Quantitative Analysis and Detection of Adulteration in Crab Meat Using Visible and Near-Infrared Spectroscopy

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Visible and near-infrared spectroscopy (VIS/NIR) has been used to detect economic adulteration of crab meat samples. Atlantic blue and blue swimmer crab meat samples were adulterated with surimi-based imitation crab meat in 10% increments. Waveform evaluation revealed that the main features seen in the spectral data arise from water absorptions with a decrease in sample absorbance with increasing adulteration level. Prediction and quantitative analysis was done using raw data, a 15-point smoothing average, a first derivative, a second derivative, and 150 wavelength spectral data gathered from a correlogram. Regression analysis included partial least squares (PLS) and principal component analysis (PCR). Both models were able to perform similarly in predicting crab meat adulteration. The best model for both PLS and PCR used the first derivative spectral data gathered from the correlogram, with a standard error of prediction (SEP) of 0.252 and 0.244, respectively. The results suggest that VIS/NIR technology can be successfully used to detect adulteration in crab meat samples adulterated with surimi-based imitation crab meat.

KEYWORDS: Adulteration; VIS/NIR spectroscopy; crab meat; PLS; PCR

INTRODUCTION

Economic adulteration of food products involves the substitution of cheaper and inferior, i.e., lower quality, ingredients for high-cost ingredients. Even though economic adulteration rarely presents a health hazard, it is an issue because it defrauds the consumer and undercuts legitimate industry prices. For this reason, there is a need for improved detection to address the problem of economic adulteration in the food industry, especially for "value-added" products, such as juices (orange and apple), honey, olive oil, and seafood.

Most of the initial research to detect adulteration has focused on detailed and expensive methodologies involving gas chromatography and mass spectrometry to identify unique chemicals that distinguish one ingredient from another (*1*). However, visible and near-infrared (VIS/NIR) spectroscopy is an objective tool that can be very powerful when properly applied and requires minimal sample preparation and destruction. VIS/NIR technology has been used to determine the authentication of olive oil that was adulterated with vegetable oils (*2*, *3*), to detect, along with chemometrics, honey adulterated with sugar solutions (*4*), and apple adulteration in diluted and sulfited strawberry and raspberry purees (*5*). While previous research has been done to detect adulteration in honey, juices, and olive oil, little research has focused on seafood products, such as crab meat.

Seafood is one of the many high-value products that are targets of intentional adulteration and economic fraud. Examples include overbreading shrimp and overglazing lobster tails, thus providing inaccurate net weights, and species substitution (*6*). Due to the reduced number of Atlantic blue crabs and the increase in foreign imports, the Atlantic blue crab meat market has become a target for economic adulteration. The greatest concentration of the blue crab industry is in the Chesapeake Bay, an area that once provided more crabs than anywhere else in the world (*7*). In 2002, 36.4 million pounds of crabs, valued at $29.3 million, were harvested in North Carolina, one of the top blue crabs producers in the nation (*8*). A decrease in the blue crab population, several major hurricanes, and an increase in crab meat imports have resulted in a dramatic reduction in the number of commercial crabbers because of the substantial negative economic impact (*9*). To continue in the business and increase revenues, some companies have gone as far as economically adulterating their crab meat products. One such example is Miss Sally's Stuffed Crabs from Sam's Club membership stores (*10*). The packages contained pictures of crab shells stuffed with chunks of white meat, and the product claimed to have "more crab meat than ever". Upon FDA inspection, however, instead of being stuffed with crab meat, the shells were found to contain surimi-based imitation crab meat.

The main objective of this study was to use VIS/NIR spectroscopy to determine the level of adulteration with surimi-based imitation crab meat in two types of crab meat, Atlantic blue (*Callinectes sapidus*) and an imported pasteurized frozen blue swimmer crab meat (*Portunus pelagicus*). The imitation

