FRESHNESS ASSESSMENT OF FISH FILLETS BY VISIBLE AND NEAR INFRARED (VIS/NIR) SPECTROSCOPY

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ABSTRACT

This study was carried out to examine the ability of visible/near infrared (VIS/NIR) spectroscopy to predict the freshness of Catfish (Icatalurus punctatus) and Atlantic halibut (Hippoglossus hippoglossus) fillets for estimating their storage time. The fillets of both species were stored at 4°C in vacuum-packages. Spectral properties of individual fillets were extracted using VIS/NIR spectroscopy throughout a period of 16 days to construct models for predicting storage time of fillets. Multivariate data analysis using partial least-squares regression (PLSR) was used to determine the correlation between NIR/VIS spectra and storage time. The correlation coefficient between actual and predicted storage time was 0.998 and 0.996, and the root mean square error estimated by cross validation (RMSECV) was found to be 1.33 and 1.89 days for catfish and halibut respectively. The accuracy, rapidity and the low complexity of the obtained models suggested spectroscopy technique as a promising method for rapid assessment of the degree of freshness in fish fillets by identifying their storage period.

Keywords: Spectroscopy, fish, freshness, storage, Catfish, Atlantic Halibut, multivariate analysis, PLSR, PCA, NIR.

INTRODUCTION

Fish are one of the most highly perishable food products, which have limited shelf life (Erkan *et al.*, 2007). Also, fish are a rich source of high-quality protein, essential vitamins and healthful polyunsaturated fatty acids (Ashie *et al.*, 1996). Quality evaluation has come to be viewed as a highly useful tool for determining the consumer acceptability of fish, and researchers have therefore been working to improve evaluation technique of assessing fish quality. The problem was that the quality of fishery products has always been hard to define, and is

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typically based on the general perception of the consumer evaluating the product. One general definition of fish quality is the 'degree of excellence'. One thing that remains almost certain for any food product is that quality decreases over time. In most cases, storage time is used as a quality indicator and will be used as a sign of fish freshness throughout this paper. That is because during storage time the freshness is reduced until spoilage occurs due to biochemical, chemical, physical and microbiological processes (Sivertsen *et al.*, 2011). Expiration dates serve as a guide, but the sensory appeal of a fishery product is generally the deciding factor as to whether a product is deemed acceptable or not by the end consumer. Expiration dates give the consumer a clear indicator by which to tell that a food may no longer be safe for consumption (Dodd, 2006).

Some of the more important factors that determine quality from the customer's point of view are species; ease of preparation; appearance; odor; flavour; freshness; size; presence or absence of bones, blood and filth; absence of specific microorganisms; packaging and composition. Freshness is the most important quality factor to the consumer; thus assessment of freshness is vital in quality control process. Fish freshness is not a distinct parameter; it is a complex interaction of several parameters where each one to some extent may explain freshness. Freshness can be explained to some extent by some objective sensory, biochemical, microbial and physical parameters, which could be defined as an objective attribute (Ólafsdbttir *et al.*, 1997). As fresh fish is a highly perishable product, it is essential for proper commercialization to be able to estimate accurately its freshness (Huidobro *et al.*, 2001). Commercial decisions to accept or reject a product can be made by means of physicochemical, microbiological and sensory methods (Arvanitoyannis, *et al.*, 2005). Unfortunately, there is no entirely satisfactory physical method to determine fish freshness as the main parameter of the quality of fish products (Kent, *et al.* 2004).

The standard method for monitoring fish quality is using a sensory panel. Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret characteristics of food as perceived by the senses of sight, smell, taste, touch and hearing (Ólafsdbttir *et al.*, 1997).

While using human senses has the advantage of being able to consider a large variation of factors, it has limitations due to great efforts required to perform a sensory panel analysis on a large number of samples. This drawback makes it cost prohibitive and impractical to be used on a large scale at processing plant for most food manufacturers. On the other hand, while chemical tests provide quantifiable results, they are normally destructive, time consuming, and expensive. Instruments that can overcome both disadvantages of sensory and chemical methods as well as rapidly and economically assess the quality of food products would be a practical choice for quality control implementation. Recent developments in multivariate analysis techniques along with advances in data acquisition and sensing capabilities have led to the creation of new technologies that can better monitor complex situations such as the freshness of fishery products.

