Evaluation of the Quality of Frozen Minced Red Hake: Use of Fourier Transform Infrared Spectroscopy

Judith Pink,*,† Marian Naczk,‡ and David Pink†

Food Science Laboratory and Department of Human Nutrition, St. Francis Xavier University, P.O. Box 5000, Antigonish, Nova Scotia, Canada B2G 2W5

The suitability of FTIR spectroscopy for evaluating changes in the quality of frozen minced red hake was studied. The spectral data between 1600 and 890 cm^{-1} were correlated with the chemically determined dimethylamine (DMA) content of the fish using chemometric techniques. This spectral region contains all of the DMA and trimethylamine oxide (TMAO) absorbance bands. Discriminant analysis of the data results in a 90% success of distinguishing fresh from frozen fish and classifying frozen hake into three quality categories. The RPD value of 4 shows that the PLS model predicts the DMA content of the frozen fish well enough for quality assessment.

Keywords: *Fish quality; FTIR; hake; dimethylamine; discriminant analysis; PLS*

INTRODUCTION

The deterioration in the quality of gadoid fish upon prolonged frozen storage has been extensively studied, and the development of a tough, rubbery texture and the formation of off-flavors have been reported (Crawford et al., 1979; Gill et al., 1979; Kelleher et al., 1981; Owusu-Ansah and Hultin, 1986; Perez-Villarreal and Howgate, 1991; Haard, 1992; Sotelo et al., 1994). This deterioration has been attributed to the presence of high levels of trimethylamine oxide (TMAO) and of an enzyme, trimethylamine oxide demethylase (TMAO-ase), in these fish. TMAO-ase catalyzes the splitting of TMAO into equimolar amounts of dimethylamine (DMA) and formaldehyde (FA) (Sikorski et al., 1976; Regenstein et al., 1982). The FA so produced is thought to be the major cause of the toughening of frozen fish by bringing about protein denaturation (Sikorski et al., 1976; Gill et al., 1979; Ang and Hultin, 1989; Sotelo et al., 1994). Gill et al. (1979) reported the formation of higher molecular weight myofibrillar protein aggregates in frozen red hake, a result of covalent linking of troponin and myosin light chains. Subsequent studies showed that myosin heavy chain was the major protein that became unextractable in hake (Owusu-Ansah and Hultin, 1986, 1992) and in cod fillets (Ang and Hultin, 1989). Gill et al. (1979), Crawford et al. (1979), Owusu-Ansah and Hultin (1986), and Rehbein (1988) also reported the existence of a statistically significant correlation between DMA accumulation and textural deterioration of gadoid fish as measured by Instron and sensory evaluation. LeBlanc et al. (1988) also demonstrated that the frozen storage history of samples (time and temperature) does not affect this correlation.

DMA content is the most useful chemical index of frozen fish quality and may be assayed by GC (Keay and Hardy, 1972), HPLC (Gill and Thompson, 1984), and the copper dimethyldithiocarbamate assay (Dyer and Mounsey, 1945). The last of these is most commonly used and is accepted by the Nova Scotia Department of Fisheries and Oceans as the standard method for the quantification of DMA in fish (Woyewoda et al., 1986). However, these assays are laborious and, in addition, generate toxic waste. It is desirable, therefore, to develop a rapid, reliable analytical method for the classification of frozen fish that does not require the use of chemical assays.

Atlantic red hake (*Urophycis chuss*), selected as the subject of this study, is still relatively abundant in the North Atlantic. In addition, this fish deteriorates more rapidly than any other gadoid fish, especially in the minced state, and the deterioration is most rapid at around −10 °C (Gill et al., 1979; Kelleher et al., 1981). This temperature range is often encountered in retail cabinets and domestic freezer compartments. Representative sampling is a major problem because of the small sample size used in spectral measurements. Accordingly, in our study we chose minced white muscle of hake as the model system and stored the fish samples at around −10 °C. The freezing conditions were deliberately not well controlled to generate a database that would approximate the commercial situation of unknown sample history.

The objective of this study was to determine the suitability of Fourier transform infrared (FTIR) spectroscopy for the evaluation of changes in the quality of frozen minced red hake. The aim of this study was also that this new procedure may find a practical application in industrial laboratories. The procedure developed was the correlation of the IR spectrum with the DMA concentration of the fish, using chemometric techniques (Beebe and Kowalski, 1987; van de Voort, 1992; Defernez et al., 1995).

