

# Analysis of visible reflectance spectra of stored, cooked and diseased chicken meats<sup>☆</sup>

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## Abstract

Visible spectra of cold stored, cooked, and diseased chicken meats were collected. Changes in ratios of  $R_1 = A_{485 \text{ nm}}/A_{560 \text{ nm}}$  and  $R_2 = A_{635 \text{ nm}}/A_{560 \text{ nm}}$ , which are related to absorbances of the bands at 485 (metmyoglobin), 560 (oxymyoglobin), and 635 nm (sulphyoglobin), were observed to be useful for studying the variation of meat color under the conditions of cold storage and cooking process. Such a strategy was also applied to classify fresh-raw wholesome and unwholesome meats into respective classes, and the result was compared with that produced from a chemometric model. The strategy might be used as a simple methodology for monitoring the color variation of meats where the development of the chemometric model is either impractical or not desirable. Published by Elsevier Science Ltd.

*Keywords:* Visible spectroscopy; Chicken meat; Meat color; Myoglobin; Chemometrics

## 1. Introduction

Visible spectroscopy, with a scanning extension to the near-infrared (NIR) region, has been found considerable applications in safety and quality control issues of chicken meat products (Chen & Marks, 1997, 1998; Chen, Huffman, Park, & Nguyen, 1996; Chen, Park, Huffman, & Nguyen, 1998; Fumiere, Sinnae, & Dardenne, 2000; McElhinney, Downey, & Fearn, 1999; Rannou & Downey, 1997). The applications include the quantitative prediction of the physical characteristics of heat-treated chicken patties (Chen & Marks, 1997, 1998), the identification of the chicken species from other meats (McElhinney et al. 1999; Rannou & Downey, 1997), the discrimination of “slow-growing” chickens from “industrial” ones (Fumiere et al., 2000), and the classification of chicken carcasses into wholesome and unwholesome classes at the slaughter plant (Chen et al., 1996, 1998).

The major pigment in well-bled meat is myoglobin, and the color of meat is largely determined at the meat surface by the relative amounts of three forms of myoglobin, i.e. deoxymyoglobin (DeoxyMb), metmyoglobin (MetMb), and oxymyoglobin (OxyMb; Fennema, 1996; Francis & Clydesdale, 1975; Kinsman, Kotula, & Breidenstein, 1994; Lawrie, 1985; Price & Schweigert, 1987; Zhu & Brewer, 1998). During external processes such as cooking, storage, and irradiation, the three forms of myoglobin interconvert and are degraded through oxygenation, oxidation and reduction reactions, ultimately influencing the appearance of meat color. The subsequent changes can be detected non-destructively and sensitively by visible absorption characteristics (Liu & Chen, 2000; Nanke, Sebranek, & Olson, 1998; Swatland, 1989).

In order to improve the application of visible spectroscopy, we used the recently developed two-dimensional (2D) correlation analysis to systematically investigate the spectral features of chicken meats under various conditions such as thermal treatment, cold storage, and bacterial infection of meats (Liu & Chen, 2000; Liu, Chen, & Ozaki, 2000a, b). It has turned out that the 2D visible approach can not only establish the spectral band assignments but also elucidate the complex sequence of occurrences for DeoxyMb, OxyMb, and MetMb species. For example, four visible bands

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around 445, 485, 560, and 635 nm are identified as DeoxyMb, MetMb, OxyMb, and sulfmyoglobin (SulfMb), respectively. The results also revealed that cooking and storage cause the degradation of DeoxyMb and OxyMb components into MetMb, SulfMb, and small molecules through oxidation and reduction reactions, and ultimately lead to the discoloration of meats. In addition, it showed that DeoxyMb, MetMb, and OxyMb species coexist in all fresh-cut wholesome and unwholesome (diseased) meats, with a clear indication that wholesome meats have more variation in OxyMb and DeoxyMb and less variation in MetMb than do diseased meats (Liu et al., 2000a). However, 2D correlation analysis could not provide the information for an individual spectrum, as it utilizes a data set comprised of several spectra and compares the spectral intensity variations of one spectrum to those of others.