During the past few years, spectroscopic methods have gained great attention in the evaluation of food quality parameters (Sun, 2008). The advantages of spectroscopic methods are their ability to provide rapid analysis and simultaneous evaluation of several parameters, and their potential for on-line use. Spectroscopic techniques have been progressed dramatically, particularly with the use of fiber optics, lasers and solid-state components, which can facilitate the miniaturization of spectrometers (Scotter, 1997). Spectroscopic analysis exploits the interaction of electromagnetic radiation with atoms and molecules to provide qualitative and quantitative chemical and physical information that is contained within the wavelength or frequency spectrum of energy that is either absorbed or emitted. (Scotter, 1997; Pasquini, 2003; Garini *et al.*, 2006; Cen and He, 2007). It is intrinsic that when a spectroscopic technique is calibrated against a traditional chemical measurement, the traditional technique is described as the 'direct' method and the spectroscopic analysis as 'indirect' (Scotter, 1997). Therefore, the versatility of NIR spectroscopy in food science has been demonstrated in many applications. It is a non-destructive and rapid technique in the assessment of food quality with the advantage of being easy to use in combination with chemometrics analysis for qualitative and quantitative analysis (Bøkæns *et al.*, 2002). NIR spectroscopy reveals information

related to the vibrational behavior of molecular bonds and therefore can give details of the varieties of molecules present in the food (Cen and He, 2007). However, development of NIR methods for fish and fish products has been limited (Wold *et al.*, 1996). Despite that NIR spectroscopy has gained a foothold as a quantitative method in food analysis, relatively little is known concerning the applicability to fish freshness and estimating its storage time.

Since both fish freshness and its overall quality decrease over time and since spectroscopy measurements represent the fingerprint of fish condition, this study was carried out to investigate the ability of detecting changes in fillets of catfish and Atlantic halibut fish over storage time by means of VIS/NIR spectroscopy and multivariate analysis techniques. In this study, the main aim was to monitor spectral response of fish fillets in the wavelength region of 400-2500 nm as a function of time. Multivariate models are aimed to be used for predicting storage period as the indication of physicochemical changes occurred during storage.

MATERIAL AND METHODS

Thirty fish fillets of two different species: Catfish (16 fillets) and Atlantic halibut (14 fillets) were purchased from a local fish retailer. The fish fillets were fresh and of superior quality. The average water contents were 72.60 and 74.50%, and the average fat contents were 9.15 and 10.14% for catfish and halibut fillet samples, respectively (ElMasry and Wold, 2008). Each fillet was vacuum-packed individually in polyethylene bags and stored at 4°C for 16 days. In the first day of the experiment 6 fillets from catfish and 4 fillets from Atlantic halibut were scanned using spectrometer described below and its corresponding spectra represent spectra of both species at zero time of storage. Two fillets from each species were randomly chosen to be scanned after 3, 6, 10, 13 and 16 days respectively.

At 0, 3, 6, 10, 13 and 16 days of storage, fillets were removed from the vacuum packages and then scanned with NIRSystems 6500 monochromatic spectrometer (Foss NIRSystems, Silver Spring, MD, USA) in reflectance mode. The instrument was equipped with a fiber-optic reflectance probe (NIRSystems no: NR-6539) and measurements were collected as an average of 32 scans in the spectral range of 400-2500

nm with 0.5 nm increments yielding 4200 data points per spectrum. As shown in Figure (1), the spectrometer mainly consisted of light source, beam splitter unit (tunable gratings), sample detector, optical detector, data processing system and a computer. The system was supplied with software for data acquisition, communication and unit control. Each fillet was placed in the instrument and the reflectance spectrum in the visible and near infrared regions (VIS/NIR) was recorded from the surface of the fillet. Five spectra per one fillet were recorded from five different locations of each fillet which then averaged to give one spectrum for each fillet. The locations of these five points where the spectra were acquired were selected along the lateral line of the loin to express the whole fillet and to consider any expected changes occurred in any part of the fillet. The absorbance at a certain wavelength (λ) was calculated as [log (1/Reflectance)] at this wavelength (λ) based on Beer Lambert law which states that the log reciprocal absorbance is directly related to the concentration of the absorbing bond pairs, or combination absorbances, and is inversely related to the pathlength of light through the sample. A reference spectrum of a white Teflon surface was taken at the beginning of every testing time throughout the storage period of 16 days. Also, dark current was measured automatically prior to each measurement. The dark and white spectra were used for calibrating each spectrum individually.

