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Effect of Varying Postmortem Deboning Time and Sampling Position on Visible and near Infrared Spectra of Broiler Breast Filets

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Abstract: Visible-Near Infrared spectroscopy (Vis-NIR) was used to characterize broiler breast filets with varied deboning times and identify how the side and position of the sampling affects the chemometric analysis and prediction capabilities. This study served to identify what differences, if any, exist when collecting spectra from the skin side and the medial side of the breast filets. In addition to the side of the filet, two different positions, anterior and posterior, on the filet were also probed spectroscopically. The comparison of the region and side of sampling of the breast filets has been previously unreported. The breast filets under investigation were subjected to different post-mortem deboning times. The right and left breast filets from each carcass were both used, but were deboned at different times. The results of this study show that the side of the filet has more impact on the spectra than does the position of the sampled area. The data analysis also shows that the spectra from the skin side are more useful for separation of samples by deboning time.

Key words: Visible-near infrared spectroscopy, deboning time, broiler breast meat, chemometrics, sample position

INTRODUCTION

The ubiquitous nature of spectroscopic analytical instrumentation, along with advanced software and sophisticated computers, has led to a revolution in the way that scientists can characterize agricultural commodities. Using spectroscopy, it may be possible to identify adulteration, contamination and disease in meat, plants and other agricultural products (Davis *et al*., 2010; De la Haba *et al*., 2007; Hawkins *et al*., 2010a; Hawkins *et al*., 2010b; Li *et al*., 2008). Spectroscopic data collection can be rapid and depending on the specific sample and method, sample preparation can be minimal. Technology has also made a lot of instruments affordable without sacrificing reliability or resolution. Many current instruments that may be considered entry level or do not require a skilled scientist to operate, far surpass the capabilities of their "research grade" predecessors. When paired with a robust prediction model, this may allow industry to identify problems in their products quickly without having a chemist, microbiologist, etc. on the production floor. An initial characterization of sample spectra along with correlation to other measured parameters may lead to a fast method for prediction of many qualities quickly and simultaneously. This could lead to a cost and time savings, as well as provide a safer and higher quality product.

Visible-Near Infrared spectroscopy (Vis-NIRS) has shown a lot of promise for use in prediction modeling for food products (Fluckiger *et al*., 2011; Givens *et al*., 1997; Prieto *et al*., 2009a). It has been used for quality and safety studies on meats, grains and fruit, to name a few (Fontaine *et al*., 2001; Gonzalez-Martin and Hernandez-Hierro, 2008; Owens *et al*., 2009; Windham *et al*., 2003a; Windham *et al*., 2003b; Yancey *et al*., 2010; Yang *et al*., 2010). Vis-NIRS has been used both for identification of quality parameters and for the detection of bacteria and toxins (Chao *et al*., 2003; Chao *et al*., 2008; Ding *et al*., 2006; Park *et al*., 2007; Windham *et al*., 2003b). The applications for the meat industry, in particular, include measurement of fat content, moisture, protein content, texture and tenderness. These studies have been performed on a wide range of meats, including beef, lamb, pork and chicken (Prevolnik *et al*., 2009; Prieto *et al*., 2009b; Valdes and Leeson, 1994; Valdes and Summers, 1986; Yancey *et al*., 2010). According to the reported data, spectroscopy can be used to correctly classify 80% or more of the samples against the categories under investigation.

Tenderness is one of the most important factors determining the palatability and consumer acceptance of poultry breast meat filets (pectoralis major) (Hayman, 2004). It is significantly affected by postmortem deboning time, with delayed deboning time resulting in more tender meat (Cavitt *et al*., 2005; Cavitt *et al*., 2004; Lyon and Lyon, 1990; Lyon *et al*., 1985; Xiong *et al*., 2006). Tenderness, traditionally, is measured using destructive methods, including Warner-Bratzler shear force and sensory evaluation techniques (Lyon and Lyon, 2001). These methods involve multiple steps and

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are time consuming. Vis-NIRS in conjunction with chemometric analysis can non-destructively, rapidly and accurately predict the quality characteristics of food products (Sun, 2009). Therefore, spectroscopic methods are of great interest and have been studied in order to predict the tenderness of deboned chicken filets (Liu *et al*., 2004a; Liu *et al*., 2004b; Meullenet *et al*., 2004). However, the results from these studies are inconsistent. This could be the result of variations in breast muscle composition on the filet, location position on the filet from which the sample spectra were collected, or differences in experimental conditions. The objective of the present study was to investigate what effects variations of the sampling position will have on the data analysis and separation of the broiler breast filets by postmortem deboning times.