The objective of this study is to find visible marker bands which are sensitive to meat color variation in an individual spectrum. We examined the possibility that the three visible bands at 485, 560, and 635 nm, respectively, due to MetMb, OxyMb, and SulfMb, might be useful as indicators of meat color variation during cooking and cold storage. In addition, we presented a comparison in the classification of fresh-raw wholesome and unwholesome chickens into individual classes on the basis of such a strategy and a chemometric model.

## 2. Materials and methods

### 2.1. Meat samples

Fresh chicken carcasses were selected by Food Safety and Inspection Service (FSIS) veterinarians from the processing line at a poultry slaughter plant located on the Eastern Shore of Maryland (Cordova, MD, USA). The carcasses were packaged into polyethylene bags which were placed in a plastic cooler filled with ice, and transported to USDA's Instrumentation and Sensing laboratory in Beltsville, MD. In the afternoon, fresh breast meat (1 cm thick and 3.8 cm diameter) was cut from each chicken carcass, sized to fit into the spectrophotometer's quartz-windowed cylindrical cup.

#### 2.1.1. Cooking

The cut samples were cooked in a small convection oven (Equipex Sodor Model No. FC-26/34, Providence, Rhode Island, USA) at a constant air temperature of 150°C for 3, 6, 9, 12, 15, and 18 min. Once the cooking sample reached the target time, it was removed from the oven and resealed in a polyethylene bag. After cooling to room temperature, the spectra were collected (Liu et al., 2000b).

#### 2.1.2. Cold storage

The meats were sealed in polyethylene bags and held in a cooler filled with ice, and the cooler was placed in a 0°C cold room and dark environment. Next day (designated as Day 2), the spectra were obtained. After the measurement, the slices were returned to the storage condition until the measurements were taken again. The same procedures were repeated at third day postmortem (Day 3), fourth day postmortem (Day 4), up through the 18th day postmortem (Day 18; Liu & Chen, 2000).

#### 2.1.3. Fresh-raw wholesome and unwholesome meats

Their conditions were examined in the plant by FSIS veterinarian inspectors (Liu et al., 2000a). The carcasses included 80 wholesome, 27 cadaver, 12 tumor, 26 septicemia, 22 air-sacculitis, and 12 ascites. The slices were sealed in polyethylene bags and kept at 0°C, prior to spectral collection.

### 2.2. Spectroscopic measurements and data analysis

All the reflectance spectra were recorded on a scanning monochromator NIRSystems 6500 spectrophotometer (NIR Systems, Silver Spring, MD, USA) equipped with a rotating sample cup. Each spectrum was collected over the 400–2500 nm wavelength range at 2 nm intervals and 32 scans (Liu & Chen, 2000; Liu et al., 2000a, b).

The obtained spectra were imported into Grams/32 by using Grams/32 software (Galactic Industries Corp., Salem, NH, USA), and the second derivative spectra were calculated with the Savitzky-Golay derivative function and 11 smoothing points. All the evaluations of the intensities for the bands due to MetMb, OxyMb, and SulfMb species were made using the wavelength region between the ranges of 458–520, 520–615, and 620–664 nm, respectively, from the individual second derivative spectrum with the use of Grams/32 software. They are, respectively, denoted to  $A_{485 \text{ nm}}$ ,  $A_{560 \text{ nm}}$ , and  $A_{635 \text{ nm}}$ .

Chemometric analysis was also performed for a total of 179 fresh-raw wholesome and unwholesome meats using Soft Independent Modeling of Class Analogy (SIMCA) of principal component analysis with a Mahalanobis distance metric in Grams/32 (Galactic, 1996). Briefly, 53 wholesome and 63 unwholesome chickens were randomly selected to form calibration sets, and the remaining 27 wholesome and 36 unwholesome chickens formed validation sets. By applying two SIMCA classes (wholesome and unwholesome) to each spectral data set, and employing the class assignment rules of either a lower Mahalanobis distance or a lower spectral residual if the ratio of two Mahalanobis distances was between 0.85 and 1.15, the sample was identified as the species being modeled, i.e. either the wholesome or the unwholesome class.