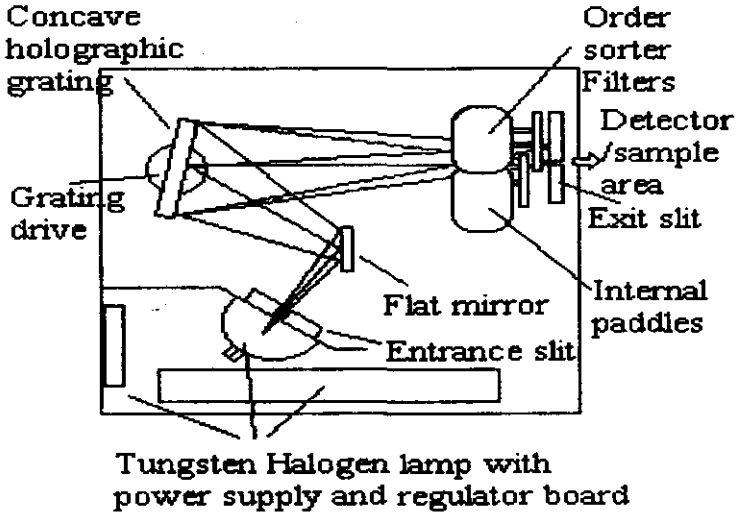

Fig. (1). Schematic diagram of the main components of the VIS/NIR spectrometer

It has been proven that despite the precision and accuracy of robust analytical techniques, detection of authenticity and freshness of food stuffs could not be claimed without resorting to multivariate analysis (Arvanitoyannis *et al.*, 2005). Due to the large hidden information in the spectral data and its high co-linearity, it is intrinsic to find a reliable method to process and extract the characteristics of the spectra using one or more of chemometrics tools which improve the potential application of VIS/NIR spectroscopy technique in food industry. As stated by Cen and He (2007) chemometrics in VIS/NIR spectroscopy analysis includes three aspects as: (1) spectral data pre-processing; (2) building calibration models for quantitative and qualitative analysis; and (3) model transfer. In this study, different types of pre-treatment (such as mean centering, auto scaling, Savitzky Golay smoothing, multiplicative scatter correction, standard normal variate, and 1^{st} and 2^{nd} derivatives) of the resulting VIS/NIR spectra were tested, but only the mean centering and then auto scaling pre-treatments were chosen due to their simplicity and its successful results in the final models. Multivariate calibration models for quantitative or qualitative analysis in food analysis play a key role in the analytical measurement. The reliability of measurement from VIS/NIR spectroscopy method depends on the calibration model. The calibration model built by VIS/NIR spectroscopy and its accompanying multivariate analysis can be used for predicting the properties of unknown samples nondestructively without any tedious preparations of these samples. Thus, realizing the transfer of multivariate calibration models can help to improve the VIS/NIR spectroscopic technology. In essence, the on-line application of this system basically requires a carefully planned execution of this off-line calibration models to ensure successful on-line implementation.

First, principal component analysis (PCA) was used to obtain an overview of the systematic spectral variations among classes/storage times (0, 3, 6, 10, 13, 16 days). PCA is an unsupervised chemometrics tool because there is no information about the samples was provided throughout PC's computations. This process determines most of the variation among samples regardless of its source. PCA as multivariate statistics was used to reduce a large multidimensional array down to its most important

principal components. The first component is the combination representing the largest variation in the data, thereby giving the data the best class separation. Whereas the second component gives the next best class separation, and so on. Typically, the first few components are usually graphed against each other for a visual representation of the data. Therefore, fish fillet samples having similar spectral features tend to be projected together along a principal component.

