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

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The quality of frozen minced red hake was assessed by FTNIR spectroscopy in transmission mode. The region 1530−1866 nm was best correlated to dimethylamine (DMA) concentration, which is an accepted quality index for frozen gadoid fish. Partial least-squares (PLS) analysis showed that this technique predicts the DMA content sufficiently well for quality assurance (SD/SEP > 3). The PLS calibration was equally successful when five narrow spectral regions were chosen instead of the continuous spectrum, indicating that a cheap filter instrument may be developed for industrial use.

Keywords: *Fish quality; FTNIR hake; dimethylamine; PLS*

INTRODUCTION

In the last 10 years, improvements in instrumentation and data analysis led to a tremendous increase in the use of near-infrared (NIR) spectroscopy, because of the simplicity of sample preparation, rapidity of analysis and the nondestructive nature of the technique (Bokobza, 1998). This technique was used successfully, for example, to determine the protein and fat content in meat and fish (Isaksson et al., 1992; Sollid and Solberg, 1992), to assess physical and chemical characteristics of beef cuts (Mitsumoto et al., 1991), and to evaluate previous thermal treatment of meats (Chen and Marks, 1997; Ellekjaer and Isaksson, 1992). Measurements in the NIR transmittance mode allow for much greater sample thickness than is possible using mid-IR. This is particularly useful for making representative measurements on nonhomogeneous samples, e.g., fish.

In our previous paper (Pink et al., 1998), we referred to the published data correlating dimethylamine (DMA) accumulation with textural deterioration in frozen gadoid fish. DMA is formed, along with an equimolar amount of formaldehyde (FA), from trimethylamine oxide (TMAO) in the frozen state by the action of the enzyme, trimethylamine oxide demethylase, which is present in these fish (Sikorski et al., 1976; Regenstein et al., 1982). FA brings about protein denaturation, which can be detected spectroscopically. We demonstrated that mid-IR spectroscopy and chemometric treatment of the data can be used to predict DMA concentration sufficiently well for quality assessment of frozen minced red hake (Pink et al., 1998). In this paper, we shall evaluate the use of NIR for the same purpose.

MATERIALS AND METHODS

Sample Preparation. Fresh, iced red hake (*Urophycis chuss)* was purchased at the wharf or in a supermarket. All fish samples were acquired after an unknown time of resolution of rigor mortis. Sample preparation has been reported previously (Pink et al., 1998).

Spectral Measurements. The spectral analysis was carried out using a Nicolet Magna 750 FTIR spectrophotometer (Nicolet Instrument Inc., Madison, WI), equipped with a PbSe detector and quartz beam splitter. Measurements were made in the transmission mode, at a resolution of 8 cm^{-1}, using silica windows, in the spectral range of 1000−1876 nm. Although the spectral range of 1000−2500 nm is available on the instrument, the high absorbance water peak centered at 1927 nm limits sample thickness. The presence of sample inhomogeneities, on the other hand, necessitates thicker samples, which, in turn, reduce transmitted light intensity. Accordingly, we excluded the region 1877−2500 nm and thus were able to use about 0.9 mm thick samples. Slices of this thickness were cut from the frozen fish samples and were warmed to room temperature (10 min) prior to running the spectra. The Magna 750 is designed with a vertical sample presentation. To simplify getting representative spectra and to avoid drip loss during measurement, a new device was designed and built. This allows for horizontal sample presentation and rotates the sample holder, so that the average spectrum of a significantly larger area can be obtained. For each full circle of rotation 64 scans were collected to give one spectrum. Then the center of the rotation was shifted, so that a new area was recorded in the next rotation. A set of eight spectra, which reflect the variabilities in sample thickness, were collected for each sample.

Chemical Analysis. The DMA content of the fish, expressed as mg of DMA-N/100 g of fish, was determined as reported previously (Pink et al., 1998).

Mathematical Analyses. Spectral data were averaged using the Nicolet Omnic v. 1.2 software. WIN_IR v. 3.04 and its PLSplus/IQ v. 3.0 software (Galactic Industries Corp., Salem, NH) were used for measuring the area under the spectra and for the partial least-squares (PLS) analysis, respectively.

RESULTS AND DISCUSSION

DMA Content. The DMA-N content of the red hake samples used in this study has been reported previously (Pink et al., 1998).