### 3. Results and discussion

#### 3.1. Visible spectra of cold stored chicken meats

During nonfrozen storage, raw meats undergo several changes that can affect their quality attributes (Kinsman et al., 1994; Lawrie, 1985; Price & Schweigert, 1987). These changes are reflected through the color, tenderness, flavor, and juiciness of the meats. Color is one of the primary meat sensory factors, and its variation can be characterized by visible absorption (Liu & Chen, 2000; Nanke et al., 1998; Swatland, 1989).

Fig. 1 shows the representative visible reflectance spectra of the chicken meats stored at 0°C from Day 2 to Day 18. With increasing storage time, the peak intensity increase of two broad bands near at 485 and 635 nm and the peak intensity reduction of the broad band around 560 nm are clearly observed. In the present study, the carcasses were well-bled and thus myoglobin is the primary heme pigmentation responsible for the color of meats (Fennema, 1996; Francis & Clydesdale, 1975; Kinsman et al., 1994; Lawrie, 1985; Price & Schweigert, 1987; Zhu & Brewer, 1998). Spectral intensity variations of the peaks at 485, 560, and 635 nm likely indicate a dynamic conversion and decomposition for a number of myoglobin derivatives.

It also reveals that the peak at 635 nm is undetectable in Day 2, and then becomes stronger and distinctive thereafter, implying that fresh cut meats contain little of the species represented by the 635 nm absorption. Another notable feature in Fig. 1 is the appearance of two peaks at 545 and 575 nm and the disappearance of the 560 nm peak.

Earlier literature had assigned the peak at 555 nm to deoxymyoglobin (DeoxyMb), the two peaks at 545 and 575 nm to oxymyoglobin (OxyMb), and the two peaks

at 505 and 630 nm to metmyoglobin (MetMb; Price & Schweigert, 1987; Swatland, 1989). This assignment might not be quite correct and reasonable at least for chicken meats, because it is unlikely that MetMb absorbs at both 505 and 630 nm with a difference of 125 nm, and that OxyMb appears at 545 and 575 nm while DeoxyMb has an absorption between them.

Our systematic study on visible spectra of chicken meats under a variety of conditions, such as cooking, cold storage, wholesome and unwholesome, has revealed that the bands at 445, 485, 560, and 635 nm arise mainly from DeoxyMb, MetMb, OxyMb, and sulfmyoglobin (SulfMb) species, respectively (Liu & Chen, 2000; Liu et al., 2000a, b). The conclusions were established from the following observation. (1) During the cooking and cold storage process which associated with meat discoloration, two bands around 445 and 560 nm decrease in intensity. The 445 nm band assignable to DeoxyMb is due to its significant spectral intensity reduction. (2) The band at 485 nm is attributed to MetMb species which accumulated with the meat storage, as it comes from the degradation/oxidization of DeoxyMb and OxyMb components. (3) DeoxyMb, OxyMb, and MetMb coexist in all meats, but with a clear indication that there were more variations in OxyMb and DeoxyMb and less variations in MetMb in the wholesome cadaver meats than in the diseased meats. (4) Each of the bands at 445, 485, and 560 nm has at least three close but separated bands. Probably, changes in secondary structure of myoglobin proteins (such as from  $\alpha$ -helix to  $\beta$ -sheet) and the interactions between proteins and other components such as water and lipids contribute to the variations in the molecular environment of the heme vibrations. (5) The 635 nm absorption is different from those at 440, 485, 500, and 585 nm bands, and it could be due to the presence of SulfMb, a derivative of interaction between myoglobin and H<sub>2</sub>S generated by bacteria.

