseased birds on high-speed processing lines, ultimately to increase inspection efficiency, reduce labor and cost, and produce significant benefits for poultry processing plants.

2.1.2 Fecal contaminated poultry carcasses inspection

Contamination of meat and poultry with food-borne bacterial pathogens can potentially occur as a result of exposure of the animal carcass to fecal materials during or after slaughter. Microbial pathogens can be transmitted to humans by consumption of contaminated undercooked or mishandled meat and poultry. Bacterial pathogens in food cause an estimated 76 million cases of human illness and up to 5000 death annually in the

U.S. To ensure a healthy and safe meat supply, the USDA FSIS has established a zero tolerance policy to minimize the likelihood of bacterial pathogens on the surfaces of meat and poultry carcasses during the slaughter. Applicable HACCP programs require individual meat processor to identify all food safety hazards in the process and to identify critical control points adequate to prevent them. Preventing carcasses with visible fecal contamination from entering the chlorinated ice water tank (chiller) is critical for preventing cross-contamination of other carcasses. Thus, the final carcass wash to remove all surface-adhering feces, before entering the chiller, has been adopted by many poultry processors as an HACCP system critical control point.

Compliance with zero tolerance in meat processing is routinely verified through human visual observation where the criteria of color, consistency, and composition are used for the identification. Trained inspectors use the established guidelines to verify that carcasses with visible fecal contaminations must be removed prior to entering the chillers. Current visual inspection is both labor intensive and prone to both human error and inspector-to-inspector variation. Therefore, investigators at the USDA ARS have been developing multi- and hyper- spectral imaging systems in real-time on-line detection of fecal contaminated chicken carcasses (Lawrence et al., 2003; Park et al., 2004).

Aiming to improve the detection performance of imaging systems with optimum settings, Windham et al. (2003) reported the use of multivariate data analysis on visible/NIR reflectance spectra to determine specific wavelengths for analyzing the imaging spectra from relative intensities of PC loadings. As a complementary approach, Liu et al. (2003a) presented a novel methodology to analyze and then classify the visible/NIR spectra of uncontaminated chicken skins and pure chicken feces as well as hyperspectral imaging spectra of fecal contaminated chicken skins. By examining the spectral difference, they identified several characteristic bands and subsequently developed simple two- or three-band subtraction and ratio algorithms. Their results revealed that both algorithms could be used to perform the classification analysis between skins and feces class with a great success, which was in good agreement with 2-class PCA/SIMCA models (skins vs. feces).

Recently, Park et al. (2011) have updated their efforts in commercializing the imaging system. Latest research demonstrated the feasibility of the system in terms of processing speed and detection accuracy for a real-time, in-line fecal detection at current processing speed (at least 140 birds per min) of commercial poultry plant. The preliminary results showed the real-time hyperspectral imaging system could detect small amount (about 10 mg) of fecal and ingesta contaminants, and the system performance could be improved by optimizing line lighting system especially NIR bands for quality images and additional spectral images to minimize false positive detection errors.

2.1.3 Sanitation efficiency at poultry processing plant

Relative to possible fecal contamination of meats during the slaughter processing, there is also a concern that meats might be contaminated from fecal remains on the surfaces of equipment, utensils, and walls at slaughter plants. To this regard, the USDA FSIS's mandatory HACCP systems require all meat and poultry plants to develop written sanitation standard operating procedures to show how they will meet daily sanitation requirements. This is important in reducing pathogens on poultry because unsanitary practices in plants increase the likelihood of product cross-contamination. Thus, slaughter plants are required not only to document daily records of completed sanitation standard operating procedures, but also to undergo hands-on sanitation verification by the USDA FSIS inspectors.

Evaluations and inspections of sanitation effectiveness are usually performed through one or more of the following methods; organoleptic (e.g., sight and feel), chemical (e.g., checking the chlorine level), and microbiological (e.g., microbial swabbing and culturing of product contact surface). As poultry feces are the most likely source of pathogenic contamination, the USDA FSIS inspectors use the established guidelines to identify fecal remains on the surfaces of equipment, utensils, and walls at slaughter plants. Certainly, the inspection duty is not easy, both labor intensive and prone to human error. Scientists and engineers at the USDA ARS have been looking into low-cost, reliable, and portable sensing devices, such as head-wear goggles and binoculars, by extending the scope of hyper- and multi- spectral reflectance and fluorescence imaging systems. One key factor in successful applications is to use a few essential spectral bands that meet the discrimination expectations.

