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Chicken Disease Characterization by Fluorescence Spectroscopy

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Abstract. *Fluorescence spectroscopy was used to characterize chicken carcass spectra. Spectral signatures of three different disease categories of poultry carcasses (airsacculitis, cadaver and septicemia) were obtained from fluorescence emission measurements in the wavelength range of 360 to 600 nm with 330 nm excitation. Principal Component Analysis (PCA) was used to select the most significant wavelengths for the classification of poultry carcasses. These wavelengths were analyzed for pathologic correlation of poultry diseases. Using a Soft Independent Modeling of Class Analogy (SIMCA) of principal components with a Mahalanobis distance metric, poultry carcasses were individually classified into different classes with 97.9% accuracy.*

Keywords. Fluorescence, spectrofluorometer, poultry carcass diseases, classification

Introduction

The per capita consumption of chickens increased rapidly from 33.3 pounds in 1965 to 84 pounds in 1997, and it is expected 91 pounds in 2001. All chickens consumed to U.S. consumers are required to be inspected post-mortem by the United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) inspectors for the wholesomeness (USDA, 1984). The number of chicken under federal inspection has increased from 2.8 billion in 1965 to 8.3 billion in 1999 (USDA, 2001) by about 2,200 poultry inspectors from FSIS. The poultry carcasses and viscera on-line at processing plants are inspected visually and manually by the inspectors. Each inspector can inspect the maximum of 35 birds per minute and works at least 8 hours per day. These working conditions have developed repetitive motion injuries and attention, and fatigue problems (OSHA, 1999).

There are six major defects that cause chicken carcasses to be removed from the chicken processing line. They are septicemia, cadaver, bruise, tumor, airsacculitis, and ascites. Under the current Hazard Analysis and Critical Control Point (HACCP)-based Inspection Models Project (HIMP), FSIS requires that any poultry showing evidence of septicemia, which is a systemic condition and a manifestation of infectious disease caused by pathogenic microorganisms in the blood, to be condemned.

The Instrumentation and Sensing Laboratory (ISL) of the USDA has developed a visible/near-infrared (Vis/NIR) system to measure spectral characteristics of the poultry systemic diseases in both the visible light and NIR regions. This system has been shown feasible for on-line chicken carcass inspection (Chen and Massie, 1993; Chen et al., 1998; Chen et al., 2000; Park et al., 1995). The Vis/NIR system has been used to distinguish among wholesome, septicemic, and cadaver. It would complement the current system, if additional disease categories can be differentiated.

Fluorescence techniques have a broad scientific application, ranging from microscopic imaging (Harris, and Hartley, 1976) to environmental air-borne measurements (Albers et. al, 1995). However, its use in on-line application for food safety inspection has been limited. A number of compounds in poultry meat emit fluorescence when excited with electromagnetic radiation in the ultraviolet (UV) to visible (VIS) regions. Studies have reported emission characteristics of meat products (Swatland and Barbut, 1991; Swatland et. al, 1996; Li and King, 1996; Wold et. al, 1999; Swatland, 2000; Wold and Mielnik, 2000; Wold and Kvaal, 2000). Chicken meat products have been shown to emit both UV and blue-green fluorescence. In this study, we demonstrated the potential of fluorescence techniques for chicken carcass inspection.

Objectives

The objectives of this paper were to 1) evaluate emission characteristics of chicken carcass spectra of three different disease categories (airsacculitis, cadaver, and septicemia), and 2) examine the feasibility of using fluorescence spectroscopy to classify chicken carcasses. If successful, a rapid, fluorescence-based classification procedure could find application in detecting or inspecting operations for the food safety and processing.

Material and Methods

Measurement of Fluorescence Spectra

The spectroflourometer (Fluorolog III, Horiba Industries, Edison, NJ, USA) was used to measure fluorescence spectra of poultry samples. The measuring equipment has additive dispersion double grating and two 0.22 m Czerny-Turner double monochrometers. One monochrometer is attached to a 450 W xenon lamp with variable excitation from 220 to 700 nm. Another one is attached to a photon counting photomultiplier tube (PMT) which allows acquisition of fluorescence emissions from 250 nm to 900 nm. The radiometric calibration factors were provided by the manufacture to correct excitation and emission spectra from 250 nm to 850 nm for wavelength bias caused by lamp emission characteristics, PMT response, and optical components.

A bifurcated quartz fiberoptic attachment from the spectroflourometer was used to measure spectra. The distance between the excitation and measurement tip of the attachment and sample materials was 3 cm and angled at 90 degree. The effective area of illumination by excitation light was approximately 2 cm in diameter. The sample materials and the attachment were sit inside a light-tight sample chamber (50 H X 40 W X 50 cm L) equipped with a height-adjustable laboratory jack to minimize stray light and to accommodate variation in the size and shape of sample materials. Fluorescence emission spectra were collected from 360 nm to 600 nm at 2 nm intervals with excitation wavelengths at 330 nm.