MATERIALS AND METHODS

Sample Preparation. Fresh, iced red hake (*U. chuss)* was purchased at the wharf or in a supermarket. All fish samples were acquired after an unknown time of resolution of rigor mortis. The fish was filleted and skinned, and the white

* Author to whom correspondence should be addressed [telephone (902) 867-2248; fax (902) 867-2389; e-mail jpink@stfx.ca].

† Food Science Laboratory.

‡ Department of Human Nutrition.

S0021-8561(98)00395-1 CCC: $15.00
Published on Web 08/29/1998

muscle was carefully separated from the dark. The white muscle was ground twice using a Moulinex HV6 meat grinder and then homogenized in a Cuisinart food processor for 1 min. The homogenized fish was vacuum packaged in 50 g batches in polyethylene pouches (0.08 mm wall thickness) (Rondeau Agencies, Dieppe, NB) using an Audionvac VM 101 HG (Audion Elektro B.V., Weesp, Holland). The packets of fish, each of thickness not exceeding 10 mm, were frozen in a single layer in the freezer of a domestic refrigerator (Kenmore) held at −10 to −12 °C. Some samples were transferred to a Westinghouse upright freezer, held at −14 to −15 °C.

Spectral Measurements. The spectral analysis was carried out using a Nicolet Magna 750 FTIR spectrophotometer (Nicolet Instrument Inc., Madison, WI), equipped with a 40° ZnSe crystal (subsequently referred to as the ATR crystal) in-compartment attenuated total reflectance (ATR) accessory. Prior to analysis, the ATR crystal was soaked for 10 min in saline solution (Bausch and Lomb) containing Unizyme (Ciba Vision) to remove any protein deposit, then rinsed with deionized water, and dried. A reference background absorbance spectrum was taken of the clean crystal. The crystal surface was fully covered with a single layer of thawed thin fish slices (0.7−1.0 mm), and the top of the sample was tightly covered with cling foil to prevent dehydration. The ATR accessory with a fish sample was placed in the instrument optical cavity. The sample compartment was purged for 5 min with dry air from a Balston 75-45 FTIR dryer (Balston, Lexington, MI) to minimize water vapor and carbon dioxide interference. The spectrum of the fish sample was recorded from 4000 to 800 cm^{-1} at 4 cm^{-1} resolution at room temperature. Spectra of four to five samples from a given packet of fish were collected to take into account sample inhomogeneities. Each collected spectrum was made up of 256 co-added scans and ratioed against the reference background spectrum to obtain the spectrum of a fish sample.

Chemical Analysis. The unused portion of the frozen fish packet was partially thawed at 4 °C for 3−4 h and then minced in a Handy Chopper (Black and Decker). The DMA content of the fish was determined following the procedure described by Woyewoda et al. (1986).

Mathematical Analyses. Spectral data were averaged using Nicolet Omnic v. 3.0 software. Areas under the absorbance spectra were calculated by using Sigma Plot v. 3.0 software (Jandel Scientific Software, San Rafael, CA). All other mathematical analyses of the spectral data were performed using PLSplus/IQ v. 3.0 software (Galactic Industries Corp., Salem, NH). Both discriminant analysis and partial least squares (PLS) methods were used to develop chemometric calibration models.

RESULTS AND DISCUSSION

DMA Content. The samples of refrigerated red hake used in this study were found to contain from 1 to 12 mg of N-DMA/100 g of white muscle. The samples of fish purchased at the supermarket had higher levels of DMA, indicating longer refrigeration times. The DMA content in frozen fish samples ranged from 5 to 84 mg of N-DMA/100 g of fish after 2 and 554 days of frozen storage in domestic freezers, respectively. These data are in good agreement with those published by Gill et al. (1979).

