Characterization of Visible Spectral Intensity Variations of Wholesome and Unwholesome Chicken Meats with Two-Dimensional Correlation Spectroscopy

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Generalized two-dimensional (2D) correlation analysis of visible spectra (400-700 nm) was performed to characterize the spectral intensity variations of wholesome and five different classes of unwholesome chicken meats. The meats were obtained from the chicken carcasses that were judged to be wholesome or condemned by a Food Safety and Inspection Service (FSIS) veterinarian at a poultry processing plant. The unwholesome carcasses were condemned either because they were improperly bled (cadaver) or showed a disease symptom such as air-sacculitis, ascites, septicemia, or tumors. The results showed that there are at least three prominent bands around 445, 485, and 560 nm that could be attributed to deoxymyoglobin, metmyoglobin, and oxymyoglobin absorption, respectively. The results also demonstrated that deoxymyoglobin, metmyoglobin, and oxymyoglobin components coexist in all meats. There is, however, a clear indication that there were more variations in oxymyoglobin and deoxymyoglobin and less variations in metmyoglobin in the wholesome and cadaver meats than in the diseased meats. The asynchronous spectral analysis of the wholesome and unwholesome meats revealed that the spectral intensity change at the 485 nm band occurs later than those of the 445 and 560 nm bands. It indicates that metmyoglobin, the degraded species of both the deoxymyoglobin and oxymyoglobin, mainly existed in the diseased meats.

Index Headings: Two-dimensional correlation analysis; Visible spectroscopy; Chicken meats; Chicken disease; Myoglobin.

INTRODUCTION

In recent years, U.S. consumers have increased their consumption of poultry products. To ensure a healthy and safe meat supply to the consumers, U.S. legislation requires each poultry carcass at poultry slaughter plants be inspected by Food Safety and Inspection Service (FSIS) inspectors.1 The average speed of inspection by an inspector is approximately 30 to 35 birds per minute. Experienced inspectors visually inspect the carcass exterior, the inner surfaces of the body cavity, and the visceral organs for apparent diseases such as air-sacculitis (a lung disease), ascites (fluid in the abdominal cavity), septicemia (an influenza-type diseased state), and tumor (cartilaginous nodules). Inspectors also condemn cadaver carcasses, which are chickens not properly slaughtered and bled, a condition not associated with any diseases. The unwholesome carcasses demonstrate a variety of obvious changes in skin and meat color.

Myoglobin, the heme-protein, comprises 50–80% of meat pigment mass. It is primarily responsible for the absorbance in the visible region.2-5 The heme group within myoglobin is a planar chemical structure containing a centrally located iron atom (Fig. 1).3-6 The iron atom has six coordination sites available for chemical bonds. Four of these bonds anchor the iron atom within the heme structure. A fifth bond connects the iron atom to the globin protein. The sixth coordination site is available for binding a variety of chemical groups. Both the chemical group bound at the sixth site and/or the oxidation state of heme iron determine meat color and type of myoglobin. When heme iron is in the ferrous (+2 valences) form and lacks a ligand at the sixth position, it is referred to as deoxymyoglobin. The color of deoxymyoglobin is purplish-red and is a characteristic of recently sliced fresh meat. Ferrous myoglobin that is exposed to air will bind oxygen at the sixth coordination site and form oxymyoglobin, which is cherry red and typical of fresh meat displayed in the supermarket.

The process of oxidation/degradation occurs when ferrous iron (deoxymyoglobin or oxymyoglobin) is converted to ferric (+3 valences) iron and leads to the third form of myoglobin, metmyoglobin. Metmyoglobin is brownish-red in color and is represented by ferric iron with a water molecular bound at the sixth position. Meat color is an extremely important sensory characteristic that consumers and FSIS inspectors use to make judgments of meat quality. It becomes essential to understand the fundamental spectroscopic features of wholesome and unwholesome meats with the consideration of deoxymyoglobin, oxymyoglobin, and metmyoglobin components.