MATERIALS AND METHODS

Sample collection: Eighteen broiler carcasses (~42 days old) were obtained from a local processing plant. The carcasses were collected after the flow-through chillers on the processing line, which occurs at 60-65 min. postmortem. The carcasses were deboned at 2-, 4-, or 24- hrs postmortem. The breast filets were numbered and left and right filets on each carcass were deboned at a different time so that there was less experimental variation due to the use of multiple carcasses. For example, the right breast of carcass 1 was deboned at 2 hrs and the left breast was deboned at 4 hrs. In this scheme, 12 breasts were deboned at 2 hrs, 11 breasts were deboned at 4 hrs and 10 breasts were deboned at 24 hrs. Three broiler breast filets were not used due to several experimental factors including size and excess blood spots.

Vis-NIRS: The Vis-NIR spectra were collected using a Foss XDS scanning monochrometer (FOSS North America, Eden Prairie, MN) in the diffuse reflection mode. All samples were scanned at approximately 26 hours post mortem, regardless of the de-boning time. Each reflectance spectrum is an average of 32 scans with 0.5 nm resolution in the range of 400 to 2500 nm. The spectral collection time is less than 2 min per spectrum. Samples were cored from the breast filets, 38 mm diameter and placed in a sample cup with a quartz window. Four spectra were collected from each breast filet. The samples were collected from an anterior position (P1) and a posterior position (P2). Each sample position was then scanned on both the medial side (B) and the skin side (S), as shown in Fig. 1. Thus, a total of 132 spectra were collected for this study. These spectra were further simplified by averaging together the spectra collected from like samples (i.e. the 12 spectra collected from samples deboned 2 hrs postmortem, skin side and anterior position were averaged to produce one spectrum, designated S2HP1).

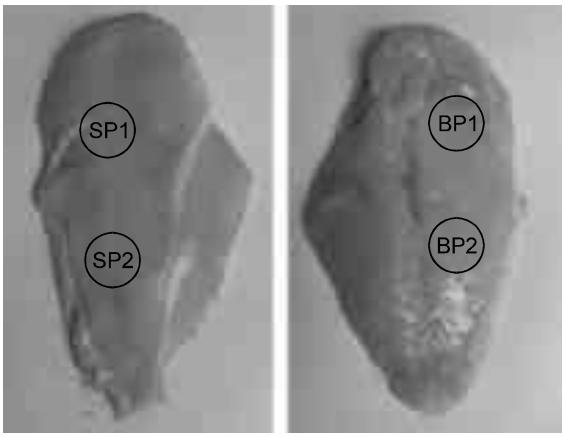

Fig. 1: This figure illustrates the positions that the 38 mm samples were taken from. S is skin side, B is bone side, P1 is anterior and P2 is posterior

Chemometric analysis: The spectra collected were analyzed using The Unscrambler™ software (Camo Software Inc, Woodbridge, NJ). The data were mean centered before analysis. Savitzky-Golay second derivative processing was also applied to the data. Principal component analysis (PCA) was employed to determine if the breast filets could be classified by their deboning time using the spectra. Additionally, the analysis was used to determine what differences, if any, could be attributed to sample location.

RESULTS AND DISCUSSION

Figure 2 shows the raw Vis-NIRS reflectance spectra of the broiler breast filets deboned at 2-, 4-, and 24- hrs postmortem in this study. The most distinguishable peaks are at 430, 492, 560, 980, 1200, 1450, 1800 and 1920 nm. The two broad peaks centered near 1450 and 1920 nm are attributed to water absorption bands. The peak at 1450 nm is the first overtone of the OH stretching vibration of water molecules present in the broiler meat and the peak near 1920 nm is a water combination band; specifically a stretching and a bend combination (Liu and Chen, 2000). Overtone and combination bands of NH groups present in muscle proteins are commonly found at 1500 and 2000 nm. Due to the proximity of these bands to those of water it is difficult to identify how much of each band is due to each functional group. Peaks located at 430, 492 and 560 nm have been assigned to deoxymyoglobin, metmyoglobin and oxymyoglobin, respectively (Liu and Chen, 2000). In the samples collected for this study, it was found that the skin-side samples showed lower absorption peaks for all of the myoglobin bands, regardless of deboning time or whether the sample was collected from the anterior or posterior position of the filet. The peaks located at 1200 and 1800 nm are commonly attributed to overtone (stretch) bands of CH groups present in lipid (fat) molecules found in foods.