Partial least square regression (PLSR) is an extension of the multiple linear regression (MLR), but the main advantage of this technique is to avoid co-linearity or inter-correlation problems and to deal with a number of variables that is greater than the number of samples. Partial least squares regression (PLSR), with full cross validation, was used to find the correlations between spectroscopic spectra of the tested fillets (X-matrix) and its corresponding duration of storage period (Y-matrix). PLSR finds a mathematical relationship between a set of independent variables, the X-matrix ($N_{samples} \times \lambda_{wavelengths}$), and the dependent variable, the Y-matrix ($N_{samples} \times 1$). The X-matrix represents the absorbance values at 4200 wavelengths per spectrum in the range of 400-2500 nm for tested fillets (N). The Y-matrix represents the tested storage periods (0, 3, 6, 10, 13, and 16 days at 4°C). The optimal number of latent variables (LV) for establishing calibration model was determined using the minimum value of predicted residual error sum of squares (PRESS). Full cross-validation was used to provide a predicted storage time for each sample ($\hat{y}_i$), which was then compared to the actual duration of the storage time (y_i) for all samples from i to N. Cross-validation by leave-one-out method removed one sample from the calibration data set and construct the model without this data. The sample left out then has its storage time predicted by the model and a calibration error is calculated. The procedure is repeated for each sample and a root mean square error estimated by cross-validation (RMSECV) was computed as:

$$RMSECV = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\hat{y}_i - y_i\right)^2} \qquad (1)$$

In addition, samples for which the difference between actual (y_i) and predicted ($\hat{y}_i$) values of storage time exceeded three times standard

deviation were considered as outliers. By the way, no outliers were detected using both spectral and storage time residuals. The predictive ability of the calibration model was then evaluated by calculating some statistics such as root mean square error of calibration (RMSEC), coefficient of determination in calibration R_C^2, root mean square error from cross-validation (RMSECV) and coefficient of determination in cross-validation R_{CV}^2. The best model should have high determination coefficients (R_C^2 and R_{CV}^2) and the low errors (RMSEC and RMSECV) as well as a small difference between RMSEC and RMSECV. All multivariate data analyses were performed with Matlab 7.3 (The Mathworks Inc., MA, USA) and the PLS toolbox (Eigenvector Research, Inc., Wenatchee, WA).

RESULTS AND DISCUSSION

The average spectra of fish fillets at different storage times collected from 16 samples of catfish and 14 samples of Atlantic halibut fillets are shown in Figures (2a) and Figure (2b), respectively. It can be observed that the spectral characteristics of the fillets are different from fillet to fillet according to its storage time. The absorbance spectra are rather smooth across the spectral region. Even with limited chemical composition and structural information of tested fillets, preliminary band assignments in the 400-2500 nm region could be easily recognized for water, protein, and fat. Two bands appeared at 430 and 560 nm in the visible region are related to the presence of various forms of myoglobin (Liu *et al.*, 2000). The broad peaks at about 980, 1440 and 1940 nm are assigned to the second overtones of O–H stretching mode related to water H_2O. Features between 1100 and 1300 nm are from the second overtones of the C–H stretching modes, whereas their first overtones appear in the 1600-1850 nm region. Bands in the 1300-1400 nm region are ascribed to combination bands of the C–H vibrations. Broad bands in the 1400-1600 nm region are due to the overlaps of the first overtones of the O–H/N–H stretching modes of self-associated and water-bonded OH/NH groups in fish compositions (Liu *et al.*, 2000).

The bands in the 1850-2490 nm region arise from the combination modes of different vibrations of C-H, O-H, and N-H as well as other functional

groups such as C=O group (Liu *et al.*, 2004). Protein has an absorbance peak around 2200 nm corresponding to the third overtone N-H stretch, which is difficult to discern in the figure especially in halibut samples. The prominent offset variation between the spectra is mainly due to light scattering phenomena caused by varying fillet thickness. However, the main difference among samples is attributed to differences in the distribution of crude chemical contents, in different fiber structures and changes in chemical composition in the fillets.