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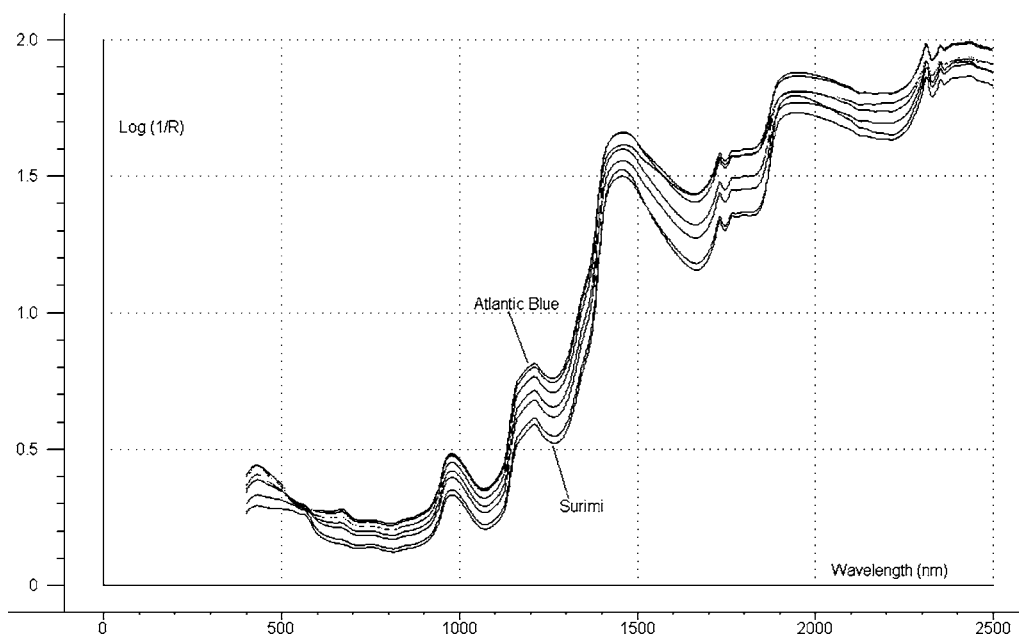

Figure 1. Average absorbance spectra of adulteration samples of Atlantic blue crab meat. The top spectrum represents class 0 (0% adulteration), the bottom spectrum represents class 10 (100% adulteration), and classes 1–9 (10%–90% adulteration) are in order from top to bottom.

crab meat was chosen as the adulterant product due to its low cost, similar flavor and consistency, and widespread commercial availability. In addition, several data pretreatments (a 15 point moving average, first derivative, and second derivative), as well as the use of a smaller data set showing high correlation with adulteration level, were used to determine the effect on model performance.

MATERIALS AND METHODS

Sample Preparation. Three pounds of Atlantic blue crab meat were obtained from a regional crab meat supplier (Sea Safari, Ltd., Belhaven, NC), as well as three pounds of an imported pasteurized frozen blue swimmer crab meat. A total of six one-pound packages (three for each type of crab meat) were stored in a refrigerator (4 °C). Six pounds of surimi-based imitation crab meat (Emerald Sea) were obtained from a local supermarket and also stored at 4 °C until sample preparation and analysis.

Prior to analysis, pure and imitation crab meat samples (3 lbs each) were tempered to room temperature (25 °C) in a water bath. The three pounds of pure crab meat were then pooled in a clean container and thoroughly mixed with gloved hands. The gloves were washed with water to get rid of powdery residue and dried with paper towels to remove excess moisture prior to hand mixing the pooled crab meat. The same method was used to mix imitation crab meat in a different container. Pure and imitation crab meats were each weighed and mixed to obtain a total sample weight of 75 g. Adulteration of pure crab meat was completed in 10% increments (imitation crab meat weight/total sample weight). For example, samples in the 10% adulteration class consisted of 67.5 g of pure crab meat and 7.5 g of surimi-based imitation crab meat for a total of 75 g. Each pure-to-imitation weight combination corresponded to one of 11 adulteration classes: class 0, 0% adulteration; class 1, 10% adulteration; and so forth, until class 10, 100% adulteration.

Once weighed, each adulteration sample was placed in a blender and mixed for five 1-s intervals. Homogenized samples were placed in polyethylene bags, labeled, and stored at room temperature (25 °C) until analysis. Samples were prepared in triplicate for each of the 11 classes: pure crab meat (0), 9 classes of adulteration (1–9), and imitation crab meat (10), giving a total of 33 samples (3 samples/adulteration class) per type of crab meat. To minimize the effect on the percentage of adulteration, the samples were prepared sequentially, from lowest to highest, according to adulteration class. The blender container was cleaned before samples composed entirely of pure or imitation crab meat were prepared.

Data Analysis. Spectra from 400 to 2498 nm at 2 nm intervals (a total of 1050 wavelengths) were recorded in $\log(1/R)$ units at ambient room temperature (25 °C) using a NIRSystems 6500 spectrometer (FOSS NIRSystems, Silver Spring, MD) equipped with a rectangular sample chamber. For each crab meat sample, the recorded spectra consisted of the average of 32 sample scans. Each sample was randomly scanned four different times. The four replicates per sample were averaged to obtain a new data set consisting of 33 sample scans (3 sample scans/adulteration class) per type of crab meat.