Sample materials

A total of 131 chicken carcasses (34 normal, 30 cadaver, 32 septicemia, 35 airsacculitis) were collected from a poultry processing plant (Allen Family Foods Inc., Cordova, MD) approximately bi-weekly from October 2001 to March 2002. The FSIS veterinarians at the plant identified the conditions of the chicken carcasses. The carcasses were tagged according to the condemnation categories and placed in plastic bags to minimize dehydration and color changes. Then the bags were placed in coolers, filled with ice, and transported to the Instrumentation and Sensing Laboratory within two hours for the measurements.

Multivariate Analysis

A commercial spectral analysis software (GRAMS32/AI Version 6.0, Thermo Galactic, Inc., Salem, NH) was used to analyze the spectra of normal and the three different disease categories of poultry carcasses with the principal component analysis (PCA), and the Soft Independent Modeling of Class Analogy (SIMCA) of principal components with the Mahalanobis distance metric (SIMCA-PCA/MD) method was used to classify and validate the chicken carcass category. First-20 spectra from each chicken carcass category were used as PCA and SIMCA-PCA/MD training sets, and the remaining spectra (i.e., N=14 for normal; N=10 for cadaver; N=12 for septicemia; and N= 10 for airsacculitis) were used to validate the SIMCA-PCA/MD model.

Principal Component Analysis (PCA)

PCA is a projection method to extract the systematic variations in a dataset resulting in principal component models. Principal components, new coordinate axes, are ordered by variance size to describe the spectral variations. For each principal component, the scores of samples can show the relative locations of the samples.

Each 20 spectra data for airsacculitis, cadaver, normal and septicemia was chosen for PCA with cross validation diagnostic type and mean centering. From PCA, eigenvalue, F-ratio, F-test, and total variance for individual chicken category were obtained to determine the minimum factors (components) that explain variation in the spectra due to disease conditions.

Soft Independent Modeling of Class Analogy (SIMCA) of principal components with a Mahalanobis distance metric (SIMCA-PCA/MD)

SIMCA is a supervised classification technique that builds a distinct confidence region around each class after applying PCA. Each PCA model of airsacculitis, cadaver, normal, and septicemia was developed from each 20 spectra data. The optimal number of principal components was determined by an F-test of the normalized eigenvalue associated with each factor. Developed PCA models were used to classify the remaining sample spectra (N=14, normal; N=10, cadaver; N=12, septicemia; N= 10, airsacculitis).

Results and discussion

Fluorescence Spectra of the poultry carcasses

Figure 1 shows the average fluorescence emission spectra of normal chicken and three different disease categories of chicken carcasses (airsacculitis, cadaver, and septicemia). The emission spectra exhibited a multiple emission peaks in the blue-green regions of the spectrum which suggested a multi-compound interactions. In general, emission maxima were observed at approximately 385 and 445 nm with emission shoulders at 475 and 515 nm. The emission peak in the blue for cadaver appeared to be red-shifted (e.g., 450 nm) compared to other samples at 445 nm. Emission spectra from the normal and diseased chicken carcasses were quite similar in shape, but relative intensity differences were observed among them. In addition, normal chicken carcasses exhibited more pronounced emission shoulders at 515 nm compared to the other chicken samples. At emission maxima, airsacculitis had the highest intensity values followed by normal, septicemic, and cadaver.

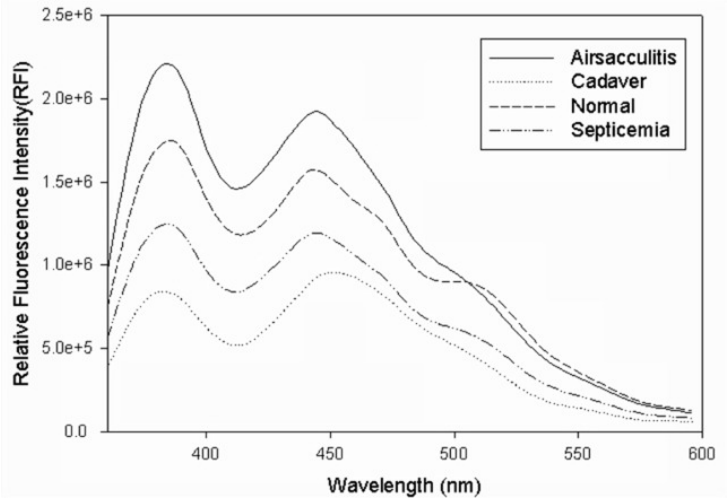

Figure 1. Average fluorescence spectra of normal chicken and three different disease chicken carcasses (Airsacculitis, Cadaver, and Septicemia).