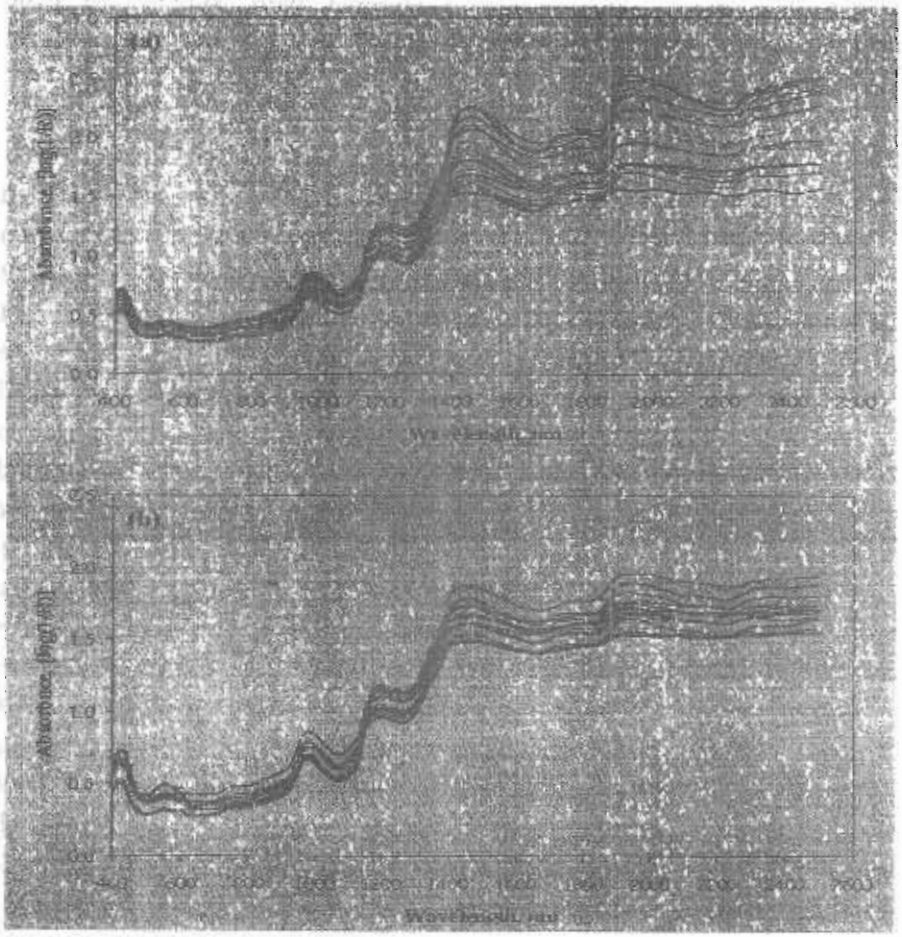

Fig. (2). Typical VIS/NIR spectra in the range of 400-2500 nm collected from (a) catfish fillets and (b) atlantic halibut fillets at different storage time.

As declared in Figure 2, it is noted that the intensities of two visible bands at 430 and 560 nm decreased but are insignificant, whereas those of NIR bands at higher wavelength (>1460 nm) decreased clearly with increasing storage time. The difference in intensity among samples occurred in the VIS/NIR spectral regions suggest color, physical, and chemical changes in fish fillets (due to change in its freshness) which will help in sample discrimination to different storage classes. Therefore, these spectral measurements leaded to believe that there is a connection between VIS/NIR spectra and fish fillet characteristics in terms of its freshness and consequently its safe storage time.

Since PCA is an unsupervised technique and there is no prior information about the sample was provided throughout the computations, the sample having the same features tend to be projected together along the principal component. This process determines the most variation regardless of its source by retaining as much as possible of the information present in the original data. Scores and explained variance were studied for the first principal components. Score plots were used to visualize the relation between samples for the corresponding PCs. In order to ease the interpretation of the principal component analysis, catfish and Atlantic halibut fillets were analyzed separately. Figure (3a) illustrates the score plot of the principal component analysis of catfish-fillet spectra at 0, 3, 6, 10, 13, 16 days. The analysis indicated that most variation among samples (spectra) was retained within the first two principal components (96.16%). The first principal component retained 87.78 % of the total variation and the second component retained 8.38% of the variation.

As depicted in Figure 3a, spectra of fresh samples at the beginning of the experiment at zero time (0d) were projected together because they have the same characteristics. The samples (fillets spectra) stored at 3, 6, 10, 13 days (3d, 6d, 10d and 13d) are projected together as if they have almost the same features. The difference between these samples and zero time samples was distinct due to losing some freshness attributes with storage. However, this difference should be accentuated with chemical and microbiological evaluation to ensure the validity of these fillets for consumption. The samples tested after 16 days have exceptional trend with different features and they are completely separated in one class.