On a direct analysis of visible and NIR spectral differences between feces/ingesta objectives and rubber belt/stainless steel backgrounds, Liu et al. (2006a) identified a number of significant bands and then developed simple three-band ratio algorithms for discriminant analysis. They observed that the three-band based algorithms could classify feces/ingesta objectives from rubber belt/stainless steel backgrounds with a success of over 97%, which was at least the same accuracy as those from the 2-class SIMCA models (feces/ingesta objectives vs. rubber belt/stainless steel backgrounds). Meanwhile, PCA was performed on both spectral sets, and the score-score plot showed a clear separation between feces/ingesta objectives and rubber belt/stainless steel backgrounds. However, the optimal loadings did not provide any specific characteristic bands that could further improve the classification rate. The finding of three visible or NIR bands is most promising in the development of simple goggle and binocular sensing system for in-situ inspection of fecal and ingesta contaminants at slaughter plants.

2.1.4 “Tender” / “Tough” poultry meat and meat quality

To provide poultry processors with accurate, reliable, and rapid information on the evaluation of meat quality attributes and, further, to facilitate the efficiency of new processing techniques, food scientists have been focusing on the relationships between sensory attributes and changes in the production process (Lyon & Lyon, 1991, 1996). In these pioneering studies, trained sensory panels and instrumental measurements were used together to draw conclusions and make decisions about meat quality. Instrumental methods, such as the Warner-Bratzler (W-B) shear force, can measure characteristics that are directly related to the physical components of meat products and can provide reliable information about meat quality. However, human subjects go beyond the physical components to describe a wide range of factors involved in mastication and afterfeel/aftertaste sensations, such as appearance, flavor, and texture. Sensory panels provide complementary information to instrumental method, and neither can be replaced. For example, instruments do not account for the juiciness and other moisture-related characteristics that panelists may perceive while chewing, and panels may identify and quantify more specific texture attributes that are not measured instrumentally. Meanwhile, relationships might exist between instrumental measurements and sensory panel evaluations. Previous studies have established a range of instrumental shear force values corresponding to different portions of the consumer texture scale, which enables commercial processors to relate the meaning of instrumental shear values to terms of relative toughness/tenderness of broiler breast meats (Lyon & Lyon, 1991).

Although instrumental W-B shear force measurement and sensory evaluation techniques can provide reliable information about poultry meat quality, it is destructive, time-consuming, and unsuitable for on-line application. The development of fast, non-destructive, accurate, and on-line / at-line techniques is critical to increase processing efficiency. Visible / NIR spectroscopy could form the basis for such techniques due to the speed, ease of use and less interference from color of meat samples.

The preliminary study, reported by Liu et al. (2004b), suggested that visible/NIR technique might have the potential to predict W-B shear force value, color, pH, and sensory characteristics in broiler muscles. As expected, the predictive models of meat color indices (L*, a*, b*), pH, and W-B shear force have better accuracies than those of individual sensory attributes. From visible/NIR predicted tenderness values in PLS model, breast samples were classified into "tender" and "tough" classes with a correct classification of 74.0% if the boundary was set to be 7.5 kg. As an alternative, a model based on PCA/SIMCA of measured shear force values as an indication of tenderness was attempted, and it showed nearly the same classification success.

A variety of chemical, physical, color, and sensory analyses are necessary to completely describe the characteristics of meats. Each type of analysis contributes specific and important information on overall meat quality. In other words, a meat sample might be characterized with more than one technique, resulting in many diverse parameters (variables). Generally, it is difficult to obtain a comprehensive overview of many meat samples with a number of variables. Hence, it might be useful to reduce the number of variables to describe the meats. As a strategy, Liu et al. (2004a) applied PCA to characterize the variations of a total 24 variables representing the objective and sensory properties of broiler breast meats deboned at different times. They observed several significant correlations among these variables, and W-B shear force had high positive correlations with 5 sensory texture attributes. Although PCA score plot showed no clear separation of the breast muscles deboned at different postmortem times, it could be still possible to differentiate them. The loading biplot suggested that 18 variables were effective in meat differentiation, including W-B shear force. However, the means to obtain either objective or sensory properties are destructive, time-consuming, and unsuitable for meat quality grading at large-scale operation or on-line implementations. As a part of conclusion, they suggested the development of fast, nondestructive, and on-line/at-line optical or imaging techniques for qualitative and quantitative determination of poultry meat eating qualities.