Spectral Changes during Frozen Storage. Figure 1A shows the spectra of a refrigerated and of a thawed sample of minced white muscle of red hake after prolonged freezing. Peak broadening and increased absorbance upon frozen storage are evident.

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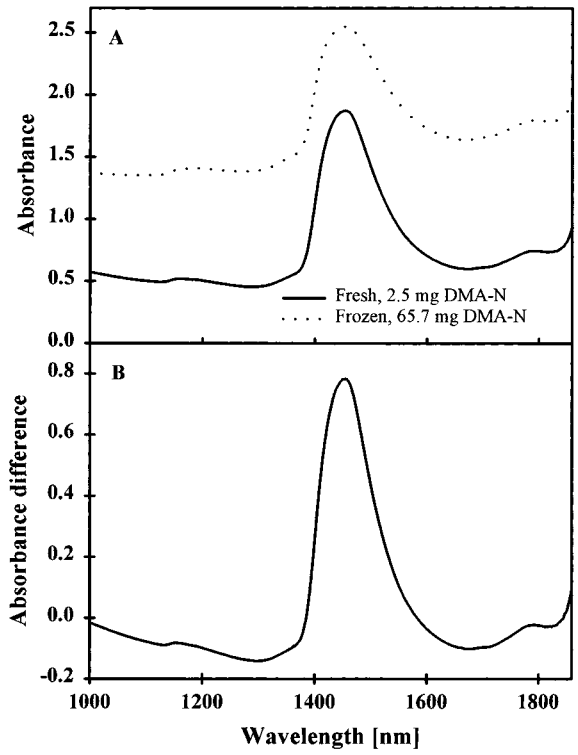

Figure 1. (A) Near-infrared spectra of a refrigerated and of a frozen-thawed sample of minced red hake. DMA-N contents are indicated. (B) Difference spectrum obtained by subtraction of the spectrum of frozen-thawed fish from that of the refrigerated. Absorbances were normalized to zero at 1000 nm.

Table 1. Statistics of DMA Concentration Predictions by PLS

	CS-PLS model[a]		SW-PLS model[b]	
	cross validation	validation	cross validation	validation
no. of samples	38	54	38	54
DMA-N range[c]	2.5−84.0	2.5−78.0	2.5−84.0	2.5−78.0
log DMA-N range[d]	0.398−1.924	0.398−1.892	0.398−1.924	0.398−1.89
mean log DMA-N[d]	1.374	1.273	1.374	1.273
SD[e]	0.400	0.472	0.400	0.472
r^2 [f]	0.939	0.947	0.927	0.916
SEP[g]	0.098	0.109	0.107	0.137
RPD[h]	4.08	4.33	3.74	3.44

[a] PLS model based on continuous spectrum, 1531−1866 nm range. [b] PLS model based on five selected wavelength regions. [c] mg/100 g fish. [d] log(mg/100 g fish). [e] SD standard deviation. [f] r^2 correlation coefficient squared. [g] SEP standard error of performance. [h] RPD ratio of standard deviation of data to standard error of performance, SD/SEP.

Discriminant Analysis. Discriminant analysis is a pattern-recognition procedure used to classify unknowns into groups based on similarities to the characteristics of the training group (Friedman, 1989). Spectral changes in the mid-IR region caused by frozen storage of minced hake allowed classification into various quality groups (Pink et al., 1998). In the near-IR, however, the changes in spectral pattern (as illustrated by the difference spectrum, Figure 1B) are not sufficient to make this method successful.

Analysis of Spectral Data by the Partial Least-Squares Method. The partial least-squares (PLS) method (Beebe and Kowalski, 1987; van de Voort, 1992) was used to develop a calibration model by establishing a correlation between the spectral data and a chemical variable, the DMA-N content. The database consisted of 92 samples that were randomly divided between a calibration set of 38 and a validation set of 54 samples, so that the whole DMA-N range was well represented in each set (Table 1). Each spectrum representing a sample was an average of all the spectra taken for that sample. Figure 2 illustrates a set of spectra recorded for a given sample.