To further confirm the 635 nm band establishment, we formed SulfMb by exposing the commercial myoglobin (from horse skeletal muscle: 95–100%, Sigma Chemical Co., St. Louis, MO) to the atmosphere of H<sub>2</sub>S, which in turn was generated by adding sodium sulfide (nonahydrate, Sigma Chemical Co., St. Louis, MO) into deoxygenated and deionized water. After 60 min of exposure to the H<sub>2</sub>S gas, the visible reflectance spectrum of the treated myoglobin was collected. Fig. 2 compares the visible spectra of untreated and H<sub>2</sub>S-treated myoglobin. Notably, with the H<sub>2</sub>S treatment, the intensity around 635 nm clearly increased, and those of broad bands from 540 to 590 nm decreased. The relative intensity and band position in Fig. 2 are generally and reasonably similar to those of chicken meats in Fig. 1. This result suggests that SulfMb complexes such as Sulf-OxyMb, Sulf-DeoxyMb, and/or Sulf-MetMb contribute to the 635 nm band absorbance.

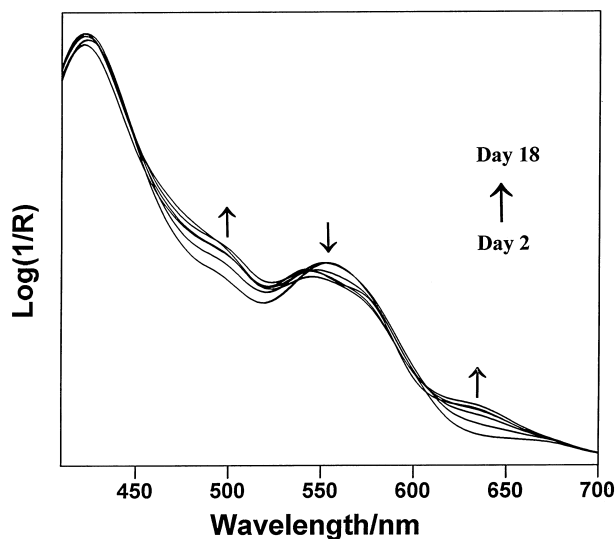


Fig. 1. Visible reflectance spectra of chicken meats during cold storage (0°C) from Day 2 to Day 18.

Our assignment of the 635 nm band to SulfMb seems to be contradictory with the reported 617 nm wavelength (Nicol et al., 1970). In fact, different spectral collection modes (transmission or reflectance) cause this discrepancy. Meanwhile, chromophore molecular environmental changes/alternations will occur before and after biochemical extraction, which, in turn, may lead a loss of information in real meat samples.

The differences in band shape and intensity are highlighted in the second derivative presentation (Fig. 3). The bands lower than 450 nm would not be utilized due to the anomalies and distortions. More distinctly, at

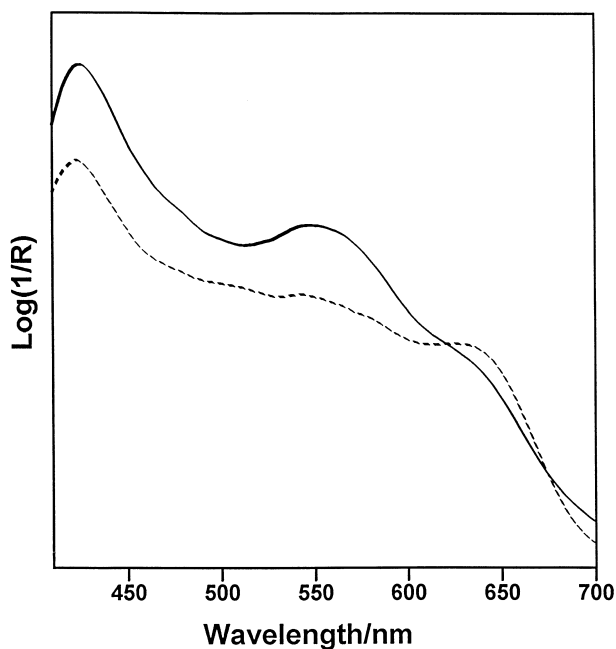


Fig. 2. Visible reflectance spectra of untreated (solid line) and  $H_2S$ -treated (dotted line) myoglobin.