2.2 Grain safety and quality

2.2.1 DON contaminant screening

Deoxynivalenol (DON), also known as vomitoxin, is a type B trichothecene mycotoxin. It is one of major secondary metabolites produced by fungi of the *Fusarium* genus and occurs predominantly in grains such as wheat, barley, and corn (Leonard & Bushnell, 2004). The presence of DON has been reported to cause the quality degradation of grain and also a variety of very real toxic effects in humans and livestock who have consumed DON contaminated grain products. Authorities in a number of countries and organizations have established regulatory levels or guidelines for DON in food and feed. For example, the U.S. Food and Drug Administration (FDA) has proposed advisory levels for DON at 1 mg kg^{-1} for finished wheat products for humans, 5 mg kg^{-1} for swine and other non-ruminants, and 10 mg kg^{-1} for cattle and poultry feed. Though the milling process typically reduces DON concentration by approximately one-half, subsequent baking or heating processes cannot destroy DON toxin due to its thermal stability.

A number of analytical methods, such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), and GC- / HPLC- coupled MS, have been developed to measure DON concentration in grain. Clearly, these traditional methods involve expensive and time-consuming steps, including solid-phase extraction, separation, detection, and sample cleanup. New development of biotechnological approaches (e.g., biosensors and immunoassays) has been reported for rapid and specific detection of DON at trace levels, but these attempts still involve extraction and washing steps as well as the extra time for binding process.

Fast DON screening requires minimal sample preparation (e.g., to avoid the extraction / centrifugation), permits routine analysis of a number of samples with minimal use of reagents, requires fewer procedures, and is easy to operate. Vibrational spectroscopy is an alternative approach, since it can be applied directly to the solid grain in the state of single-kernel and ground without any DON extraction steps (Delwiche & Hareland, 2004; Delwiche, 2008). In general, grain contains a large portion of moisture that, in turn, yields intense and broad water bands in both the IR and NIR regions and can substantially hide other useful bands attributable to protein and carbohydrate species. Conversely, the Raman technique, which is based on the polarizability of bonds and not their dipoles like IR, is insensitive to water and provides fewer overlapping bands.

Due to DON toxin at the ppm concentration level, it is unlikely that the Raman method is directly sensitive to differences in DON levels. Rather, as DON is produced as a metabolite of *Fusarium* fungi during the growth of grain, it causes side effects on chemical, physical, color, and structure of grain. In turn, these effects could result in minor but significant changes in relative intensity, position and shape of Raman bands between low DON grain and high DON grain. Based on such spectral distinctions, Liu et al. (2009) suggested the use of two Raman bands near 1560 and 904 cm^{-1} in creating simple intensity-intensity plot for discrimination analysis. Their observation from a limited set of samples revealed that the simple intensity-intensity algorithm could be used to classify low DON grains from high DON ones, which were well confirmed by the PCA/SIMCA models. Notably, the use of Fourier transform (FT) methodology and a 1064 nm NIR excitation laser provides precise wavenumber measurement and good-quality Raman spectra by reducing the interference from fluorescence and photodecomposition of chemical components in wheat and barley.

2.2.2 *Fusarium* damage assessment

Fusarium head blight (scab) is a worldwide fungal disease that affects the small grains such as wheat and barley. Affecting the spikelets during plant development, the fungus causes a reduction of yield and further compromise the grain quality. Importantly, secondary metabolites that often accompany the fungus, such as DON, are health concerns to humans and livestock. Conventional grain inspection procedures for *Fusarium* damage are heavily reliant on human visual analysis. As an inspection alternative, Delwiche et al. (2011a) investigated the potential of hyperspectral image systems (1000 -1700 nm NIR vs. 400-1000 nm visible) in the detection of *Fusarium*-damaged wheat kernels. On a limited set of wheat samples that their conditions were subjectively assessed and also using a linear discriminant analysis (LDA) classifier, they found that hyperspectral imaging in either visible or NIR regions was able to discriminate *Fusarium*-damaged kernels from sound kernels at an average accuracy of approximately 95%.