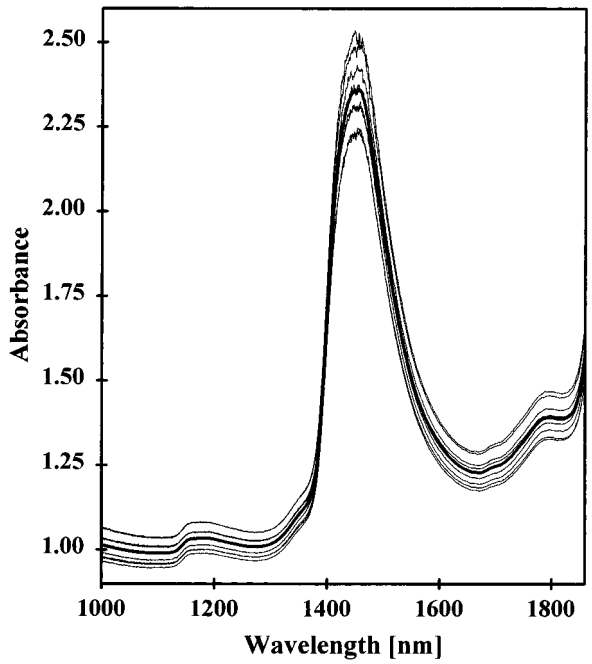

Figure 2. Typical set of eight spectra recorded per sample of fish, 64 scans each, resolution 8 cm^{-1}. The average spectrum is shown as a thicker line.

Various data pretreatments were evaluated. The best predictions were obtained by using mean centering (MC) and path length correction based on area measurements (PLSplus/IQ Manual, 1996). Poorer results were obtained by using derivatives, deconvolution, baseline correction, standard normal variate (SNV) with or without detrending (DT) transformation, and multiplicative scatter correction (MSC).

The best correlation with DMA-N content was found in the spectral region 1531−1866 nm. Using log DMA-N concentration instead of DMA-N concentration for calibrating gave much better results and was, therefore, selected. A similar transformation was also used by Chen and Marks (1997) to achieve the best correlation in evaluating previous heat treatment of chicken patties by near-IR spectroscopy. According to these authors, the optical changes due to protein denaturation caused by cooking are not linearly related to the measured chemical indices. Frozen storage of hake also denatures proteins (Gill et al., 1979; Haard, 1992), and our findings parallel those of Chen and Marks (1997).

The optimum number of factors was established on the basis of the PRESS criterion (prediction residual error sum of squares) (Haaland and Thomas, 1988) and was found to be 9. This PLS model, referred to as the continuous spectrum (CS-PLS) model, was tested by cross-validation in which one sample of the calibration set was rotated out in each prediction. The results of the cross-validation prediction are plotted in Figure 3 in terms of predicted versus measured log DMA-N content for the 38 calibration set samples. The model was

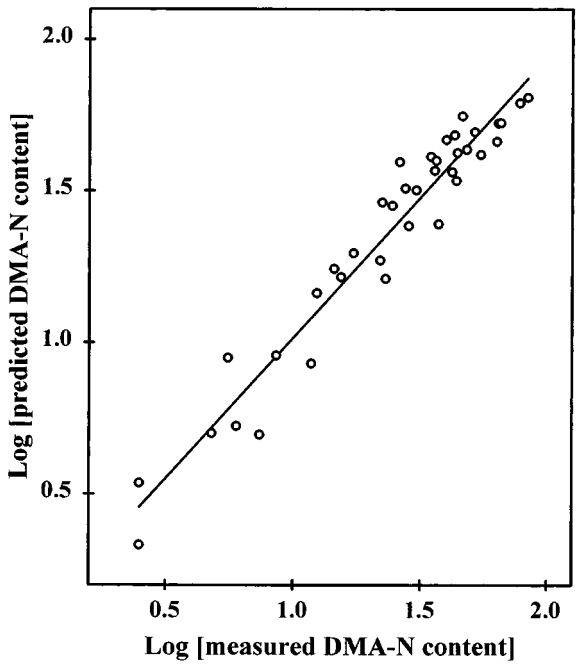

Figure 3. Plot of log DMA-N content predicted by the CS-PLS model versus chemically determined log DMA-N content. Cross validation results. Equation of the line: predicted log DMA-N = 0.087 + (0.928 × measured log DMA-N). DMA-N content: mg/100 g of fish.