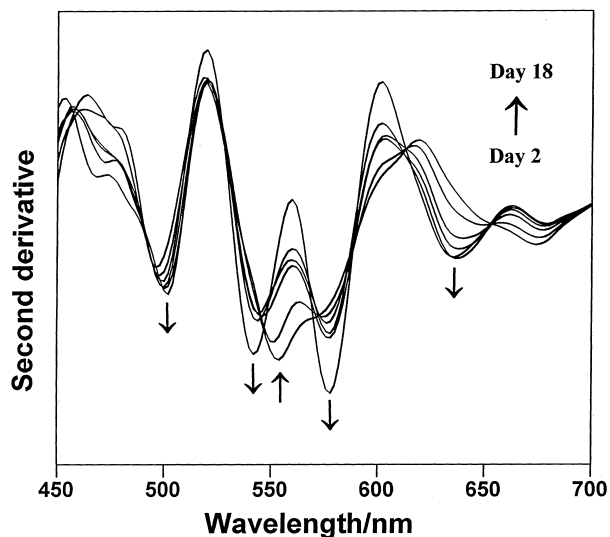


Fig. 3. The second derivative of visible spectra of chicken meats during cold storage ( $0^\circ C$ ) from Day 2 to Day 18.

least two bands around 555 and 675 nm decrease in peak intensity, and four bands at 495, 545, 575 and 635 nm increase in peak intensity. Accordingly, the bands at 495 and 635 nm are from MetMb and SulfMb species, whereas the bands around 515–600 nm are assignable to various types of OxyMb components (Liu & Chen, 2000). Meanwhile, the origination of the weak feature at 675 nm wavelength is unknown, and its assignment is beyond this study.

Fig. 4 plots the relative intensity ratios of  $R_1 = A_{485 \text{ nm}}/A_{560 \text{ nm}}$  and  $R_2 = A_{635 \text{ nm}}/A_{560 \text{ nm}}$  versus storage time, linking the variations in the amount of MetMb (485 nm), OxyMb (560 nm), and SulfMb (635 nm) for individual meat, respectively. The  $R_1$  value increases quickly from Day 2 to Day 8, then decreases afterwards; whereas  $R_2$  continues to increase before turning into a minor decrease around Day 11. It suggests that MetMb and SulfMb are produced promptly at the initial storage, and then they are in a relatively steady-state between their production and further decomposition into small molecules (Voet & Voet, 1995). Moreover, the track of  $R_1$  versus storage time differs from that of  $R_2$ , indicating that the bands at 485 and 635 nm arise from two completely different species. This observation is in consistent with the previous 2D correlation analysis (Liu & Chen, 2000). Hence, the accumulation of both MetMb and SulfMb species results from the oxidation/degradation reaction of DeoxyMb and OxyMb components. In other words, the steady elimination of DeoxyMb and OxyMb fractions causes the meat discoloration.

### 3.2. Visible spectra of cooked chicken meats

Fig. 5 shows the second derivative of the visible spectra of chicken meats cooked at a constant air temperature of  $150^\circ C$  for 3, 6, 9, and 18 min. With cooking,

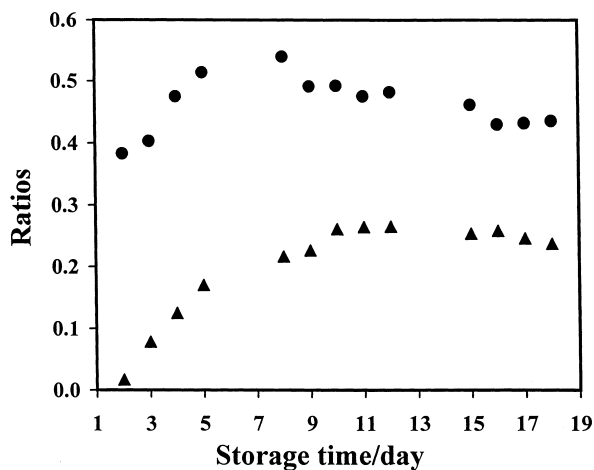


Fig. 4. Plot of the intensity ratios of  $R_1 = A_{485 \text{ nm}}/A_{560 \text{ nm}}$  (circle) and  $R_2 = A_{635 \text{ nm}}/A_{560 \text{ nm}}$  (triangle) in the second derivative spectra versus storage time.