2.2.3 Grain discrimination

Canada is one of the most wheat producers and exporters in the world. In Canada, wheat is classified based on color (red or white), hardness (soft or hard), and growing season (winter or spring). A specific wheat class is used as a primary raw material for specific products, such as Canada Western Red Spring (CWRS) wheat is processed for loaf bread. At present, visual method which requires extensive training and experience, is commonly used to identify wheat classes in grain handling facilities. A machine vision technique has been used to differentiate two wheat classes (Canada Western Red Spring vs. Canada Western Amber Durum). However, human inspection cannot be utilized for identifying wheat of different moisture levels because of subjectivity of the method. Higher moisture wheat (>15%) needs to be dried to an optimal level (12-13%) to be stored safely so as to prevent its spoilage and/or sprouting prior to processing.

A NIR hyperspectral imaging system (960–1700 nm) has been explored to identify five western Canadian wheat classes at varying moisture levels (Mahesh et al., 2011). Besides the generation of scores images and loadings plots from PCA, the linear and quadratic discriminant analyses were used to classify wheat classes giving accuracies of 61–97 and 82–99%, respectively, independent of moisture contents. They also observed that the linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) could classify moisture contents with classification accuracies of 89–91 and 91–99%, respectively, independent of wheat classes. Once wheat classes were identified, classification accuracies of 90–100 and 72–99% were observed using LDA and QDA, respectively, when identifying specific moisture levels. From this study, it was concluded that hyperspectral imaging technique can be used for rapidly identifying the wheat classes even at varying moisture levels.

2.2.4 Differentiation of waxy wheat

Wheat (*Triticum aestivum L.*) breeding programs are currently developing varieties that are free of amylose (waxy wheat), as well as genetically intermediate (partial waxy) types. Successful introduction of waxy wheat varieties into commerce is predicated on a rapid methodology at the commodity point of sale that can test for the waxy condition. In meeting this trend, Delwiche et al. (2011b) examined the ability of NIR reflectance spectroscopy to differentiate the starch waxy genotypic groups in hard winter wheat breeders' lines representing all eight genotypic combinations. By applying the LDA of PC scores, they noted that fully waxy wheat is identifiable at typically 90-100% accuracy. Since the fully waxy trait can be easily identified by NIR, regardless of the genetic background (population) within which it resides, hopefully breeders could easily use NIR to select waxy lines from early generation materials. Further, they pointed out the potential that end users could also utilize NIR to differentiate waxy crops at harvesting sites.

2.3 Fruit and vegetable safety

2.3.1 Fecal contaminant inspection on apples

Apple products could be contaminated with bacteria pathogens due to the contact with fecal materials in the phases of growing and harvesting. Animal feces are the most likely source of pathogenic *E. coli* O157: H7 contamination. In addition, the potential of contamination increases with physical damages on apples, such as lesions and bruises, which provide a site for bacterial growth. Cleaning processes can reduce, but are unlikely to eliminate, pathogens from the surfaces of produce even if antimicrobial chemicals are contained in the wash water. Bacterial pathogens can be presented in contaminated apples or raw (unpasteurized) apple juice / cider. There have been several reported foodborne illness outbreaks attributed to unpasteurized apple juice and cider. These outbreaks have raised the concerns of public health officials and apple cider / juice producers.

Responding to this interest, the U.S. FDA has issued an HACCP system to minimize the likelihood of any pathogens in fruit juices and identified an urgent need to develop methods for detecting fecal matters on apples. One element of the guidelines, on good agricultural practices (GAPs) and good manufacturing practices (GMPs) for fruits and vegetables, suggests the removal of fecal contaminated apples before entering the washer tank.

At present, inspection of fecal contamination is through visual observation over an inspection table. Inspectors use the GMPs guidelines to prevent apples with visible fecal contaminants from entering the next step. Current visual inspection is labor intensive and prone to human error and inspector-to-inspector variation. A research team led by Kim has been developing both hyperspectral reflectance and fluorescence image systems for the detection of fecal contaminated apples (Kim et al., 2002a, 2002b; Yang et al., 2012). In the systematic approach, they utilized a hyperspectral reflectance imaging technique in conjunction with the use of PCA to define several optimal wavelength bands. The investigation illustrated that, with the use of the PCA, high spectral dimension reflectance image data were reduced to several optimal wavelengths (multispectral) images. Also, they suggested three visible–NIR bands that could potentially be implemented in multispectral imaging systems for detection of fecal contamination on apples.