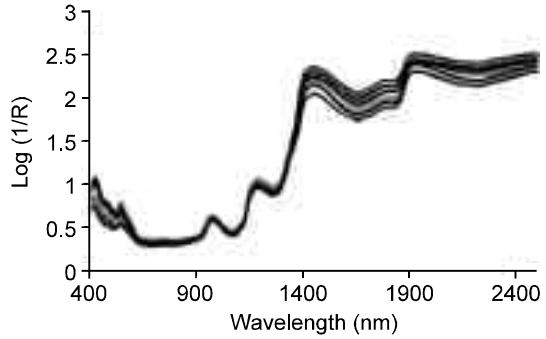

Fig. 2: A representative selection of several raw spectra of the chicken meat samples taken using visible-near infrared spectroscopy

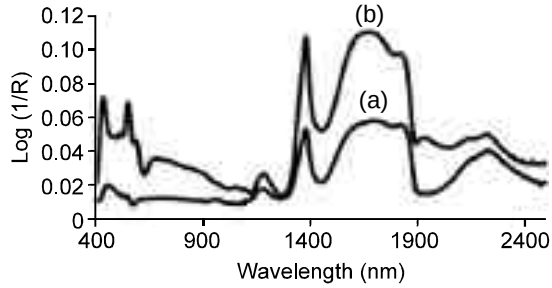

Fig. 3: Mean difference spectra of skin side samples from position 1 that were deboned at (a) 2 hrs and 4 hrs postmortem (S2HP1-S4HP) and (b) at 2 hrs and 24 hrs postmortem (S2HP1-S24HP1)

Prior to further spectral analysis, the spectra for each treatment combination (i.e. all samples that were from a single side, position and deboning time) were averaged together, thus resulting in 12 spectra. Figure 3 shows the difference between the average spectra for the skin side samples in the anterior position from 2- to 4- hrs (S2HP1-S4HP1) and from 2- to 24- hrs (S2HP1-S24HP1) postmortem deboning time. The difference between 2- to 4- hrs is most apparent at ~1385 and 1500-1900 nm, both of which are regions where the overtones of CH stretches from fats occur. The difference from 2- to 24-hrs deboning time also has a big difference due to the CH fat peaks, but also a more noticeable difference in the visible region occurs. The peaks at 430 and 560 nm, due to deoxymyoglobin and oxymyoglobin, respectively, show a marked change with increasing deboning time.

Principal Component Analysis (PCA) was chosen as the most appropriate chemometric method to analyze the spectra of sampled broiler breast filets in this study. PCA is a valuable tool when trying to analyze data sets with a lot of variables because this method compresses the a large set of variables into a much smaller set, referred to as principal components, PCS (Sun, 2009). By using this mathematical tool, data sets can be reduced into PCS with the most useful information while still being able to account for variance in the data. This method is useful for more clearly showing how the absorbance varies with wavelength in spectral data, while at the same time it decreases the importance of sloping and slight offsets of the spectra (Sun, 2009). This treatment resulted in a clear separation of both deboning times and sampling sides when analyzed with PCA.

The PCA score chart for these 12 sets of samples is shown in Fig. 4. Score charts show how the spectra variables correspond to the variables of the principal component. The principal component analysis of this sample set resulted in two principal components that account for 91% of the data variance, 78% for PC1 and