extensive changes in the appearance and physical properties of meats occur (Chen & Marks, 1997, 1998; Kinsman et al., 1994; Swatland, 1983). These changes include the toughening and discoloration of the meats, with toughening due to the denaturation of proteins and discoloration due to the oxidization of pigment heme groups (Kinsman et al., 1994). So, a marked drop in peak absorbance around 560 nm could be associated with the loss of meat color (Liu et al., 2000b; Swatland, 1983). In addition, Fig. 5 exhibits less significant visible bands than Fig. 3; for example, the bands at 545, 575, and 635 nm are too weak to be seen. Possibly, the spectral difference between Figs. 5 and 3 indicates the time-related occurrence of the oxidization/degradation process of OxyMb component; that is, heating accelerates the oxidization/degradation of OxyMb into MetMb and small molecules.

Fig. 6 plots the relative intensity ratios of  $R_1$  and  $R_2$  versus cooking time. Notably, the value of  $R_1$  increases gradually with cooking time, while that of  $R_2$  shows little increase and is lower than 0.1. It indicates that the relative amount of MetMb continues to increase with heating, whereas SulfMb species is in a nearly steady-state between its production and decomposition equilibrium.

Comparison of the trend for either  $R_1$  or  $R_2$  in Figs. 4 and 6 provides an interesting point. Regardless of the scale of increment, both  $R_1$  and  $R_2$  increase with the treatment time, suggesting that the relative amount of MetMb and SulfMb to OxyMb increases. In other words, quantity of OxyMb decreases, which, in turn, is responsible for the discoloration of meats in both circumstances. On the other hand, the trend in Fig. 4 differs from that in Fig. 6, implying that different mechanisms of chemical, biochemical and physical changes occur in meats during cooking and cold storage.

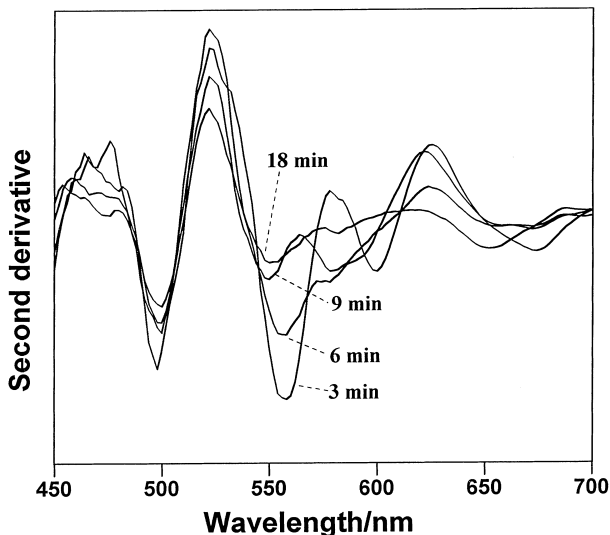


Fig. 5. The second derivative of visible spectra of chicken meats cooked for 3, 6, 9, 18 min under the air temperature of 150°C.

### 3.3. Visible spectra of fresh-raw wholesome and unwholesome chicken meats

The above observation suggests that the variations in relative amount of OxyMb, MetMb, and SulfMb species may be useful as an indicative of meat color change responding to external treatments. Therefore, it is useful to examine whether fresh wholesome and unwholesome chicken meats could be discriminated on the basis of such a strategy. In the present trial, unwholesome meats included five different types: cadaver, air-sacculitis, ascites, septicemia, and tumor. They were condemned either because they were improperly bled (cadaver), or showed a disease symptom (such as air-sacculitis, ascites, septicemia and tumor) at slaughter plants by FSIS inspectors (USDA, 1989). These carcasses demonstrated a variety of obvious changes in appearance, skin color, and/or meat color.