In supporting the selection of minimum and effective spectral bands in the image processing, Liu et al. (2007) characterized the distinctions in Region of Interest (ROI) spectral features between fecal contaminated areas and uncontaminated apple surfaces, and found the occurrence of large spectral differences in the 675-950 nm visible/NIR region, which provided the basis for developing universal algorithms in the detection of fecal spots. Comparison of a number of processed images (including those from PCA), they determined that a dual-band ratio ($Q_{725/811}$) algorithm could be used to identify fecal contaminated skins effectively. The observation was most important as the two bands are away from the absorptions of natural pigments (such as chlorophylls and carotenoids), and hence can reduce the influence from color variations due to different apple cultivars.

Complementary to acquire images and also to overcome the low sensitivity of detecting thin fecal smears (or low concentrations) in visible/NIR region, Kim et al. (2002b) proposed the use of hyper- and multi- spectral fluorescence imaging for classification of fecal contaminated apples. They utilized both PCA and visual examination to determine the optimal bands that allow the effective recognition of fecal contaminations on apple surfaces.

2.3.2 Fecal contaminant detection on cantaloupes

Since its first notice in early September 2011, the *listeria* outbreak linked to a crop of cantaloupes has now claimed the lives of at least 23 people and sickened more than 109 people across 23 U.S. states as of mid-October. The deadliest foodborne illness in more than a decade matches the death toll from a multi-state *listeria* outbreak linked to hot dogs and deli turkey that started in 1998 and stretched into 1999.

Cantaloupes become contaminated with pathogens through direct contact with manure, contaminated soil, animals or humans during any stage of the food-handling chain, including the growing and harvesting operations as well as while in the processing plants. In general, the pathogens originate from the intestinal tracts of animals and humans, thus making fecal matter a major source of contamination. For instance, contaminated cantaloupes were found to be responsible for 2 deaths and 18 hospitalizations due to *Salmonella* bacteria between 2000 and 2002, and a thorough investigation revealed the cause of unsanitary conditions in processing and packaging plants (Anderson et al., 2002).

In 2005, Kim's research group probed the feasibility of hyperspectral fluorescence images in detecting fecal spots on cantaloupes that were artificially contaminated with bovine feces at varying concentrations (Vargas et al., 2005). To improve the detection algorithms, they presented several image processing tactics (such as single-band and two-band ratio images) and found the potential of the PCA processing of hyperspectral images in the detection of fecal contaminated spots (a minimum of 16-μg/mL dry fecal matter) on cantaloupes with minimal false positives. With the examination of PC weighing coefficients, they identified several dominant wavelengths that could be implemented to a multispectral imaging system for further on-line applications.

2.3.3 Bruise detection

Bruises are of great concern to the fruit and vegetable industry and the retailer because they lower the quality grade of the produce and can cause significant economic losses. Bruising normally happens to the tissue beneath the fruit skin. After the fruit tissue is damaged, its cells are initially filled with water and turn brownish. As time elapses, the damaged cells start to lose moisture and eventually become desiccated. It is a challenging task to detect bruises on the fruit, in part because of the presence of fruit skin and in part because detection accuracies are affected by factors such as time, bruise type and severity, apple variety, and fruit pre– and post–harvest conditions. Not long ago, Lu (2003) developed a NIR hyperspectral imaging based bruise detection system for detecting bruises on apples in the spectral region between 900 nm and 1700 nm. His results indicated that the spectral region between 1000 nm and 1340 nm was most appropriate for bruise detection. He also observed that bruise features changed over time from lower reflectance to higher reflectance, and the rate of the change varied with fruit and variety. Using both PC and minimum noise fraction transforms, his system was able to detect both new and old bruises, with a correct detection rate from 62% to 88% for Red Delicious and from 59% to 94% for Golden Delicious. The optimal spectral resolution for bruise detection was between 8.6 nm and 17.3 nm, with the corresponding number of spectral bands between 40 and 20.

Later, Ariana et al. (2006) applied the same imaging system to capture hyperspectral images from pickling cucumbers at 0–3, and 6 days after they were subjected to dropping or rolling under load which simulated damage caused by mechanical harvesting and handling systems. PCA, band ratio, and band difference were applied in the image processing to segregate bruised cucumbers from normal cucumbers. They reported that bruised tissue had consistently lower reflectance than normal tissue and the former increased over time. Best detection accuracies from the PCA were achieved when a bandwidth of 8.8 nm and the spectral region of 950–1350 nm were selected. The detection accuracies from the PCA decreased from 95 to 75% over the period of 6 days after bruising, which was attributed to the self-healing of the bruised tissue after mechanical injury. The best band ratio of 988 and 1085 nm had detection accuracies between 93 and 82%, whereas the best band difference of 1346 and 1425 nm had accuracies between 89 and 84%. From general classification performance analysis, they concluded that the band ratio and difference methods had similar performance, and both were better than the PCA.