Exploratory data analysis, model development and verification, and calibration and validation were performed using Unscrambler software version 7.6 (CAMO Software Inc., OR). Preliminary waveform evaluation of the raw data set, i.e., no data pretreatment, was performed using a correlogram, i.e., a plot between the wavelength data and adulteration class in terms of correlation coefficients. Correlograms are useful tools for determining wavelength importance in terms of a given attribute (*11*). On the basis of this waveform evaluation, a smaller data set (150 wavelengths), corresponding to a continuous wavelength range exhibiting the highest correlation coefficient ($-0.941 < R^2 \leftarrow 0.982$) between wavelength and adulteration class, was also selected for quantitative analysis.

Quantitative VIS/NIR analysis was developed using partial least squares (PLS) and principal component regression (PCR). The full spectrum data set was divided into a training set (two-thirds) for calibration and a testing set (one-third) for prediction, with each set containing 44 samples (22 for each crab meat type) and 22 samples (11 for each crab meat type), respectively, across the range of adulteration classes. Three data pretreatments (a 15-point smoothing moving average, first derivative, and second derivative) and the raw data were used to determine the effect of data pretreatment on regression model prediction. The smaller data set obtained from the correlogram was also used to determine the effect on model prediction. The accuracy of the prediction models was examined in terms of a low standard error of calibration (SEC), a low standard error of prediction (SEP), and a high correlation coefficient (R^2).

RESULTS AND DISCUSSION

Spectra. The average absorbance spectra for the 11 classes of adulterated Atlantic blue and blue swimmer crab meat samples are shown in **Figure 1** and **Figure 2**, respectively. The main features of the spectra arise from water absorptions. High water absorptions are found in the NIR range. At 20 °C, pure water has maxima at 970, 1190, 1450, and 1940 nm (*12, 13*).
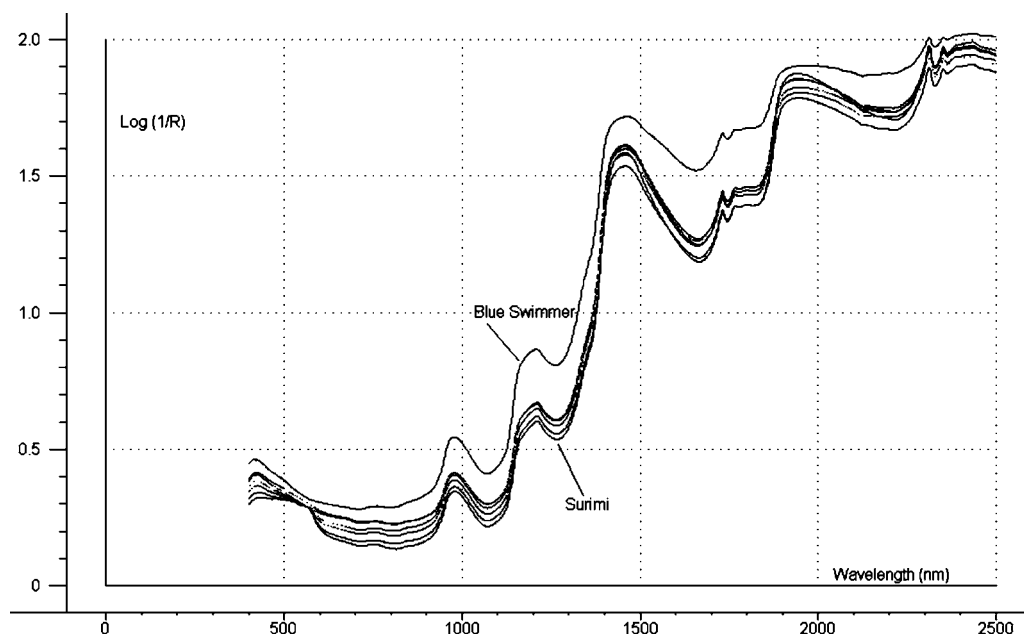
Figure 2. Average absorbance spectra of adulteration samples of blue swimmer crab meat. The top spectrum represents class 0 (0% adulteration), the bottom spectrum represents class 10 (100% adulteration), and classes 1–9 (10%–90% adulteration) are in order from top to bottom.

According to previous studies, three overtones, 1450, 970, and 760 nm, correspond to the first, second, and third overtones of water, respectively. A combination of O−H stretching and bending also occurs around 1940 nm, and the 1190 nm maximum is attributed to the second overtones of C−H stretching. With the exception of the third water overtone, which was not seen, the peaks of the crab meat spectra (**Figures 1** and **2**) are consistent with published literature concerning water absorption. Even though the main features correspond to water absorption, VIS/NIR spectra usually describe many other interactions that give high absorptions at specific wavelengths, such as combination bands of C−H in carbohydrates or N−H in proteins, as well as double bonds of C=O and C=C (*14*). Biomolecules often contain these types of bonds, and many biological materials contain a high water composition; therefore, these absorptions are to be expected. Tables of chemical assignments to spectral peak absorptions are easily found in NIR application books, such as *ref 14*.