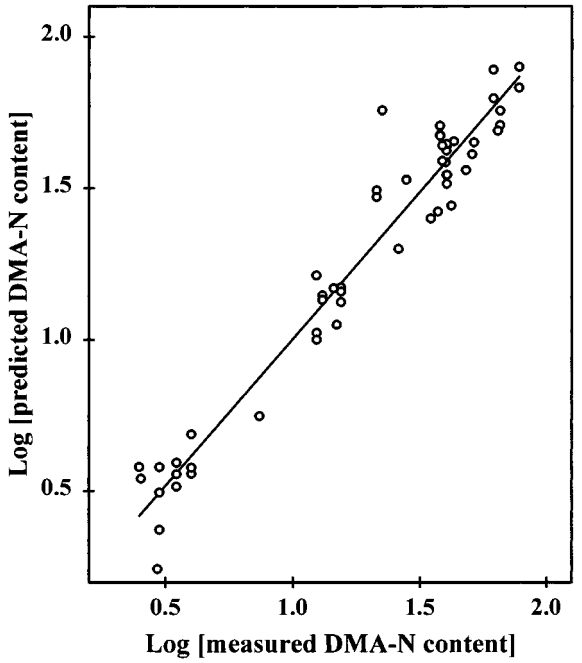

Figure 4. Plot of log DMA-N content predicted by the CS-PLS model vs chemically determined log DMA-N content. Validation results. Equation of the line: predicted log DMA = 0.032 + (0.979 × measured log DMA-N). DMA-N content: mg/100 g of fish.

validated using a test set consisting of 54 spectra. The log DMA-N concentrations predicted by the model are plotted against the corresponding actual values in Figure 4.

Figure 5A shows the β-coefficient (Haaland and Thomas, 1988) spectrum obtained using the PLSplus/IQ software. This spectrum shows the wavelengths at which log DMA-N is positively or negatively correlated at factor 9. In the mid-IR, we noted previously that the positive β-coefficient peaks corresponded to DMA absorbances and the negative ones to those of TMAO. In the near-IR, the DMA and TMAO peaks overlap more. However, we found that the most prominent peaks in the β-coefficient spectrum correspond to maxima and minima in the difference spectrum obtained by subtracting the NIR spectrum of TMAO from that of DMA (Figure 5B).

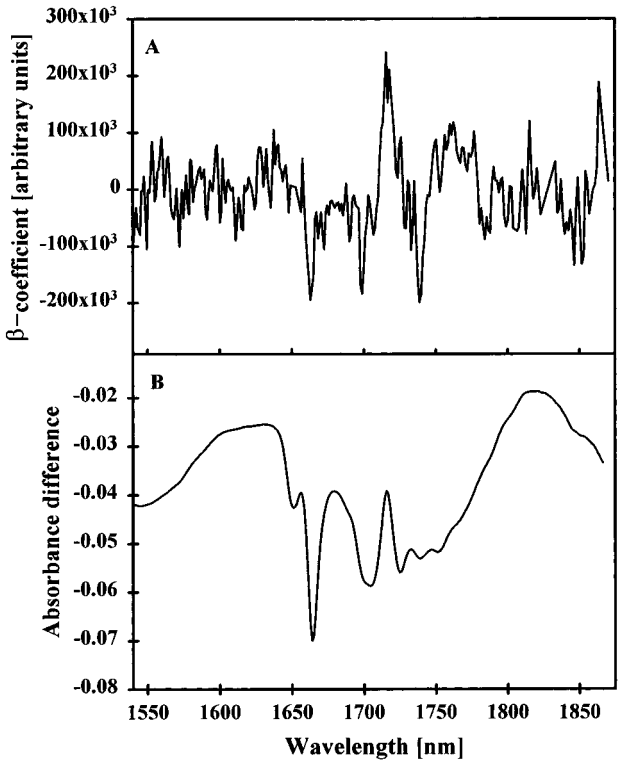

Figure 5. (A) β-Coefficient spectrum of factor 9 obtained by the CS-PLS method. (B) Difference spectrum obtained by subtracting the spectrum of aqueous TMAO from that of aqueous DMA.

Based on the β-coefficient spectrum, we selected five narrow spectral regions, namely, 1662−1666, 1697−1700, 1711−1721, 1736−1742, and 1862−1866 nm, for another PLS calibration. The first four of these regions correspond to peaks in the difference spectrum. The 1862−1866 nm region is also necessary to give a good calibration. Spectral changes in this region may be due to protein denaturation brought about by frozen storage. This PLS model (referred to as the selected wavelengths or SW-PLS) was developed, cross-validated, and validated using the same data set as described above for the CS-PLS calibration. Seven factors were found to be optimal in this case. The results of the cross-validation prediction are shown in Figure 6, while the validation predictions are plotted in Figure 7.