To improve the effectiveness of the formal inspection program, researchers at the USDA Agricultural Research Service have developed a visible/near-infrared (NIR) spectrophotometer system for use in on-line, real-time classification of poultry carcasses in slaughter plants.7-9 The system can separate chicken carcasses into wholesome and unwholesome classes on-line with over 95% accuracy.

Visible/NIR spectroscopy has also been developed to perform meat identification and to estimate the chemical and physical properties of chicken products.10 12 In recent studies, researchers evaluated the integrated time-temperature history and physical properties of heat-treated chicken patties.11 12 These studies indicate that visible/NIR spectroscopy could be used for identification of meats and for quality control of raw and thermally pro-
cessed poultry products. However, little information on the composition and structure differences of wholesome, unwholesome, and thermally processed meats is available, partly due to the complexity of visible/NIR spectra.

The 2D correlation analysis for the vibrational spectroscopy was first developed by Noda more than ten years ago. The spectral peaks are spread over a second dimension, thereby amplifying the visualization of spectral peaks and enhancing spectral resolution. Two-dimensional (2D) correlation spectroscopy has now been widely adopted as a useful analytical tool in many areas. It has the ability to monitor the spectral intensity variation as a function of many variables such as time, temperature, and concentration. Recently, we applied the 2D correlation technique to the study of visible/NIR spectra of cooked wholesome chicken meats. Most current 2D work, however, has been associated with spectral intensity changes induced by specific external perturbations. This condition leads to the spectral intensity either continuously increasing or decreasing at different wavelengths.

In a previous study of 2D visible/NIR correlation spectroscopy of cooked wholesome chicken meats, temperature was used as a perturbation variable. This paper reports the results of applying 2D correlation spectroscopy to characterize visible spectra of the meats from different classes of chickens having similar health conditions. We analyzed the intensity variations among samples representing a sampling perturbation. We also analyzed intensity variations between classes of meats, representing disease and defect perturbation. The present 2D correlation analysis was limited to spectral features of chicken meats in the visible region (400–700 nm).

**MATERIALS AND METHODS**

**Meat Samples.** A total of 57 chicken carcasses (15 wholesome, 8 septicemia, 9 cadaver, 9 tumor, 8 airsacculitis, and 8 ascites) were obtained from a processing line at a poultry slaughter plant on the Eastern Shore of Maryland (Cordova, MD). The conditions of these carcasses were identified in the plant by an FSIS veterinarian. Fresh breast meat was acquired from each chicken carcass. Each slice (1 cm thick and 3.8 cm diameter) was then cut to fit into a spectrophotometer’s quartz window-clad cylindrical cup and was sealed in a polyethylene bag and kept at 0 °C, prior to spectral collection.

**Spectroscopic Measurement and 2D Correlation Analysis.** Visible reflectance spectra were obtained by using a scanning monochromatic NIR Systems 6500 spectrophotometer (NIRSystems, Silver Spring, MD) equipped with a rotating sample cup. Each spectrum, an average of 32 scans, was collected over the 400 to 700 nm wavelength range at 2 nm intervals. Before a spectrum of meat was measured, reference and background spectra were acquired.

The spectra were transformed into .spc files (Grams file format), and, with the use of Grams/32 software (Galactic Inc., Salem, NH), the spectra were then offset to zero at the wavelength of 715 nm where the absorbance was minimum. The subsequent 2D correlation analysis was performed with the use of the KG2D correlation program developed by the School of Science, Kwansei-Gakuin University, Japan. In the 2D approach, dynamic (or difference) spectra, obtained by subtracting the average spectrum of the individual set from each spectrum, were used to develop the generalized 2D correlation spectra.

The mathematical background for generalized 2D correlation spectroscopy has been described in detail by Noda elsewhere, and the robust application of the generalized 2D correlation approach to IR, NIR, Raman, and other spectral data has been widely reported.

The generalized 2D correlation spectra consist of synchronous and asynchronous correlation spectra. A synchronous 2D correlation spectrum characterizes the similarity between the sequential variations of spectral intensities. Autopeaks located at the diagonal position represent the extent of dynamic variations of spectral intensity at different wavelength. Synchronous cross peaks appear at off-diagonal positions if the basic trends of dynamic variations observed at two different wavelengths of the cross peak spectral coordinate are similar. Positive cross peaks (shown in solid lines) indicate that intensities at both wavelengths are either increasing or decreasing together, while negative peaks (shown in dashed lines) mean that one intensity is increasing and the other decreasing.