In both cases, the spectra of the samples pertaining to 100% crab meat (0% adulteration) have a higher absorbance than the adulterated or surimi-based imitation crab meat samples. As the level of adulteration increases, the absorbance decreases at every wavelength. The one exception occurs for the Atlantic blue crab meat (**Figure 1**), which shows no visible distinction between the spectra of the 0% adulteration class and the spectrum for 10% adulteration. The blue swimmer crab meat (**Figure 2**), however, reveals a large gap between the sample with 0% adulteration (class 0) and that with 10% adulteration (class 1). This difference in spectra can probably be attributed to the fact that the blue swimmer crab meat was pasteurized while the Atlantic crab meat did not undergo this processing treatment. Previous research has shown that NIR analysis is able to detect pasteurized versus unpasteurized crab meat (*15*).

Even though pasteurization might explain the observed spectral difference between types of crab meat, it does not explain why there is the large gap between the 0% adulteration sample and the 10% adulteration sample of the blue swimmer crab meat. It is possible this was due to interactions between the surimi-based imitation crab meat and the blue swimmer pasteurized crab meat. During sample preparation, it was noted

that the blue swimmer crab meat contained more moisture than the Atlantic blue crab meat. This is to be expected because the blue swimmer crab meat was an imported frozen food item. Most nondried imported foods contain a higher moisture level, a postharvest food safety measure, to ensure that the food quality is maintained while in storage. The imitation crab meat, however, is relatively free of moisture; therefore, it probably absorbed water from the blue swimmer crab meat, reducing the amount of moisture in the overall sample. After the 10% adulteration sample, however, the spectra resemble the pattern seen in the Atlantic blue crab meat samples.

Figure 3 shows the correlogram between the raw wavelength data and those for the adulteration class. Overall, the correlation is negative with a high negative correlation to adulteration level in the 600−1400 nm region. In the case of VIS/NIR applications, the use of a small set of optimal wavelengths that contains the most important information is desired. From this correlogram, a smaller data set containing only 150 wavelengths (602−900 nm), i.e., those with higher negative correlations ($-0.941 < R^2 \leftarrow 0.982$), was chosen for quantification analysis.

Quantification of Adulteration Level. To quantify the level of adulteration, 16 models were developed for the data set that contained both the Atlantic blue and the blue swimmer crab meat. The raw data (no pretreatment), data from the three pretreatments (a 15-point smoothing moving average, the first derivative, and the second derivative), and the correlogram data set composed of only the 602−900 nm wavelength data were used to develop regression models using PLS and PCR.

The number of factors, i.e., principal components, used in each model was chosen to optimize model performance and minimize model errors, such as those caused by underfitting and overfitting the data, by looking at the residual variance. **Figure 4** shows a typical plot of the residual variance in terms of the number of factors. For this example, five factors were required to reach a stable minimum residual validation variance; hence, five factors were used in the PLS model. Choosing more factors than required often leads to overfitting the data. In this case, even with a low SEC, the model will be unable to accurately predict unseen samples and will generate a high prediction error.

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Figure 3. Correlogram between wavelength data and adulteration class for the blue swimmer and Atlantic blue crab meat data set.
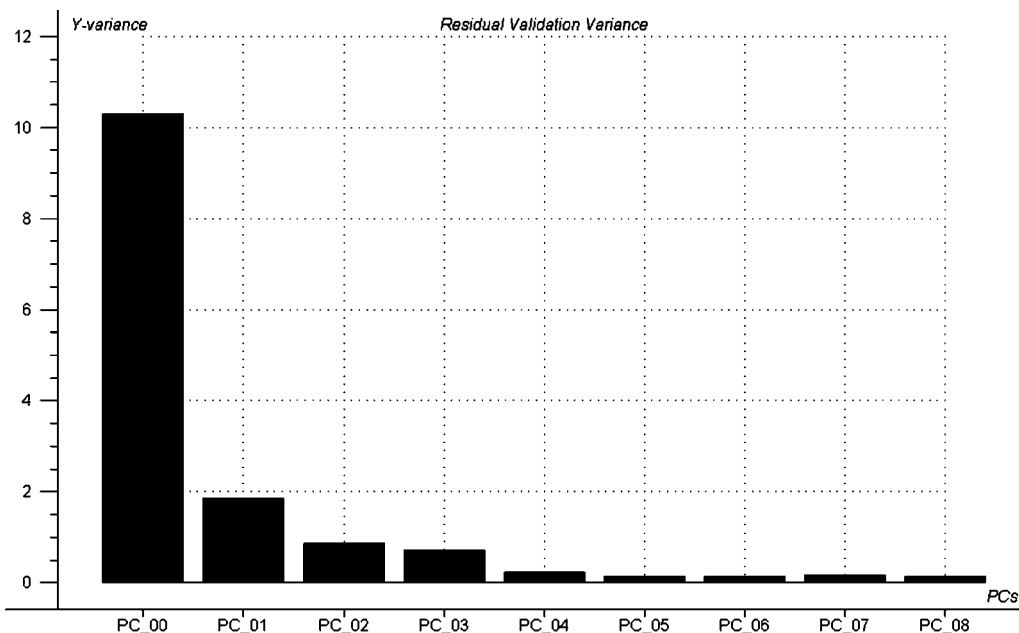