Spectral Changes during Frozen Storage. Figure 1 illustrates the ATR spectra of one refrigerated and two frozen−thawed samples of minced white muscle of red hake. The most evident differences between the spectra of unfrozen and frozen fish samples were found in the regions from 3800 to 2800 cm^{-1} and from 1700 to 800 cm^{-1}. An increase in absorbance in the region from 3800 to 2800 cm^{-1} might be associated with an increase in free water content in white muscle due to prolonged frozen storage. This is in accordance with the observations of Ciarlo et al. (1985) and Kelleher et al. (1981), who reported that the amount of free drip increases during frozen storage. Because of its high absorbance and noisiness, the water peak centered at 3300 cm^{-1} was not used in the analysis of the data. Nevertheless, the spectrum indicates that frozen storage of fish causes changes in the magnitude of water peaks. This in turn suggests that the changes in spectral data in the region from 3800 to 2800 cm^{-1} may be correlated to changes in the water-holding capacities of fish. More research is required to establish the existence of such a correlation. The difference spectra (Figure 2), obtained by the subtraction of frozen fish spectra from the spectrum of the refrigerated fish, show changes in absorbance in the region from 1800 to 800 cm^{-1}. The assignment of the absorbance peaks present in Figure

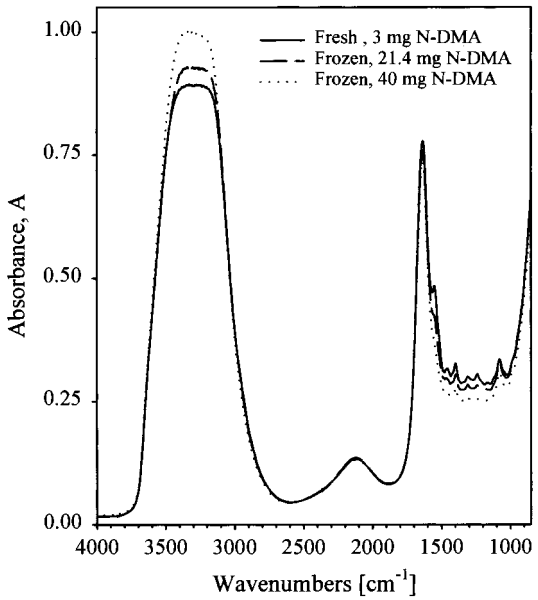

Figure 1. ATR FTIR spectra of refrigerated and frozen−thawed samples of minced red hake. DMA contents are indicated.

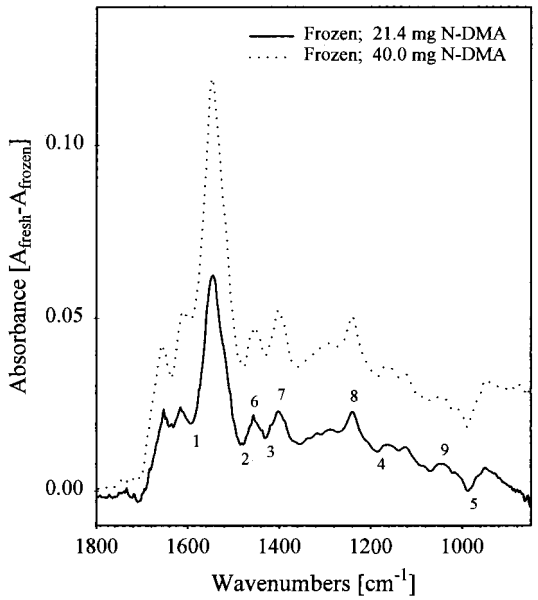

Figure 2. Difference spectra obtained by subtraction of the spectrum of frozen−thawed fish, A_{frozen}, from the spectrum of refrigerated minced red hake, A_{fresh}. Troughs labeled 1−5 are DMA absorption bands; peaks 6−9 are TMAO absorption bands.