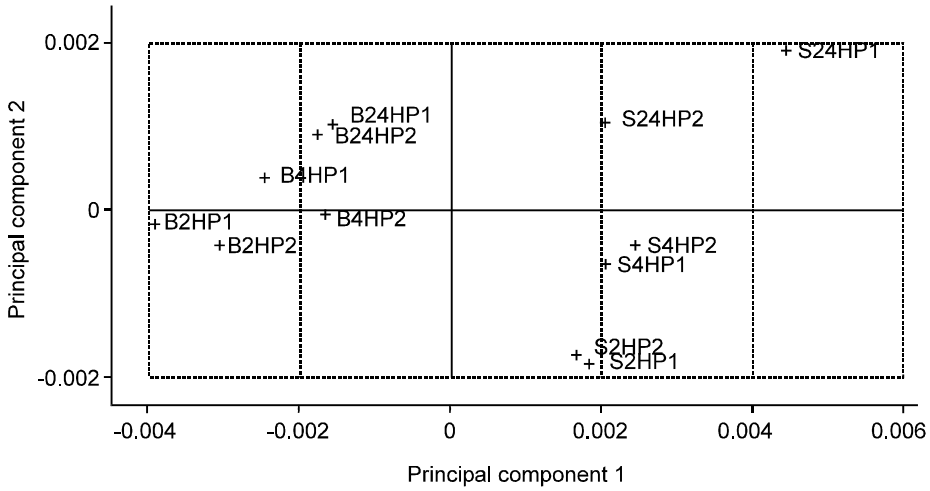

Fig. 4: The score chart resulting from the principal component analysis of the Vis-NIR spectra of the broiler breast filets deboned at 2 hrs, 4 hrs and 24 hrs, sampled from anterior (P1) and posterior (P2) positions of the skin side (S) and bone side (B) of the filets