Figure 4. Plot of adulteration level residual variance versus number of factors used in model calibration.

Table 1. Summary Results of Quantitative Analysis Performed with PLS, with the Best Model Indicated in Bold

data treatment	factors	calibration		prediction	
		SEC	R^2	SEP	R^2
none	5	0.386	0.993	0.468	0.989
none + corr	5	0.459	0.990	0.564	0.985
15-pt	6	0.396	0.992	0.480	0.989
15-pt + corr	6	0.449	0.990	0.549	0.986
first der	5	0.483	0.989	0.545	0.986
first der + corr	**5**	**0.251**	**0.997**	**0.252**	**0.997**
second der	10	0.112	0.999	1.926	0.820
second der + corr	5	0.609	0.982	0.838	0.966

A summary of the PLS results obtained from the quantification analysis is given in **Table 1**. Overall, the SEC of the models is low (<0.460) with the exception of the second derivative data of the correlation data set (SEC = 0.609), and all have high (>0.980) correlation of the calibration data. The SEP is higher than the SEC for each model but still shows, with the exception of the second derivative data sets, an error less than 0.57. The 15-point smoothing average did not optimize model performance (SEC = 0.396, SEP = 0.480) over models with the raw data (SEC = 0.386, SEP = 0.468). A slight decrease in both SEC and SEP was noted in the smoothing average model using the correlation data set; however, due to the small difference, this type of data pretreatment was not useful in enhancing the model's ability to predict adulteration level.

Generating a low SEC does not necessarily imply that a model is adequate and able to predict future unseen data samples. An example is the model performance of the second derivative pretreatment data set. Even though the model generated the lowest SEC (0.112), it also generated the highest SEP (1.926). It is possible that this model overfitted the data, hence the low error in the calibration data set. Verification of the model's
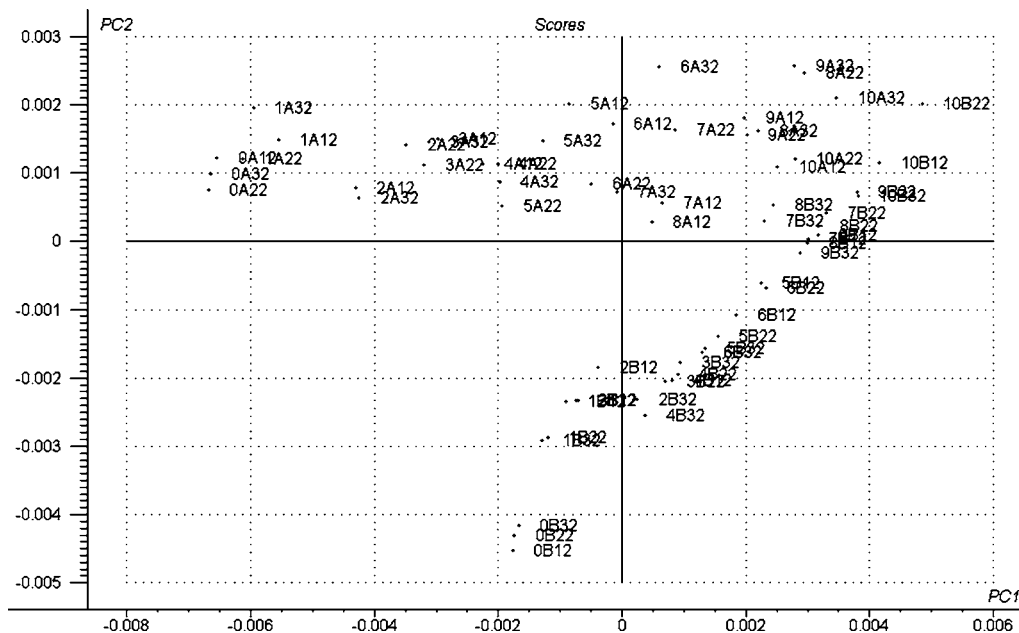
Figure 5. Plot of the first two principal components of the PLS model with first derivative correlation data. The explained variations in PC1 and PC2 were 73% and 23%, respectively. Samples are named according to adulteration level (from 0, i.e., no adulteration, to 10, i.e., 100% adulteration), crab meat type (A = Atlantic blue, B = blue swimmer), and sample number (1–3). Due to repetitions being averaged, the last number is constant throughout the samples.