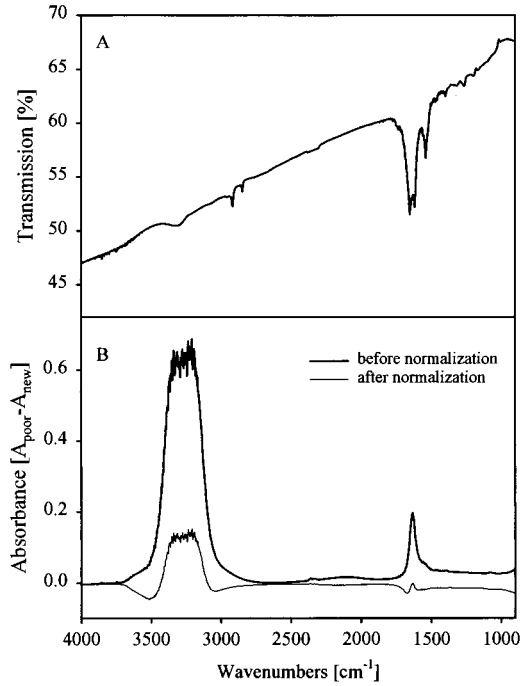

Figure 3. (A) Single-beam spectrum for a poor ATR crystal ratioed against that of a good ATR crystal and (B) difference spectrum of red hake (21.4 mg of N-DMA/100 g of fish) obtained by subtracting the spectrum recorded using a good ATR crystal, A_{new}, from the spectrum obtained using a poor ATR crystal, A_{poor}. The effect of normalization is shown.

2 will be discussed in the section describing the results of the analysis of spectral data by the PLS method.

Prolonged exposure of the ATR crystal to fish samples causes deterioration of the crystal surface as indicated by the reduction in the signal-to-noise ratio. This in turn may cause variability in the spectral data. The ATR crystal was arbitrarily called "poor" when the energy of the single-beam background spectrum was reduced by ~50%. Figure 3A shows the change as the transmittance spectrum. ATR crystals not classified as "poor" are referred to as "good". Figure 3B shows difference spectra of the same fish recorded using poor and good ATR crystals. The spectra were normalized by dividing the absorbances by the total areas under the spectra. This technique decreases the variability in the spectral data. To study the effect of the residual variability, the spectra for most samples were recorded using both poor and good ATR crystals. Both types of spectra were included in the mathematical analysis in all training (calibration) and test sets.

Discriminant Analysis of Spectral Data. Discriminant analysis of spectral data is a pattern-recognition procedure in which unknown samples are classified into groups based on similarities to the characteristics of the training group. This procedure consists of three steps. In the first step, the spectral data are subjected to principal component analysis (PCA). PCA highlights the maximum variations in a set of apparently similar spectra and reduces the representation of spectral data to a small set of well-defined numbers (factors) representing the major variations common to all spectra in the set (Fredericks et al., 1985). In step two, each spectrum is reconstructed by a linear combination of the PC scores, which are weighting factors, describing how much of each factor is needed to reconstruct the original spectrum. The reconstructed spectrum is subtracted from the original to obtain the spectral residual. In the third step, the PC scores and the sum of the squared spectral residuals are used in a Mahalanobis group matrix calculation to obtain a normalized Mahalanobis distance space. This distance is then used to classify the unknown spectra (Mark and Tunnel, 1985).

Table 1. Classification of Hake Samples and Spectral Database for Discriminant Analysis

class/ group	DMA content (mg of N-DMA/100 g)	training set: no. of samples	training set: no. of spectra	validation set[a]
A[b]	≤12.0	8	41	21
B[c]	≤25	11	47	49
C[c]	>25, ≤40	10	44	28
D[c]	>40	8	35	16

[a] No. of samples = no. of averaged spectra. [b] Unfrozen hake. [c] Frozen hake.

Table 2. Percentage of Samples Correctly Classified into Groups A−D, Predicted by the Discriminant and PLS Models, with Optimal Factor Numbers in Parentheses

class/ group	discriminant analysis	PLS	class/ group	discriminant analysis	PLS
A	86.0 (9)[a]		C	89.3 (8)[c]	72 (10)[b]
A + B		90 (10)[b]	D	81.3 (5)[c]	94 (10)[b]
B	93.8 (4)[c]				

[a] Factor selected on the basis of total percent variance. [b] Factor selected on the basis of PRESS. [c] Factor selected on the basis of *F* test on eigen values.

The data pretreatment consisted of ATR correction, path length correction based on the ATR corrected area measurement, and mean centering (*PLSplus/IQ Manual*, 1996).

To develop the discriminative calibration model, the samples were classified into four training sets (groups). All unfrozen fish samples were placed into group A. The frozen fish samples were divided into groups B−D based on the arbitrarily chosen DMA content ranges (Table 1) to test the suitability of FTIR for the classification of frozen fish. Each training set of ~40 spectra (see Table 1) comprised all of the spectra collected for a given sample, to include spectral variability. The optimal number of factors that were responsible for most of the variations in each training set of spectra were determined either by the minimum of total (percent) variance or by using the *F* test on eigenvalues (*PLSplus/IQ Manual*, 1996) and are listed in Table 2 in parentheses.