An asynchronous 2D correlation spectrum consists exclusively of off-diagonal cross peaks. It characterizes the difference between the perturbation-dependent sequential variations of spectral intensities. Asynchronous cross peaks appear if the basic trends of dynamic variations observed at two different wavelengths of the cross peak spectral coordinate are dissimilar. From the sign of an asynchronous cross peak, it is possible to assign the specific sequence of events occurring at different times. A negative cross peak indicates that the spectral intensity change observed at λ1 occurs after that at λ2, and positive peaks indicate the opposite. It should be noted that, in the cases of asynchronous spectra, only the spectral data resulting from either monotonic increasing or decreasing perturbations can be used to interpret the sequence of spectral changes meaningfully. Hence, in this paper, the positive or negative signs of asynchronous cross peaks derived from individual sets of samples of similar condition were not discussed, except in a case where the perturbation was ordered in a sequence of wholesome, cadaver, ascites, airsacculitis, septicemia, and tumor. A subjective arrangement of such a sequence is to focus on...
the relationship between the spectral intensity change and disease perturbation.

RESULTS AND DISCUSSION

Figure 2 shows representative reflectance spectra of the wholesome and unwholesome chicken breast meats in the visible region (400–700 nm). This region is one of the most interesting regions due to the presence of various forms of myoglobin, which plays an important role in the chemical and physical characteristics of meats.2-6 On the other hand, some amount of hemoglobin, another important pigmentation responsible for meat color, may be retained in the cadaver meats.

Figure 2 shows that it is very difficult, if not impossible, to visually find any differences between the wholesome and various unwholesome meats in the entire spectral region. It is also very difficult to identify any special spectral absorption bands. Hence, the application of 2D correlation analysis was first attempted on the individual sets of six classes of chicken breast meats. The application was then attempted on the system consisting of all samples in the wholesome, cadaver, ascites, airsacculitis, septicemia, and tumor sequence.

2D Correlation Analysis of Individual Meat Class.

Figure 3 shows the three-dimensional (3D) representation of the synchronous 2D visible correlation spectrum of wholesome chicken meats. It represents the spectral intensity variations among wholesome meats in the region between 400 and 700 nm. Figure 4A shows the contour line map of Fig. 3. In Fig. 4A, major autopeaks at the diagonal position are observed around 445 and 560 nm, and cross peaks associated with the autopeaks are also observed at the off-diagonal position. The sign of the cross peaks (445 vs. 560 nm) or (560 vs. 445 nm) is positive (solid lines), indicating that the spectral intensity change at the 445 nm band was similar to that of the at 560 nm. The appearance of the autopeaks suggests that the intensities of these two bands vary greatly within various wholesome chicken meats. Hence, it implies that the two bands may be influenced significantly by variations in chemical composition and muscle structure.

In a recent investigation of cooked wholesome chicken meats,20 it was observed that the intensities at the 445 and 560 nm bands decrease with increasing temperature due to cooking, and that their intensity reduction occurs before (at a lower temperature) the intensity change at 475, 520, or 585 nm bands. The result strongly suggested that the 445 and 560 nm bands are related to deoxymyoglobin and oxymyoglobin components, which are easily oxidized and degraded into metmyoglobin and other small molecules.2-6 Therefore, Fig. 4A indicates that the relative amount of the deoxymyoglobin and oxymyoglobin components varies significantly from one carcass to another.