performance, however, is done by predicting samples that were not used in the model development during calibration. The SEP is indicative of the model's ability to accurately predict samples in terms of adulteration class. The high SEP indicates that the model is not adequate or effective in accurately predicting samples according to level of adulteration. In VIS/NIR quantitative analysis, $2 \times$ SEP is regarded as a 95% confidence interval (*4, 16*). Therefore, this model is able to predict adulteration within ±1.926 classes. Since each class corresponded to a 10% increase in adulteration, the true estimate of the sample lies within almost ±2 classes (or $\pm20\%$ adulteration). In other words, this model cannot accurately estimate samples below 20% adulteration. In addition, the model will falsely classify a sample [i.e., a sample actually in class 4 (40%) classified as a class 6 (60%)]; hence, it is not an accurate model for determining crab meat adulteration.

The best model used the first derivative data of the correlation data set, as data pretreatment, and generated the lowest SEC and SEP, 0.251 and 0.252, respectively, while also generating the highest correlation for both calibration and prediction data ($R^2 = 0.997$). The errors associated with this model imply that future unseen samples will be able to be predicted within ±0.252 of a level or 2.52% of the true adulteration percentage.

A plot of the first two principal components (96% of the total variation) of the first derivative correlation data (**Figure 5**) shows an interesting relationship not only in the adulteration level but also in the type of crab meat. There is a visible trend (left to right) describing level of adulteration, with samples belonging to 0% adulteration on the left and 100% adulteration on the right. In addition, there is another trend, one for each type of crab meat, which describes adulteration level. On the basis of this observation, the first factor (PC1: 73% variance) contains information that describes adulteration level, whereas the second factor (PC2: 23% variance) seems to contain information describing crab meat type since it almost separates the two types of crab meat. The horizontal linear trend (top part of the graph) corresponds to samples from the Atlantic blue crab meat, while the almost 45° linear trend (bottom right)
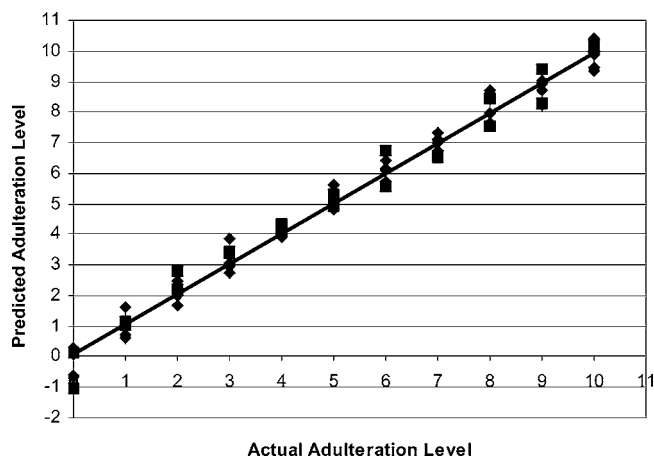

Figure 6. Actual versus predicted adulteration level of the raw, i.e., no pretreatment, spectral data. PLS model with five factors showing the calibration data (♦) and the prediction data (■).

corresponds to the blue swimmer crab meat. Even though the two types of crab meat are differentiated at the lower adulteration levels, they seem to converge at a common region in the upper right quadrant of the graph, which occurs at the samples containing 100% adulteration, i.e., surimi-based imitation crab meat. Hence, the first two factors contain enough information to be able to determine a relationship not only for adulteration in general, but also for adulteration in terms of each type of crab meat. The convergence of the surimi-based imitation crab meat samples is to be expected because all the samples in this adulteration class should depict a similar spectral pattern.