The calibration model was validated using a test set of 114 samples (see Table 1). In the validation procedure all of the spectra taken of a given sample were averaged to ensure that the test spectrum is truly representative of that sample. Spectra with a Mahalanobis distance of ≤3.0 (*PLSplus/IQ Manual*, 1996) were classified as belonging to a group defined by the training set. The validation set spectra were discriminated using the optimal number of factors listed in Table 2. The results of the validation test were analyzed stepwise as follows. First, the total number of spectra classified as A was determined. Then, the total number of spectra classified as B was identified in the non-A group of spectra. Next, the total number of spectra classified as C in the non-B group of spectra and then the total number of spectra classified as D in the non-C group of spectra were obtained. The remaining spectra were considered as unclassifiable. Table 2 lists the results of the validation test. The correctly classified samples are expressed as percentage of the total number

Table 3. Statistics of the Cross-Validation and Validation Predictions of the PLS Model

test	no. of samples	DMA range[a]	mean DMA content[a]	SD[b]	r^2 [c]	SEP[d]	RPD[e]
cross-validation	36	1.0−83.0	28.5	20.2	0.955	4.36	4.75
validation	54	2.5−78.0	33.4	18.7	0.942	4.50	4.16

[a] Milligrams of N-DMA per 100 g of fish. [b] SD, standard deviation. [c] r^2, squared correlation coefficient. [d] SEP, standard error of performance. [e] RPD, ratio of standard deviation of data to standard error of performance, SD/SEP.

of samples in the specific groups; 2.6% of the spectra belonging to the validation set were considered to be unclassifiable by the calibration model. The percentage of samples correctly classified into all four groups ranged from 81.3% (group D) to 93.9% (group B) (Table 2). The deterioration of fish is a gradual process, so it is impossible to achieve a clear separation between adjacent groups. Accordingly, some samples of adjacent groups, having a borderline quality, may be classified incorrectly. The data shown in Table 2 also indicate that the combination of optimal factors for groups A and B differentiates frozen and unfrozen fish samples. These results are similar to those reported by Thyholt and Isaksson (1997), who differentiated frozen and unfrozen beef by using near-IR spectroscopy.

Analysis of Spectral Data by the PLS Method. A multivariate calibration model was developed using the PLS method. This procedure (Beebe and Kowalski, 1987; van de Voort, 1992) establishes a correlation between the spectral data and DMA content. The database consisted of 90 samples that were randomly divided between a calibration set of 36 and a validation set of 54, so the whole DMA range was well represented in each sample set (Table 3). Each spectrum in the set was an average of three to six spectra taken per sample. The data pretreatment consisted of path length correction based on the area measurements and mean centering (*PLSplus/IQ Manual*, 1996). The spectral region 1600−890 cm^{-1} was used to develop and then to cross-validate and validate the PLS calibration model. The optimum number of factors was established on the basis of prediction residual error sum of squares (PRESS) (Haaland and Thomas, 1988) and was found to be 10. In the cross-validation one sample was rotated out in each prediction. The results of the cross-validation prediction are plotted in Figure 4 in terms of predicted versus actual DMA content for the 36 calibration set samples. The PLS model was then validated using a test set consisting of 54 spectra. The DMA concentrations predicted by this model are plotted against the corresponding actual values in Figure 5.

The statistics of both the cross-validation and the validation tests, expressed as the squared coefficient of correlation (r^2) between the spectral and chemical values, and the standard error of performance (SEP) are similar (Table 3). This indicates that the PLS model developed in this study is adequate for the prediction of DMA content in red hake. According to Williams (1995), a model is considered to be good if it yields an RPD (ratio of the standard deviation of data to the standard error of performance) value in the range of 5−7 and is considered to be fair and suitable for screening if the RPD value lies in the range of 3−5. The calculated RPD values (Table 3) indicate that the method that we have developed is suitable for fish quality assessment. The SEP values show that poor- and good-quality hake can be differentiated, although the borderline DMA value as a criterion will always be arbitrary.