The asynchronous spectrum in Fig. 4B reveals an interesting point. An asynchronicity is observed between the 485 nm band and the 445, 540, and 570 nm bands, whereas no corresponding synchronous cross peaks are observed at the 485, 540, and 570 nm coordinates. The most likely explanation here is that the spectral intensity changes at 485, 540, and 570 nm are too weak. The development of asynchronous cross peaks around the 485 nm coordinate indicates that the 485 nm hand arose from neither deoxymyoglobin nor oxymyoglobin, but probably from the other degraded derivative of myoglobin (i.e., metmyoglobin). The assignment of the 485 nm absorption to metmyoglobin will become clear in the following discussion. Incidentally, the bands at 415 and 425 nm were from the Soret absorbance of oxymyoglobin and metmyoglobin.2

Figures 5A and 5B show the synchronous and asynchronous 2D visible correlation spectra derived from the visible spectral set of cadaver meats. As expected, the pattern of autopeaks and cross peaks in Fig. 5A is similar to that in Fig. 4A, except that the autopeak was shifted from 560 to 568 nm. Although Fig. 5B is somewhat similar to Fig. 4B, the notable differences are the appearances of the 568 and 605 nm bands. This finding may be due to the existence of hemoglobin in the cadaver meats, because cadavers are condemned because of an improper bleeding process during slaughter rather than disease-caused condemnation.
Figures 6A and 7A show the synchronous 2D visible spectra produced from the visible spectra of the ascites and the air-sacculitic meats, respectively. These 2D correlation spectra are quite different from the correlation spectra of wholesome or cadaver meats (Figs. 4A and 5A), with the appearance of the autopeak at 485 nm. The corresponding asynchronous features presented in Figs. 6B and 7B show an obvious asynchronicity between the 485 nm band and the 445 and 560 nm bands, confirming that the nature of absorption at 485 nm differs from that at 445 and 560 nm.

Figures 8A and 9A, respectively, show the synchronous 2D correlation of visible spectra generated from the visible spectra of meats infected by septicemia and tumor disease. The most noticeable differences between these 2D correlation spectra and the previous four spectra of wholesome, cadaver, ascites, and air-sacculitis (Figs. 4A through 7A) are the disappearance of the autopeak at 445 nm and the appearance of the dominant autopeak at 485 nm. The corresponding asynchronous spectra in Figs. 8B and 9B again indicate that the spectral intensity variation at 485 nm was different from those at the 445 and 560 nm.

Fig. 4. (A) Synchronous 2D visible correlation spectrum of wholesome chicken meats only. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.

Fig. 5. (A) Synchronous 2D visible correlation spectrum of cadaver chicken meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.
nm bands. In Figs. 8A and 9A, there are no autopeaks or cross peaks at the 445 nm band, possibly because the spectral intensity variations at 445 nm among the septi-
cemic and tumorous meats were very low.

The 2D correlation approach among the different sample classes allows the observation of the spectral intensity variations for the 445, 485, and 560 nm bands. Although it is impossible to compare intensity change between two 2D synchronous autopeaks, it is possible to analyze the relative intensity change of the autopeaks within an individual 2D synchronous spectrum. Table I summarizes the observations from the three-dimensional representation of Figs. 4A through 9A. It becomes clear that (1) the intensity change around 445 nm becomes weaker or is not observed compared to results for other autopeaks from wholesome to unwholesome meats; (2) the intensity variation at the 560 nm band is common among various meats, albeit to different degrees; and (3) the intensity variations at the 485 nm band are strong for diseased classes (air-sacculitis, ascites, septicemia, and tumor).

Fig. 6. (A) Synchronous 2D visible correlation spectrum of ascites meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.

Fig. 7. (A) Synchronous 2D visible correlation spectrum of air-sacculitis meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.
Also, it should be noted that, although the cadaver meats show two autopeaks similar to those for the wholesome meats and have distinct differences from the diseased meats, the wavelengths of these two autopeaks shifted slightly toward a longer wavelength region, as compared to that of wholesome meats, possibly due to the existence of hemoglobin in blood retained in the meats.

Table I summarizes the strengths of spectral intensity variations at the three visible bands for various meats. The cross peaks of synchronous and asynchronous spectra shown in Figs. 4 to 9 clearly suggest that deoxymyoglobin, metmyoglobin, and oxymyoglobin components exist in all wholesome and unwholesome meats. However, little variation in metmyoglobin is seen in wholesome and cadaver meats, whereas for diseased meats, large variations of metmyoglobin were observed. It was also observed that the variations of deoxymyoglobin among diseased meats were small.