Figure 6 shows the actual versus predicted adulteration level for calibration and prediction corresponding to the first model of **Table 1** (i.e. no data treatment and five factors). The model has a SEC = 0.386 ($R^2 = 0.993$) and a SEP = 0.468 ($R^2 = 0.989$), giving a very good data fit, not only for the calibration but also for the prediction. The high correlation of the SEP is a good indicator that the results of the model are reliable and the model is able to detect adulteration level. For the PLS
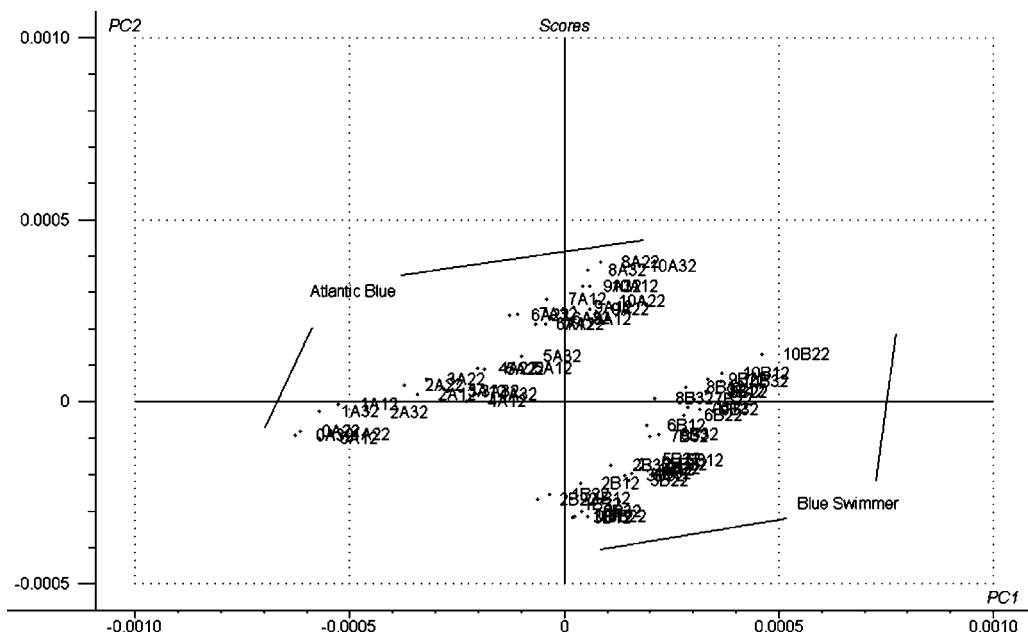
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Figure 7. Plot of the first two principal components of the PLS model with the second derivative correlation data. The explained variations in PC1 and PC2 were 58% and 18%, respectively. Samples are named according to adulteration level (from 0, i.e., no adulteration, to 10, i.e., 100% adulteration), crab meat type (A = Atlantic blue, B = blue swimmer), and sample number (1–3). Due to repetitions being averaged, the last number is constant throughout the samples.

Figure 8. Plot of the first two principal components of the PCR model with the second derivative data. The explained variations in PC1 and PC2 were 28% and 8%, respectively. Samples are named according to adulteration level (from 0, i.e., no adulteration, to 10, i.e., 100% adulteration), crab meat type (A = Atlantic blue, B = blue swimmer), and sample number (1–3). Due to repetitions being averaged, the last number is constant throughout the samples.
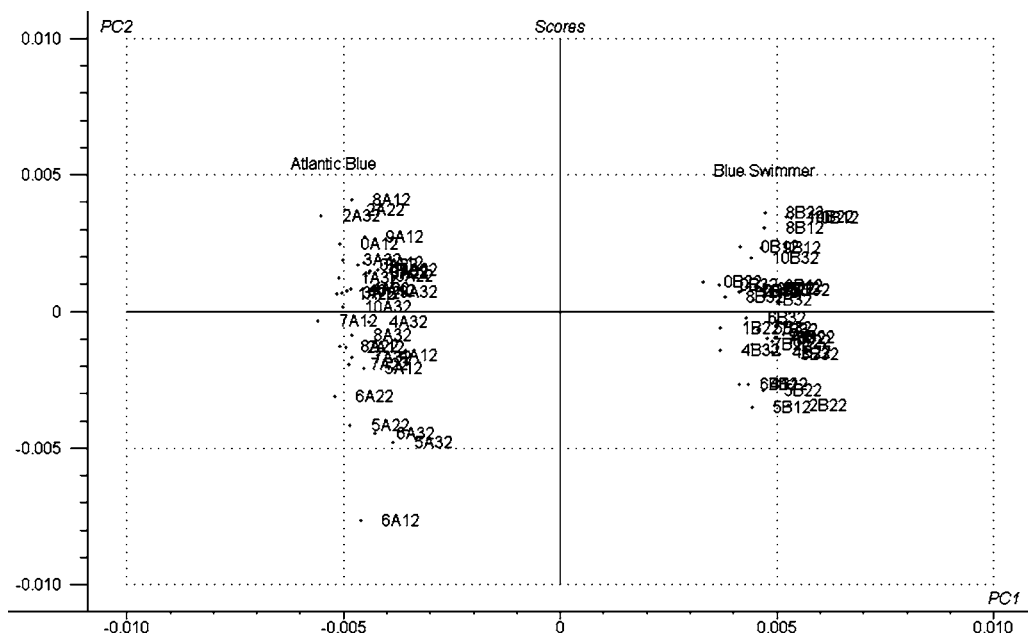
models, the first derivative of the correlation data set gave the best performance (boldface in **Table 1**).