2.3.4 Chilling injury inspection

It is well-known that many fruits and vegetables are sensitive to chilling and are damaged by low temperatures during the storage and transportation process. Cucumber is one such produce, apt to suffer chilling injury from relatively short periods of time at low temperatures. Extensive decay occurs when chilling injured cucumbers are returned to warmer temperatures, and damaged areas can become locations for further fungal decay and bacterial infection. Accumulated bacterial pathogens from these areas can be harmful to humans by consumption of uncooked or mishandled cucumbers.

Chilling injury has been of great attention to the fruit and vegetable industry, and a diversity of methods has been developed to reduce the occurrence of chilling injury for cold-sensitive produces (Wang, 1993). These techniques include low temperature preconditioning, intermittent warming, waxing, genetic modification, and chemical treatments. Nevertheless, the development of rapid, non-destructive, and accurate methodologies that are suitable for on-line and at-line operations is critical to increase the efficiency of cucumber safety/quality evaluation. Hyperspectral imaging spectroscopy can be the basis for the development of such techniques, thanks to its non-invasive nature and capacity for large spatial sampling areas.

Usually, the amount of information contained in hyperspectral images is excessive and redundant, and data mining for waveband selection is needed. In the applications such as fruit and vegetable defect inspections, effective spectral combination and data fusing methods are required in order to select a few optimal wavelengths without losing the crucial information in the original hyperspectral data. Cheng et al. (2004) proposed a novel method that combines PCA and Fisher's linear discriminant (FLD) method to show that the hybrid PCA−FLD method maximizes the representation and classification effects on the extracted new feature bands. The method was then applied to the detection of chilling injury on cucumbers. Based on tests on different types of samples, their results showed that this new integrated PCA−FLD method outperforms the PCA and FLD methods when they were used separately for classifications.

As a differing approach, Liu et al. (2006b) tested a variety of image processing and visually compared the detection efficiency of chilling injury. Firstly, they examined the ROI spectral features of chilling injured areas that showed a reduction in reflectance intensity during multi-day post-chilling periods of room temperature (RT) storage. Next, they determined the large spectral difference between good-smooth skins and chilling injured skins occurred in the 700 to 850 nm visible/NIR region. Then, a number of data processing methods, including simple spectral band algorithms and PCA, were attempted to discriminate the ROI spectra of good cucumber skins from those of chilling injured skins. The observation indicated that using either a dual-band ratio algorithm ($Q_{811/756}$) or a PCA/SIMCA model from a narrow spectral region of 733 to 848 nm could detect chilling injured skins with a success rate of over 90%. Further, they applied the dual-band algorithm to the analysis of images of cucumbers at different conditions, and the resultant images showed more correct identification of chilling injured spots than PCA method. The results also suggested that chilling injury was relatively difficult to detect at the stage of the first 0 to 2 days of post-chilling RT storage, due to insignificant manifestation of chilling induced symptoms.

2.4 Single *Bacillus* spores detection

Under stressed conditions such as lack of key nutrients, certain *Bacillus* cells in the vegetative state will spontaneously develop into a dormant state known as an endospore. The spore is organized into a series of concentrically arranged structures, each of which contribute in a different way to resist against environmental stresses such as heat, radiation, desiccation and chemical disinfectants. Detection of *Bacillus* spores is of considerable importance in agricultural and food industries, since the ubiquity of these spore-forming bacteria allows it to potentially threaten the safety of a wide range of foods, including dairy products, meats, cereals, vegetables, spices, and ready-to eat meals. In addition, Bacillus spores can survive standard processing and sanitation treatments for foods and food processing equipment.

There has been growing interest in the applications of optical methods such as Raman spectroscopy for microbial characterization. Major advantages of Raman approach are that samples can be analyzed with minimum preparation in aqueous state and measured non-destructively on-line, and in real time. However, Raman signals are normally very weak and can only be used for bulk samples or concentrated solutions. The problems can be overcome by using a much more sensitive method, namely surface enhanced Raman spectroscopy (SERS), which could reach the limit of detection (LOD) to a single spore or cell. When the targeted molecules are attached to noble metal substrates (typically, Au or Ag), SERS effect will occur with the enormously enhanced Raman signals.