Table 1 summarizes the statistics of all of these predictions. Values of $r^2 > 0.9$ were found in all cases, and the ratios of standard deviation to standard error of performance, SD/SEP, indicate that both methods are suitable for quality assurance. Values of 2.5−3.0 are considered adequate for screening samples for quality, but values of at least 3−5 are required for quality assurance (Sinnaeve et al., 1997). The method is not as sensitive as the mid-IR range (Pink et al., 1998), but the SEP values show that poor and good quality hake can be easily differentiated. Our results compare very favorably with those published by Jorgensen and Jensen (1997), who used NIR to determine the water holding

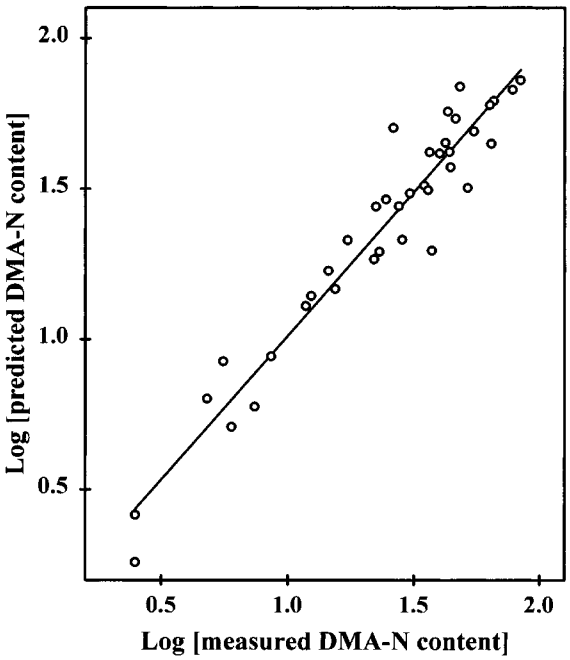

Figure 6. Plot of log DMA-N content predicted by the SW-PLS model versus chemically determined log DMA-N content. Cross validation results. Equation of the line: predicted log DMA-N = 0.0873 + (0.928 × measured log DMA-N). DMA-N content: mg/100 g of fish.

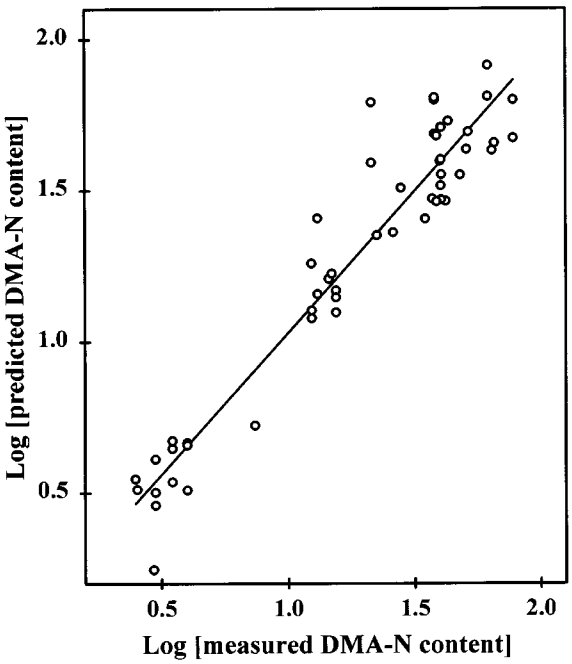

Figure 7. Plot of log DMA-N content predicted by the SW-PLS model versus chemically determined log DMA-N content. Validation results. Equation of the line: predicted log DMA-N = 0.093 + (0.936 × measured log DMA-N). DMA-N content: mg/100 g of fish.

capacity of minced frozen cod. Their value of $r^2 = 0.85$ is lower than ours, and their sample preparation requires centrifugation of the thawed mince.

CONCLUSIONS

The results of this study indicate that FTNIR spectroscopy can be used to determine quality attributes in frozen red hake. Although it is not as quantitative as the mid-IR region, it would be quite adequate for on-line screening. These results parallel our findings in the mid-IR region, i.e., that the deliberate lack of control of freezing storage conditions does not affect the ability of the technique to characterize frozen fish. The success of the selected wavelengths (SW-PLS) calibration suggests the possibility of developing a simple filter instrument, suitable for an industrial environment.

Future research should be carried out on minced whole fillets and other types of gadoid fish and to explore the performance of cheap, rugged NIR instruments.

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