Figure 2 is a scatter plot of principal components 1, 2, and 3 where four distinctive clusters for airsacculitis, cadaver, normal, and septicemia are seen. The first-three components accounted for 98.8% of the total spectral variation. Scores from the first principal component showed normal and airsacculitis had positive values, whereas cadaver and septicemia had negative scores. Airsacculitis was distributed widely along the second principal component axes compare to the other samples. Less spectral variations from cadaver and normal compared to airsacculitis and septicemia were seen in this figure. The scatter plot suggests fluorescence spectra in conjunction with principal components 1 through 3 may provide a means to classify airsacculitis, cadaver, normal and septicemia.

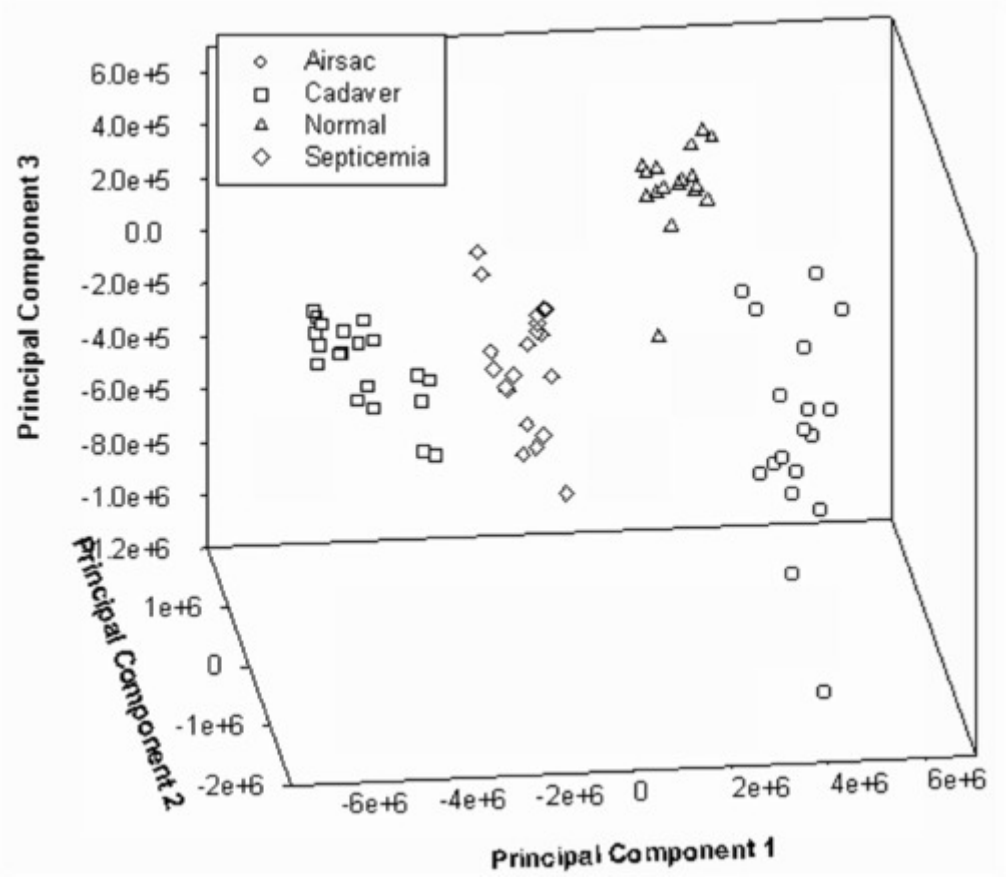

Figure 2. The scatter plot of principal components 1, 2, and 3 for chicken carcasses of airsacculitis, cadaver, normal, and septicemia.

Each principal component is a linear combination of the original spectral data and spectral loading weights, which indicate how much each wavelength contributes to explaining the response variation along each principal component. Hence, significant wavelength regions responsible for creating the clusters of the disease classes in the scatter plot (Figure 2) can be assessed.

The loading weights of the first-three principal components are shown in Figure 3. The loading weight for principal component 1 resembles the mean fluorescence spectrum of samples.