For practical applications it is useful to classify fish samples, on the basis of the PLS model predictions, into

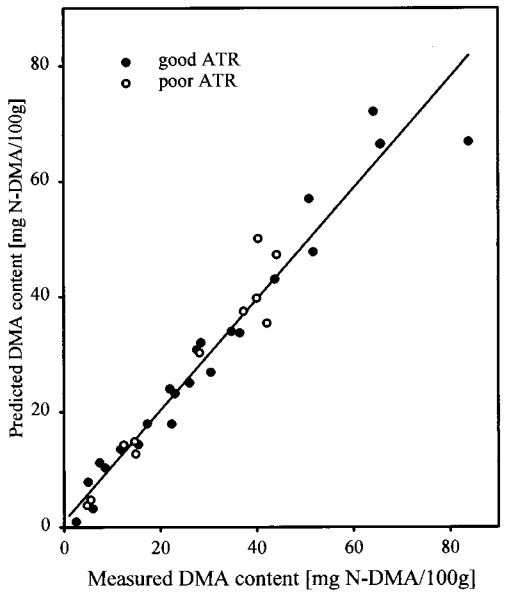

Figure 4. Plot of DMA content predicted by the PLS model versus chemically determined DMA content as shown by cross-validation results. Equation of the line: predicted DMA = 1.04 + 0.96 × (measured DMA).

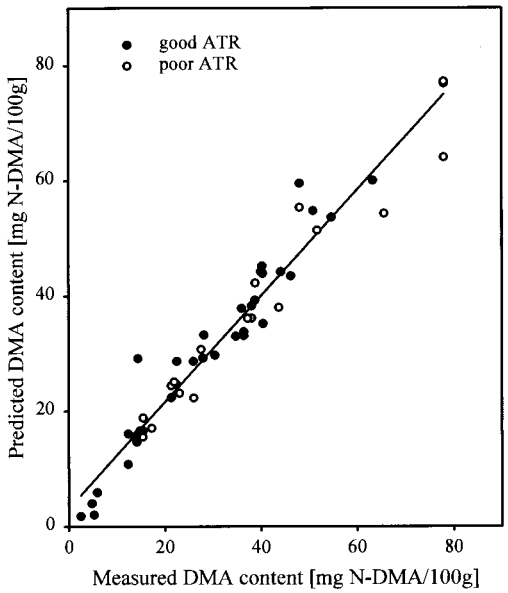

Figure 5. Plot of DMA content predicted by the PLS model versus chemically determined DMA content as shown by validation results. Equation of the line: predicted DMA = 3.14 + 0.92 × (measured DMA).

quality groups (see Table 1). Table 2 shows the results of classification of fish into groups A + B, C, and D based on the predicted DMA contents. The samples belonging to groups A and B cannot be separated by this model due to overlapping ranges of DMA contents. It should be emphasized that even a small prediction error may cause misclassification in a borderline case. The results of classifying fish samples using the PLS model are comparable to those obtained by the discriminant model.

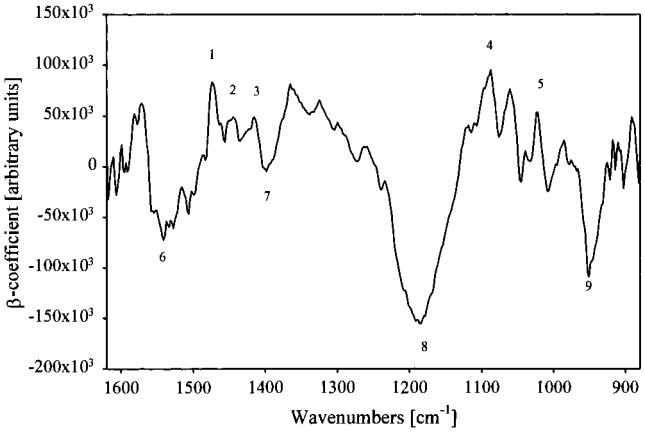

Figure 6. β-Coefficient spectrum of factor 10 obtained by the PLS method. Peaks labeled 1−5 are DMA absorption bands; peaks labeled 6−9 are TMAO absorption bands.

Table 4. Absorbance Peaks of an Aqueous DMA Solution, Positive Correlation Peaks in the β-Coefficient Spectrum, and Troughs in the Difference Spectrum

DMA solution[a]		spectra	
cm^{-1}	peak type	β-coefficient[b] (cm^{-1})	difference[c] (cm^{-1})
1600	s, br	1600, br	1600
1450	s	1450	1436, v br
1420	m	1420	
1169	m		1169
1115	s	1090	1130
1024	s	1024	1024

[a] Aqueous DMA·HCl, cited from Pouchert (1985). [b] Positive peaks in Figure 6. [c] Troughs in Figure 2.