This finding could be elucidated to the sharp change occurred in characteristics of these fillets. This could lead to conclude that the fillets stored for 16 days have abnormal features compared with fresh fillets (zero time samples) and fillets stored at 3, 6, 10, and 13 days.

In case of Atlantic halibut spectra shown in Figure (3b), the cumulative variance explained by the first two principal components was 92.62%, with the first component providing 66.49% of total variation among spectra and 26.13% explained by the second principle component.

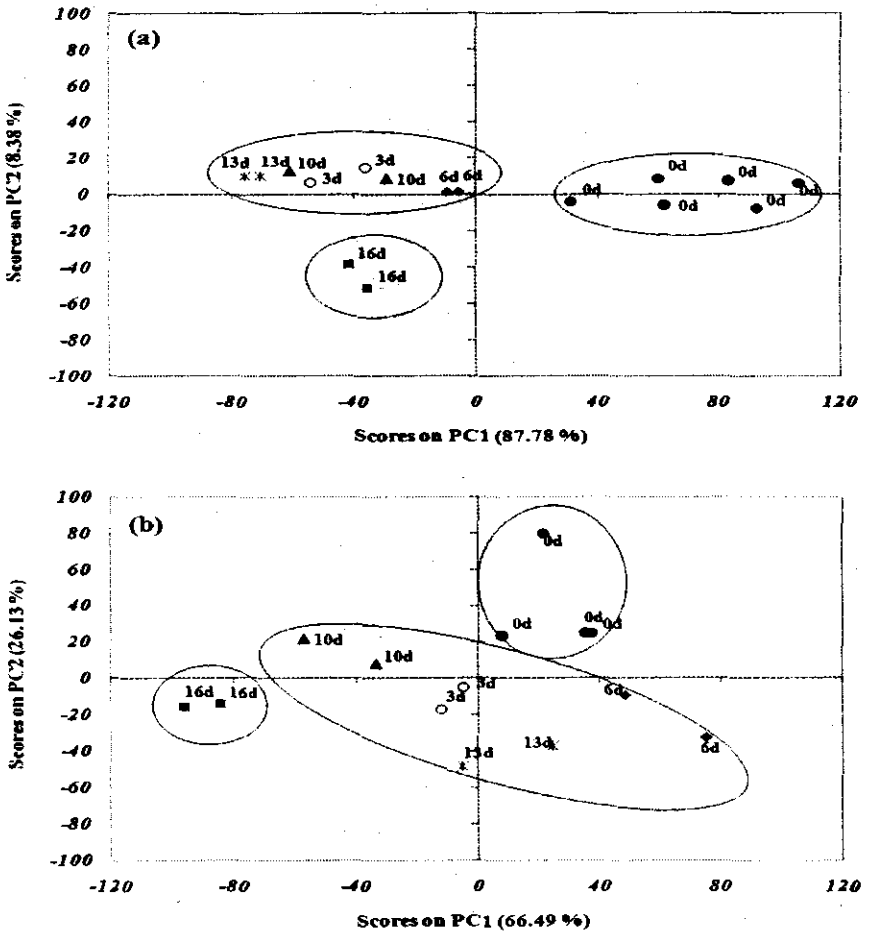

Fig. (3). Score plot of principal component analysis (PCA) conducted on VIS/NIR spectra of fish samples (a) catfish-fillets and (b) atlantic halibut-fillets during storage time.

The number in parentheses indicates the proportion of the total variance explained by each principal component (PC).

Similarly, the samples of fresh fillets (0-days of storage) are projected with each other in one group as clearly shown in Figure (3b). Fillets tested after 3, 6, 10 and 13 days are projected beside each other and could be considered as one group indicating that fillets stored up to 13 days have similar spectral behavior. The acceptability of these samples for consumption would be permitted if chemical and microbiological tests were proofed. The exceptional trend of samples tested after 16 days was also observed because they are isolated in one group. This was ascribed to the distinct difference between spectral features of these samples and those of fresh and/or the other samples. One of the most reasonable conclusions that could be inferred from these results is that the fillets stored for 16 days are entirely different from the other samples due to unfavorable nature and unusual loss of freshness of these samples.