With the use of gold SERS-active substrates, He et al. (2008) were able to observe distinct spectral differences among five different *Bacillus* spores at single spore level. In the following, hierarchical cluster analysis (HCA) and PCA were applied and the results showed clear data segregations at the species level between five Bacillus spores. The corresponding PC values indicated that the Raman range between 900 and 1200 cm^{-1}

contributed significantly to the total data variance in the PCA plot. In particular, a prominent band of dipicolinic acid (DPA) was observed at 998 cm^{-1} and served as a biomarker for bacterial spores. Their study demonstrated that SERS method is a promising tool for rapid, ultra-sensitive, and selective detection of bacterial spores in foods and other complex biological matrices.

2.5 Cotton quality grading

Micronaire property has been recognized as one of key cotton quality indices for fiber classers and processors. It is a measure of fiber fineness and maturity, and is determined by measuring the air permeability of a constant mass of cotton fiber compressed to a fixed volume. Previous studies have implied the ability of NIR technique to determine cotton micronaire with a relatively high degree of success (Liu et al., 2010). Apparently, NIR predicted micronaire values could be near the boundaries separating three cotton classes of "Discount Range", "Base Range", and "Premium Range", which might be a problem and the source of error during the cotton classification.

Following the assignment of fibers into "Discount Range", "Base Range", and "Premium Range" classes according to predicted micronaire values from optimal PLS model, 16, 12, and 9 samples were correctly classified at respective classes of 18, 13, and 10 cotton samples, with a 90.4% of overall classification (Liu et al., 2010). For a comparison, the 3-class based SIMCA/PCA discriminant models were created, and a better separation power than respective PLS model was observed.

3. PCA and 2D correlation analysis

Two-dimensional (2D) correlation spectroscopy, a universal and modern technique of vibrational spectral analysis, was originally developed as 2D IR correlation spectroscopy by Noda (1986). In this initial concept, a system was excited by an external perturbation that includes dynamic fluctuations of IR signals, and then a simple cross-correlation analysis was applied to sinusoidally varying dynamic IR response to generate a set of 2D IR correlation spectra. Years later, Noda (1993) introduced a more applicable and simple mathematical formalism to perform the generalized 2D correlation analysis, which has been considerably and successfully applied not only to a variety of optical spectroscopic techniques (IR, NIR, Raman, visible, fluorescence), but also for a number of different types of simple external perturbations (electrical, thermal, magnetic, chemical, acoustic, mechanical, spatial positions etc) and waveforms. When an external perturbation was applied to a sample system, specific components within the system could be selectively excited and, subsequently, be monitored with many different types of electromagnetic probes. Over the period, generalized 2D correlation spectroscopy has been established as a viable means to analyze and extract useful information from conventional one-dimensional (1D) spectral data.

Major advantages of generalized 2D correlation spectroscopy include the enhancement of spectral resolution by spreading peaks over the second dimension, the band assignments through the correlation analysis, and probing the complex sequence of events arising from the changes in a system. To obtain generalized 2D correlation spectra and also to interpret them in a reasonable manner, a limited number of spectral data was arranged in an increasing or decreasing variable. On the other hand, one of the specific challenges might be how to implement generalized 2D correlation analysis in large and diverse spectral sets, which are common in chemometric model developments and actually include multivariate variations in chemical and physical components, and to acquire useful information from them. As an approach to large samples with at least one known attribute, the use of the average spectra that had close physical or chemical values has been reported (Liu et al., 2004c). Being another method to explore the variations within a diverse spectral set, PCA was utilized to classify the spectra of samples (Liu & Chen, 2000; Kokot et al., 2002). Further, 2D characterization was attempted to understand the PC loading spectra on large spectral data set with multi-variables (Liu et al., 2003b).

3.1 Cluster of samples

Most 2D applications are limited to one perturbation (or variable) dependent spectral intensity change within a simple system. To extend the scope of 2D analysis to more complicated biological samples, Liu & Chen (2000) proposed a strategy of utilizing PCA procedure to identify two NIR spectral clusters of chicken meats at different periods during cold storage for the first time in 2000, in which one group representing the storage process from day 2 to day 9 and another one from day 10 to day 18. It opens a way to implement 2D study in more applications and, anticipated, this PCA process has been successfully attempted to other agricultural products, such as cotton fibers at various elevated temperatures (Kokot et al., 2002).