Fig. 7 shows representative second derivative of visible spectra of fresh-cut wholesome meats. The appearance and relative intensity change of the bands at 550, 560, and 575 nm suggest the diversity of meats even with the same condition, as the meat color is influenced greatly by a variety of factors, such as sex, pH values, species, breed, postmortem handling and temperature (Francis & Clydesdale, 1975; Kinsman et al., 1994; Lawrie, 1985; Price & Schweigert, 1987; Zhu & Brewer, 1998). We also carefully examined second derivative spectra of various unwholesome meats and found that there was no clear spectral difference between individual wholesome and unwholesome meat samples.

The  $R_1$  and  $R_2$  values for all wholesome and unwholesome meats were evaluated using the same procedure as above. It was found that  $R_1$  and  $R_2$  for all meats fall in 0.1–0.45 and 0.02–0.07 ranges, respectively. Attempts were made to distinguish the wholesome meats from unwholesome class based on either  $R_1$  or  $R_2$  or the

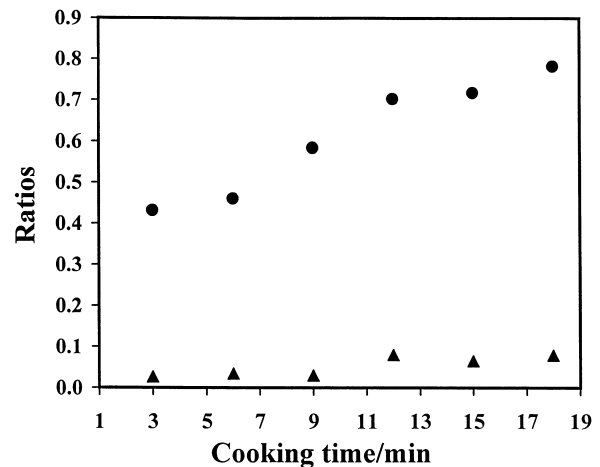


Fig. 6. Plot of the intensity ratios of  $R_1 = A_{485 \text{ nm}}/A_{560 \text{ nm}}$  (circle) and  $R_2 = A_{635 \text{ nm}}/A_{560 \text{ nm}}$  (triangle) in the second derivative spectra versus cooking time.

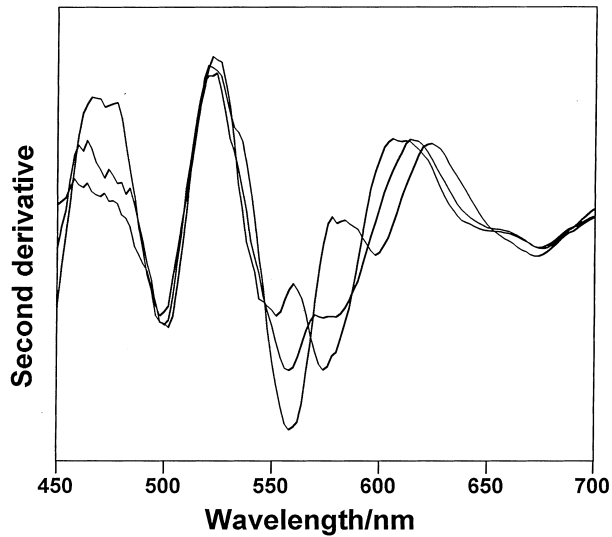


Fig. 7. Representative of the second derivative of visible spectra of wholesome chicken meats.

combination of  $R_1$  and  $R_2$ . It turned out that  $R_2$  could be hardly used because all meats have small and similar  $R_2$  values, due to the fact that SulfMb fraction is little in newly fresh-cut meats (Liu & Chen, 2000). However, if a threshold value of  $R_1$  was set to 0.24 (Table 1), i.e. a sample was classified as wholesome when  $R_1 > 0.24$  and as unwholesome otherwise, the correct classification of wholesome meats was 87.5% from the visible spectrum. Meanwhile, it also showed that nearly one-third of unwholesome meats, especially those with tumors, is mistakenly classified as wholesome.