Since the samples were isolated in distinct classes based on their spectral features as proofed by principle component analysis, the next step is to predict the exact storage time of each fillet according to its spectral signature in the VIS/NIR region. Since characteristics of fish fillets are changing continuously over time, a model was produced using PLS to accentuate this fact. In essence, the storage time has an indirect relationship with chemical, physical, and sensory attributes of the stored fillets. With increasing storage time all of these features are expected to be altered in certain manners depending on the initial characteristics of the samples. The PLSR method is a modeling technique that uses predictive data (actual storage period) to select latent variables having the variability among samples. Figure 4 shows the predicted storage times by PLSR model applied to VIS/NIR spectra of catfish and Atlantic halibut fillets. The PLSR is a bilinear modeling technique that extracts the most relevant information for the prediction of chemical, physical, and sensory attributes of the sample by considering its storage time as the predictive criterion.

The PLS prediction model was developed using the absorbance at all 4200 wavelength channels of spectra as X-variables and the actual storage period (0, 3, 6, 10, 13, and 16 days at 4°C) as Y-variable. In case of catfish fillets the storage time was predicted with a coefficient of

determination in calibration (R_C^2) of 0.98, root mean square error of calibration (RMSEC) of 0.24 days, coefficient of determination by cross-validation (R_{CV}^2) of 0.91 and root mean square error from cross-validation (RMSECV) of 1.33 days using 8 latent variables as shown in Table (1).

Table 1. PLSR models for storage time prediction of fish fillets.

Fish Fillet	PLS model				
	Latent variables (LV)	R_C^2	R_{CV}^2	*RMSEC*	*RMSECV*
Catfish	8	0.98	0.91	0.24	1.33
Atlantic halibut	9	0.95	0.90	0.35	1.89

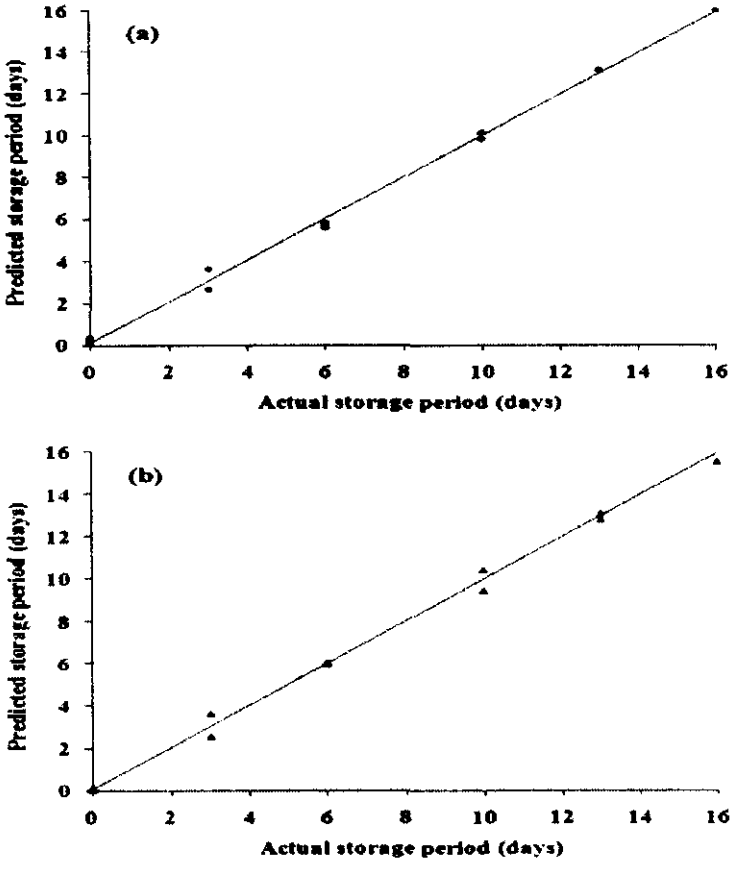

Fig. (4). Predicted versus actual storage time using pls model applied to VIS/NIR spectra of the (a) catfish-fillets and (b) atlantic halibut-fillets.