The second derivative data alone generated the lowest SEC (0.112) but was the worst model in terms of SEP (1.926), and it required a higher number of factors. The correlation data using the second derivative, on the other hand, produced lower errors of calibration and prediction (**Table 1**). A plot of the first two principal components of the second derivative data of the correlation data (**Figure 7**) indicates that the variation found in the data set was explained by the difference in type of crab meat, not necessarily adulteration class. While there is a visible adulteration pattern in the Atlantic blue crab meat samples, the

blue swimmer crab meat samples seem to have three main groups in which adulteration classes are mixed (0 to 2, 3 to 5, and 6 to 10), indicating a lack of a sequential relationship of adulteration in the blue swimmer crab meat samples. Even though this model (2nd der + corr) gives errors less than 0.9 for both SEC and SEP and high correlations (>0.96), it is not likely to be an acceptable method for detecting level of adulteration since it cannot accurately predict adulteration of blue swimmer crab meat.

A summary of the PCR results obtained from the quantification analysis is given in **Table 2**. Of the eight models, the one with the first derivative of the correlation data set (with six

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Table 2. Summary Results of Quantitative Analysis Performed with PCR, with the Best Model Indicated in Bold

data treatment	factors	calibration		prediction	
		SEC	R²	SEP	R²
none	8	0.383	0.993	0.414	0.992
none + corr	8	0.329	0.995	0.488	0.989
15-pt	8	0.390	0.993	0.420	0.992
15-pt + corr	7	0.323	0.995	0.476	0.990
first der	6	0.610	0.982	0.576	0.984
first der + corr	**6**	**0.278**	**0.996**	**0.244**	**0.997**
second der	10	1.393	0.900	2.211	0.770
second der + corr	10	0.751	0.972	0.912	0.960

factors) also resulted in the lowest SEC (0.278) and SEP (0.244) and the highest correlation for both the calibration and prediction data sets ($R^2 > 0.996$). On the basis of the SEP, PCR is able to predict the true value of an unseen sample within a level of ±0.244, or 2.44% adulteration. Graphical representation of the first two factors shows results similar to those in **Figure 5**.

Overall, PCR generated a lower SEC and SEP than PLS in all the models, with the exception of the model using the first derivative data and any model that used the second derivative data. A big difference between PLS and PCR is the number of factors that were required to achieve a constant minimum variance, with PCR requiring more factors than PLS. The number of factors for PLS was relatively constant (5−6), with the exception of the second derivative, whereas the factors for PCR range from 6 (for the first derivative) to 10 (for the second derivative). The worst models are also those developed from the second derivative data, though, as seen with PLS, the correlated data give better results than the model with just second derivative data.

A plot of the first two principal components of the second derivative data (**Figure 8**) reveals two clusters, one pertaining to Atlantic blue crab meat (left cluster) and the other to the blue swimmer crab meat (right cluster). While PLS was able to model the level of adulteration of the Atlantic blue crab meat (**Figure 7**), PCR, using the same data set, was not able to model the samples according to adulteration class though it is able to group them into type of crab meat. For the purpose of this experiment, which was to determine the amount of adulteration with surimi-based imitation crab meat, this type of model was found to be inadequate and cannot predict adulteration, regardless of the type of crab meat.

Adulteration of high quality and high priced food products, such as crab meat, is a commercial and economic problem. The widespread availability of surimi-based imitation crab meat makes it an ideal adulterant for crab meat products. In this study, VIS/NIR spectroscopy was used to detect adulteration level in two types of crab meat. PCR generated lower SEC and SEP for the models generated with the raw data (no pretreatment) and the 15-point smoothing moving average data. Overall, however, the PLS models were preferred because they used less factors in model development than PCR. Data pretreatments are often used to optimize model performance. On the basis of the results from this study, a 15-point smoothing average did not enhance the model's ability to predict samples and created no advantage over models derived with the raw data set. Even though PLS using the second derivative data generated lower calibration and prediction errors than PCR, this data pretreatment was not beneficial to optimize the models due to the large error associated with the prediction (SEP) of new adulterated samples. Additionally, the second derivative data sets seemed to contain more information about the type of crab meat rather than

adulteration class. The results here indicate that using the first derivative is more successful in modeling adulteration than using the second derivative or the 15-point smoothing average. A smaller set of correlation data was found to enhance model performance, especially with the first derivative data, since it contained information that pertained to both types of crab meat and also adulteration class. The results from this study indicate that it is possible to detect adulteration using VIS/NIR analysis in crab meat samples adulterated with surimi-based imitation crab meat.

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