Note that wholesome meats have larger  $R_1$  values than unwholesome ones, which seems to be contradictory to the cases of meats in cooking and storage. However,  $R_1$  values provide information on the relative

amount of MetMb to OxyMb for specific meats. With cooking and storage treatment, increasing  $R_1$  indicates a decrease in the relative amount of OxyMb species, which is associated with the discoloration of meats. Meanwhile, large  $R_1$  values imply that fresh-raw wholesome meats have relatively more MetMb and/or less OxyMb than unwholesomes do. Attentively, contribution from DeoxyMb species was not under consideration because of the anomalies and distortion for the DeoxyMb absorption band.

Because of the failure in discriminating wholesome and unwholesome meats at a satisfactory level on the basis of the relative intensity ratio, the application of a chemometric model, namely principal component analysis (PCA), was attempted (Galactic, 1996). A total of 179 visible spectra representing 80 wholesome and 99 unwholesome meats were loaded into PLSplus/IQ package in Grams/32 to perform discriminant analysis. Fifty-three of 80 spectra collected for wholesome and 63 of 99 spectra for unwholesome meats were used for calibration sets; the remaining 63 spectra used for model validation sets. Classification models were developed using two classes (wholesome and unwholesome) and second derivative spectra, based on SIMCA (Soft Independent Modeling of Class Analogy) principal component analysis with a Mahalanobis distance metric and a residual spectral measurement (Galactic, 1996). For each of the two classes, the optimal number of factors was determined to be 9 or 10, and each model was applied to all 179 samples. The summary for SIMCA analysis is shown in Table 2. The results of calibrations involving both the visible and NIR regions (400–2500 nm) and only the NIR region (700–2500 nm) are also summarized in Table 2 for comparison. The result from visible spectral region is almost as effective as that from

Table 1

Classic classification of wholesome and unwholesome chicken meats with intensity ratio in visible region

	Wholesome	Unwholesome				
		Cadaver	Tumor	Septicemia	Air-sacculitis	Ascites
$R_1 > 0.24$	70	4	12	7	5	1
$R_1 < 0.24$	10	23	0	19	17	11
Correct classification (%)	87.5	85.2	0	73.1	77.3	91.7

Table 2

Summary results of SIMCA models for wholesome and unwholesome meat classification

Wavelength range (nm)	% Correct classification calibration samples <sup>a</sup>	% Correct classification validation samples <sup>b</sup>	Average (%)
400–700	95.1	92.6	93.8
400–2500	95.8	95.8	95.8
700–2500	90.4	81.9	86.2

<sup>a</sup> Mean of wholesome and unwholesome calibration sets.

<sup>b</sup> Mean of wholesome and unwholesome validation sets.

the entire 400–2500 nm region, with a correct classification of 93.8%. Consequently, the visible wavelength region provides satisfactory differentiation of wholesome and unwholesome chickens, echoing the observation reported earlier that visible spectroscopy is useful in studying the variation of meat color and in discriminating various homogenized meat species (Liu & Chen, 2000; McElhinney et al., 1999).

#### 4. Conclusions

This study presents an alternative methodology to analyze and characterize visible spectra of meats under a variety of conditions. For example, the changes in the intensity ratios of  $R_1$  ( $A_{485 \text{ nm}}/A_{560 \text{ nm}}$ ) and  $R_2$  ( $A_{635 \text{ nm}}/A_{560 \text{ nm}}$ ), which are linked to the absorbances of MetMb, OxyMb, and SulfMb species, could be used to describe the meat color variation with cooking and storage treatments. Although fresh-raw wholesome and unwholesome meats could be hardly discriminated acceptably on the basis of such a strategy, they could be correctly classified with over 93.8% success from the chemometric model. Nevertheless, the classic approach is still attractive since, in its simplest form, there is no calibration model which is commonly built from a large set of spectral data.

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