For Atlantic halibut fillets, the PLS model predicted storage time with coefficient of determination in calibration $R_C^2 = 0.95$, root mean square error of calibration RMSEC = 0.35 days, and root mean square error estimated by cross validation RMSECV = 1.89 days using 9 latent variables. The resulted high correlation between actual and predicted values of storage time justified the suitability of this technique for predicting the storage time and freshness in fish fillets. However, the big difference between root mean square error of calibration (RMSEC) and root mean square error estimated by cross validation (RMSECV) in both cases put some restrictions about the validity of this model. Strictly speaking, this awkward confusion is attributed to the limited number of samples used in this study. Lower RMSECV could be obtained by examining greater data set which spanned the natural variations more completely and gave a more stable prediction model. To obviate such problem and to implement this technique in a large scale, increasing number of samples accompanied with physicochemical, microbiological and sensory tests are essential. In addition, testing various fish verities and different levels of storage conditions for prolonged period of time is very important before generalizing this technique.

Because this study was just to investigate the ability of VIS/NIR spectroscopy to predict storage time as an indication of various changes occurred in the fillet during storage, the qualitative modeling indicated that VIS/NIR spectroscopy is a useful tool for estimating storage period in an objective way. While this does not tie to a specific chemical indicator, time has the advantage of taking into account many different changes that might be missed by any assay. From a customer and industrial points of view, it is essential for proper commercialization to accurately estimate freshness of fish fillets. The loss of major freshness characteristics of fish during storage leads to remarkable changes in its physicochemical properties. Measuring these properties for quality assurance is very critical to guarantee safe products. Assessment of quality parameters in a rapid and non-destructive way became a priority in food industry. Knowing the major fish composition and its evolution relating to different storage and preservation conditions is an important in quality control.

CONCLUSIONS

This paper presents one of the most significant applications of near infrared spectroscopy to identify fish freshness as an indication of its safety. Visible and Near-Infrared (VIS/NIR) spectroscopy in the range 400–2500 nm was investigated for classification and for identifying fish freshness estimated by predicting storage time of catfish and Atlantic halibut fillets. Multivariate data analysis using principal component analysis (PCA) and partial least-squares regression (PLSR) were used for these purposes. The current study suggests that establishing this technique in fish quality assurance and quality control programs will provide more consistent and expeditious analysis. The correlation coefficient between actual and predicted duration of storage was 0.91 and 0.90, and the root mean square error estimated by cross validation (RMSECV) was found to be 1.33 and 1.89 days for catfish and halibut respectively. The results of current study motivated more research for further application VIS/NIR spectroscopy in quality change assessment in fish fillets using various samples for elucidating more robust models.

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الملخص العربى

تقدير درجة طزاجة الأسماك باستخدام التحليل الطيفى فى المدى المرئى والقريب من التحت حمراء

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أجريت هذه الدراسة بغرض إختبار مقدرة نظام التحليل الطيفى فى تقدير طزاجة نوعين من الأسماك (Catfish وHalibut) وذلك عن طريق التنبؤ بفترة تخزين كل منها، حيث تم تجهيز شرائح الأسماك وتعبئتها تحت تفريغ فى أكياس بوللى ايثلين ثم تخزينها على درجة حرارة ٤ درجات مئوية. بعد ذلك تم مسح Scan شرائح الأسماك باستخدام جهاز التحليل الطيفي فى المدى المرئي والقريب من التحت حمراء على فترات تخزين مختلفة لمدة ١٦ يوماً بهدف إستخراج الخصائص الطيفية لشرائح الأسماك عند كل فترة تخزين. تم بناء نموذجاً رياضياً للتنبؤ بفترة التخزين وذلك بتحليل البيانات الطيفية المستخرجة باستخدام التحليل المتعدد للبيانات بطريقة إرتداد أقل التربيعات الجزئية PLSR. ولقد أظهرت نتائج هذه الدراسة أنه يمكن التنبؤ بفترة تخزين شرائح الأسماك المختبرة بقيم معامل ارتباط بين فترة التخزين الفعلية والمتنبأ بها وصلت الى ٠.٩٩٨ و ٠.٩٩٦ و بخطأ مقداره ١.٣٣ و ١.٨٩ يوماً لشرائح أسماك Catfish و Halibut على الترتيب. إن دقة وسرعة وبساطة هذه التقنية للتقدير الفورى لدرجة طزاجة الأسماك بدون تحليل كيميائى أو ميكربيولوجى عن طريق التنبؤ بفترة تخزينها يعطى أفضلية كبيرة جداً لهذه الطريقة عن الطرق التقليدية لتحليل الأسماك.

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