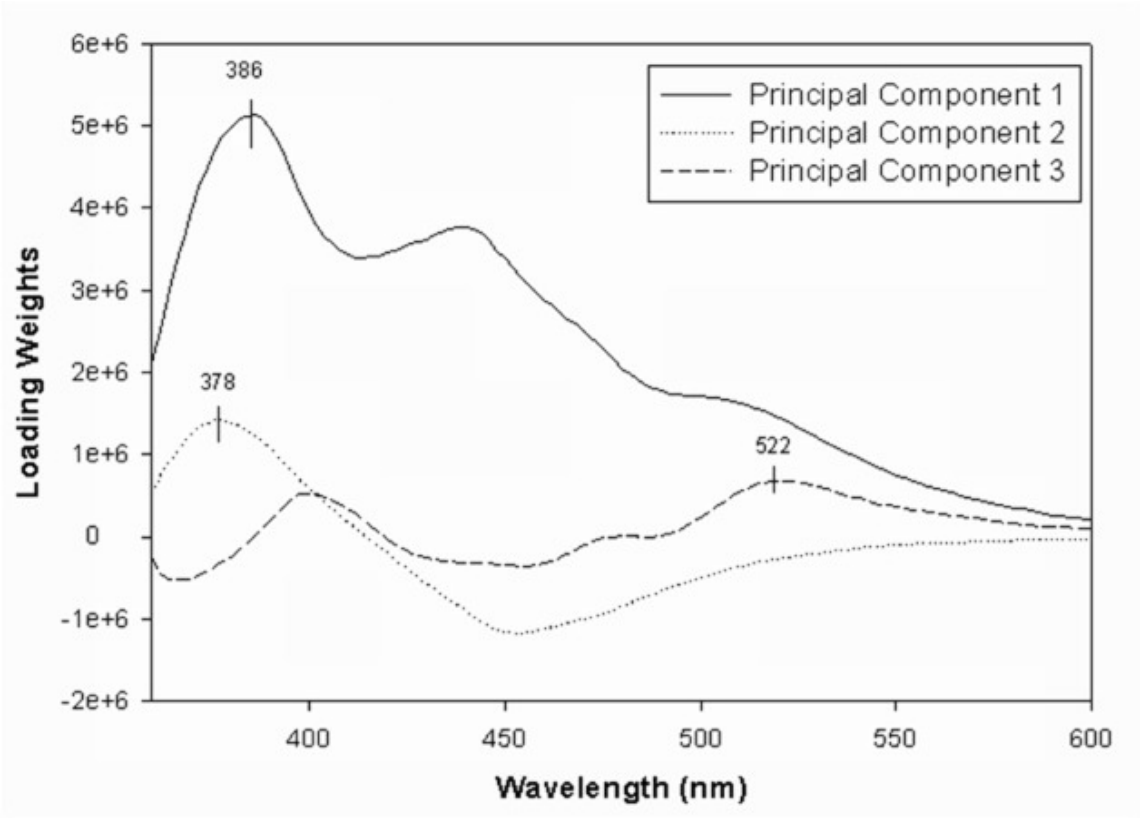

Figure 3. Loading weights of principal components 1, 2, and 3 for chicken carcasses of airsacculitis, cadaver, normal, and septicemia.

Delwiche and Graybosch (1999) showed that SIMCA-PCA/MD was superior spectral classification method compared to PCA. On the basis of SIMCA-PCA/MD results, the optimal number of principal components for classification of disease categories was determined to be either 6 or 7, depending on the individual disease categories. Therefore, six principal components from each category were used to validate classification models in the remaining 51 sample spectra. Four PCA models were applied to each sample, and employed a lowest Mahalanobis distance rule for the disease category assignment. The results summarized in Table 1 indicated that only one septicemia sample was misclassified as a normal sample. The overall classification accuracy rate was 97.9 % for the SIMCA-PCA/MD model.

Table 1. Classification by SIMCA-PCA with Mahalanobis distance

		Assigned Category				
Actual Category	Total	Airsacculitis	Cadaver	Normal	Septicemia	Type II % Error
Airsacculitis	15	15	0	0	0	
		(100.0)	(0.0)	(0.0)	(0.0)	0
Cadaver	10	0	10	0	0	
		(0.0)	(100.0)	(0.0)	(0.0)	0
Normal	14	0	0	14	0	
		(0.0)	(0.0)	(100.0)	(0.0)	0
Septicemia	12	0	0	1	11	
		(0.0)	(0.0)	(8.3)	(91.7)	8.3
Type I % Error		0	0	6.7	0	

Table 1 shows that, among the 15 airsacculitis, 10 cadaver, and 14 normal cases, none were misclassified. Among 12 septicemia, 1 sample (8.3%) was misclassified as normal. Of the 14 normal carcasses, none was misclassified as abnormal (Type II Error), while among the 37 abnormal carcasses, 1 sample (2.7%) was predicted as normal carcass. Of the 15 classified as normal, 1 sample (6.7%) was septicemia case (Type I Error). Among the 36 cases predicted to be abnormal, none was normal carcass.

Summary and conclusions

Fluorescence spectroscopy can be used to discriminate among airsacculitis, cadaver, normal, and septicemia for chicken carcasses. A SIMCA-PCA with a Mahalanobis distance metric from the spectral data was used to classify airsacculitis, cadaver, normal, and septicemia with a success rate of 97.9%.

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