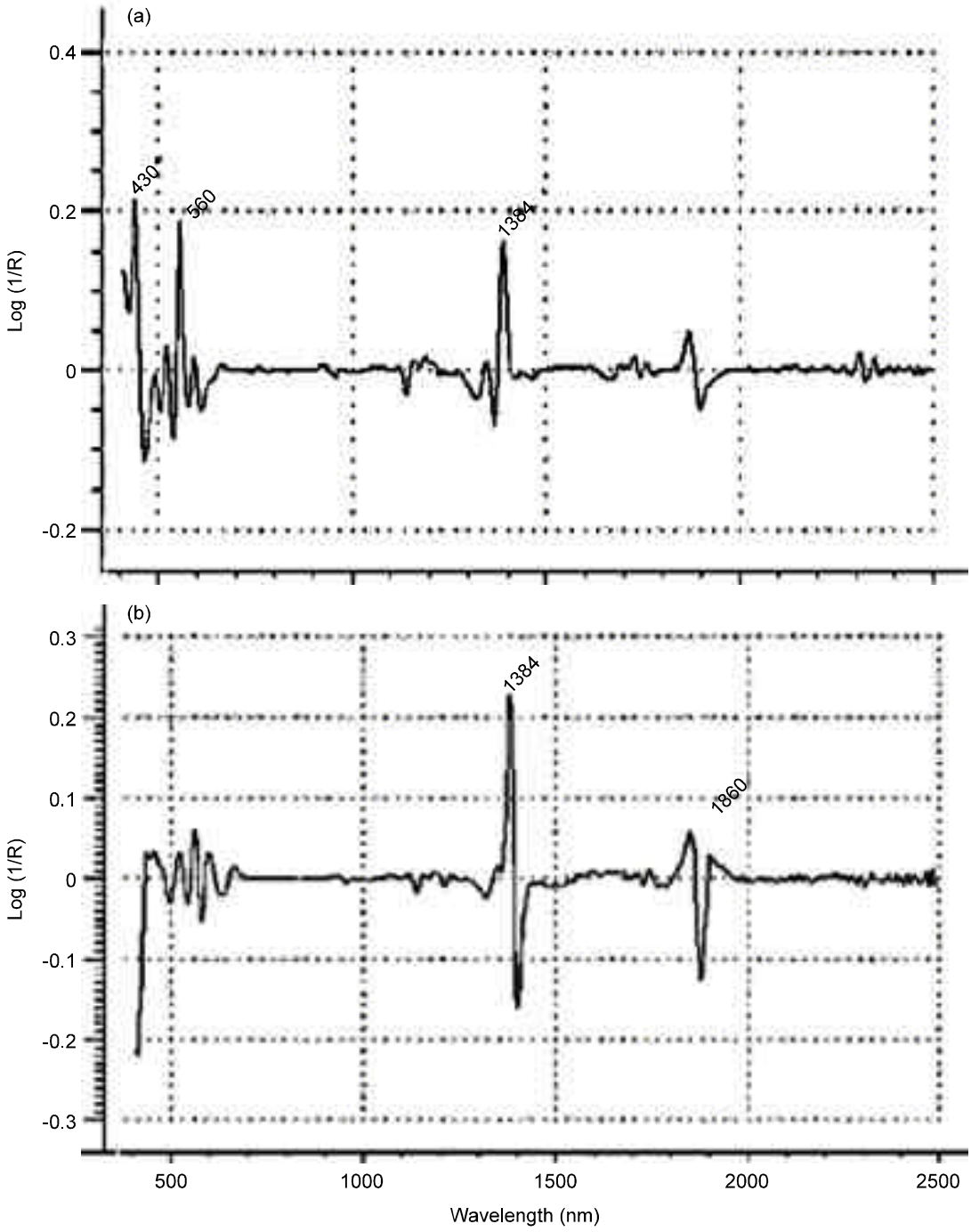

Fig. 5: (a) PC1 loading chart (b) PC2 loading chart

13% for PC2. This score chart shows that the position of the sample, either anterior or posterior, matters very little when using this method for separation of deboning times. All three deboning times and both sides of the broiler breast filets are fairly consistent with two positions being near each other on the score chart. The spectra which were collected from the skin side of the broiler breast filets are well separated from those of the bone side of the filets in this score chart. Figure 5 illustrates why the two sides are so easily separable. The principal component loading charts shown in Fig. 5 demonstrate which variables were the most useful in the data for explaining the data variance for these samples. Figure 5a is the loading chart for PC1 (principal component 1, the x-axis in Fig. 4) and Fig. 5b is the loading chart for PC2 (principal component 2, the y-axis

in Fig. 4). Loading refers to a weighted importance of each variable for the particular principal component. Figure 5a, when viewed in conjunction with PC1 of Figure 4, shows that the myoglobin peaks at 430 and 560 nm and the lipid peak at 1384 nm are instrumental variables for the separation of the spectra for the skin side and the bone side of broiler breast filets. This result is not unexpected given that chicken breasts are heterogeneous and are known to have higher concentration of myoglobin derivatives on the bone side (Liu and Chen, 2000).

The comparison of the y-axis of Fig. 4 with Fig. 5b reveals that the variables at 1384 and 1860 nm are much more significant in the separation of the deboning times in this set of samples. These peaks are attributable to the CH combination band and stretch overtones, respectively, of lipid molecules that are present in the meat. As is indicated by the lower percentage of explanation of data variance of PC2, these variables have less variance between the spectra of the different samples. Even with a lower measure of variance, these variables change enough to separate the 2-, 4- and 24- hr deboning times used in this study. A previous study that focused on the variation of NIR spectra of chicken that was placed in cold storage suggested that there was an earlier change in the water and amine (protein) peaks than in the peaks that were due to CH groups (Liu and Chen, 2000). That study was conducted over the period of several days on samples that all received the same treatment. The current study shows that small changes in the fats that occur first may be due to the muscle postmortem aging on the bone. The changes of all of these peaks has been correlated with meat quality and this data shows that the spectra can be used to separate out meat by deboning time and thus by the increase or decrease of the presence of chemicals that enhance or detract from the tenderness of the poultry meat.

The separation of the data in Fig. 4 can help us to draw several conclusions about the importance of sampling techniques when collecting visible-near infrared spectra. First, chicken broiler breast filets are heterogeneous with respect to side. The spectra appear to have more of a separation due to sampling at either the skin side or the bone side than to either the anterior or posterior position. This suggests that researchers could use several samples from the same filet for correlation of different measurements as long as they are consistent with the side of the filet that is used. Secondly, the skin side samples are more useful in this study for separation of deboning time than the bone side samples. Since the loading chart of PC2 shows that the variables at 1385 and 1860 nm have more of an effect on the data variance and it is seen in Fig. 4 that PC2 separates the deboning times more effectively, it is reasonable to assume that the lipid molecules present on the skin side have a greater change introduced with the variation in the postmortem deboning time. The third conclusion from this data is that both visible and near infrared absorptions contribute to the separation of the filet surfaces, but the near infrared absorptions contribute more for the separation of deboning times. The results from this study have shown that sampling techniques introduce differences in the Vis-NIR spectra of broiler breast filets and that Vis-NIRS can be used with chemometrics to successfully separate filets that had 2-, 4- and 24- hr postmortem deboning times. Alternatively, the results suggest that this technique may be able to be used to successfully separate filets with different shear force or tenderness, since it has been shown that there were consistent differences in average shear force values between filets deboned at 2-, 4-, or 24-hr postmortem (Cavitt *et al*., 2005; Cavitt *et al*., 2004; Lee *et al*., 2008; Liu *et al*., 2004a; Xiong *et al*., 2006).

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