Table 5. TMAO Absorbance Peaks, Negative Correlation Peaks in the β-Coefficient Spectrum, and Positive Peaks in the Difference Spectrum

TMAO		spectra	
in Nujol[a] (cm^{-1}), peak type	in water[b] (cm^{-1}), peak type	β-coefficient[c] (cm^{-1})	difference[d] (cm^{-1})
1470 (s)	1481 (s), 1465 (s)	1500, v br	1460
1402 (m)	1404 (m)	1400	1402
1240 (s)	1238 (s)	1190, v br	1240
950 (s)	950 (s)	950, v br	950

[a] Cited from Pouchert (1895). [b] Our results, TMAO from Sigma (T-0514). [c] Negative peaks in Figure 6. [d] Positive peaks in Figure 2.

Figure 6 shows the β-coefficient (Haaland and Thomas, 1988) spectrum obtained using the PLSplus/IQ software. This spectrum shows the wavelengths at which DMA is positively and negatively correlated with factor 10. The range 1600−890 cm^{-1} encompasses all of the significant absorbance peaks characterizing DMA and TMAO. The DMA bands occur as positive peaks in the β-coefficient spectrum and as troughs in the difference spectrum (Figure 2). Table 4 lists the peaks of aqueous DMA solution (Pouchert, 1985) along with the corresponding absorptions in the β-coefficient and subtraction spectra. The TMAO peaks are negatively correlated with DMA; hence, they correspond to negative peaks in the β-coefficient spectrum (Table 5). They appear as positive peaks in the difference spectrum (Figure 2), because the TMAO content of fresh hake is higher than that of frozen hake. The TMAO peaks listed in Table 5 are from a spectrum taken in Nujol (Pouchert, 1985) and in aqueous solution.

CONCLUSIONS

The results of this study indicate that FTIR spectroscopy can be used to differentiate frozen and unfrozen minced white muscle of red hake using discriminant analysis. The classification of frozen hake into various quality categories was also possible with ∼90% success using either discriminant analysis or the PLS method. Our results also indicate that the deliberate lack of control of freezing storage conditions does not affect the ability of the models developed to classify fish. These findings are similar to those reported by LeBlanc et al. (1988), who found that the correlation between DMA content and texture was independent of freezing history.

The prediction of DMA content using the PLS method is fair and suitable for quality assessment. The best correlation was obtained in the 1600−890 cm^{-1} region, which contains all of the DMA and TMAO absorbance bands. The research should be expanded to (a) use minced whole fillets and other types of gadoid fish and (b) determine whether other chemical/physical quality indices could be correlated with the observed changes in the spectral characteristics of fish.

We have found that the classification of fish, based on discriminant or PLS analyses of spectral data, is as reliable as the lengthy chemical DMA analysis used for evaluating the quality of frozen fish. To obtain spectra for three samples of the same fish takes ∼22 min, with very simple sample preparation and cleaning up. In the case of white muscle of red hake three to five samples per fish were sufficient to obtain representative spectral data. The additional mathematical analysis, using the multivariate calibration models, takes only 5 min. In comparison, the chemical DMA analysis requires ∼3 h, including cleaning up, and generates toxic waste. Therefore, the FTIR methodology described here is potentially suitable for application in industrial laboratories.

In the next paper we shall discuss the results of our studies on the development of a robust near-IR method for industrial applications in on-line testing.

ACKNOWLEDGMENT

The technical assistance of Sheryl Boucher is gratefully acknowledged. We thank Dr. Henry Buijs (Bomem Inc., Quebec City), Dr. Tom Gill (Department of Food Science and Technology, Daltech, Halifax), Dr. Ashraf Ismail and Dr. Fred van de Voort (Department of Food Science and Agricultural Chemistry, McGill University, Montreal) for stimulating discussions.

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Received for review April 20, 1998. Revised manuscript received July 20, 1998. Accepted July 22, 1998. This work was carried out under a contract between the Atlantic Canada Opportunities Agency (ACOA) and St. Francis Xavier University (Project 2060-60-21543-1). M.N. thanks the Natural Sciences and Engineering Research Council of Canada for partial support of this research.

JF980395U