2D Correlation Analysis of the System Consisting of the Entire Meats. Although Figs. 4 through 9 provide

Fig. 8. (A) Synchronous 2D visible correlation spectrum of septicemia meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.

Fig. 9. (A) Synchronous 2D visible correlation spectrum of tumor meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.
TABLE 1. Characteristic visible spectral intensity variations of various chicken meats from the 2D correlation approach.

<table>
<thead>
<tr>
<th>Characteristic visible spectral intensity variations</th>
<th>Visible region (400–700 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholesome</td>
<td>445 X 560</td>
</tr>
<tr>
<td>Cadaver</td>
<td>448 (w) X 568 (s)</td>
</tr>
<tr>
<td>Air-saccultis</td>
<td>440 (w) 485 (s) 560 (s)</td>
</tr>
<tr>
<td>Unwholesome</td>
<td>Ascites 445 (w) 485 (s) 565 (s)</td>
</tr>
<tr>
<td></td>
<td>Septicemia X 485 (s) 560 (w)</td>
</tr>
<tr>
<td></td>
<td>Tumor X 485 (s) 565 (w)</td>
</tr>
</tbody>
</table>

\(^{a}\) X: no autopeaks appearing around 445, 485, or 560 nm.

\(^{b}\) ( ): indicating the relative intensities among the autopeaks in 3D representation of the synchronous 2D correlation spectrum; s = strong; w = weak.

A better overall view of the intensity variations of 2D correlation spectra among the individual spectral groups, it is unclear which type of meat has the obvious intensity change of the 485 nm absorbance. In the following analyses, we combined all the above spectra into a new data set with the ordered sequence of wholesome, cadaver, ascites, air-saccultis, septicemia, and tumor. This system was subjectively arranged because wholesome and cadaver meats show two 2D correlation autopeaks at 445 and 560 nm; ascites and air-saccultis show three at 445, 485, and 560 nm, while septicemia and tumor show two at 485 and 560 nm. Such an arrangement allows the monitoring of the sequential change of spectral intensity variations of the three bands at 445, 485, and 560 nm with the consideration of disease perturbation.

The asynchronous spectrum in Fig. 10B suggests that the pattern of the band intensity variation near 485 nm, associated with diseased meats, is different from the variations at 445 and 560 nm bands. The positive/negative signs of asynchronous correlation peaks show that the variations at the 485 nm band occurred after the 445 and 560 nm, indicating that the chemical constituent with strong light absorption at 485 nm could have come from those with strong absorption at 445 and/or 560 nm. It is well recognized that disease alters meat composition with the change of colors as a visible manifestation of heme degradation. Hence, the 485 nm band could be assigned to metmyoglobin, a degraded derivative of both the deoxymyoglobin and oxymyoglobin components.

**CONCLUSION**

Visible spectra of meats are intrinsically rich in information for studying the chemical and quality characteristics of meat and for qualitative and quantitative analyses of meat quality. However, because of the spectral complexity, it is not easy to extract useful information from heavily overlapped one-dimensional spectra. With the 2D correlation technique, it is clearly revealed that there were at least three absorption bands at around 445, 485, and 560 nm, which could be assigned to deoxymyoglobin, metmyoglobin, and oxymyoglobin absorptions, respectively. The results obtained from synchronous and asynchronous spectra of individual spectral classes suggest that the deoxymyoglobin and oxymyoglobin components coexisted with metmyoglobin in both wholesome and unwholesome meats, but with different amounts. However, there is a clear indication that there were more variations in oxymyoglobin and deoxymyoglobin and fewer varia-

![Fig. 10](image-url) (A) Synchronous 2D visible correlation spectrum of both wholesome and unwholesome meats. (B) The corresponding asynchronous 2D correlation spectrum in the same spectral region.
tions in metmyoglobin in the wholesome and cadaver meats than in the diseased meats. Also, the asynchronous spectral analysis of the wholesome and diseased and defective meats revealed that the intensity change at the 485 band occurred later than those of the 445 and 560 nm bands, indicating that metmyoglobin, the degraded species of both the deoxymyoglobin and oxymyoglobin, mainly existed in the diseased meats.

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