In the most recent 2D study of characterizing the attenuated total reflection (ATR) spectral intensity fluctuations of immature and mature cotton fibers, their conditions were identified subjectively and then were verified by the PCA (Liu et al., 2011a). As a traditional practice, the cotton bolls were taken at various days post-anthesis (DPAs) to unravel a number of interests in structure, maturity, and physical properties among developing fibers. Abidi et al. (2010) have employed PCA to analyze the FT-IR/ATR spectra of fibers as a function of developmental DPAs, and observed that the PC1 scores, in general, increased with DPAs for two cotton varieties. Although the PC1 scores were not linear with DPAs, they identified two groups of spectra with negative PC1 scores for shorter DPAs and positive PC1 scores for longer DPAs, and further concluded the transition phase at 17 and 21 DPAs for the respective cotton varieties. The PCA results were consistent between two sampling approaches, visual inspection (Liu et al., 2011a) and differing DPAs (Abidi et al., 2010).

The unique bands identified through the 2D study could be used to interpret two key bands at 1032 and 956 cm^{-1} that were utilized to develop simple algorithm for the classification of immature fibers from mature ones (Liu et al., 2011b). For example, by setting a ratio value of 0.4, it is possible to detect immature fibers positively with over 95% success rate, and, furthermore, the degree of fiber maturity was assessed.

3.2 PC loadings

Liu et al. have systematically investigated the 2D visible/NIR spectral correlation analysis of chicken breasts under various conditions, such as cooking, thawing, and cold storage as well as diseases (Liu & Chen, 2000, 2003; Liu et al., 2000). These results have shown that the visible bands identified through the 2D studies have been found to be useful as an indicator of meat color variation during cooking, irradiation and cold storage. They have displayed the significance of 2D approach in analyzing overlapped and broad bands of meat products, and also established the relationship between spectral absorption and meat color structure that has been indicative in creating the next-generation sensing devices.

To understand the variations within the large spectral set, Liu et al. (2003b) analyzed the loadings spectra of PCA. They presented three examples of visible/NIR spectra of chicken breast muscles under a variety of treatments, as muscles are one of the complicated agricultural commodities that vary greatly in color, chemical, physical and sensory attributes from one portion to another. The 2D results indicated that characteristic bands from the loadings spectra are in good agreement with those from a small number of spectra induced by simple external perturbations in previous investigations. They concluded that although some advantages of 2D correlation analysis (such as sequential changes in intensity) were not available, it might still be useful for the interpretation of large and complex spectral data set with multi-variable variations.

4. Conclusions

The Chapter reviews the recent developments of PCA in optical and imaging spectroscopy for agricultural and food safety and quality. Food safety is one of most important issues for public health, and authorities have zero tolerance performance standards for various food products. Driven by this increasing interest of protecting food supply, the ability of optical and imaging spectroscopic techniques to rapidly, routinely, potentially to be portable and on-site, as well as non-destructively screen agricultural commodities sets them apart from traditional analytical or inspection methods that are labor intensive and time-consuming.

Optical and imaging inspection systems have been used increasingly for inspection and evaluation purposes as they can provide rapid, economic, hygienic, consistent and objective assessment. However, difficulties still exist, evident from the relatively slow commercial uptake of these machine vision systems. Even though adequately efficient and accurate algorithms have been generated, processing speeds still fail to meet modern manufacturing requirements, for example. With few exceptions, researches in this field have dealt with trials on a laboratory scale thus the nature of complex biological samples has been neglected. Hence, it needs more focused and detailed study of data mining on agricultural and food matrixes with the aid of such advanced multivariate data analysis as PCA.

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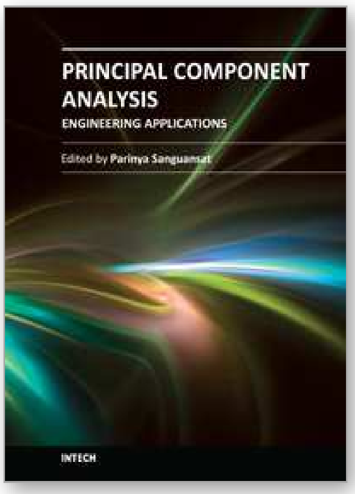

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This book is aimed at raising awareness of researchers, scientists and engineers on the benefits of Principal Component Analysis (PCA) in data analysis. In this book, the reader will find the applications of PCA in fields such as energy, multi-sensor data fusion, materials science, gas chromatographic analysis, ecology, video and image processing, agriculture, color coating, climate and automatic target recognition.

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